Peroxidase activity develops in the micropylar endosperm of tomato seeds prior to radicle protrusion

Yukio Morohashi

Department of Regulatory Biology, Faculty of Science, Saitama University, Urawa, Saitama 338-8570, Japan

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Abstract

Peroxidase activity developed specifically in the micropylar region of the endosperm of imbibed tomato seeds prior to radicle emergence. The activity was first detected approximately 24 h after the start of imbibition (6 h before radicle emergence) and increased markedly thereafter. In the lateral portion of the endosperm, peroxidase activity was undetectable for the first 2 d after the start of imbibition. Although the activity in the lateral endosperm became detectable 3 d after imbibition, the extent of the development of the activity was slight. The localization of peroxidase activity in the micropylar endosperm 2 d after the start of imbibition was confirmed by tissue printing analyses. When the endosperm tissues were wounded, there was an enhancement of the enzyme activity at the wounded region. H$_2$O$_2$ was formed at the expense of NADH only in the presence of Mn$^{2+}$ and dinitrophenol by the extract from the micropylar endosperm in which peroxidase activity was present. The presence of H$_2$O$_2$ in the micropylar portion of the endosperm was shown histochemically. The possible functions of the peroxidases that develop in the endosperm of tomato seeds are discussed.

Key words: Endosperm, germination, hydrogen peroxide, peroxidase, tomato seed.

Introduction

It was reported in a previous paper that the activity of a basic $\beta$-1,3-glucanase develops markedly in the micropylar region of the endosperm of germinated tomato seeds (Morohashi and Matsushima, 2000). Wu et al. (2001) have recently shown by using a more sensitive assay method that enzyme activity is already expressed prior to radicle emergence. However, the physiological significance of the development of the enzyme in the micropylar tissues of germinating and germinated tomato seeds is not well understood at present. In tobacco seeds, Vogeli-Lange et al. (1994) and Leubner-Metzger et al. (1995) have shown that $\beta$-1,3-glucanase is induced in the micropylar part of the endosperm prior to radicle protrusion and have suggested that the enzyme contributes to the weakening of the cell walls of the micropylar endosperm by helping to hydrolyse cell wall $\beta$-1,3-glucans and thereby contributes to facilitating penetration of the radicle. However, substrates for $\beta$-1,3-glucanase do not seem to be present in the cell wall of tomato seeds (Wu et al., 2001). Therefore, it is unlikely with tomato seeds that the enzyme contributes to the weakening of the cell walls of the micropylar part of the endosperm.

When the radicle of tomato seeds penetrates the micropylar portion of the endosperm, that endosperm part is ruptured. The ruptured part will be an avenue for the invasion of pathogens. It is possible that some mechanism(s) is present for protecting the ruptured micropylar endosperm against pathogen entry. It is well known that $\beta$-1,3-glucanase is induced as part of the defence reaction of plants to pathogen attack (Simmons, 1994). It has been reported that $\beta$-1,3-glucanase is induced in response to wounding in plant tissues including tomato endosperm (Mauch et al., 1988; Derckel et al., 1998; Morohashi and Matsushima, 2000). Radicle penetration through the micropylar portion of the endosperm may accompany the breakdown of the cell wall, that is, wounding. The possibility, therefore, is suggested that $\beta$-1,3-glucanase accumulating in the micropylar endosperm may play a role in protecting tomato seeds from pathogen infection through ruptured endosperm tissues, as has been proposed previously (Petruzelli et al., 1999; Morohashi and Matsushima, 2000; Wu et al., 2001). It is known that
chitinases are induced coordinately with β-1,3-glucanases and act in synergy with β-1,3-glucanases in protecting plants from pathogen infection (Simmons, 1994). Interestingly, Wu et al. (2001) have shown that chitinase is expressed, together with β-1,3-glucanase, in the micropylar tissues of tomato seeds.

Many studies have shown that peroxidase is induced by wounding in plants (Desbiez and Boyer, 1981; Espelie et al., 1986; Svelheim and Robertsen, 1990; Kawaoka et al., 1994; Hiraga et al., 2000; Kato et al., 2000). Peroxidase is probably implicated in lignin and/or suberin formation during the polymerization of monolignols or aromatic monomers (Kolattukudy, 1981; Whetten et al., 1998). Lignification and suberization was believed to play a role in the defensive responses to the entry of pathogenic microorganisms through a wounded part by developing physical barriers (Espelie et al., 1986; Kolattukudy et al., 1992; Siegel, 1993; Quiroga et al., 2000). On the other hand, peroxidase has been reported to be involved in generation of H₂O₂ from NADH (Maeder et al., 1980; Maeder and Amberg-Fisher, 1982). There are quite a few studies reporting that H₂O₂ serves as an important factor controlling pathogen-resistance responses and programmed cell death in plants (Lamb and Dixon, 1997; Wu et al., 1997; Bestwick et al., 1998; Joseph et al., 1998; Grant and Loake, 2000; DeRafael et al., 2001). Taking these into consideration, it is of interest to know if peroxidase, like β-1,3-glucanase, is expressed specifically in the micropylar part of the endosperm of tomato seeds and whether it is induced by wounding in the endosperm. The pattern of the development of peroxidase activity in the endosperm of tomato seeds is reported here in connection with the possible physiological significance of the enzyme.

**Materials and methods**

**Plant material**

Tomato (*Lycopersicon esculentum* [L.] Mill. cv. First Up) seeds, a kind gift from Sakata Seed Corp. (Yokohama, Japan), were used. For germination, they were placed on wet filter paper in Petri dishes and incubated at 25 °C in the dark.

**Endosperm isolation**

A seed was transversally cut into halves to produce the micropylar half-seed and the lateral half-seed. After cutting, the embryo parts were carefully removed by pushing out using forceps. The de-embryonated micropylar half-seed and lateral half-seed were denoted as micropylar endosperm half and lateral endosperm half, respectively, although they contained part of the testa. In some cases, the micropylar seed tip was excised from seeds as previously described (Nonogaki et al., 1992) and the embryo parts were pushed out with forceps. The de-embryonated micropylar seed tip was referred to as the micropylar endosperm. A wound was inflicted on the endosperm by cutting the tissue. One-day-imbibed seeds were transversally cut in the middle and the lateral half-seed was incubated on wet filter paper at 25 °C in the dark. After incubation for 1 d, embryo parts were removed from the wounded half-seed and the de-embryonated lateral half-seed (wounded lateral endosperm) was subjected to analyses for peroxidase activities and isoforms.

**Enzyme extraction and assay**

Endosperm parts (30 endosperm halves or 60 micropylar endosperms) were homogenized in 0.8–1.2 ml of 1/3× McIlvaine buffer (0.1 M citric acid–0.2 M disodium phosphate; pH 6) containing 1 M NaCl in a chilled mortar and pestle. The homogenate was centrifuged at 10 000 g for 2 min. The supernatant was used for enzyme assays. Peroxidase activity was assayed in 6 mM guaiacol, 4 mM H₂O₂ and 1/3× McIlvaine buffer (pH 6.8). The formation of tetraguaiacol was followed by recording the absorbance at 470 nm. One unit of the enzyme activity represents the activity catalysing the formation of 0.01 μmol tetraguaiacol min⁻¹. NADH oxidation was monitored by recording the absorbance at 340 nm in 50 mM Na-acetate buffer (pH 4.8), 0.15 mM NADH, 1 mM MnCl₂ and 1 mM 2,4-dichlorophenol (DCP).

**Isoelectric focusing**

Endosperm extracts were dialysed against 1/10× McIlvaine buffer (pH 6) and were subjected to flat bed isoelectric focusing (IEF) on precast polyacrylamide gels in the pH range 3.5–9.5 (Ampholine PAG plate; Pharmacia Biotech) according to the manufacturer’s instructions. The peroxidase isozymes were visualized by soaking the gels in 20 mM Na-phosphate buffer (pH 6.8) containing 4-chloro-1-naphthol (0.6 mg ml⁻¹) and 0.18% H₂O₂, according to Lagrimini and Rothstein (1987).

**Tissue printing**

Seeds imbibed for 1 d, seeds of 2-d-old seedlings and wounded lateral half-seeds were laterally cut into halves and the embryo parts were removed using forceps. De-embryonated seed parts were immediately laid with the cut surface down on the polyacrylamide...
Table 1. Peroxidase activities in the endosperm of tomato seeds

(A) The micropylar endosperm and the section contiguous to it was excised from seeds of 2-d-old or 3-d-old seedlings and peroxidase activities determined (see the text for details). (B) Seeds imbibed for 1 d were transversally cut in the middle and the lateral half-seed was incubated. After incubation for 1 d, the embryo parts were removed from the wounded half-seed and the resultant wounded lateral endosperm was transversally halved in the middle, and peroxidase activity in each section was determined (see the text for details). Shown is the mean of, and difference between (in parenthesis), two replicates. ND, not detectable.

<table>
<thead>
<tr>
<th>Activity (units part−1)</th>
<th>(A) Seeds of 2-d-old seedlings</th>
<th>(B) Section containing wounded region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micropylar endosperm</td>
<td>Section contiguous to the micropylar endosperm</td>
</tr>
<tr>
<td></td>
<td>2.51 (0.14)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Seeds of 3-d-old seedlings</td>
<td>Section contiguous to the micropylar endosperm</td>
</tr>
<tr>
<td></td>
<td>3.23 (0.27)</td>
<td>0.10 (0.07)</td>
</tr>
<tr>
<td></td>
<td>Micropylar endosperm</td>
<td>Section away from the wounded region</td>
</tr>
<tr>
<td></td>
<td>6.03 (0.16)</td>
<td>ND</td>
</tr>
</tbody>
</table>

Effect of wounding on development of peroxidase activity

The effect of wounding on the development of peroxidase activity in the endosperm was studied by cutting the tissue (see Materials and methods). After 1 d of incubation, high activity of peroxidase was found in the wounded lateral endosperm (1.03±0.16 units part−1), while the activity was undetectable in the lateral endosperm half of intact seeds at the corresponding stage (2-d-old seedlings) (Fig. 1). This indicates that peroxidase activity is induced by wounding in the endosperm of tomato seeds. To study in which part of the wounded lateral endosperm the activity developed, the wounded lateral endosperm was transversally cut into halves to produce the section containing the wounded surface and the section away from the surface, and the activity in each section was determined. As seen from Table 1B, the activity was detected in the wounded region, but not in the part away from it. This indicates that the site where the peroxidase activity is induced by wounding is limited to the injured part and that the wounding effect is not transmitted to other parts away from the wounded region. This was confirmed by tissue printing analyses of the localization of peroxidase activity (see below).

NADH oxidation by the endosperm extract

The time-course of NADH oxidation by the extract from the micropylar endosperm of seeds of 2-d-old seedlings is shown in Fig. 2. The reaction was carried out in the presence of Mn2+ and DCP, because it has been shown that these cofactors enhance the peroxidase-catalysed oxidation of NADH (Halliwell, 1978). Requirement of these two factors for NADH oxidation by the endosperm extract was also confirmed in the present study; only slight oxidation of NADH occurred in the absence of either factor (data not shown). The oxidation of NADH was non-linear, and the rate was gradually accelerated with reaction time. This reaction pattern was similar to that reported with tobacco peroxidases (Maeder and Amberg-Fisher, 1982). H2O2 formation during NADH oxidation was monitored by
Adding guaiacol to the reaction mixture at the end of the reaction of NADH oxidation and by following the change in absorbance at 470 nm. As seen from Fig. 2, guaiacol, added after NADH oxidation, was oxidized. When the reaction mixture for NADH oxidation was preincubated in the absence of NADH for 5 min and then guaiacol was added, no oxidation of guaiacol was observed (data not shown). Thus H2O2-formation was dependent on NADH oxidation.

Localization of peroxidase activity

As shown above, peroxidase activity was present exclusively in the micropylar endosperm of tomato seeds during the first 2 d after imbibition (Table 1A). To confirm this further, tissue printing experiments were done. In tissue prints of seeds imbibed for 3 h, no signal of the enzyme activity appeared (data not shown). On the other hand, in 1-d-imbibed seeds or seeds of 2-d-old seedlings, the signals of the activity were clearly detected specifically in the micropylar endosperm (Fig. 3A, 1, 2). These histochemical observations are in accord with the results obtained by assaying the activities spectrophotometrically (Table 1A).

In the wounded lateral endosperm, the activity was detected exclusively at the wounded (cut) region (Fig. 3A, 3, 4). This is in good agreement with the observation that peroxidase activity is induced by wounding and that the wound-effect is manifested only at the wounded part (Table 1B).

Presence of H2O2 in the micropylar endosperm

Seeds of 2-d-old seedlings were laterally halved and embryo parts were removed from the halved seeds. When the de-embryonated half-seed was incubated in the presence of TMB, a blue staining appeared in the micropylar endosperm (Fig. 4A). The staining was completely precluded in the presence of an H2O2 scavenger (KI, 10 mM) or an antioxidant (ascorbic acid, 1 mM) (Fig. 4B). Thus, it is clear that H2O2 accumulated in the micropylar endosperm. Since this method for H2O2 detection is based on the H2O2-dependent oxidation of TMB by peroxidases, the results do not necessarily exclude the possibility that H2O2 is also present in the regions other than the micropylar endosperm.

Fig. 2. Time-course of NADH oxidation (A340) in the presence of Mn2+ and DNP, and H2O2 reduction (tetruguaicol formation; A470) by the extract from the micropylar endosperm of seeds of 2-d-old seedlings. The arrow indicates the time when guaiacol was added. See the text for details.

Fig. 3. (A) Tissue prints for peroxidase activities of a seed imbibed for 1 d (1), a seed of a 2-d-old seedling (2) and wounded lateral endosperms (3, 4). Schematic drawings of prints are presented beside each photograph. Vertical bars show cut positions. The active regions are marked in black. (B) Schematic presentation of the structure of a tomato seed.
Many peroxidase isozymes of tomato endosperm were visualized on an IEF gel using 4-chloro-1-naphthol as the substrate, as shown in Fig. 5. The pattern of the isozymes in the micropylar endosperm changed with time after imbibition (Fig. 5A). A band at the origin could be due to an artefact as a result of the application of tissue extracts at this area of the gel, since such artefact bands appeared wherever the samples were applied (data not shown). A few minor isozymes with pI values between 5.0 (origin) and 3.5 were observed to be present, but the reproducibility of their appearance and of their separation on IEF gels was poor. These minor isozymes were not analysed in the present study. In the micropylar endosperm of imbibed seeds for 1 d, two isozymes with pI's lower than 3.5 were the most active (dots in Fig. 5A, lane 1). In addition to these, three isozymes (pI 7.1, 7.5 and 8.0) were noticeable. In the micropylar endosperm of seeds of 2-d-old seedlings, the activities of pI 7.1 and pI 8.0 isozymes were remarkably enhanced (Fig. 5A, lane 2). The isozyme with a pI of 7.5 that was detected in 1-d-imbibed seeds disappeared in the micropylar endosperm of seeds of 2-d-imbibed seedlings, and pI 9.4 isozyme newly appeared. In the micropylar endosperm of seeds of 3-d-old seedlings, basic isozymes (pI 9.1, 9.2 and 9.3) were markedly expressed (Fig. 5A, lane 3).

The isozyme pattern in the lateral endosperm was studied with seeds of 3-d-old seedlings. Before this stage, peroxidase isozymes in the lateral endosperm were difficult to analyse because of their low activities. The major isozymes detectable in the lateral endosperm of seeds of 3-d-old seedlings were those with pI <3.5, 4.7 and 7.1 (Fig. 5B, lane 1). This isozyme profile of the lateral endosperm was different from that of the micropylar endosperm of 1-d-imbibed seeds (Fig. 5A, lane 1); the notable differences are that the pI 7.5 isozyme present in the micropylar endosperm was not detectable in the lateral endosperm and that the pI 4.7 isozyme detected in the lateral endosperm was not present in the micropylar endosperm. Thus, the isozyme profiles observed at the time when peroxidases began to be synthesized in the endosperm were different between the micropylar portion (1 d after imbibition) and the lateral one (3 d after imbibition). In a previous paper (Nonogaki et al., 1998), it was shown that the processes of the biochemical activation of the endosperm after imbibition are qualitatively different between the micropylar region and the lateral one. The difference in peroxidase isozyme profiles between the two endosperm regions may reflect this situation.
Effect of wounding on the expression of peroxidase isozymes

In the wounded lateral endosperm, five peroxidase isozymes represented the majority of the peroxidase activity (Fig. 5B, lane 2); two isozymes with pIs lower than 3.5 and isozymes with pIs of 3.5, 7.1 and 7.9. Among them, the pI 3.5 and 7.9 isozymes were not detectable in intact lateral endosperm. These isozymes seemed to be newly induced in response to wounding in the lateral endosperm. Anionic peroxidases have been shown to be induced in response to wounding in several plant species (Espelie et al., 1986; Kolattukudy et al., 1992; Christensen et al., 1998; Hiraga et al., 2000). On the other hand, it has also been reported that cationic peroxidases are induced by wounding (Desbiez and Boyer, 1981; Lagrimini and Rothstein, 1987; Svelheim and Robertsen, 1990; Kawaoka et al., 1994; Smith et al., 1994; Hiraga et al., 2000; Quiroga et al., 2000).

Discussion

In tomato seeds, the embryo is surrounded by the endosperm and, when the radicle of germinating seeds penetrates the micropylar portion of the endosperm, that endosperm part is ruptured. Radicle penetration through the endosperm, therefore, results in the exposure of the inner tissues of the seed to environmental factors such as pathogenic micro-organisms; pathogens in the surrounding soil can easily enter into the inner tissues through the ruptured micropylar endosperm. There is the possibility that tomato seeds are endowed with some mechanism(s) for defence against such an invasion of pathogens. In this connection, the fact is suggestive that β-1,3-glucanase and chitinase are expressed specifically in the micropylar endosperm in germinating and germinated tomato seeds, in spite of the absence of their substrates in the endosperm tissue (Morohashi and Matsushima, 2000; Wu et al., 2001). Since these enzymes are well known to help plants defend against fungal infection (Simmons, 1994), the induction of these enzymes in the micropylar endosperm might be a defensive mechanism of tomato seeds during and following germination.

In the present study it was shown that peroxidase activity markedly developed in the micropylar endosperm, but not in the lateral endosperm, of germinating and germinated tomato seeds (Fig. 1; Table 1). This developmental pattern is very similar to that observed with β-1,3-glucanase and chitinase (Morohashi and Matsushima, 2000; Wu et al., 2001). There are quite a few reports showing that peroxidase is induced in response to wounding in plants (Espelie et al., 1986; Lagrimini and Rothstein, 1987; Kolattukudy et al., 1992; Siegel, 1993; Quiroga et al., 2000). The present study showed that peroxidase activity in tomato endosperm was greatly enhanced when the tissue was wounded (Table 1B; Fig. 3). Radicle penetration through the micropylar endosperm is accompanied by the rupture, or wounding, of the micropylar endosperm. It is possible that the induction of peroxidase activity in the micropylar endosperm is brought about by the radicle penetration of the tissue and is associated with defensive reactions. However, the endosperm rupturing (wounding) does not seem to be the direct cause of the induction of peroxidase activities in the micropylar endosperm, because peroxidase activity had already developed prior to radicle protrusion (Fig. 1). It has been shown that cell wall hydrolysis by endo-β-mannanase occurs in the micropylar endosperm prior to radicle protrusion, resulting in the endosperm weakening at the site of radicle penetration (Bewley, 1997). The degradation of the cell wall may have an effect similar to wounding. Therefore, the micropylar endosperm may be wounded in a sense during germination (prior to radicle emergence). This may be the reason why the peroxidase activity develops in the micropylar endosperm prior to radicle protrusion. On the other hand, the cell wall of the lateral endosperm cells is degraded after the completion of germination (Bewley, 1997). This may result in the enhancement of peroxidase activity in the lateral endosperm at rather later stages (Fig. 1). However, this view does not explain why peroxidase activity in the micropylar endosperm is much more strongly enhanced than that in the lateral endosperm.

It is noteworthy that the isozyme pattern was different between the intact and the wounded (cut) endosperm; pI 3.5 and 7.9 isozymes that were not detected in the intact endosperm were highly expressed in the response to wounding (Fig. 5B). This may indicate that the signal which triggers the induction of peroxidase in the intact endosperm is different from that resulting from wounding (cutting).

What is the function of peroxidases expressed in the micropylar endosperm of tomato seeds? It has been reported that there is an association between peroxidase activity in plant tissues and their resistance to pathogen infection (Hammerschmidt et al., 1982; Joseph et al., 1998). There is increasing evidence suggesting that the joint exudation of H2O2 and peroxidase plays an important role in the defence system of plants against pathogens (Scott-Craig et al., 1995; Bestwick et al., 1998; Schopfer et al., 2001). In plants, the increased production of the superoxide radicals and H2O2 is a common feature of defence responses to pathogen attack (Lamb and Dixon, 1997). H2O2 released into the apoplast is directly toxic to pathogens (Wu et al., 1997; Joseph et al., 1998; De Rafael et al., 2001). It is also suggested that, in addition to direct action on the growth of pathogenic microorganisms, H2O2 (and other active oxygen species) may induce complex programmed cell death at sites attacked by pathogens, the hypersensitive reaction (Grant and Loake, 2000; Fath et al., 2001).
Peroxidase activity in tomato seeds

2001). The presence of H$_2$O$_2$ in the micropylar endosperm was shown by the histochemical observation (Fig. 4). Taking this into consideration, the possibility can be suggested that peroxidases induced specifically in the micropylar endosperm play a critical role, along with H$_2$O$_2$, in protecting the exposed inner part of the endosperm against the invasion of pathogenic organisms.

As shown above (Fig. 2), tomato peroxidase catalyses the synthesis of H$_2$O$_2$ at the expense of NADH. However, there is no evidence that this oxidase activity is physiologically significant in the tissues. Recently, a model of pH-dependent generation of H$_2$O$_2$ by a cell-wall peroxidase has been proposed; although the reductant of the peroxidase in this model has not been identified, NADH is not likely to be the reductant (Bolwell et al., 1995; Wojtaszek, 1997). There are also reports indicating that H$_2$O$_2$ generation can be mediated by enzymes other than peroxidase (Bolwell and Wojtaszek, 1997; Frahry and Schopfer, 1998).

It is noteworthy that peroxidase activity develops in the micropylar endosperm of tomato seeds prior to radicle protrusion, i.e. prior to the exposure of the inner tissues to a hazardous environment. It has recently been shown that active oxygen species are released to the apoplast from embryos and aleurone layers of radish seeds prior to radicle emergence (Schopfer et al., 2001). It is, therefore, conceivable that peroxidase expression accompanied by H$_2$O$_2$ generation is carried out in anticipation of radicle penetration through the endosperm and gives the seed a pre-emptive protective mechanism for survival in hazardous environments. The fact that peroxidase activity begins to develop in germinating seeds without any environmental cues such as pathogen attack or wounding, may indicate that peroxidase expression may be a developmentally regulated phenomenon and a constitutive biochemical process in germinating seeds. It has recently been shown that roots of soybean, sunflower and maize seedlings are able to produce H$_2$O$_2$ in the absence of pathogen attack (Frahry and Schopfer, 1998).

The developmental stage (days 1 and 2) when germination completes and the radicle ruptures the endosperm, is thought to represent the time most sensitive for pathogen infection, as pointed out by Schopfer et al. (2001). At later stages, the upper part of the seedling (hypocotyl and cotyledon) appears above the soil and the seed part (endosperm remnants and testa) is shed from the seedling. However, peroxidase activity in the micropylar endosperm of tomato seeds continued to increase markedly until day 3 and reached a level more than 200-fold higher than that at day 1. Is it necessary for peroxidase activity to continue to increase in the micropylar endosperm until rather later stages? Furthermore, pH 9.1, 9.2 and 9.3 isozymes were newly expressed in the micropylar endosperm between days 2 and 3 (Fig. 5A). If they play a defensive role, why are they not expressed at earlier stages? It is difficult to explain the reason from the viewpoint of defensive responses why the pattern of peroxidase isozymes in the micropylar endosperm changes so markedly with time (Fig. 5A). These problems remain to be solved in future. It is necessary to isolate each isozyme, characterize it and identify its function.

Lignification or suberization is thought to be an important wound-healing mechanism (Espelie et al., 1986; Angelini et al., 1993). Peroxidase has been proposed to play a role in the oxidation of monoglinols prior to their polymerization during lignin formation, although other oxidases may also be involved (Whetten et al., 1998). Peroxidases are also suggested to be critical in the polymerization of aromatic monomers to suberin (Kolattukudy, 1981). Lignin formation in the endosperm was examined histochemically by the phloroglucinol–HCl method (Barcelo, 1998). No signal for the presence of lignin in the micropylar endosperm was obtained.

Further investigations are needed in order to elucidate the physiological roles of peroxidases that develop in the endosperm of germinating and germinated tomato seeds.

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