Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 65 (2004) 2181–2187
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

# Polyphenol oxidase expression in potato (*Solanum tuberosum*) tubers inhibited to sprouting by treatment with iodine atmosphere

Francesco Eolini [a], Alejandro Hochkoeppler [a], Andrea Credi [b], Antonio Gonzàlez Vara Y Rodríguez [a], Valeria Poggi [a,*]

[a] *Department of Industrial Chemistry, University of Bologna, Viale Risorgimento 4, I-40136 Bologna, Italy*
[b] *Modena Centro Prove Laboratories, Via Sallustio 78, I-41100 Modena, Italy*

Received 21 April 2004; received in revised form 9 June 2004
Available online 4 August 2004

**Abstract**

Iodine-saturated atmosphere was found to inhibit the sprouting of potato (*Solanum tuberosum* L.) tubers. The iodine concentration in tuber tissues increased as a function of exposure length, and the onset of inhibition of sprouting was found to depend on tubers genotype. During the time-course of the treatment, the transcription of polyphenol oxidases (EC 1.10.3.1 and EC 1.14.18.1) was undetectable in tuber peel, whereas in bud tissues featured an increase, followed by a decrease occurring simultaneously with the suppression of sprouting. The treatment of tubers with iodine strongly affected the expression of polyphenol oxidases at the transcriptional level. Polyphenol oxidase activity in buds poorly reflected the corresponding level of transcription; similarly, little differences were found among the enzyme isoforms expressed in buds as a function of length of exposure to iodine. These findings suggest that the induction of polyphenol oxidases mRNAs transcription could probe the inhibition of sprouting by iodine.
© 2004 Elsevier Ltd. All rights reserved.

*Keywords:* *Solanum tuberosum*; Solanaceae; Potato tuber; Enzyme expression; Polyphenol oxidase; Inhibition of sprouting; Iodine

## 1. Introduction

Sprouting is responsible for remarkable economic losses during potato storage: sprouted potatoes are subjected to high water evaporation and quality deterioration, which reduce both their weight and marketability. Accordingly, the inhibition of sprouting is crucial in potato industry, as it provides flexibility in storage or transport and extension of the marketing period. Since the storage at low temperatures causes starch degradation and sweetening of tubers (Ross and Davies, 1992; van Es and Hartmans, 1987a), chemical inhibitors are widely used to control sprouting. Recently, due to environmental and health safety concerns, the use of 1-methylethyl-3-chlorophenylcarbamate (CIPC), the most widely used sprout inhibitor, and of other chemicals (van Es and Hartmans, 1987b) have called under question, and alternative methods to inhibit sprouting are under study. In particular, the exposure of tubers of potato to iodine-saturated atmospheres was found to be effective in the suppression of sprouting. This outcome led recently to file an international patent application (Pifferi, 2001).

Sprouting occurs in potato tubers at the end of the rest period (also known as endodormancy), when exogenous conditions like temperature or photoperiod are favorable (Hemberg, 1985). The duration of the rest period and its termination are primarily regulated by plant hormones (Wiltshire and Cobb, 1996; Hemberg, 1985), but little is known about physiological pathways or metabolic factors involved in this transition. Moreover, the metabolic alterations connected to the inhibition of sprouting are scarcely understood. Knowledge of the

* Corresponding author. Tel.: +39-051-20-93670; fax: +39-051-20-93673.
*E-mail address:* poggi@ms.fci.unibo.it (V. Poggi).

0031-9422/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2004.07.002

processes occurring in potato tubers in which this physiological event is restrained could be useful in order to reveal the stages critical for commitment to sprouting.

Polyphenol oxidases (PPOs; EC 1.10.3.1 and EC 1.14.18.1) are metalloenzymes, which catalyze the oxygen-dependent oxidation of phenols to quinones. Quinones can polymerize and cross-link many cellular nucleophilic compounds through 1,4 addition mechanism: these secondary reactions lead to the formation of brown or black pigments, which are responsible for tissues alteration of different fruits and vegetables (Mayer, 1987). Since these reactions decrease the vulnerability of plants or plant organs, different authors have suggested a close connection between the expression of polyphenol oxidases and plant defense against wound and pathogens (Mayer, 1987; Li and Steffens, 2002; Stout et al., 1999; Thipyapong and Steffens, 1997; Bashan et al., 1987; Mayer and Harel, 1979). In particular, the induction of polyphenol oxidases in response to wounding was studied at the physiological level (Constabel et al., 1995), showing that the expression of these enzymes triggered by wounding is regulated via the octadecanoid signal trasduction pathway. This observation suggests that the physiological regulation of polyphenol oxidases expression is part of the plant defense system and is related to other metabolic pathways; moreover, it suggests that these enzymes are involved in the response of plants to different stressing conditions.

In the present work, the suppression of sprouting by means of exposure of potato tubers to iodine-saturated atmosphere was challenged. Contextually, a role for tuber polyphenol oxidases as a metabolic marker for sprouting inhibition was investigated, considering a potential unspecific damaging effect of iodine. In particular, the relationship between the transcription in potato buds of polyphenol oxidases genes and the damage of tissues upon treatment with iodine was investigated.

## 2. Results and discussion

### 2.1. Sprouting inhibition in tubers exposed to iodine atmosphere

The exposure of potatoes to iodine-saturated atmosphere induced inhibition of sprouting; nevertheless, multifaceted results were obtained. Effective treatment conditions were strongly affected by the particular genotype considered; furthermore, the iodine-treated tubers were stored at 5 °C (usual storage temperature) and at 18 °C to evaluate the persistence of sprouting inhibition independently of the conservation temperature.

Interestingly, three months after the treatment and upon storage at 5 °C, tubers featuring inhibited sprouting ability could be clearly identified (Fig. 1, cf. column a and column b, see arrows). These tubers can be maintained in vegetative stasis up to five months of storage at 5 °C (Fig. 1, column b).

Persistent sprouting inhibition was induced by iodine in tubers cv. Agata stored at both 5 and 18 °C after an 8 days-exposure to iodine-saturated atmosphere (Fig. 1). A different response to the treatment was found both in cv. Monalisa and in cv. Primura: sprouting was inhibited in tubers stored at 5 °C and exposed to iodine for 8 and 2 days, respectively (Table 1). Moreover, for these two cultivars sprouting was inhibited permanently at 18 °C in tubers exposed to iodine for 16 days (Table 1). Tubers cv. Innovator featured the capability to sprout if stored at 18 °C, although the exposure for 7 days was inhibitory when tubers were stored at 5 °C (Table 1). As expected, the inhibition of sprouting was found to be dependent on the length of tubers exposure to iodine atmosphere, but the wide differentiation in results obtained for different cultivars pointed out the importance of the genotypes under study. Nevertheless, all the tubers responsive to iodine treatment featured necrosis in buds but not in peel tissues.

### 2.2. Iodine concentration in potato tissues

Fig. 2 reports the concentration of iodine determined in whole tubers and in peel of potatoes cvs. Agata, Monalisa, Innovator and Primura ((a)–(d), respectively) exposed to iodine atmosphere for different time intervals. Some observations were common to all cultivars under study. In particular, the concentration of iodine

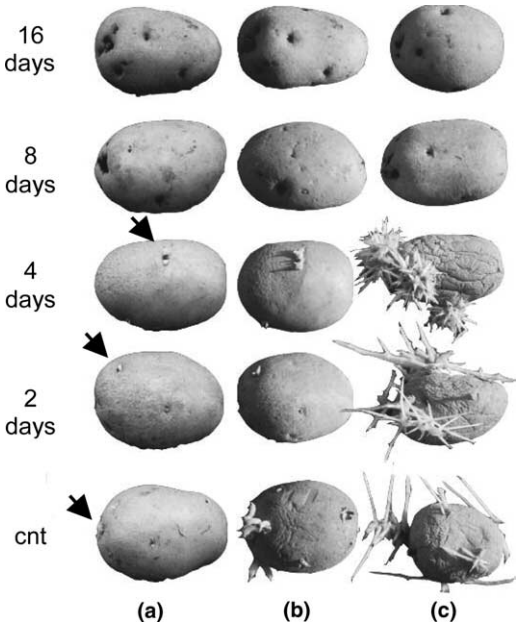


Fig. 1. Tubers of cv. Agata exposed to iodine atmosphere for different time intervals (as indicated, cnt represents the untreated control) stored for three months at 5 °C (a), for five months at 5 °C (b) or for five months at 18 °C (c).

Table 1
Sprouting in potato tubers exposed to iodine-saturated atmosphere after storage for five months at different temperatures

| | | Exposure time | | | | | | | | Control |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 h | 18 h | 1 d | 2 d | 4 d | 7 d | 8 d | 16 d | |
| Innovator | 5 °C | + | + | + | + | + | – | – | – | + |
| | 18 °C | + | + | + | + | + | + | + | + | + |
| Monalisa | 5 °C | + | + | + | + | + | + | – | – | + |
| | 18 °C | + | + | + | + | + | + | + | – | + |
| Primura | 5 °C | + | + | + | – | – | – | – | – | + |
| | 18 °C | + | + | + | + | + | + | + | – | + |

+ Tubers sprouted.
– Tubers not sprouted.

was always higher in peel than in the entire tuber; moreover, as expected, the concentration of iodine in both peel and tuber increased as a function of the exposure time in potatoes of each cultivar. Similarly, buds from tubers cvs. Monalisa and Primura (Fig. 2(b) and (d), respectively) featured a consistent increase of iodine concentration over the entire time-length of the treatment with iodine. However, variable trends were observed in different cultivars.

In cv. Agata (Fig. 2(a)) the sharpest increase in iodine concentration was observed after 4 days of exposure in peel, but only after an exposure of 8 days in the entire tuber. Similarly, in cv. Monalisa (Fig. 2(b)) a marked increase in iodine concentration was observed after 1–2 days of exposure in both peel and buds, but only after 7 days in tubers. These observations suggest that, in these genotypes, iodine absorbed by peel diffuses through tissues with a certain delay, but constantly. In cv. Innovator (Fig. 2(c)) iodine concentration increased consistently as a function of time in peel, but not in the entire tuber. Similarly, in cultivar Primura (Fig. 2(d)) a low increment of iodine in tuber tissues was observed, even when high iodine concentrations were determined in both peel and buds. In these genotypes, iodine diffusion could be hampered by specific structural characteristics of tuber tissues.

In general, no correlation was found between the inhibition of the sprouting and the increase, as a function of time, of iodine concentration in potato tissues, suggesting complex interactions between tuber metabolism and the iodine concentration in tissues. Iodine reached high concentrations in tubers in which the

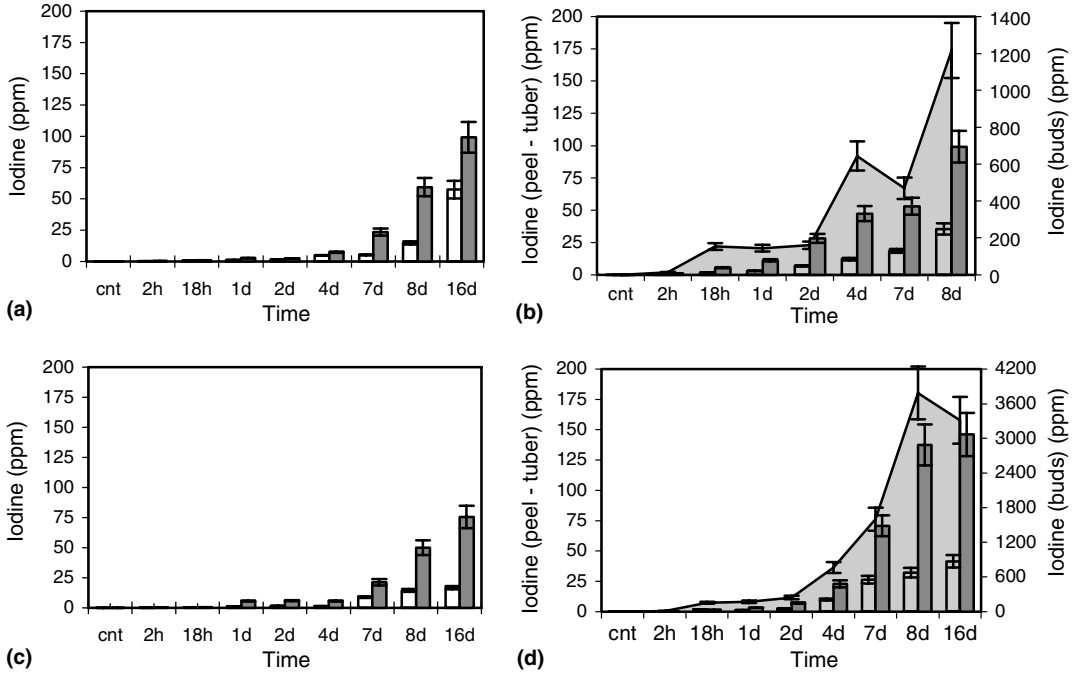


Fig. 2. Iodine concentrations in the entire tuber (empty bars) and in peel (filled bars) of potatoes cvs. Agata (a), Monalisa (b), Innovator (c), and Primura (d) as a function of exposure length to iodine atmosphere. Iodine concentrations in buds (light grey area) are reported for potatoes cvs. Monalisa (b) and Primura (d). Error bars represent standard deviation ($n = 3$).

sprouting was inhibited. Presumably, the iodine treatment could be modified in order to reduce the level of the iodine absorbed by tubers (e.g., the exposure could be performed in atmosphere saturated at different temperatures), until leveling off at values consistent with nutritional requirements.

### 2.3. Transcription of polyphenol oxidases in potato tissues

The transcription of PPOs coding genes in tissues of both buds and peel tissues was studied during the exposure of potato tubers to iodine-saturated atmospheres. The probe used was directed to a well-conserved portion of the gene coding for POT32, an isoform of polyphenol oxidase especially expressed in potato tubers, as described by Thygesen et al. (1995). Importantly, this probe features sufficient aspecificity to hybridize different transcripts of potato PPO, all characterized by similar molecular mass (Thygesen et al., 1995). In particular, 2 kb transcripts were observed in Northern blots performed with RNA extracts of buds tissues after hybridization with the biotinylated probe (Fig. 3(a)).

During the treatment with iodine, the polyphenol oxidases mRNAs were undetectable in tuber peel (Fig. 3(b)), whereas in buds a corresponding increase followed by a decrease was observed in every cultivar under study. Interestingly, no significant differences in PPOs mRNA levels were observed in buds subjected for 8 days to the same experimental conditions but not exposed to iodine (data not shown). Fig. 3(a) reports the levels of polyphenol oxidases mRNAs in buds from potato cv. Primura exposed for different time intervals to iodine atmosphere; the intensity of the bands corresponding to polyphenol oxidases transcripts features a transient increase (when no difference is observed among the corresponding levels of 18S ribosomal RNA). This trend is quantitatively reported in Fig. 4, which reports the levels of PPOs transcripts observed for all the cultivars under study.

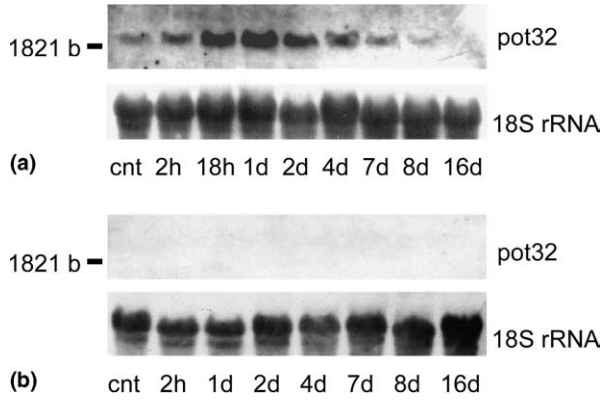


Fig. 3. Polyphenol oxidases mRNAs in extracts of buds and peel (upper panels (a) and (b), respectively) of tubers cv. Primura exposed to iodine-saturated atmosphere for different time intervals. The levels of the corresponding 18S rRNA are reported in the lower parts of each panel.

An increase in the transcription of polyphenol oxidases in tissues subjected to wound and injuries was observed by Thipyapong and Steffens (1997). In a more recent study, Li and Steffens (2002) substantiated a role of PPOs in plant self-defense, demonstrating that their overexpression in tomato reduces the development of bacterial diseases. The observation that iodine triggers the transcription of PPOs coding genes suggests that, in plants, the expression of polyphenol oxidases can be induced in response to a wide range of stressing conditions. In particular, confirming what reported by Constabel et al. (1995) for wound-inducible tomato leaf PPO, the findings of the present work suggest that the control of the expression of polyphenol oxidases in potatoes exposed to iodine atmospheres is exerted at the transcriptional level.

The maximum level of polyphenol oxidases mRNAs was observed in different cultivars after different time intervals of exposure to iodine. Interestingly, in each cultivar the peak of transcript(s) was reached immediately before the onset of inhibition of sprouting in tubers, during storage at 5 °C (see arrows in Fig. 4). This means that the commitment of iodine-treated tubers to incapability to sprout was characterized by a decrease of the transcription of PPOs coding genes and/or by an increase of mRNA degradation. The increase and subsequent decrease of PPOs transcripts in buds, induced directly by the treatment, could be an interesting marker of the inhibition of the sprouting in tubers of potato exposed to iodine. In particular, the characterization of a specific level of polyphenol oxidases expression correlated with sprouting inhibition could be used in order to identify effective treatments conditions. Moreover, it is interesting to note that the concentration of iodine in buds neither correlates with PPOs mRNA levels nor is diagnostic for inhibition of sprouting.

### 2.4. PPO activity and isoforms in potato buds

PPO activities determined in extracts of buds from different cultivars exposed to iodine-saturated atmosphere are reported in Fig. 4. In each cultivar, the trend observed for enzymatic activity seemed to reflect partially the corresponding fluctuations in polyphenol oxidases mRNAs. In particular, in cultivars Innovator (Fig. 4(c)) and Primura (Fig. 4(d)) the highest activity value was reached, during the treatment, few days after the maximum level in polyphenol oxidases mRNAs was attained. This observation suggests that transcription of gene(s) coding for PPO(s) is rapidly coupled to translational and post-translational processes. In cv. Agata (Fig. 4(a)) and in cv. Monalisa (Fig. 4(b)) a light correlation, if at all, could be observed between the level of polyphenol oxidases mRNAs and enzyme activity.

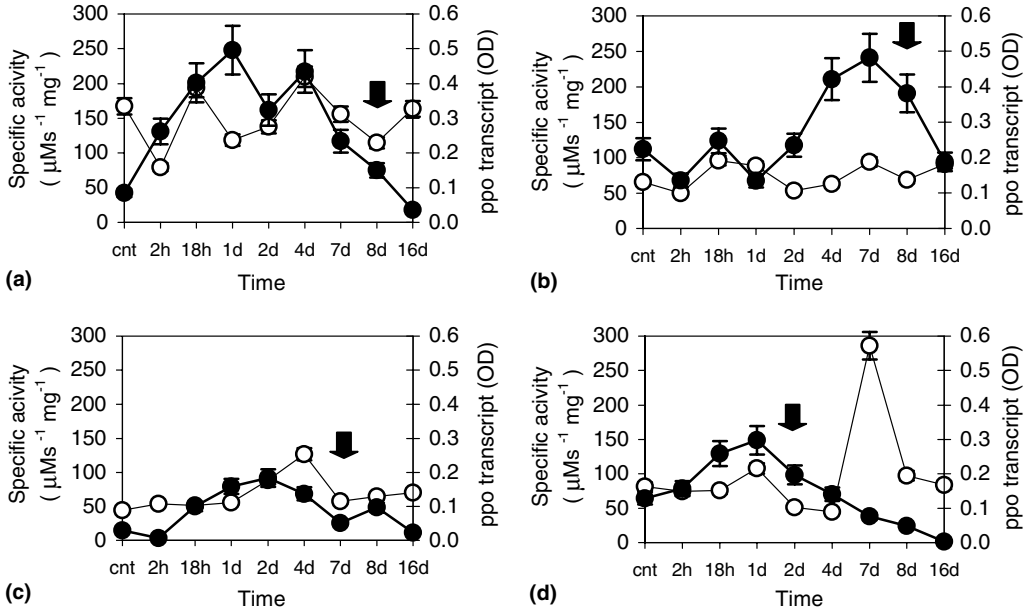


Fig. 4. Polyphenol oxidases mRNAs level (closed circles) and specific PPO activity (open circles) in bud extracts of tubers cvs. Agata (a), Monalisa (b), Innovator (c) and Primura (d), exposed to iodine-saturated atmosphere for different time intervals. The arrows indicate the minimal length of treatment effective in sprouting inhibition. Error bars represent standard deviation ($n = 3$).

Native electrophoresis stained for PPO activity and performed with extracts of buds from cultivar Agata and Monalisa are shown in Figs. 5(a) and (b), respectively. Similarly to the results obtained for cvs. Innovator and Primura (data not shown), the importance of the anodic forms of PPO decreased with the increase of the time exposure of tubers to iodine. However, this observation was not directly related to the reduction of the ability of tubers to sprout.

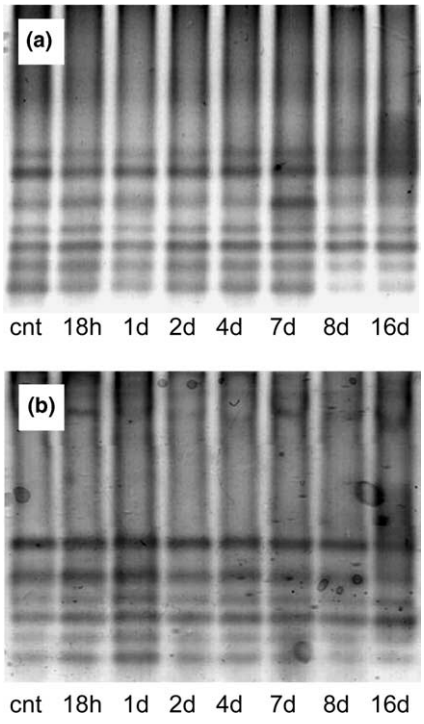


Fig. 5. Native electrophoresis, stained for PPO activity, of bud extracts, obtained from tubers cvs. Agata (a) and Monalisa (b) exposed to iodine-saturated atmosphere for different time intervals.

In conclusion our observations demonstrate that the transcription of PPOs coding genes is responsive to the treatment of potato tubers with iodine, and that the induced suppression of sprouting is concomitant with a transient increase of the level of polyphenol oxidases mRNAs. Unfortunately, this relationship does not generate a genotype-independent pattern of expression of polyphenol oxidases which can be simply related to the suppression of sprouting or, in other words, which can be easily used to probe the onset of sprouting inhibition. Nevertheless, the level of polyphenol oxidases mRNAs could be associated with other metabolic parameters which are, at present, under study to identify a biochemical pattern useful as a marker of sprouting inhibition.

## 3. Experimental

### 3.1. Materials

Potato (*Solanum tuberosum* L., cultivars Monalisa, Agata, Primura and Innovator) tubers were obtained from field cultivations and stored at 6–8 °C. Tubers from each cultivar (cv.), featuring buds inferior than 3 mm, were exposed to iodine-saturated atmosphere at 5 °C for 2, 18 h, or 1, 2, 4, 7, 8, 16 days. Tubers cv. Agata were subjected for up to 8 days to the same conditions

(wash, air-drying, storage in boxes), but avoiding exposure to iodine. Tissues from both buds and peel were immediately frozen in liquid nitrogen after sampling, and then preserved at −80 °C.

### 3.2. Protein extraction and determination

Buds were kept frozen in liquid nitrogen and powdered in mortar. Proteins were extracted anaerobically in 1:10 (w/v) 0.1 M sodium phosphate buffer, pH 6.8, containing 50 mg $ml^{-1}$ polyvinylpyrrolidone, 5 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride. The mixture was shaken for 2 h at 4 °C and the suspension was centrifuged at 14,000*g* for 10 min. All operations were conducted at 4 °C. Protein concentration was determined according to Bradford (Bradford, 1976), using bovine serum albumin as standard.

### 3.3. Enzyme assay

PPO activity was determined spectrophotometrically at 20 °C in triplicate. The reaction mixture consisted of 0.1 M sodium phosphate buffer, pH 6.8, 20 mM 3,4-dihydroxy L-phenylalanine (L-DOPA, Merck) and 50 µl of the sample. The increase in absorbance was measured in a 1 cm light path cuvette at 475 nm, in a final volume of 1 ml. PPO activity was calculated considering a molar extinction coefficient for dopaquinone of 3600 $M^{-1}$ $cm^{-1}$ (Boyer, 1977).

### 3.4. Native polyacrylamide gel electrophoresis (native PAGE)

Native electrophoresis was performed with PPO crude extracts (4 µg of protein was loaded for each sample) according to Laemmli (1970), using a Miniprotean II dual slab cell unit (Bio-Rad) and 10% polyacrylamide gels. Gels were stained for PPO activity in 0.1 M potassium phosphate buffer (pH 5.7) containing catechol and *p*-phenylenediamine (Yu et al., 1992) at the concentrations of 10 mM and 0.05% (w/v), respectively.

### 3.5. Northern blotting

Genomic DNA was extracted and purified from potatoes using the Nucleospin Plant kit (Macherey Nagel, Düren, Germany). A 1495 bp fragment of the gene *pot32* described by Thygesen et al. (1995) was amplified through PCR, using genomic DNA as template and the primers 5′CGCCTAAGCCTGCAGATATGGAG (for) and 5′CAGAGCAGGATCCATCAAAGTG (rev). After digestion with *Pst*I and *Bam*HI, the fragment was cloned into pUC19. A 562 nucleotides fragment of the pea 18S ribosomal RNA gene, cloned in pBluescript II KS (Mittler and Zilinskas, 1994), was gently provided by Pierre Golubinoff (Alexander Silberman Institute, Jerusalem, Israel).

Probes were prepared by PCR amplification of the described inserts in the presence of 0.15 mM biotin-$N_4$-dCPT (Detector PCR DNA Biotinylation Kit, KPL, Gaithersburg, MD, USA) and using universal primers.

Total RNA from frozen plant tissues was extracted and isolated using the Nucleospin RNA kit (Macherey-Nagel) and RAP (Macherey-Nagel) as extraction buffer. RNA was electrophoresed, transferred to Hybond N+ (Amersham Pharmacia Biotech, Sweden) and detected after hybridization of the biotinylated probes with a conjugate streptavidin-alkaline phosphatase, by using a DNA detector Northern Blotting Kit (KPL) and CDP-Star (KPL) as substrate.

### 3.6. Determination of iodine concentration in potato tissues

Either peel or the whole potato tuber was homogenized with Ultra-Turrax (IKA Werke GmbH & Co, Germany) in ultrapure water (Millipore); the same procedure was followed for buds from tubers cvs. Monalisa and Primura. The suspension was incubated in the dark at 40 °C for 30 min and filtered through a Millipore 0.45 µm membrane; the supernatant was then analyzed by ion chromatography using an amperometric detector (Dionex ED50, GS50 column AS11). Determination was carried out after calibration with standards at increasing KI concentration.

## Acknowledgments

This work was supported by the Consorzio delle buone idee (Bologna, Italy). We thank Paola Filippini for her skillful technical support and Barbara Clò for her assistance in iodine analysis.

## References

Bashan, Y., Okon, Y., Henis, Y., 1987. Peroxidase, polyphenoloxidase, and phenols in relation to resistance against *Pseudomonas syringae* pv. *tomato* in tomato plants. Can. J. Bot. 65, 366–372.

Boyer, R.F., 1977. A spectrophotometric assay of polyphenoloxidase activity. J. Chem. Educ. 54, 585–586.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Constabel, C.P., Bergey, D.R., Ryan, C., 1995. Systemin activates synthesis of wound-inducible tomato leaf polyphenol oxidase via the octadecanoid defense signaling pathway. Proc. Natl. Acad. Sci. USA 92, 407–411.

Hemberg, T., 1985. Potato rest. In: Li, P.H. (Ed.), Potato Physiology. Academic Press, London, pp. 353–388.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Li, L., Steffens, J.C., 2002. Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. Planta 215, 239–247.

Mayer, A.M., 1987. Polyphenol oxidases in plants – recent progress. Phytochemistry 26, 11–20.

Mayer, A.M., Harel, E., 1979. Polyphenol oxidase in plants. Phytochemistry 18, 193–215.

Mittler, R., Zilinskas, B., 1994. Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. Plant J. 5, 397–406.

Pifferi, P.G., 2001. Method for inhibiting the sprouting and for avoiding the disease proliferation in tubers, preferably potatoes. International Patent Application WO 02069719.

Ross, H.A., Davies, H.V., 1992. Sucrose metabolism in tubers of potato (*Solanum tuberosum* L.): effect of sink removal and sucrose flux on sucrose-degrading enzymes. Plant Physiol. 98, 287–293.

Stout, M.J., Fidantsef, A.L., Duffey, S.S., Bostock, R.M., 1999. Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. Physiol. Mol. Plant Pathol. 54, 115–130.

Thipyapong, P., Steffens, J.C., 1997. Tomato polyphenol oxidase (PPO): differential response of the PPO F promoter to injuries and wound signals. Plant Physiol. 115, 409–418.

Thygesen, P.W., Dry, I.B., Robinson, S.P., 1995. Polyphenol oxidase in potato. A multigene family that exhibits differential expression patterns. Plant Physiol. 109, 525–531.

van Es, A., Hartmans, K.J., 1987a. Dormancy, sprouting and sprout inhibition. Post-harvest behavior, store design, storage practice, handling. In: Rastovski, A., van Es, A. (Eds.), Storage of Potatoes. Pudoc, Wageningen, pp. 114–132.

van Es, A., Hartmans, K.J., 1987b. Starch and sugar during tuberization, storage and sprouting. Post-harvest behavior, store design, storage practice, handling. In: Rastovski, A., Es, A. (Eds.), Storage of Potatoes. Pudoc, Wageningen, pp. 79–113.

Wiltshire, J.J.J., Cobb, A.H., 1996. A review of the physiology of potato tuber dormancy. Ann. Appl. Biol. 129, 553–569.

Yu, H., Kowalski, S.P., Steffens, J.C., 1992. Comparison of polyphenol oxidase expression in glandular trichomes of *Solanum* and *Lycopersicon* species. Plant Physiol. 100, 1885–1890.