High-Performance Liquid Chromatography Determination of Phenolic Acids in Potato Tubers (*Solanum tuberosum*) during Wound Healing

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The content of phenolic acids formed during wound healing of γ-irradiated and nonirradiated potato tubers was determined by HPLC. Chlorogenic acid, caffeic acid, *p*-coumaric acid, and ferulic acid were detected in small quantities in resting whole tubers of irradiated and nonirradiated potatoes. During wound healing their content increased many fold, and in addition, the neo and crypto isomers of chlorogenic acid accumulated in the wound healing tissue. The increased formation of phenolics was accompanied by a parallel rise in phenylalanine ammonia-lyase activity. Chlorogenic acid contributed about 56% and together with the neo and crypto isomers accounted for 88% of the phenolics formed. Tubers irradiated to 100 Gy for sprout inhibition showed significantly lower levels of chlorogenic acid and its isomers during the first 8 days of wound healing. The results point to an impairment of wound-induced biosynthesis of phenolics in general and chlorogenic acid and its isomers in particular by irradiation.

INTRODUCTION

Phenolics are believed to be an important part of the general defense mechanism of many plants to infection (Kosuge, 1969; Friend, 1985) and have long been postulated to play a role in the resistance of potatoes to soft rot bacteria (Lovrekovich et al., 1967; Lyon, 1989). Potato tubers contain a small amount of free and conjugated phenolic acids, the major constituent being chlorogenic acid (Malmberg and Theander, 1984). A major factor limiting the storage of potato tubers under tropical conditions is bacterial soft rot. Apart from infection through the lenticels (Perombelon, 1973), wounds inflicted on the tubers during harvest, handling, and transport can provide easy entry to rot-producing pathogens. Losses due to disease and desiccation can be minimized by proper wound healing as soon as potatoes are harvested and placed in storage.

The wound healing process involves deposition of suberin, a lipid phenolic polymer (Mader, 1958; Dean and Kolattukudy, 1977) on cell layers below the wound surface followed by formation of wound periderm. γ irradiation at sprout-inhibiting dose levels (100 Gy) is known to prevent the formation of the wound periderm in potatoes (Penner, 1970; Thomas, 1982), although suberization is not affected (Thomas and Delincee, 1970). Increase in total phenolic constituents of potato tubers subjected to stress like γ irradiation or wounding has been reported (Pendharkar and Nair, 1987; Thomas and Delincee, 1970; Thomas, 1982; Mondy and Gosselin, 1989). However, very little information is available on the formation of individual phenolic constituents in potato tubers during wound healing. In our studies on the storage aspects of potato tubers subjected to γ irradiation for sprout inhibition, it was of interest to know the factors that contribute to the increased rotting tendency of irradiated tubers in response to mechanical or wound injury. As part of these studies the quantitative changes in individual phenolic acids of irradiated and nonirradiated tubers when subjected to wounding are reported in this paper.

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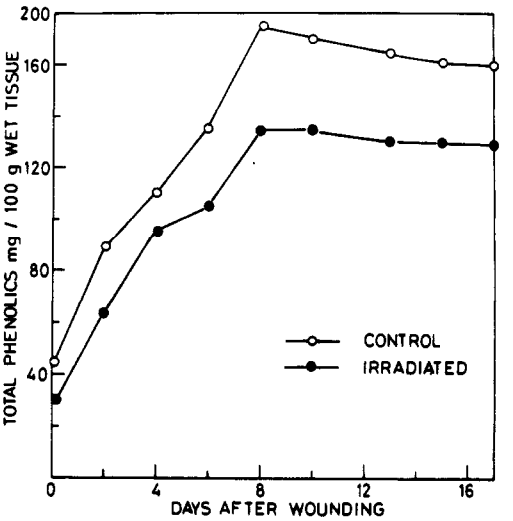

Figure 1. Changes in total phenolic acid content during wound healing of control and irradiated potato tubers.

MATERIALS AND METHODS

Mature potatoes of the Kufri Chandramukhi cultivar, 3–4 weeks old after harvest, were obtained from the local market and held at ambient conditions for 8–10 days for proper curing. Tubers with a well-set skin and free from diseases and mechanical injury were irradiated to 100 Gy in a package irradiator (Atomic Energy of Canada Ltd.) at a dose rate of 10 Gy/min. In earlier investigations half-potatoes have been found to provide a convenient system to study the wound metabolism in potato tubers (Thomas and Delincee, 1979; Thomas, 1982). Hence, a similar methodology was adopted in the present investigation. Irradiated and nonirradiated tubers of uniform size were sliced in half longitudinally from bud end to stem end and allowed to wound heal at 25 °C by keeping them in enameled trays covered with perforated polyethylene sheets of 150 gauge. The relative humidity inside the trays was maintained at 90% by wetting pieces of foam rubber kept alongside the tuber halves with distilled water. No microbial contamination was noticed in the experimental setup.

Preparation of Phenolic Extract. After 0, 2, 4, 6, 8, 10, 13, 15, and 17 days of wound healing period, 1 mm thick slices below the wound surface including the suberized layer were cut from six tuber halves with a stainless steel knife. The natural peri-

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Table I. Quantitative Changes in Phenolic Compounds (mg/100 g of Fresh Weight) in Control Potatoes during Wound Healing

		wound healing period, days								
no.	phenolic compound	0	2	4	6	8	10	13	15	17
1	3-*O*-caffeoylquinic acid (neochlorogenic acid)		12.60	13.56	24.24	31.45	15.66	14.15	11.72	10.56
2	4-*O*-caffeoylquinic acid (cryptochlorogenic acid)		5.49	5.78	10.37	13.43	6.80	5.95	4.88	4.44
3	5-*O*-caffeoylquinic acid (chlorogenic acid)	4.50	31.90	34.10	60.75	78.75	38.25	34.50	28.50	26.25
4	caffeic acid	1.85	4.02	5.68	6.42	7.75	6.75	5.65	4.90	4.50
5	*p*-coumaric acid	1.62	3.49	4.48	4.98	5.12	4.36	4.00	3.46	3.40
6	ferulic acid	1.33	2.65	3.06	4.02	4.38	3.98	3.66	3.25	3.12
	total	9.30	60.15	66.66	110.78	140.88	75.80	67.91	56.71	52.77

Table II. Quantitative Changes in Phenolic Compounds (mg/100 g of Fresh Weight) in Irradiated Potatoes during Wound Healing

		wound healing period, days								
no.	phenolic compound	0	2	4	6	8	10	13	15	17
1	3-*O*-caffeoylquinic acid (neochlorogenic acid)		5.72	7.10	8.06	9.45	8.46	6.30	5.53	5.14
2	4-*O*-caffeoylquinic acid (cryptochlorogenic acid)		3.41	4.16	4.14	4.38	4.52	3.38	3.16	2.86
3	5-*O*-caffeoylquinic acid (chlorogenic acid)	6.00	26.25	32.28	34.50	37.80	35.25	30.00	26.25	24.50
4	caffeic acid	2.88	4.02	4.86	5.30	5.76	5.18	4.78	4.16	3.88
5	*p*-coumaric acid	1.95	1.98	2.26	2.88	3.22	2.95	2.66	2.24	2.12
6	ferulic acid	1.78	1.75	2.08	2.46	2.68	2.24	2.05	1.98	1.88
	total	12.61	43.13	52.74	57.74	64.29	58.60	49.14	43.42	40.38

derm layer (skin) along the circumference of each slice was removed with a blade and the remaining part of the slice cut into small pieces. Ten grams of fresh tissue pooled from six tuber halves was extracted with 75 mL of 85% aqueous methanol in an Omni mixer at setting 3 for 3 min. The extract was filtered through Whatman No. 541 filter paper in a Büchner funnel. The residue was reextracted with 50 mL of 85% aqueous methanol as above, the filtrates were pooled, and the volume was made up to 150 mL. Complete extraction of total free phenolics as tested by Folin–Denis reagent (Swain and Hillis, 1959) was observed by this procedure. An aliquot of 2 mL was passed through a precolumn (0.6 cm i.d. × 12 cm) packed with 200 mg of Corasil for the purpose of removing nonphenolic components that otherwise wound have been strongly retained by the HPLC column.

HPLC Analysis. HPLC separation was carried out as described by Maiti et al. (1989) on a Du Pont Model 8800 series instrument with a gradient controlled pump module, a Rheodyne 7125 valve injector, a Spectro 400 variable-wavelength UV detector, and a 25 cm × 4.6 mm i.d., 5-μm particle size, Zorbax octadecylsilane (ODS) reversed-phase column (Du Pont). A linear gradient elution was carried out by using solvent A, acetic acid/water (2:98 v/v), and solvent B, acetic acid/acetonitrile/water (2:30:68 v/v). During analysis the solvent gradient was programmed from 10 to 100% B in A in 30 min with a flow rate of 1.5 mL/min. The UV detector was set at 280 nm.

Peak Confirmation and Quantification. Chromatographic peaks were tentatively identified by two procedures: (1) comparison of retention times of sample chromatographic peaks with those of authentic standards (Sigma Chemical Co., St. Louis, MO) using the same HPLC operating conditions and (2) cochromatography of the extract with the standard components. Individual phenolic acids were quantified by reference to a calibration curve for authentic standards. A linear relationship was obtained in each case.

Total Phenolics. Total phenols in the methanolic extract were estimated using the Folin–Denis Reagent (Swain and Hillis, 1959).

Phenylalanine ammonia-lyase activity was estimated according to the method of Zucker (1965).

RESULTS AND DISCUSSION

The time course of accumulation of total phenolic constituents during wound healing of potato tuber halves is shown in Figure 1. Total phenolics content increased by 3–4-fold during wound healing, reaching maximal levels on day 8 followed by a gradual decline. Irradiated potatoes showed lower amounts of phenolics throughout the 17-day wound healing experiment period.

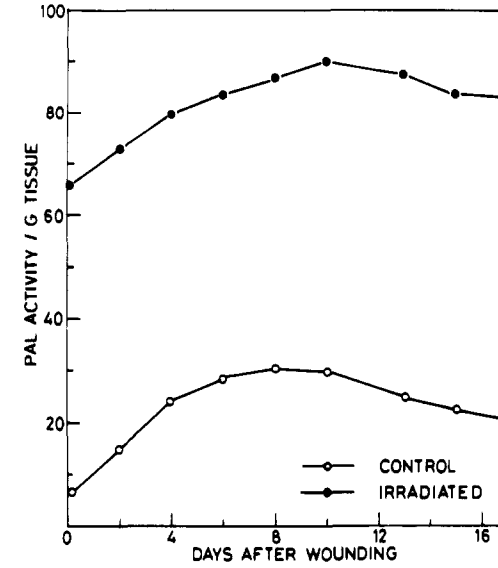

Figure 2. Phenylalanine ammonia lyase activity (PAL) during wound healing of control and irradiated potato tubers.

The rise in phenolic content during wound healing was accompanied by a parallel increase in phenylalanine ammonia-lyase (PAL) activity (Figure 2), the first enzyme of the phenylpropanoid pathway. Irradiated potatoes consistently showed higher levels of PAL activity than controls; however, no correlation was observed between PAL activity and the phenolic content since irradiated potatoes showed higher levels of PAL activity but recorded lower amounts of phenolics than control tubers. These results suggest the possible inhibition of subsequent enzymes involved in the biosynthesis of phenolics by

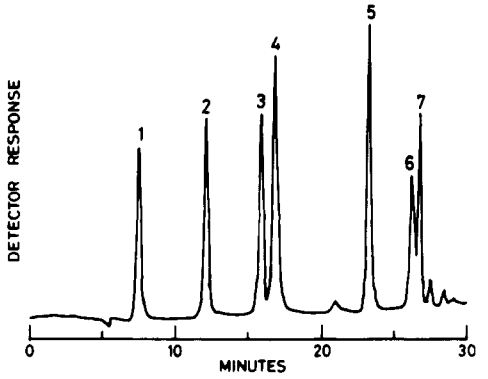

Figure 3. HPLC chromatogram of standard phenolic compounds. Peaks: 1, protocatechuic acid (7.67); 2, *p*-hydroxybenzoic acid (12.46); 3, chlorogenic acid (16.50); 4, vanillic acid (17.39); 5, caffeic acid (24.34); 6, *p*-coumaric acid (27.22); 7, ferulic acid (28.15). Figures in parentheses indicate retention time in minutes.

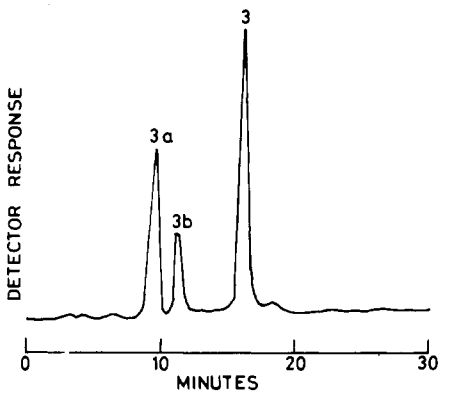

Figure 4. HPLC profile of isomers of chlorogenic acid. Peaks: 3, chlorogenic acid (16.54); 3a, neochlorogenic acid (9.62); 3b, cryptochlorogenic acid (11.50). Figures in parentheses indicate retention time in minutes.

irradiation. A study of the first two enzymes involved in chlorogenic acid biosynthesis in excised potato tuber tissue by Pendharkar and Nair (1987) has shown that irradiation impaired the induction of the second, cinnamic acid 4-hydroxylase (CA-4-H) enzyme, but not PAL, the first enzyme. They found a reduction in chlorogenic acid synthesis in irradiated potato which was attributed to the impaired CA-4-H activity. The continued increase in PAL activity during wound healing observed in our studies using tuber halves as test systems was quite different from that reported from potato tissue slices aged in a shallow layer of buffer solution (Zucker, 1965). In the latter system PAL activity was reported to peak 24 h after slicing followed by a steep decline (Zucker, 1965). This points to the striking effect of variations in experimental condition on the time course of PAL activity. This may be of practical significance since experimental systems using thin tissue may behave altogether differently from wounded whole tubers under commercial storage conditions or cut tuber pieces which are used as seed material for planting.

The HPLC separation of a mixture of seven standard phenolic acids is shown in Figure 3, and the separation of chlorogenic acid (5-*O*-caffeoylquinic acid) and its isomers crypto- and neochlorogenic acids (4-*O*- and 3-*O*-caffeoylquinic acids, respectively) prepared according to the method of Nagels et al. (1980) is shown in Figure 4. In our chromatographic system, both the crypto and neo isomers were eluted before chlorogenic acid as reported by Brandl and Herrmann (1983) and Malmberg and Theander (1985). Typical HPLC separations of phenolic acids from tuber halves of control and irradiated potatoes allowed to wound-

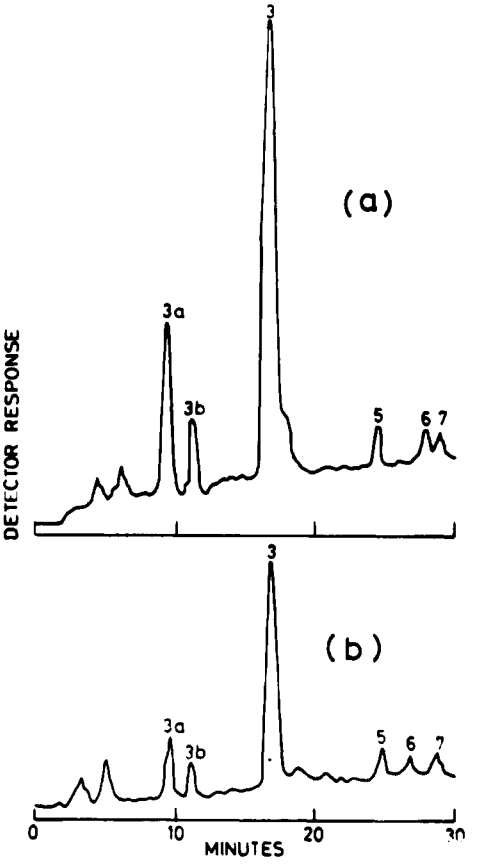

Figure 5. HPLC profiles of phenolic acids of control (a) and irradiated (b) potato tuber extracts during wound healing. Peaks: 3a, neochlorogenic acid; 3b, cryptochlorogenic acid; 3, chlorogenic acid; 5, caffeic acid; 6, *p*-coumaric acid; 7, ferulic acid.

heal for 8 days are shown in Figure 5. Tentative identifications were made by comparing relative retention times of the standards with those of the peaks of the potato extracts. Further, standards for the tentatively identified peaks and crypto- and neochlorogenic acids prepared according to the method of Nagels et al. (1980) were then coinjected with the potato extract to determine whether a single amplified peak or two peaks resulted. In all cases single amplified peaks were observed when standards were coinjected with the potato extract. On this basis six phenolic acids were tentatively identified, of which the major constituent was chlorogenic acid followed by its neo and crypto isomers in that order. Caffeic acid, *p*-coumaric acid, and ferulic acid were the other phenolic constituents detected. Some peaks in the HPLC chromatograms remained unidentified.

The quantitative results on the pattern of accumulation of individual phenolic acids during wound healing of control and irradiated potato tuber halves determined by HPLC are given in Tables I and II, respectively. In resting whole tuber of control and irradiated potatoes only chlorogenic acid, caffeic acid, *p*-coumaric acid, and ferulic acid were detected. The content of these acids increased many fold, reaching maximal levels on the eighth day after wounding. In addition, synthesis and accumulation of neo and crypto isomers of chlorogenic acid were noted during the wound healing process. Chlorogenic acid accounted for about 56% of the total phenolics, and together with its neo and crypto isomers comprised 88% of the phenolics formed in the wound healing tissue. Although the patterns of accumulation of individual phenolic acids in control and irradiated potato tuber halves were identical, major quantitative differences were noted. A comparison of the levels of various phenolic acids at

different days of wound healing showed significant reduction in the content of individual phenolic acids in irradiated tubers. Thus, after 8 days of wound healing, when maximal levels of phenolics were recorded, the contents of chlorogenic acid and total phenolic constituents as determined by HPLC were 79 and 141 mg for control compared to 38 and 64 mg for irradiated tubers. A similar trend is also seen in the total phenolics content determined according to the Folin–Denis method (Figure 1). Our results thus point to an impairment of wound-induced biosynthesis of phenolics in general and chlorogenic acid in particular by irradiation.

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Registry No. Chlorogenic acid, 327-97-9; caffeic acid, 331-39-5; *p*-coumaric acid, 7400-08-0; ferulic acid, 1135-24-6; neochlorogenic acid, 906-33-2; cryptochlorogenic acid, 17608-52-5; PAL, 9024-28-6.