Distribution of Horseradish Peroxidase Activity in Horseradish Plants

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Abstract. Thirty horseradish (*Armoracia rusticana* Gaertn., Mey., & Scherb.) cultivars from eight countries in Europe and North America and from advanced lines developed at the Univ. of Illinois were evaluated for horseradish peroxidase (HRP; electrical conductivity 1.11.1.7, donor : hydrogen peroxide oxido-reductase) activity. Nearly 86% of the activity was present in the taproot and lateral roots and 14% in the leaf petiole, but there was no activity in the leaf blade. The 30 cultivars were divided into three groups with high (eight cultivars), medium (13 cultivars), and low (nine cultivars) activities [11.58 to 16.97, 7.19 to 9.79, and 2.88 to 6.91 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ fresh weight (FW), respectively]. The cultivars with the highest activity were 819-A from the Illinois and 810-A from Switzerland with 16.97 and 16.67 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ FW, respectively. The cultivar with the lowest HRP activity was 244-A from the United States with 2.88 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ FW. Cultivar 819-A also had the highest protein concentration (4.92 $\text{mg}\cdot\text{g}^{-1}$ FW). When HRP activity was expressed per milligrams of protein, cultivar 167-A, also known as 'Bohemian', had the highest activity and cultivar 244-A had the lowest (5.35 and 0.83 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ protein, respectively).

Horseradish is a perennial rootcrop of the Cruciferae family. It is one of the oldest known condiments, valued for its extremely pungent, fleshy roots (Rhodes et al., 1965). Approximately 60% of the 8000 tons of horseradish produced annually in the United States is grown in Illinois on deep and fertile loess-originated soil near the Mississippi River east of Saint Louis (Peck et al., 1969; Rhodes et al., 1965). The bulk of the horseradish is processed into a paste and used as a food additive. The pungency of horseradish is due to the hydrolysis of sinigrin by a complex enzyme called myrosinase into allylthiocyanate, butylthiocyanate, glucose, and sulfate (Ohtsuru and Kawatani, 1979).

Horseradish production in the United States has not changed significantly in the last several decades. Recently identified uses for horseradish products, however, offer growers new opportunities to expand production. Horseradish roots are rich in horseradish peroxidase. About 82% of the commercially available enzyme is used in kits to test for levels of glucose and cholesterol in blood (Krel, 1991). The enzyme is also an invaluable tool in life sciences (Ryan et al., 1994), and has been used as an indicator in oxidase-based immunoassays (Tijssen, 1985), in estimation of the level of other enzymes (Akhavan et al., 1994), in cytochemistry (Oliver et al., 1984), and in preparation of DNA probes (Cercek et al.,

1995). Peroxidase is also important in industrial processing. It has been used in bio-bleaching (Harazono et al., 1996), degradation of wood pulp into fuel (Macek et al., 1993), and removal of carcinogenic pollutants, such as aromatic amines, phenols, and nitrate, from industrial wastewater (Dec and Bollag, 1994; Klibanov and Morris, 1981; Roper et al., 1996; Peive et al., 1972).

In 1991, world production of horseradish peroxidase (HRP) was estimated at 30 billion kilo-units, and demand is expected to double by 2010 (Krel, 1991). There are presently ≈ 10 major producers of horseradish HRP worldwide. In the United States, Sigma Chemical Co., St. Louis; ICN Pharmaceuticals Inc., Costa Mesa, Calif.; and Boehringer Mannheim Corp., Indianapolis, Ind., are the three major HRP-producing companies. These companies extract the enzyme from a few commercial cultivars selected primarily for their paste quality. However, to our knowledge, there have been no studies on the activity of HRP in different varieties of horseradish or on the

distribution of the enzyme in the horseradish plant. The objective of this study was to provide preliminary evaluation of HRP activity in 30 horseradish cultivars and advanced selections, with the objective of optimizing HRP production.

Materials and Methods

The 30 cultivars selected for this study are part of a collection housed at the Univ. of Illinois Urbana-Champaign campus; their origins are listed in Table 1. Morphological characteristics of six of these cultivars (104-A, 113-A, 125-A, 167-A, 196-A, and 777-A), as well as 45 others not listed in this study, have been described by Rhodes et al. (1965, 1969). Plants were grown in a completely randomized block design of four replicates per cultivar. To determine the distribution of HRP activity in various parts of the horseradish plant, composite samples of 50 g fresh weight (FW) from leaf blades, leaf petioles, taproot, or lateral roots of five cultivars (104-A, 125-A, 777-A, 819-A, and 1573) were assayed separately. Leaf blade and petiole samples were collected from fully developed leaves on 11 July 1996, while taproot and lateral root samples were collected at commercial harvest time on 7 Oct. 1996 and assayed for HRP activity according to a procedure outlined below.

Based on the results from the distribution study, the activity in the 30 cultivars and advanced selections was determined using a sample composed of the taproot and lateral roots in equal proportions. One plant from each of four replicates of the 30 cultivars was harvested on 7 Oct. 1996, placed in a plastic bag, top iced, and transported into the laboratory. Within 30 min of harvest, roots were cleaned with distilled water and 30 g FW was collected from the middle portion of the taproot (15 g) and lateral roots (15 g), combined, and ground into fine powder under liquid nitrogen with a mortar and pestle. The powdered tissue was suspended in 45 mL of extraction buffer consisting of 15 mM sodium phosphate (pH 6.0), 10% triton X-100, and 3% (w/v) polyvinylpyrrolidone and homogenized with a Polytron homogenizer (Brinkman Inst., Westbury, N.Y.) set at a speed of 4. The homogenate was centrifuged for 30 min at

Table 1. Origins of the 30 cultivars of horseradish tested.

Cultivar number	Origin
51-A, 53-A, 502-A, 532-A, 789-A, 813-A, 819-A, 1236, 1724-3, 1573	Illinois, U.S.A.
196-A ²	Canada
856-A	Czechoslovakia
777-A	Hungary
764-A	Poland
104-A, 106-A, 113-A	Russia
807-A, 810-A, 811-A, 863-A	Switzerland
167-A ³ , 204-A ³ , 244-A	U.S.A. ⁴
125-A, 239-A, 493-A	Wisconsin, U.S.A.
753-A	Yugoslavia
1573, 1609, 1717	Unknown

²Common name: "SASS" or "TH-107."

³Common name: "Big Top Western."

⁴Common name: "Bohemian."

⁵Exact origin in the United States is not known.

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27,000 g_n and 4 °C. The supernatant was collected, dialyzed overnight against the same buffer to remove low molecular weight compounds, and stored at -70 °C for further determination of HRP activity. Due to their high HRP activity, extracts from the root tissues were diluted (1:10) with buffer prior to assay.

Horseradish peroxidase activity was determined spectrophotometrically based on the oxidation of guaiacol (o-methylphenol) in the presence of H_2O_2 (Reuveni et al., 1992). The reaction mixture consisted of 1 mM H_2O_2 , 0.1 mM guaiacol (o-methylphenol), 15 mM potassium phosphate (pH 6.0), and 20 μ L enzyme extract in a total volume of 1 mL. The mixture was placed in a 1-mL quartz cuvette and the optical density was recorded in 1-min intervals for 3 min using a Shimadzu UV160U spectrophotometer (Shimadzu Corp., Kyoto, Japan) set at 470 nm. Horseradish peroxidase activity was determined by measuring the difference in optical density between reactions with and without guaiacol as a function of time (the extinction coefficient of guaiacol is 25.5 $mm^{-1} \cdot cm^{-1}$). Changes in optical density were linear during the first 3 min of the reaction.

Activity was expressed in $\mu mol \cdot min^{-1} \cdot g^{-1}$ FW and also as specific activity in $\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein. Protein concentration was determined according to Bradford (1976). Data were subjected to analysis of variance (ANOVA) using the SAS statistical program (SAS Institute, 1985) and differences among treatment means were compared using the least significant difference (LSD) mean separation test.

Results and Discussion

Peroxidase activity differed in the different parts of the plant, with 49% of the total activity in the taproots (Table 2), and 37% in the lateral roots. Activity in the leaf was very low; only 14% of the total activity occurred in the leaf petiole and no activity was detected in the leaf blade (Table 2). Our results are in agreement with those of Kawaoka et al. (1992), who examined expression and promoter activities of genes for horseradish peroxidase isozymes and reported expression of one gene group (prxC1a and prxC1b) in the leaf petiole, two genes (prxC2 and prxC3) were expressed in the root, and no peroxidase genes in the leaf. Dunleavy and Urs (1978) examined the distribution of peroxidase in soybean leaves and roots during plant development, and reported that activity in the roots was between 4- and 17-fold that in the leaves. They found no

correlation between peroxidase activity in the roots vs. the leaves, suggesting that the enzyme activities in the two tissues are independently regulated. This lack of activity in the leaves could be an economic setback to the growers because leaves constitute $\approx 20\%$ of the total plant FW (data not shown).

Activity in the roots also differed among cultivars (Table 3). The cultivars 819-A and 810-A contained the highest activity (16.97 and 16.67 $\mu mol \cdot min^{-1} \cdot g^{-1}$ FW, respectively) and 244-A contained the lowest activity (2.88 $\mu mol \cdot min^{-1} \cdot g^{-1}$ FW).

The cultivar 167-A contained the highest activity on a unit protein basis (5.35 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein), followed by 764-A, 1724-3, and 863-A. Activity in 167-A was similar to that in horseradish callus K6DIO, but about one-seventh of that in K6/2+K (Macek et al., 1993). Cultivar 244-A also had the lowest specific activity (0.83 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein). The remaining cultivars ranged in activity from 1.09 to 3.96 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein. Protein concentrations were also variable among the cultivars (Table 2). Cultivars 819-A and 810-A, which had the highest HRP activity per gram FW, also had higher protein concentrations than did other cultivars (4.92

and 4.45 $mg \cdot g^{-1}$ FW, respectively). Cultivar 819-A is a cross between 72-A and 196-A, a Canadian selection (Table 1). Cultivar 1573, which is the most widely grown cultivar in Illinois because of its relatively high yield ≈ 8 $Mg \cdot ha^{-1}$ and good processing quality, contained $\approx 24\%$ less HRP activity per gram FW and $\approx 25\%$ less protein than did cultivar 819-A. We are in the process of comparing yield potential of cultivars 819-A vs. 1573.

Substantial peroxidase activity has also been reported in peanut, tobacco, soybean, and cultured potato cells (Buttery and Buzzell, 1968; Cairns et al., 1980; Lobarzewski and van Huystee, 1982). Buttery and Buzzell (1968) evaluated peroxidase activity in seed coats of 28 soybean cultivars, which could be classified into groups with either high or low activity. Peroxidase activity in the former was as much as 78-fold as high as that in the latter. Peroxidase activity in the highest soybean seed group is $\approx 1\%$ of that in horseradish cultivar 167-A (Table 3).

Dunleavy and Urs (1978) evaluated roots of 24 soybean cultivars and found a maximum difference among cultivars of 19-fold in young plants vs. 2.5-fold in mature plants. However, we found no significant difference in HRP

Table 3. Total horseradish peroxidase activity and protein concentration in roots of 30 cultivars of horseradish.

Illinois cultivar number	Horseradish peroxidase activity		Protein concentration
	$\mu mol \cdot min^{-1} \cdot g^{-1}$ FW ²	$\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein	$mg \cdot g^{-1}$ FW
<i>High activity</i>			
819-A	16.97	3.45	4.92
810-A	16.67	3.75	4.45
113-A	13.44	3.77	3.56
1724-3	12.51	4.72	2.65
493-A	12.47	2.90	4.30
125-A	12.18	3.46	3.52
764-A	12.16	4.80	2.53
777-A	11.58	2.81	4.12
Mean	13.50	3.71	3.76
<i>Medium activity</i>			
1573	9.79	2.64	3.71
204-A	9.51	2.19	4.35
106-A	9.50	2.18	4.35
104-A	8.78	2.27	3.87
502-A	8.73	2.29	3.81
167-A	8.67	5.35	1.62
813-A	8.61	3.32	2.59
753-A	8.47	2.31	3.66
1717	8.40	3.05	2.75
1609	7.78	2.98	2.61
856-A	7.77	2.64	2.94
239-A	7.38	1.67	4.42
789-A	7.19	2.47	2.91
Mean	8.51	2.72	3.35
<i>Low activity</i>			
807-A	6.91	1.96	3.53
532-A	6.58	3.96	1.66
811-A	6.50	1.92	3.38
51-A	5.51	1.44	3.83
1236	5.17	1.09	4.73
196-A	5.08	1.72	2.96
53-A	4.86	1.64	2.96
863-A	4.72	4.03	1.17
244-A	2.88	0.83	3.44
Mean	5.36	2.07	3.07
<i>P</i> > <i>F</i>	***	***	***
LSD _{0.05}	2.54	0.98	0.80

²FW = fresh weight.

***Significant at $P < 0.001$.

Table 2. Distribution of peroxidase activity in horseradish plant parts.

Plant parts	Peroxidase activity (% of total)
Taproot	48.8
Lateral roots	37.3
Leaf petiole	13.9
Leaf blade	0.0
<i>P</i> > <i>F</i>	***
LSD _{0.05}	9.6

***Significant at $P < 0.001$. Peroxidase activity values are from tissues pooled from five different cultivars (104-A, 125-A, 777-A, 819-A, and 1573).

activity between roots harvested in Oct. 1996 vs. May 1997 (data not shown), suggesting that root age is not a limiting factor in horseradish.

In addition to the roots, HRP has also been extracted from horseradish callus (Lotmani and Rabier, 1988), cell suspension, and hairy root cultures (Macek et al., 1993; Parkinson et al., 1993). Macek et al. (1993) reported that HRP activity was ≈ 4 -fold higher in the supernatant of suspension culture as in cells or callus and 30-fold as high as in root tissue. However, HRP from suspension cultures consists of four isozymes, while that from intact roots consists of seven. Also, pH and temperature optima of HRP from cell cultures and intact roots differed (Macek et al., 1993; Parkinson et al., 1990). Our assay procedure measures total peroxidase activity of all isozymes.

In summary, significant differences in HRP activity were observed among the 30 horseradish cultivars. Eight cultivars showed high activity, 13 showed medium activity, and nine showed low activity. Horseradish peroxidase activity was present in the roots and leaf petiole, but no activity was detected in the leaf blade.

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