flowering changes there were significant mean changes from the 2nd to the 7th generation in 5 of 23 traits. These provide a valid estimate of the kinds and magnitudes of change that might be expected in unselected traits. In this study they were of little consequence from a breeding standpoint. However, the magnitude of change in some traits would have been of concern had the trait been of economic importance. For instance, if leaf whorl purpling had been resistance to some pest, the mean change would have been very important.

Thus, in the design of breeding procedures one must strive to avoid changes of the magnitude observed in this study in order to preserve potentially useful but undefined variation. The most readily available technique for accomplishing this is the use of large effective population sizes. Based on my experience, a seed increase nursery of 700 to 1000 plants is reasonable from a field plot technique standpoint. When seeds are saved from about 100 selected plants, this provides an effective population size of about 350, which should be sufficient to avoid most problems. The increase in flowering each generation should make the job of breeding progressively easier.

**Literature Cited**


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**Enzymatic Browning of Stored Parsnip Roots**

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**Abstract.** We established that surface browning in parsnips is due to enzymatic oxidation of phenolic compounds by an active polyphenoloxidase system. Potential browning was highest in the tissue immediately below the surface. The total phenolic content was lowest on the root surface and highest in the vascular cylinder region, whereas chlorogenic acid generally was uniformly distributed throughout the entire root. Enzyme activity was highest in the surface tissue and almost absent in the vascular cylinder. No direct relationships were found between the degree of browning and the parameters studied.

Parsnip (*Pastinaca sativa* L.) roots undergo a color change from paper white at harvest to a dull yellowish brown in storage. Dark brown blemished areas often appear randomly on the surface of the root and are probably due to bruising at harvest. This discoloration, commonly called parsnip browning, makes the root unattractive as a fresh market vegetable. Parsnip browning has been reported to be a problem in Alberta (1) and Manitoba (2). Attempts to determine the cause were unsuccessful although the phenomenon was shown to be non-pathological. Browning due to enzymatic oxidation of phenolic compounds has been reported in carrots (3), potatoes (7), lettuce (12), apples (9), peaches (4) and other fruits and vegetables. Parsnips have been reported to possess polyphenolic compounds (5).

We undertook to determine if the discoloration of parsnip roots following bruising of the root surface was due to enzymatic oxidation of phenolic compounds. We investigated the susceptibility of parsnip roots to browning and the occurrence of phenolic compounds and polyphenoloxidase in different parts of the root.

**Materials and Methods**

The cv. Hollow Crown, grown at the Morden Research Station, was used. The roots were harvested in early November and stored unwashed for 4 weeks at 5°C. Prior to analysis the stored roots were washed and dried.

To determine the portion of the parsnip root most susceptible to browning, we sampled 4 consecutive layers of cortex tissue, each 1.5 mm thick, and the outer 1.5 mm layer of the vascular cylinder (v.c.). Beginning with the root surface the tissue was removed in strips from the entire length of the root using a stainless steel vegetable peeler. The core was removed by carefully slitting the cortex and prying it away. A composite sample of tissue from each layer was prepared for subsequent analyses of total phenolic content, chlorogenic acid content, degree of browning, and polyphenoloxidase enzyme activity. All sampling and preparation was carried out at 5°C.

**Enzyme extraction.** The crude parsnip polyphenoloxidase enzyme was extracted by the method outlined by Shannon and Pratt (11). One hundred g of tissue was blended for 5 min in 200 ml of 0.1 M glycine buffer at pH 10 and filtered through
cheesecloth. Six hundred ml of acetone, cooled to -24°C, was added to the filtrate. After standing for 5 min, the precipitate was filtered through Reeve Angel, grade 202 filter paper, and suspended in 25 ml pH 6.1 citrate-phosphate buffer, using a wrist action electric shaker. Preliminary extractions had shown a loss of activity in buffers more acid than pH 6.1. The resulting crude enzyme preparation was centrifuged in a refrigerated centrifuge at 20°C and stored at 1°C.

**Substrate specificity.** The parsnip enzyme preparation was reacted with the following substrates: chlorogenic acid, catechol, cinnamic acid, ferulic acid, quinic acid, quercetin, rutin, tyrosine, and dihydroxyphenylalanine (DOPA). A concn of 4 x 10^{-4} M buffered at pH 5.2 and a temp of 30°C were used throughout.

**Polyphenoloxidase pH optimum.** The rate of chlorogenic acid oxidation by polyphenoloxidase was measured over a 3.0 to 8.0 pH range.

**Effect of temperature.** Rates of browning were determined at 22, 27 and 32°C at pH 5.2 and with 4 x 10^{-4} M chlorogenic acid.

**Determination of polyphenoloxidase activity.** Parsnip polyphenoloxidase activity was measured by a colorimetric method similar to that described for apples (11). Chlorogenic acid was used as a substrate at a 4.0 x 10^{-4} M concn. The reaction vol consisted of 0.5 ml enzyme extract and 2.0 ml substrate buffered with a citrate-phosphate buffer at pH 5.2. The reaction was carried out at 30°C. The change in absorbance at 400 nm of the brown-colored solution formed by the enzymatic reaction was measured over a period of 3 min beginning at time of mixing. The results are expressed as increase in absorbance per min.

**Total phenolic determination.** The extract for phenolic content determinations was prepared by blending 10 g of tissue in 200 ml of 80% ethanol for 3 min and filtering. Total phenolic content was determined by the method of Rosenblatt and Peluso (10) using the Folin-Denis colorimetric reagent. Optical densities were read at 760 nm and calculated as mg chlorogenic acid per 100 g fresh wt.

**Chlorogenic acid determination.** Chlorogenic acid content was determined by the sodium ethoxide method outlined by Hughes et al. (6). To 3 ml of the ethanolic extract, used for total phenolic determination, 0.3 ml of 0.1 N sodium ethoxide was added and the absorbance was immediately read at 380 nm.

**Potential browning.** Tissue browning was measured by blending 50 g of tissue in 100 ml of water for 5 min in an uncovered blender at 22°C. This blending incorporated atmospheric oxygen and facilitated oxidation. An unoxidized control was obtained by blending the tissue in a 0.5% solution of sodium bisulite. The slurry was filtered until a thick paste remained. The color of the paste was measured with a Hunterlab model D25 color and color-difference meter calibrated against a standard with the following values: L = 27.2; a = +23.8; and b = +12.4. Only L-values were used since these indicated lightness and gave the best estimate of browning of the parsnip tissue (3).

Degree of browning was determined by the difference between the L-values of the control and the oxidized samples. The difference was designated as potential browning units, with each unit corresponding to an L-value of 0.1.

**Results and Discussion**

**Substrate specificity.** Chlorogenic acid, catechol, and tyrosine were found to be substrates for parsnip polyphenoloxidase. Catechol and tyrosine had only 11 and 12 percent, respectively, the activity of chlorogenic acid. No browning occurred with cinnamic acid, ferulic acid, quinic acid, rutin, quercetin, or DOPA.

**pH optimum.** Two pH optima were observed: one at pH 5.2 and the other at pH 4.4. Maximum browning rate occurred at pH 4.4 but all subsequent studies were carried out at pH 5.2 because this pH gave a more normal reaction curve and was similar to the pH of the parsnip root tissue.

**Temperature.** Enzyme activity increased with rise in temp from 22 to 32°C, although the rate of change above 27°C was minimal. A temp of 30°C was selected for continuing studies.

**Total phenol content.** The total phenol content was lowest in the tissue of the root surface and highest in that of the vascular cylinder region (Fig. 1). In general, the phenolic content increased with depth of tissue.

The distribution of total phenols in the parsnip root greatly differs from that in carrots (3), where the outer layer has a substantially higher phenolic content than the underlying tissue. Similarly, in potatoes the total phenol content is highest in the periderm and cortex region (8). The significance of the high phenol content in the vascular cylinder region of the parsnip root is not explained.

**Chlorogenic acid.** The chlorogenic acid content varied slightly among the different tissues sampled, being highest in the surface and vascular cylinder regions. Its low concn, however, indicates that it probably is not the principal browning substrate.

**Potential browning.** The susceptibility to browning of the different portions of the root was inversely related to the depth of tissue (Fig. 1). Browning intensity generally was greatest near the root surface. Browning decreased rapidly with increasing depth of tissue and the vascular cylinder tissue showed only a trace of browning which was visually indiscernible.

This finding differs from that reported earlier in carrots (3) where only the surface tissue was found to be highly susceptible to browning. As was found in parsnips, negligible amounts of browning occurred in the vascular cylinder region in carrots. In apples (9), apricots (9), peaches (4), and potatoes (7) the tissue throughout the entire organ is uniformly susceptible to enzymatic browning. In parsnips, however, the cortical tissue was found to be more susceptible to browning than tissue in the vascular cylinder region; but, even the cortical tissue was shown to vary in susceptibility with varying depths of tissue. Root tissue appears to have a different pattern of distribution of phenolic compounds and polyphenoloxidase than that reported in stem tissue and tissue of reproductive organs.

**Polyphenoloxidase activity.** Enzymatic activity, measured as absorbance change per min and termed browning rate (Fig. 1), was highest in the 0 - 1.5 mm region of the root surface and decreased considerably towards the center of the root. Only a slight amount of enzyme activity was observed in the vascular cylinder region. According to Craft and Audia (5), parsnip roots

Fig. 1. Distribution of total phenols, chlorogenic acid, potential browning, and browning rate of cortex and vascular cylinder (v.c.) tissue of the parsnip root.

do not possess the polyphenolase enzyme. Our study shows that polyphenoloxidase activity is much higher in parsnips than in carrots (unpublished data) or apples (11) when chlorogenic acid is used as a substrate. Craft and Audia (5), however, used catechol as a substrate which would explain the apparent discrepancy. As noted earlier, we found this phenol to be much less active than chlorogenic acid.

**Interrelationships of enzyme activity, phenolic content, and browning.** The decrease in polyphenoloxidase activity with depth of tissue was accompanied by an increase in total phenols. There is a general synthesis and deposition of phenolic compounds throughout parsnip roots. However, the phenols are metabolised in the presence of active enzyme plus other associated factors; so the net result is an inverse relationship between the enzyme and substrate. The lowest concn of phenols would be expected at the surface where enzyme activity is the highest.

The trend in potential browning at the various depths would depend upon both the substrate-enzyme interaction and the activity of the enzyme per se. Our study shows that little or no browning can be expected unless the enzyme is present and that browning will be the highest at the optimum combination of substrate and enzyme. In this study the highest potential browning was observed in the tissue immediately below the surface. A possible explanation of this is the manner in which the browning readings were obtained. The system used discriminates against the surface layer since the potential browning was determined as the difference between the oxidized and unoxidized tissue. This obviously does not take into account any prior oxidation and browning. Therefore, reporting potential rather than total browning somewhat reduces the browning value of the surface layer.

The degree of browning did not appear to be directly associated with either the total phenolic or chlorogenic acid content. Polyphenoloxidase activity seemed to be positively correlated with browning. Based on the observation on chlorogenic acid content, it appears that phenolic substances other than chlorogenic acid are the principal browning substrates in parsnips.

Our study demonstrated that the phenomenon of surface browning in parsnips can be attributed to the enzymatic oxidation of phenolic compounds by an active polyphenoloxidase system. In contrast to many other crops, the phenols in parsnips were found to be generally distributed throughout the entire root. However, the enzyme was restricted to the cortical tissue with the vascular cylinder devoid of activity. These findings provide a basic understanding of browning in parsnips and may lead to methods of control of the problem.

**Literature Cited**