Control of Enzymatic Browning in Potato (*Solanum tuberosum* L.) by Sense and Antisense RNA from Tomato Polyphenol Oxidase

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Polyphenol oxidase (PPO) activity of Russet Burbank potato was inhibited by sense and antisense PPO RNAs expressed from a tomato PPO cDNA under the control of the 35S promoter from the cauliflower mosaic virus. Transgenic Russet Burbank potato plants from 37 different lines were grown in the field. PPO activity and the level of enzymatic browning were measured in the tubers harvested from the field. Of the tubers from 28 transgenic lines that were sampled, tubers from 5 lines exhibited reduced browning. The level of PPO activity correlated with the reduction in enzymatic browning in these lines. These results indicate that expression of tomato PPO RNA in sense or antisense orientation inhibits PPO activity and enzymatic browning in the major commercial potato cultivar. Expression of tomato PPO RNA in sense orientation led to the greatest decrease in PPO activity and enzymatic browning, possibly due to cosuppression. These results suggest that expression of closely related heterologous genes can be used to prevent enzymatic browning in a wide variety of food crops without the application of various food additives.

Keywords: *Enzymatic browning; polyphenol oxidase; potato*

INTRODUCTION

Browning of raw fruits and vegetables during storage and processing is a significant problem in the food industry and is believed to be one of the main causes of quality loss during postharvest handling. This is a widespread phenomenon that causes loss of quality and is of major economic importance (*1*). Browning can cause deleterious changes in the appearance and organoleptic properties of the food product, resulting in reduced consumer acceptance (*1*). Several methods have been developed to inhibit browning during processing including the use of chemical additives (*2*). Previously, potato producers controlled browning by application of sulfites, which are highly effective browning inhibitors. However, because of adverse health effects, the use of sulfites for this purpose has been restricted by the U.S. Food and Drug Administration. Various sulfite substitutes, generally combinations of ascorbic acid or erythorbic acid with citric acid and cysteine, have been marketed. However, these products are oxidized irreversibly and therefore do not meet the shelf life requirements in prepeeled potatoes without special packaging or cover solutions (*3*). The limitations of some of the antibrowning agents and the pressure from regulatory agents point to the need for developing alternative technologies for the prevention of enzymatic browning that will be effective and safe.

In potato, the internal damage resulting from the effects of impact on tubers during mechanical harvesting and storage may cause severe crop losses during grading for fresh and frozen products (*4*). Potato blackspot, the brown discoloration of damaged tissue, results from enzymatic browning, which extends from the site of impact. This reaction is caused by polyphenol oxidase (PPO), which catalyzes the oxidation of phenolic substrates to quinones. These quinones spontaneously polymerize to form a brown, black, or gray pigment (*5*). In high-pressure steam peeled potatoes, this defect may be accompanied by after-cooking darkening. This heat-induced reaction results in the formation of a dark complex of ferric ion and an ortho-dihydric phenol (*6*).

Potato cultivars differ in their susceptibility to enzymatic browning (*7*). Russet Burbank, which is the major commercial potato cultivar in the United States, is very susceptible to enzymatic browning (*7*). PPO levels and activity, as well as other factors, such as the availability of substrates and the dry matter content of the tuber, have been proposed to play a role in enzymatic browning in potato (*4*). Plant PPOs are nuclear-encoded copper metalloproteins localized in the membranes of plastids and have a molecular weight of ∼59 kDa (*8*). A number of PPO genes in potato have been isolated and characterized, each with specific spatial and temporal patterns of expression (*9*). At least five different PPO genes are expressed in potato tubers. The most abundant mRNA belongs to a gene that has high sequence identity with the PPO sequence isolated from tomato leaf (*10*).

Genetic engineering techniques provide a fast and precise method for producing potato tubers with greater resistance to enzymatic browning without detrimental effects on their desirable traits. Bachem et al. (*10*) demonstrated that expression of antisense RNA from a potato PPO cDNA decreased PPO activity and inhibited enzymatic browning in European potato varieties that had previously been selected for good level of blackspot resistance. Here we show that sense and antisense RNA from a heterologous tomato PPO gene can be used to

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control the level of PPO activity and enzymatic browning in the major commercial potato cultivar in the United States, the Russet Burbank potato.

EXPERIMENTAL PROCEDURES

Isolation of PPO cDNA Clones. Two PPO cDNAs were isolated from potato (*Solanum tuberosum* cv. Russet Burbank) and tomato (*Lycopersicon esculentum*) using the polymerase chain reaction (PCR) (*11*). The template DNA in these reactions were cDNAs made from poly(A)$^+$ RNA from potato and tomato leaves, respectively. Primers were designed to match the sense and antisense strands of the tomato P2 (PPO) gene (*12*): 5′ primer (5′-CCCGGGAGATCTCAACACAATGTCT-TCTTCTT) and 3′ primer (5′-CCCGGGATCCTTTAACAATC-CTCAAGCTT).

Construction of Plant Transformation Vectors. PCR was used to incorporate convenient restriction sites (*Bgl*II at the 5′ end and *Bam*HI at the 3′ end) into the full-length cDNA encoding PPO from tomato and potato for inserting into a plant transformation vector (*13*). pNT160 and pNT162 have the tomato PPO gene in the antisense and sense orientations, respectively. pNT163 has the potato PPO B gene in the antisense orientation. These PPO genes were expressed from the enhanced 35S RNA promoter from cauliflower mosaic virus (CaMV E35S) (*14*).

Potato Transformation. *Agrobacterium tumefaciens* containing the plant transformation vectors was used to transform potato (*Solanum tuberosum* cv. Russet Burbank) by transformation of stem sections as previously described (*15*).

Plant Material Tested. Clones from 37 independent transgenic Russet Burbank potato lines were selected for field testing: 18 lines were transformed with pNT160, 9 lines were transformed with pNT162, and 10 lines were transformed with pNT163. To perform the field test, each of the 37 clones and untransformed Russet Burbank potato were micropropagated in vitro, and plantlets were transplanted into soil after rooting in tissue culture for 4 weeks. Two weeks postplanting, 15 plants per transgenic line and 45 plants from untransformed Russet Burbank potato were transplanted by hand into the field.

Field Design. The experiment was performed in a field at the University of Idaho R&E Center, Aberdeen, ID. The plot area was established as randomized complete blocks in three replications with five plants per plot spaced 12 in. apart. The transplants were planted in the field in early June during 1997. Cultural practices were based on standard University of Idaho recommendations for Russet Burbank potato production. Insect control was via soil-applied Admire at hilling. A series of late blight fungicide sprays were required from late July through August. Vines were killed in early September, and plots were harvested in October 1997 by hand picking after mechanical lifting. Tubers for blackspot testing were removed at grading and stored at 7 °C (45° F). Clones that showed significant reduction in blackspot susceptibility were propagated from seed tubers for a second field test during the summer of 1998. The second field test was set up and maintained as described for the first test. The plot area was set up as randomized complete blocks in three replications with 40 tubers per line from 12 different transgenic lines and two untransformed Russet Burbank lines.

Blackspot Test. Tubers were assessed for susceptibility to blackspot using the abrasive peeling procedure as previously described by Pavek et al. (*16*). Tuber samples were abrasively peeled in a Hobart peeler (model 6115). Each sample was abraded for 30 s with water flowing over the tubers. Following each bruising treatment, samples were arranged on a bench in a randomized complete block design and maintained at 16−18 °C for 24 h. The amount of enzymatic discoloration was evaluated after 24 h. Each tuber was scored separately on a 0−5 scale: 0 = no blackening, 1 = trace, 2 = slight blackening, 3 = moderate blackening, 4 = serious blackening, 5 = very severe black discoloration on abraded surfaces. Four tubers from each transgenic line and untransformed Russet Burbank potato were used for evaluation of blackspot susceptibility in the 1997 test, and 10 tubers per line were used for evaluation of blackspot susceptibility in the 1998 test.

PPO Extraction from Potato Leaves. Tissue from young leaves was homogenized with a Brinkman Polytron at a ratio of 200 mg of tissue per 1 mL of buffer in cold (0−4 °C) 0.5 M sodium phosphate buffer, pH 7, containing 0.1% (w/v) SDS (*17*). The homogenate was clarified by centrifugation at 12000*g* for 2 min.

PPO Extraction from Potato Tubers. Three tubers from selected potato lines were sliced, frozen immediately in liquid nitrogen, and freeze-dried (*18*). The extraction of PPO was a combination of previously described methods (*5*, *17*, *19*). From each freeze-dried tuber 3 g of tissue was finely ground at room temperature in a Waring blender, passed through a fine wire mesh, and stored at −20 °C until required. The bulked potato powder (350 mg) was homogenized with a Brinkman Polytron in 3.8 mL of ice-cold buffer (150 mM Tricine, pH 8.5, and 50 mM ascorbic acid) for 5 s. The crude extract was immediately filtered through two layers of Miracloth. Ice-cold saturated ammonium sulfate was quickly added to the resulting filtrate to 90% saturation. The mixture was then centrifuged in a 70.1 Ti rotor at 40000 rpm for 30 min, and the pellet was resuspended in 1 mL of ice-cold 50 mM sodium phosphate buffer, pH 6.8, containing 10% (v/v) glycerol. The resuspended material was centrifuged at 8000*g* for 5 min. The resulting supernatant was used to measure PPO activity. This PPO extraction was performed in duplicate.

Assay of PPO Activity. PPO activity in extracts made from leaves or freeze-dried potato powders was determined using a rapid 2-nitro-5-thiobenzoic acid (TNB) spectrophotometric assay (*20*). This assay measures the decrease in absorbance at 412 nm of the bright yellow TNB solution resulting from the oxidation of 1 mmol TNB by 1 mmol of 4-methyl-1,2-benzoquinone, generated from 4-methylcathecol by PPO activity, to form a colorless Michaels-type adduct (*20*). One unit of enzyme activity is defined as that catalyzing the conversion of 1 μmol of TNB in 1 min. Thus, the conversion of TNB indirectly measures the amount of PPO activity and, consequently, the amount of PPO produced in the transgenic tissue. Initial rates of reaction catalyzed by PPO in various samples were determined at room temperature using 5 μL of tuber extracts in 1 mL of TNB assay solution (150 μM TNB, 7 mM 4-methylcatechol in 0.1 M sodium citrate−phosphate buffer, pH 6.8) at room temperature (25 °C). The AVIV dual scan spectrophotometer used for these measurements calculates the difference in absorbance between the reference cuvette (containing only the yellow assay buffer) and the sample cuvette (containing assay buffer and plant extract) for every second in the 1 min period. The data were analyzed using EXCEL and SPSS mathematics and statistics software. The analysis required that the data saved from the AVIV spectrophotometer be converted to SPSS-readable form via Microsoft EXCEL. Once copied to SPSS, the data were replotted as a scatterplot of absorbance versus time to determine the number of points that fall within the linear portion of the curve. The points representing the linear part of the curve were then used in a linear regression analysis to determine the rate of change in absorbance. The standard error and the mean PPO activity were calculated using frequency statistics in SPSS and plotted.

Protein Concentration. The amount of soluble protein in each PPO extract was measured in duplicate using a modified Lowry assay according to the Bio-Rad protocol with bovine serum albumin as standard.

Statistics. PPO activity and blackspot data analysis of variance (ANOVA) were done using the General Linear Model procedure of PCSAS (SAS Institute, Cary, NC). $p < 0.05$ was considered to be significant.

RESULTS

Construction of Plant Transformation Vectors. We cloned PPO genes from tomato and potato by RT-

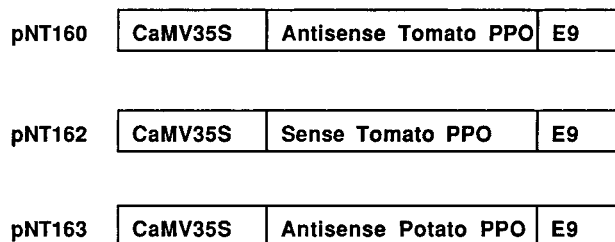

Figure 1. Plant transformation vectors containing either the tomato or potato PPO cDNAs downstream of the enhanced cauliflower mosaic virus promoter (CaMV 35S) used in potato transformation. Each vector contains the polyadenylation sequence of the small subunit of ribulose bisphosphate carboxylase (*rbcS*) E9 gene. The vectors contain NPTII gene as the selectable marker. The PPO gene from tomato was cloned between the enhanced CaMV 35S promoter and the *rbcS* E9 3′ end in sense and antisense orientation to generate NT160 and NT162, respectively. In NT163, the PPO gene from potato is cloned between the CaMV 35S promoter and the *rbcS* E9 3′ end.

PCR using RNA isolated from young tomato and potato leaves as templates. PPO mRNA is abundant in young tissues in potato and tomato plants but decreases significantly during plant development so that mature leaves and tubers have little PPO mRNA (*9*, *21*). Because the tomato leaf cDNA clone of PPO was 93.6% identical in sequence to the previously characterized potato tuber cDNA (*10*), we hypothesized that expression of the sense and antisense RNA corresponding to the tomato leaf cDNA in potato may inhibit the synthesis of PPO in potato tubers. Tomato and potato PPO cDNAs were cloned into plant transformation vectors (Figure 1) using convenient restriction sites introduced by PCR. The plant transformation vectors contained the enhanced CaMV 35S promoter (*14*) to drive the high level of expression of the sense (pNT162) and antisense RNA (pNT160) from tomato PPO cDNA and antisense RNA (pNT163) from potato PPO cDNA (Figure 1). DNA sequence analysis confirmed that the plant transformation vectors contained cDNAs corresponding to the full-length tomato PPO D gene (*12*) and the potato PPO P2 gene (*21*).

Potato Transformation. We chose to transform Russet Burbank potato because it is the major commercial potato cultivar in the United States with high susceptibility to blackspot. The challenge was to enhance the blackspot resistance of Russet Burbank potato without changing any of its important agronomic traits. Potato stem sections were transformed by cocultivation with *A. tumefaciens* containing the plant transformation vectors (*15*). About 10−20 independently transformed NPTII positive transgenic Russet Burbank lines were identified from each construct by ELISA. Southern blot analysis was used to confirm that these lines are transformed (data not shown).

Table 1. Blackspot Susceptibility of Transgenic Russet Burbank Tubers Using the Abrasive Peel Test

clone	construct	mean score[a] 1997	1998	comment[b]
control	wild-type potato	3.3	2.6[c]	
NT160-17	tomato antisense	2.4	2.5	**
NT160-27	tomato antisense	2.8	2.5	**
NT160-46	tomato antisense	2.7	2.5	**
NT162-13	tomato sense	1.6	1.7	***
NT162-29	tomato sense	2.8	2.6	**
LSD at $P = 0.05$		0.64	0.7	

[a] Average of four tubers per replication. Each tuber was scored separately on a 0−5 scale: 0 = no blackening, 1 = trace, 2 = slight blackening, 3 = moderate blackening, 4 = serious blackening, 5 = intense black discoloration on abraded surfaces (see Figure 2). The blackspot severity test was done in triplicate. [b] Duncan multiple-range test, means for lines significantly different from control at (**) 5% level, (***) 1% level. [c] The controls were relatively immature and had low solids resulting in a lower score.

Analysis of Tubers from Transgenic Plants. To evaluate genetically modified Russet Burbank tubers for reduced enzymatic browning, a random sample of 37 lines was micropropagated in tissue culture, including representatives of each construct type, and 15 plants from each line were planted in the field. Of the 37 transgenic lines planted in the field, 28 lines yielded sufficient number of tubers for sampling. The degree of abnormality of each clone was estimated by comparing total yields. Any clone that produced a significantly low yield ($P = 0.05$) and/or abnormally small tubers was assumed to be genetically impaired by the transformation or regeneration process. Tubers from these 28 lines along with tubers from untransformed plants were subjected the abrasive peel test to compare their levels of blackspot resistance. Abrasive peeling is more rapid than the conventional weight-dropping method for determining blackspot susceptibility of potato clones (*16*). Tuber samples were abraded and the amount of enzymatic discoloration was evaluated after 24 h as shown in Figure 2. The blackspot severity test was done in triplicate. Five of the 28 transgenic lines tested showed significantly reduced blackspot susceptibility compared to controls (Table 1). Tubers from NT162-13, which contains the tomato PPO cDNA in sense orientation, definitely appeared to lack the normal PPO response with a mean score of 1.6. Plant lines transformed with antisense potato P2 gene did not produce tubers with significant resistance to browning.

PPO activity in the tubers of plants that showed reduced browning was measured to determine whether the changes in blackspot severity were due to changes

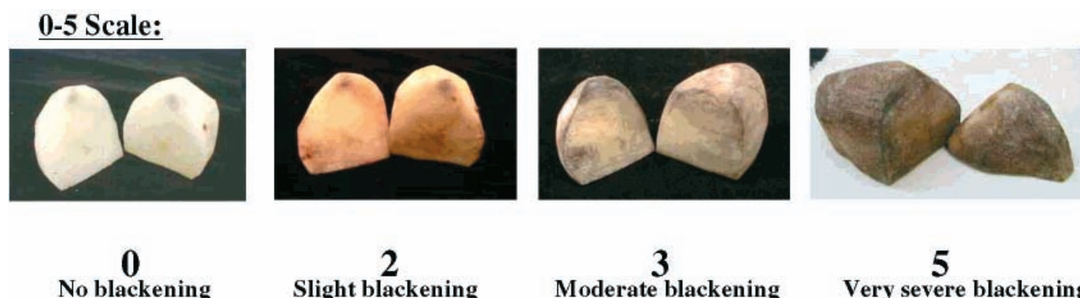

Figure 2. Abrasive peel test. Tubers were analyzed using the abrasive peeling procedure as described under Experimental Procedures, and enzymatic discoloration was evaluated visually by grouping tubers into five categories, 0 (none) to 5 (darkest), according to intensity of the discoloration observed.

Table 2. PPO Activity in Transgenic Tubers

clone	construct	units/mg of total protein[a] 1997	1998	comment[b]
control	wild-type potato	4.1 ± 0.7	3.6 ± 0.3	
NT160-17	tomato antisense	3.1 ± 0.9	2.6 ± 0.2	**
NT160-27	tomato antisense	4.3 ± 1.3	3.7 ± 1.0	**
NT160-46	tomato antisense	3.5 ± 0.6	3.7 ± 0.9	**
NT162-13	tomato sense	2.1 ± 0.9	1.6 ± 0.2	***
NT162-29	tomato sense	3.3 ± 0.9	3.6 ± 0.4	**

[a] PPO activity of selected tubers was measured spectrophotometrically following PPO extraction from freeze-dried potato powder. PPO extracts were made in duplicate, and PPO activity was measured in triplicate for each clone. [b] Student's *t*-test, (***) means of lines significantly different from control at 1% level; (**) means of lines significantly different from control at 5% level.

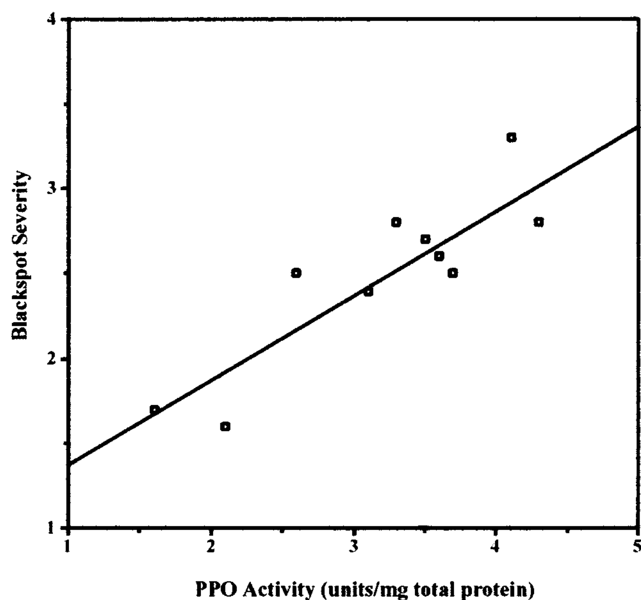

Figure 3. Correlation between blackspot severity and PPO activity in transgenic potato tubers from the 1997 and 1998 field tests. A single point is shown if the values overlap for two different lines. The regression line equation for the relationship is $y = 0.719 + 0.558x$ ($r^2 = 0.75$).

in PPO activity in transgenic potatoes. Freeze-dried potato macerates were used to isolate PPO because this method was reproducible and allowed the isolation of PPO before the formation of inhibitory compounds (*19*). As shown in Table 2, PPO activity was reduced in the transgenic tubers that showed reduced browning. Tubers from the transgenic line, NT162-13, which had the lowest blackspot susceptibility, had the lowest PPO activity. As shown in Figure 3, blackspot severity and PPO activity of the transgenic tubers and untransformed controls from both years correlated well ($r^2 = 0.75$, $p = 0.01$). This result indicated that the reduction in blackspot severity at the abraded surfaces was due to the suppression of PPO activity. The greatest reduction was observed in the transgenic line NT162-13, containing the tomato PPO cDNA in sense orientation. These results represent the first observation of cosuppression of PPO activity in potato tubers by a heterologous tomato RNA.

To confirm that the level of blackspot resistance is independent of the method of plant propagation, tubers harvested from the 1997 field test that showed reduction in blackspot susceptibility were planted in the field again in 1998. Also included in this field test were tubers from untransformed potato and four lines that showed wild-type levels of susceptibility to blackspot.

The results for blackspot resistance and PPO activity of the second field test were consistent with that of the previous year. The controls were relatively immature and had low solids. As a consequence, the blackspot score was lower than normal (Table 1). Tubers from line NT162-13 had the most significant reduction in blackspot susceptibility (Table 1) and PPO activity (Table 2).

DISCUSSION

Tomato PPO cDNA, inserted in either sense or antisense orientation behind the CaMV35S promoter, was introduced into the potato genome (*Solanum tuberosum* cv. Russet Burbank). In general, no correlation was observed between the degree of inhibition and the number of transgene copies in transgenic plants (*22*, *23*). We hypothesized that if the expression of PPO cDNA isolated from tomato leaf would affect only the PPO gene in potato with which it shares the most sequence identity, it should be possible to reduce the levels of PPO in specific tissues. We also introduced into potato a construct with an inverted PPO cDNA from potato leaves.

The level of enzymatic browning was reduced in tubers from selected transgenic lines. Transformed lines expressing the tomato PPO gene in either sense or antisense orientation produced tubers with significantly reduced levels of browning relative to wild-type controls. Lines expressing the potato PPO in antisense did not produce tubers with a significant decrease in the levels of enzymatic browning. We were unable to find tubers that were totally resistant to enzymatic browning. It is possible that a trace amount of PPO is all that is needed to produce some visible discoloration. As expected, there was a correlation between the level of browning and PPO activity ($r^2 = 0.75$). We did not observe any correlation between low PPO activity and abnormal yield or development. The transgenic line, NT162-13, which produced tubers with the lowest amount of browning, also contained the lowest amount of PPO: 59.7% PPO activity relative to wild-type controls. This PPO activity represents the total amount of PPO present in the whole tuber, which may include isozymes of PPO that normally exist in a latent form. Latency is linked to membrane association, and such PPOs are active only in damaged or senescent tissues (*8*). These latent PPOs can be activated during the isolation of PPO from the tuber and may also be active during the conditions of the browning assay.

The PPO activity in potato tissue has been shown to correlate to the level of PPO gene expression (*9*), which implies that the amount of PPO activity in vivo is controlled at the level of transcription. Both sense and antisense expressions of tomato cDNA appear to affect the level of PPO and, thus, necessarily suppress the level of mRNA from the potato PPO gene(s). Any analysis of PPO mRNA levels could be confounded by the possibility of mutual inactivation of transgenes and endogenous genes leading to lower synthesis of sense and antisense tomato PPO RNA. Moreover, control of PPO gene expression in tubers is complicated by the presence of at least five distinct PPO genes, each with its own distinct temporal and spatial pattern of expression (*9*). It is possible to distinguish which PPO gene is affected the most by transgene expression by probing total RNA from potato tissues with a series of PPO

cDNA clones. However, it remains to be shown which PPO gene product is responsible for the browning of the tuber cortex (the peeled tuber). Presently, there are three PPO genes expressed in the cortex: POT32, POT33, and POT41. These genes all have at least 80% homology with the tomato PPO gene. In this study, the tomato cDNA used to transform potato corresponded to the sequence for Tom D, a tomato PPO cDNA with 93.6% identity to the potato POT32 gene. The RNA from POT32 is the most abundant PPO RNA in the cortex of wild-type tubers. Thus, a reduction in the level of this RNA could explain the reduction in browning to trace amounts. In contrast, potatoes transformed with the potato P2 PPO antisense gene showed reduction in browning to much lesser extent. This could be explained by the fact that the level of expression from the homologous gene in the potato tuber is relatively low and detectable only in the subepidermal layer early in tuber development (*9*). The greatest reduction in blackspot susceptibility was observed in a transgenic line containing the tomato PPO cDNA in sense orientation. These results represent the first observation of cosuppression of PPO activity in potato tubers by a heterologous tomato RNA.

The results of this study compare favorably to that conducted by Bachem et al. (*10*), in which an antisense approach using potato tuber cDNAs was used to reduce the level of PPO activity to undetectable levels with a correlated decrease in blackspot formation after mechanical damage. However, the two potato cultivars used in that study, Diamant and Van Gogh, were selected for a significantly low level of browning susceptibility. Because Russet Burbank potato used in our study is very susceptible to browning, the reduction in blackspot severity in line NT162-13 would have a significant impact on the visual appearance and the shelf life of potato products. Further analysis of line NT162-13 will determine whether it can be processed commercially without the use of browning inhibitors.

Because the biological function of PPO in plants is involved with disease and pest resistance, it is important that the reduction in PPO be restricted to those tissues for which it is desirable to inhibit enzymatic browning. Assuming that closely related plant species have nearly identical PPO genes, the approach described here may allow reduction of enzymatic browning in various food crops without the need to isolate every PPO gene.

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LITERATURE CITED

(1) McEvily, A. J.; Iyengar, R.; Otwell, W. S. Inhibition of enzymatic browning in foods and beverages. *Crit. Rev. Food Sci. Nutr.* **1992**, *32*, 253−273.
(2) Martínez, M. V.; Whitaker, J. R. The biochemistry and control of enzymatic browning. *Trends Food Sci. Technol.* **1995**, *6*, 195−200.
(3) Sapers, G. M.; Miller, R. L. Control of enzymatic browning in pre-peeled potatoes by surface digestion. *J. Food Sci.* **1993**, *58*, 1076−1078.
(4) McGarry, A.; Hole, C. C.; Drew, R. L. K.; Parsons, N. Internal damage in potato tubers: a critical review. *Postharvest Biol. Technol.* **1996**, *8*, 239−258.
(5) Stevens, L. H.; Davelaar, E. Biochemical potential of potato tubers to synthesize blackspot pigments in relation to their actual blackspot susceptibility. *J. Agric. Food Chem.* **1997**, *45*, 4221−4226.
(6) Smith, O. Effect of cultural and environmental conditions on potatoes for processing. In *Potato Processing*; Talburt, W. F., Smith, O., Eds.; AVI−Van Nostrand Reinhold: New York, 1987.
(7) Sapers, G. M.; Douglas, F. W.; Bilyk, A. Enzymatic browning in Atlantic potatoes and related cultivars. *J. Food Sci.* **1989**, *54*, 362−365.
(8) Vaughn, K. C.; Duke, S. O. Function of polyphenol oxidase in higher plants *Physiol. Plant* **1984**, *60*, 106−112.
(9) Thygesen, P. W.; Dry, I. B.; Robinson, S. P. Polyphenol oxidase in potato: A multigene family that exhibits differential expression patterns. *Plant Physiol.* **1995**, *109*, 525−531.
(10) Bachem, C. W. B.; Speckmann, G.-J.; Van der Linde, P. C. G.; Verheggen, F. T. M.; Hunt, M. D.; Steffens, J. C.; Zabeau, M. Antisense expression of polyphenol oxidase genes inhibits enzymatic browning in potato tubers. *Bio/Technology* **1994**, *12*, 1101−1105.
(11) Innis, M. A.; Gelfand, D. H.; Sninsky, J. J.; White, T. J. In *PCR Protocols*; Academic Press: San Diego, CA, 1990; pp 39−53.
(12) Newman, S. M.; Eanetta, N. T.; Yu, H.; Prince, J. P.; De Vincente, M. C.; Tanksley, S. D.; Steffens, J. C. Organization of the tomato polyphenol oxidase gene family. *Plant Mol. Biol.* **1993**, *21*, 1035−1051.
(13) Sambrook, J.; Fritsch, E. F.; Maniatis, T. In *Molecular Cloning*: *A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY 1989.
(14) Kay, R.; Chan, A.; Daley, M.; McPherson, J. Duplication of CaMV 35S promoter sequences creates a strong enhancer for plant genes. *Science* **1987**, *236*, 1299−1302.
(15) Newell, C. A.; Rozman, R.; Hinchee, M. A.; Lawson, E. C.; Haley, L.; Sanders, P.; Kaniewski,W.; Tumer, N. E.; Horsch, R. B.; Fraley, R. T. Agrobacterium-mediated transformation of *Solanum tuberosum* L. cv. 'Russet Burbank'. *Plant Cell Rep.* **1991**, *10*, 30−34.
(16) Pavek, J.; Corsini, D.; Nissley, F. A rapid method for the determining blackspot susceptibility of potato clones. *Am. Potato J.* **1985**, *62*, 511−517.
(17) Sherman, T. D.; Vaughn, K. C.; Duke, S. O. A limited survey of the phylogenetic distribution of polyphenol oxidase. *Phytochemistry* **1991**, *30*, 2499−2506.
(18) Gubb, I. R.; Hughes, J. C.; Jackson, M. T. The lack of enzymatic browning in the wild potato species *Solanum hjertingii* compared with commercial tuberosum varieties. *Ann. Appl. Biol.* **1989**, *114*, 579−586.
(19) Hsu, A. F.; Thomas, C. E.; Brauer, D. Evaluation of several methods for the estimation of the total activity of potato polyphenol oxidase. *J. Food Sci.* **1988**, *53*, 1743−1745.
(20) Esterbauer, H.; Schwarzl, E.; Hayn, M. A rapid assay for cathecol oxidase and laccase using 2-nitro-5-thiobenzoic acid. *Anal. Biochem.* **1977**, *77*, 486-494.
(21) Hunt, M. D.; Eannetta, N. T.; Yu, H.; Newman; S. M.; Steffens, J. C. cDNA cloning and expression of potato polyphenol oxidase. *Plant Mol. Biol.* **1993**, *21*, 59−68.
(22) Borovkov, A. Y.; McClean, P. E.; Sowokinos, J. R.; Ruud, S. H.; Secor, G. A. Effect of expression of UDP-glucose pyrophosporylase ribozyme and antisense RNAs on the enzyme activity and carbohydrate composition of field-grown transgenic potato plants. *J. Plant Physiol.* **1996**, *147*, 644−652.
(23) Kruse, E.; Mock, H.; Grimm, B. Reduction of coproporphyrinogen oxidase level by antisenseRNA synthesis leads to deregulated gene expression of plastid proteins and affects the oxidative defense system. *EMBO J.* **1995**, *14*, 3712−3720.

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