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Molecular characterization of wheat polyphenol oxidase (PPO)

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Abstract It is well-established that the enzyme polyphenol oxidase (PPO) is involved in undesirable browning of noodles, chapattis, middle east flat breads and steamed breads. Methods for measuring PPO activity have been developed, and the variation of PPO activity among wheat (*Triticum aestivum* L.) cultivars has been well documented. However, there is no report on the identification and characterization of a wheat PPO gene. PCR performed on wheat genomic DNA with oligonucleotide primers designed from conserved copper binding regions of other PPO genes resulted in amplification of a 444-bp DNA fragment. Sequence analysis identified the conserved amino acids of PPO genes indicating that the PCR product was part of the wheat PPO gene. Screening genomic and cDNA libraries using 444- and 760-bp DNA fragments as probes failed to identify a PPO gene based on conserved sequence, even though there were very strong hybridization signals for some isolates. Rapid amplification of cDNA ends (RACE) technique was used as an alternative to obtain the remaining DNA sequences in 5′ and 3′ directions based on the 444-bp partial wheat PPO gene sequence. With the use of ThermoScript Reverse Transcriptase (which functions at higher temperatures) and Advantage-GC cDNA kit, the complete DNA sequence in the 3′ direction was obtained. A similar effort in the 5′ direction resulted in amplification of a truncated 414-bp DNA sequence. Overall, 1,509-bp of putative wheat PPO DNA sequence was obtained. Alignment of deduced amino-acid sequences revealed similarity to the other PPO gene sequences, especially in the conserved copper binding regions. Southern-blot analysis performed with four different restriction enzymes revealed two to four DNA fragments, suggesting a limited number of PPO genes in wheat. Wheat genomic DNA restricted with *Hin*dIII and hybridized using a 760-bp wheat PPO probe revealed a clear distinction between wheat cultivars with high and low PPO activities. Northern-blot analysis indicated a transcript size of about 2.0-kb. PPO DNA fragment as well as RNA transcript was observed for the durum cultivar Renville which normally has very low PPO activity. Further study is needed to explain the relationship between PPO activity and the presence of PPO gene (s).

Keywords DNA sequence · Molecular characterization · PPO · RACE · Wheat

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Introduction

Polyphenol oxidase is purported to be a major enzyme that causes undesirable time-dependent browning of noodles, chapattis, middle east flat breads and steamed breads (Faridi 1988; Kruger et al. 1994; Baik et al. 1995; Miskelly 1996; Morris et al. 2000). A recent review also emphasized the problem of browning in pasta (Feillet et al. 2000). Polyphenol oxidases catalyze the formation of quinones from phenols in the presence of molecular oxygen. The quinones react with amines and thiol groups or undergo self-polymerization to produce dark or brown products. Both growing conditions and genotype affect the expression of PPO activity (Lamkin et al. 1981; Baik et al. 1994; Park et al. 1997). Durum wheat cultivars have significantly lower PPO activities than hexaploid (bread) wheat cultivars. In a study of hard red bread wheat cultivars, growing location contributed more to variation in flour PPO activity than genotype (Park et al. 1997).

PPO genes have been cloned and sequenced in many plant species, including sugar cane (Bucheli et al. 1996),

potato (Thygesen et al. 1995), tomato (Shahar et al. 1992), *Vicia faba* (Cary et al. 1992), apple (Boss et al. 1995), grape (Dry and Robinson 1994), poplar (Constabel et al. 2000) and apricot (Chevalier et al. 1999). Plant PPO genes encode mature proteins of 52–62 kDa, and transit peptides of 8–12 kDa that are responsible for the transport of the enzyme into the thylakoid lumen. In plants, PPOs are localized in plastids and their phenolic substrates are mainly located in the vacuole so that enzymatic browning occurs only when this subcellular compartmentation is lost (Vaughn et al. 1988). PPOs are bicopper metalloenzymes and possess two conserved copper-binding domains, CuA and CuB, responsible for copper coordination and interaction with molecular oxygen and phenolic substrates (Steffens et al. 1994). Each copper atom is presumed to be coordinated by three histidine residues provided by the CuA and CuB sites. There is a very high amino-acid sequence similarity in the copper-binding regions of PPOs from different plants, but very little sequence similarity outside the copper-binding regions. For example, there is 92% amino-acid sequence similarity at the copper A binding region, but overall only 42% sequence similarity between *Vicia* and tomato PPO (Lax and Cary 1995). PPO genes reported so far do not have introns (Dry and Robinson 1994; Thygesen et al. 1995).

There have been no reports of wheat PPO gene sequence, even though 12 isozymes of PPO have been reported for wheat (Kruger 1976). PPO activity is high at early stages of wheat kernel development and decreases with kernel maturation (Taneja et al. 1974; Kruger 1976). A large part of PPO activity was localized in the endosperm at 21–25 days post anthesis (dpa) (Kruger 1976). In a dry mature wheat kernel, most of the wheat PPO activity is located in the bran, especially in the aleurone layer (Sullivan 1946). The objectives of this study were to 1. obtain wheat PPO gene sequence using molecular genetic techniques, 2. study hybridization patterns of wheat PPO using Southern-blot analysis, and 3. determine the transcript size of the gene. To our knowledge this is the first report on the molecular characterization of a wheat PPO gene.

Materials and methods

DNA extraction, PCR and DNA sequence analysis

Seeds from the cultivars Klasic (hard white spring), Penawawa (soft white spring), ID377s (hard white spring), Eltan (soft white winter), Renville (durum), IDO533 mutant (hard white spring) and Chinese Spring were grown in a green-house. Leaf samples were collected and frozen in liquid nitrogen and kept at –75 °C until needed. DNA was extracted from leaf tissues using cetyltrimethylammonium bromide (CTAB) (Doyle and Doyle 1987). Oligonucleotide primers designed from conserved copper-binding regions of plant PPO sequences were used to amplify a portion of the wheat PPO gene (see Table 1). PCR was performed in 0.5-ml microcentrifuge tubes containing 50 mM of KCl, 10 mM of Tris-HCl (pH 9.0), 1.5 mM of $MgCl_2$, 0.1% Triton X-100, 0.2 mM of each dNTP, 0.4 μM of each oligonucleotide primer, 1.0 unit of Taq DNA polymerase (Promega, Madison, Wis.) and 50–70 ng of genomic DNA. DNA amplification was performed in a Perkin Elmer Cetus 480 Thermal Cycler using 35–40 cycles of 1.0 min at 94 °C, 1.0 min at 60–65 °C, and 2.0 min at 72 °C. One additional cycle was performed at 72 °C for 10 min for complete extension of the PCR products. PCR products were cloned using TOPO TA cloning kit (Invitrogen, Carlsbad, Calif.) and sequenced by ABI prism model 377 sequencer. Some of the PCR products were also directly sequenced without cloning.

Genomic and cDNA library screening

A genomic DNA library prepared from *Triticum aestivum* L., var. Cheyenne (LICT-CNN) was kindly provided by Dr. Olin Anderson (USDA, Albany, Calif.). The vector used for cloning was EMBL-3 and the host-strain was LE392. The titer of the library was 10^9 pfu/ml and the average insert size was 15-kb. A phagemid cDNA library (TAO17E1X) prepared from spikes and seeds of Chinese Spring wheat (20–45 dpa) was kindly provided by Dr. Timothy Close (University of California, Riverside). cDNAs were directionally cloned with *Eco*RI on the 5′ and *Xho*I on the 3′ end using Uni-ZAP XR vector and XL1-Blue-MRF' (Stratagene, La Jolla, Calif.) host cells. Phagemid yield was 3.1×10^7 cfu/ml, and the insert sizes ranged from 0.6 to over 2.4-kb. Both genomic and cDNA libraries were screened using standard procedures (Ausubel et al. 1997; Sambrook et al. 1989). The genomic DNA and cDNA libraries were screened using radiolabelled 444- and 760-bp wheat PPO DNA fragments, respectively.

RACE technique to amplify 5′ and 3′ cDNA products

Rapid amplification of cDNA ends (RACE) technique was used to amplify portions of the unknown PPO DNA sequences in the 5′ and 3′ directions according to Frohman et al. (1988). Forward and reverse primers were designed from the 444-bp wheat PPO sequence that was obtained using primers designed from the copper binding regions of plant PPO sequences (see Table 1). Total RNA was extracted from either 1.0 g (large scale) or 100 mg (small scale) of immature wheat seeds (8–12 dpa) using a modified hot-phenol extraction method (Maes and Messens 1992). Messenger RNA (mRNA) was extracted from total RNA using EXPRESEP mRNA isolation kit (Biotecx Laboratories, Inc., Houston, Tex.). Thermoscript reverse transcriptase (GIBCO/BRL, Rockville, Md.) was used for cDNA preparation. Advantage-GC cDNA kit (CLONTECH, Palo Alto, Calif.) designed for amplification of GC-rich sequences was used for PCR.

Southern-blot analysis

DNA was extracted from leaves as described above. Southern-blot analysis was performed on Penawawa genomic DNA digested with the enzymes, *Apa*I, *Bam*HI, *Eco*RV and *Hin*dIII to determine the number of DNA fragments hybridizing to the wheat PPO probe. Southern-blot analysis was carried out according to Sambrook et al. (1989). A 760-bp wheat PPO DNA fragment generated with primers PPOF3 and PPOR3 (Table 1) was used as a probe. The 760-bp wheat PPO DNA fragment was randomly labeled with α-[^{32}P]-dCTP according to the manufacturer's instructions (Rediprime II random prime labeling system, Amersham Pharmacia Biotech, Piscataway, N.J.). The hybridization and prehybridization solutions contained 50% (v/v) formamide, 3 × SSC, 5 × Denhardt's solution and 1% (w/v) SDS. The hybridization solution additionally contained 4% (w/v) dextran sulphate. Hybridization was performed for 48 h at 42 °C. The hybridized membranes were washed twice for 15 min each (42 °C) in formamide wash buffer (50% formamide, 5 × SSC and 0.2% SDS). The radioactivity was checked and further washing was done with 2 × SSC and 0.1% SDS solution. Membranes were exposed to X-ray film for 3–5 days at –75 °C and developed.

Nothern-blot analysis

Total RNA was extracted from immature wheat kernels as described above. Twelve micrograms of total RNA was fractionated on a 1.5% (w/v) agarose-formaldehyde gel according to Sambrook et al. (1989). The RNA was blotted onto a Hybond-N+ nylon membrane (Amersham) by overnight capillary transfer in 10 × SSC. Solutions used for RNA work were treated with 0.1% (v/v) diethyl pyrocarbonate (DEPC) overnight and autoclaved to eliminate RNase. Probe labeling and hybridization were the same as for Southern analysis.

Results and discussion

Primer design and genomic DNA amplification

PCR performed with PPOF2 and PPOR1 primers (Table 1) that were designed from the conserved copper-binding regions of plant PPO DNA sequences amplified a 444-bp PCR product from genomic DNA (Fig. 1). No previously characterized PPO genes possess introns and thus the DNA sequence obtained from amplification of genomic DNA presumably codes for exons only. The sequence from the PCR product contained the highly conserved amino acids of histidine-rich copper-binding sites similar to other plant PPO sequences. With PPOF3 and PPOR3 primers, a 760-bp DNA fragment was observed (Fig. 1). Durum wheats generally have very low PPO activity. However, DNA fragments were obtained from Renville (durum) genomic DNA with two different sets of primers as seen in Fig. 1, indicating the presence of PPO genes in durum wheat cultivars. Similarly, the low PPO wheat cultivar ID377s also showed PPO DNA fragments with both primer sets. Further study is needed to correlate the presence of PPO gene (s) and observed PPO activity in different wheat cultivars.

Genomic and cDNA library screening

The 444- and 760-bp wheat PPO DNA fragments were used as probes to screen genomic and cDNA libraries, respectively. Positive plaques were identified in both cases. Approximately 500,000 plaques were screened from the wheat genomic DNA library and ten single plaques were isolated. Although two of the plaques exhibited strong hybridization signals, the sequences were not homologous with known PPO genes. Approximately 250,000 colonies were screened from the cDNA library, and eight single colonies were identified and characterized. But here again, the sequences were not homologous with known PPO genes. An explanation for these observed results regarding library screening is at present unknown.

Amplification of 5′ and 3′ ends with rapid amplification of cDNA ends (RACE)

Primers designed from wheat PPO DNA sequence were used for the RACE technique. The 3′ RACE produced the expected result using Thermoscript reverse transcriptase. The Thermoscript RT functions at higher temperatures (50–65 °C) than the normal reverse transcriptases which function at 42 °C. The wheat PPO gene sequence is GC rich (69%) and there was a problem of getting amplifiable DNA products with normal reverse transcriptases, presumably due to the inability of these reverse transcriptases to read through secondary structure. The 5′ RACE resulted in amplification of a short DNA sequence (414-bp) instead of the expected full-length PPO sequence of approximately 900-bp. Overall, 1,509-bp of putative wheat PPO DNA sequence was obtained (Fig. 2). The number of nitrogenous bases was 261, 529, 507 and 212 for A, C, G and T, respectively, which shows the GC-rich nature of wheat PPO gene. The 1,509-bp sequence is not a full-length PPO gene sequence as PPO genes reported so far are about 2,000-bp. Repeated attempts to obtain additional sequence in the 5′ direction using RACE, degenerate primers and inverse PCR failed. It is likely that some sort of secondary structure prevents successful PCR amplification.

The physiological role of PPO is not yet fully understood. This 1,509-bp wheat PPO DNA sequence reported for the first time could be used for antisense technology to study the role of PPO in wheat. This sequence may

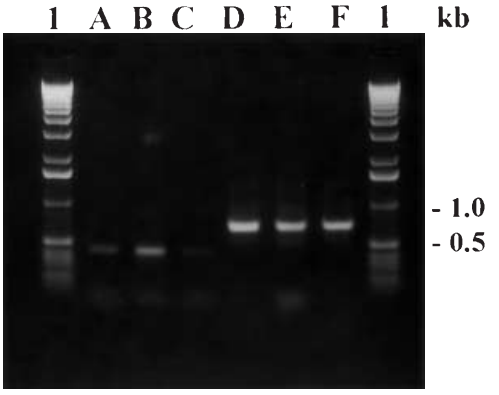

Fig. 1 PCR of wheat genomic DNA using primer pairs PPOF2 and PPOR1 (*lanes A–C*) and PPOF3 and PPOR3 (*lanes D–F*). Genomic DNA was extracted from Penawawa (*lanes A and D*), ID377s (*lanes B and E*), and Renville (*lanes C and F*). The primer pairs PPOF2 and PPOR1 produce a fragment size of about 444-bp, and the primer pairs PPOF3 and PPOR3 produce a fragment size of about 760-bp. The lane designated as '*1*' indicates a 1.0-kb DNA ladder (GIBCO, BRL)

Table 1 Sequence of primers used for PCR amplification (5′ to 3′). RACE5C and RACE3D were used for RACE PCR in the 5′ and 3′ directions, respectively

Name	Sequence
PPOF2	CTC ATC GGC GAC AAA ACC TT
PPOR1	GGA CCA GAG GTG CCA CAT
PPOF3	GAC GTC GTC GAC TTC AGC
PPOR3	GTG GTG CGA GTA GAA GAC G
RACE5C	AGT ACG GCA GCG CGA AGG T
RACE3D	AAC AAC CTC TCC GTC ATC TAC CGC CAG

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   1  Q  V  I  R  C  V  S  A  D  G  F  N  C  P  G  V  Y  R  P  E
   1  CAGGTCATCAGGTGCGTCTCGGCCGACGGCTTCAACTGCCCCGGCGTGTACCGCCCCGAG
  21  D  V  V  D  F  S  A  L  P  P  P  N  G  P  L  R  V  R  R  P
  61  GACGTCGTCGACTTCAGCGCTCTGCCGCCACCGAACGGCCCCTTGCGCGTGCGCCGGCCG
  41  A  H  L  V  A  A  D  K  E  Y  V  R  K  Y  E  A  G  V  R  M
 121  GCCCACCTCGTCGCCGCGGACAAGGAGTACGTGAGGAAATACGAGGCCGGCGTCCGCATG
  61  M  R  D  L  D  A  S  G  D  P  R  S  F  K  S  Q  A  A  I  H
 181  ATGAGGGATCTGGACGCCTCCGGCGATCCGCGCAGCTTCAAGAGCCAGGCCGCCATCCAC
  81  E  A  Y  C  N  F  H  Y  K  V  T  A  V  A  A  A  A  S  R  T
 241  GAGGCCTACTGCAACTTCCACTACAAGGTCACCGCGGTGGCCGCCGCGGCCTCAAGAACA
 101  A  G  G  G  G  G  T  P  E  I  D  F  D  V  H  F  S  S  I  F
 301  GCCGGGGGCGGCGGCGGCACGCCGGAGATCGACTTCGACGTGCACTTCTCGTCTATCTTC
 121  A  P  W  H  R  M  Y  I  Y  F  F  E  R  I  I  G  E  L  I  G
 361  GCTCCGTGGCACCGGATGTACATCTACTTCTTCGAGCGGATCATCGGCGAGCTCATCGGC
 141  D  T  T  F  A  L  P  Y  W  N  W  D  A  P  D  G  M  M  L  P
 421  GACACCACCTTCGCGCTGCCGTACTGGAACTGGGACGCGCCGGACGGGATGATGCTGCCG
 161  P  I  F  N  N  A  S  S  P  L  Y  D  A  N  R  D  Q  A  H  V
 481  CCGATATTCAACAACGCGTCCTCACCGCTCTATGACGCCAACCGCGACCAGGCGCACGTC
 181  T  A  V  M  D  L  N  K  G  P  G  A  D  N  E  L  P  L  C  S
 541  ACCGCCGTCATGGACCTCAACAAGGGCCCAGGCGCGGACAACGAGCTTCCTCTCTGCAGC
 201  D  D  A  C  V  K  E  N  N  L  S  V  I  Y  R  Q  M  A  V  D
 601  GACGACGCCTGCGTGAAGGAGAACAACCTCTCCGTCATCTACCGCCAGATGGCCGTCGAC
 221  T  A  L  Q  F  H  G  N  K  F  C  A  G  G  T  P  G  S  P  G
 661  ACGGCCCTGCAGTTCCACGGGAACAAGTTCTGCGCCGGGGGCACCCCCGGCTCCCCCGGC
 241  S  L  E  N  A  A  H  T  A  V  H  I  W  V  G  G  D  M  G  V
 721  TCACTCGAGAACGCCGCGCACACCGCCGTGCACATCTGGGTGGGCGGGGACATGGGGGTG
 261  L  G  T  A  G  R  D  P  V  F  Y  S  H  H  A  N  V  D  R  M
 781  CTCGGCACCGCCGGCCGCGACCCCGTCTTCTACTCGCACCACGCCAACGTCGACCGCATG
 281  W  H  L  W  T  T  T  L  G  N  Q  D  F  V  G  A  G  T  G  D
 841  TGGCACCTCTGGACCACCACGCTCGGGAACCAGGACTTCGTCGGCGCCGGCACCGGCGAC
 301  W  R  D  T  S  F  V  F  Y  D  E  K  R  R  P  V  R  I  S  V
 901  TGGCGCGACACCAGCTTCGTCTTCTACGACGAGAAGCGGCGGCCCGTGCGCATCAGCGTC
 321  R  D  V  L  D  A  G  R  L  G  Y  T  Y  E  E  R  E  T  L  E
 961  CGCGACGTCCTCGACGCCGGCAGGCTCGGGTACACGTACGAGGAGAGGGAGACCTTGGAG
 341  W  L  D  K  R  P  K  P  A  T  G  I  D  R  P  A  G  P  S  V
1021  TGGCTGGACAAGCGGCCCAAGCCGGCGACCGGCATCGACAGACCGGCCGGCCCAAGCGTC
 361  P  A  A  L  S  F  P  V  A  L  K  K  G  R  K  E  Y  V  T  V
1081  CCCGCCGCCCTCTCCTTCCCCGTGGCTCTGAAGAAGGGCCGGAAGGAGTACGTGACGGTG
 381  E  R  P  E  E  A  R  A  S  G  G  S  S  K  K  A  P  E  V  L
1141  GAGCGGCCGGAGGAGGCCCGGGCCAGCGGCGGCAGCAGCAAGAAGGCGCCGGAGGTGCTG
 401  V  V  D  V  T  I  D  P  C  E  Y  A  K  F  D  V  L  V  N  V
1201  GTGGTGGACGTCACCATCGACCCCTGCGAGTACGCCAAGTTCGACGTGCTCGTCAACGTG
 421  P  K  G  Q  E  A  R  V  G  P  Q  D  T  E  F  A  G  T  F  E
1261  CCCAAAGGCCAGGAGGCGCGGGTGGGGCCGCAGGACACCGAGTTCGCAGGGACCTTCGAG
 441  N  L  P  H  G  G  G  D  G  G  G  R  G  M  G  R  L  T  L  T
1321  AACCTGCCGCACGGCGGCGGCGACGGCGGCGGCCGGGGCATGGGGCGGCTGACGCTCACG
 461  Y  R  F  A  L  R  E  L  V  E  D  L  G  C  G  Q  D  R  R  L
1381  TACCGGTTCGCGCTCCGGGAGCTGGTTGAGGACCTCGGATGCGGCCAGGACCGGCGGCTG
 481  D  V  T  L  V  P  R  A  G  G  M  V  V  V  D  D  V  R  V  E
1441  GACGTCACGCTCGTCCCGCGAGCCGGTGGGATGGTCGTGGTAGACGACGTGCGAGTTGAA
 501  L  C  N  -
1501  CTTTGTAACTAACTGATATGTGCACGAGTCAGCTACTTTAGTTTTTTCAAGACGGATGAT
1561  ATACCCGGCCTCTACATCCCAGTATATAGACGAAAGTAAAATAAAACCTTAAACTTCGAA
1621  TATTG
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Fig. 2 DNA and deduced amino-acid sequence analysis of a partial wheat PPO gene. The conserved copper-binding regions are *underlined*. Amino acids 115–133 show the conserved copper A region, whereas amino acids 247–282 show the conserved copper B region

Table 2 Comparison of 503 deduced amino acids of wheat PPO to other plant PPO proteins

Species	Accession number	Number of amino acids	Identity (%)	Similarity (%)
Sugar cane	O49960	615	42	58
Sweet potato	Q9MB14	588	39	54
Spinach	P43310	639	39	52
Tomato	Q08307	587	38	55
Potato	QO6355	588	38	54
Poplar	AAG21983	563	37	54
Tobacco	O49912	592	37	53
Grape	P43311	607	36	53
Vicia faba	Q06215	606	36	53
Apple	O24057	593	34	49

also facilitate the obtaining of the remaining wheat PPO DNA sequences. The translated amino acids showed 42% sequence identity to sugar cane PPO and a similar sequence identity to other plant PPO genes (Table 2). Overall, there is low level of sequence similarity among PPO genes of different plant species. The highest level of sequence similarity is for the conserved copper-binding regions (Lax and Cary 1995).

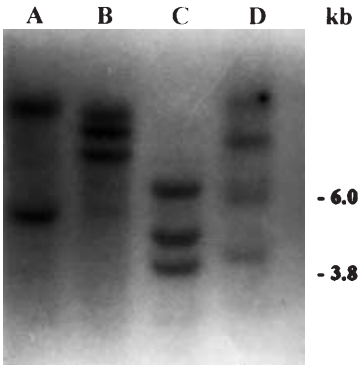

Fig. 3 Southern-blot analysis of wheat genomic DNA extracted from Penawawa and restricted with enzymes *Bam*HI (*lane A*), *Eco*RV (*lane B*), *Hin*dIII (*lane C*), and *Apa*I (*lane D*), respectively. *Bam*HI, *Eco*RV and *Hin*dIII do not cut in the 1,509-bp wheat PPO sequence, whereas *Apa*I cuts once. A 760-bp wheat PPO DNA fragment was used as probe

Southern-blot analysis

Penawawa genomic DNA restricted with four different enzymes and probed with 760-bp wheat PPO sequence revealed two to four hybridizing DNA fragments (Fig. 3). The enzymes *Bam*HI, *Eco*RV, and *Hin*dIII do not cut in the 1,509-bp DNA sequence, while *Apa*I cuts once. Kruger (1976) indicated the presence of up to 12 isozymes of wheat PPO based on polyacrylamide-slab electrophoresis of immature kernel extracts followed by incubation with gallic acid or catechin. Some of the isozymes generated by Kruger et al. (1976) may be degradation products as we were able to see only two to four hybridizing DNA fragments in our study. However, it is not uncommon to find many PPO isoforms in a given plant, as Thipyapong et al. (1997) have reported a seven-member multigene family encoding tomato PPOs.

Southern-blot analysis carried out on cultivars that have high PPO activity (Penawawa, Klasic, and Chinese Spring) and low PPO activity (ID377s, Eltan and IDO533) clearly revealed polymorphism (Fig. 4). A fragment of about 4.5-kb was only observed in cultivars with high PPO activity, whereas a fragment of about 5.6-kb was only seen in cultivars with low PPO activity. Several methods have been reported for measuring PPO activity of mature seeds (Anderson and Morris 2001; McCaig et al. 1999). However, there may be a marked environmental effect on results obtained. DNA markers are dependable as they are not affected by environment and can be conducted on any suitable tissue.

Northern-blot analysis

Total RNA extracted from immature wheat kernels (12 dpa) and hybridized with a 760-bp wheat PPO DNA

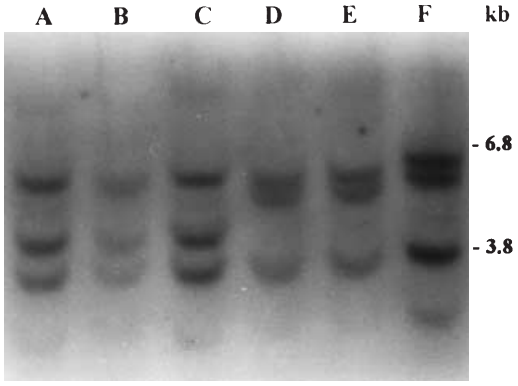

Fig. 4 Southern-blot analysis of wheat genomic DNA restricted with *Hin*dIII. *Lanes A, B, and C* are the cultivars Klasic, Penawawa, and Chinese Spring, respectively (cultivars showing high PPO activity), whereas lanes *D, E, and F* are the cultivars ID377s, Eltan, and IDO533, respectively (cultivars showing low PPO activity). Note that a DNA fragment of about 4.5-kb appears in cultivars with high PPO activity only, and a DNA fragment of about 5.6-kb appears in cultivars with low PPO activity only. A 760-bp wheat PPO DNA fragment was used as probe

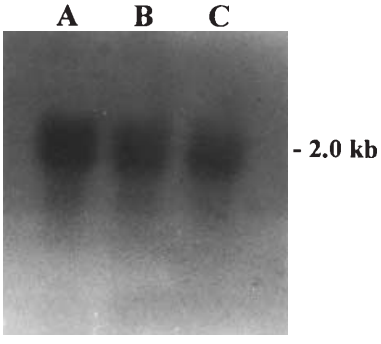

Fig. 5 Northern-blot analysis of total RNA (12.0 μg/lane) hybridized with a 760-bp wheat PPO DNA gene fragment. Total RNA was extracted from 12-dpa wheat kernels from the cultivars Penawawa (*lane A*), ID377s (*lane B*), and Renville (durum wheat, *lane C*)

probe produced a transcript size of about 1.9–2.0-kb (Fig. 5). This transcript was also seen for Renville (durum wheat), demonstrating the presence and transcription of the gene in a wheat cultivar that shows very low PPO activity.

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