Breeding and Characterization of Hexaploid Wheats with Nil Levels of Grain Polyphenol Oxidase

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Introduction:

Polyphenol oxidase (PPO) is a ubiquitous enzyme in plants, responsible for many browning reactions and reduction of food product quality. In common (bread) wheat, PPO occurs in the external layers of grain, often is carried into flour via milling, and can be responsible for the discoloration of wheat food products, especially Asian-style noodles. Previous reports have indicated durum wheats (*Triticum turgidum* var. *durum*) have low or nil levels of grain PPO, while common wheat (*T. aestivum* L.) PPO levels generally are high, with a few notable exceptions having low levels. Low levels of grain PPO are highly desirable in hard white wheat market classes.

Materials & Methods:

1. White wheat accessions were obtained from the USDA-ARS National Small Grains collection in Aberdeen, Idaho, and screened for low PPO activity by soaking seeds in a PPO activity buffer consisting of: 100 ml 1M Tris Hydrochloride pH 8.5, 225 mg Tyrosine (free base), 2 ml 10% SDS and 2 drops Tween 20, all per liter. Seed with low PPO retain their natural color – those with high PPO turn dark (Figure 1).

2. Several low PPO accessions were identified. Two low PPO accessions, PI 117635 and Seaspray (PI 134049), after mating, were found to have generated progeny with very low or nil levels of PPO. PI 117635 (Kenya/Florence//Dundee) was introduced from New South Wales, Australia, in 1936. Seaspray (Comeback/Cretan//Comeback), from Queensland, Australia, was added to the collection in 1939.

3. F1 and F2 plants were greenhouse grown in 2002 and 2003. F3 populations were grown in the field at Aberdeen, ID. Single head selections were made from the F3's, screened for PPO activity with tyrosine, and lines with nil PPO were sown as F3-derived F4 lines in the field at Yuma, AZ in 2006, along with the two parents, 'Ben' durum wheat and the following spring wheat check cultivars: Express, Leona, IDO377S, IDO580, and Jubilee. Selected lines (52) were planted in unreplicated single-row plots, and the checks and parents replicated 6 times. F5 and F6 generations were planted in 4 row plots at Corvallis, OR in 2007 and 2008. Lines again were unreplicated, and checks replicated three times.

4. A quantitative PPO assay (Anderson and Morris, 2001), using L-dopa as a substrate, was used to measure PPO from 2006-2008 harvests. Assays were conducted in duplicate using 5 seed samples. OD and OD/gram at 475 nm were recorded. Analysis of variance was used to test for differences amongst check varieties, and to test for difference in selected lines, based on DNA markers (see below). Before ANOVA, data were transformed using the equation: LOD = log10(OD+1). Regression analysis was used to evaluate heritability of the trait by regressing 2007 results on 2006 responses, and 2008 responses on 2007 values.

Alleles at the PPO2A and PPO2D loci were evaluated in all selected lines, parents, and checks using the following PCR markers: PPO18 (PPO2A; Sun et al., 2005), PPO29 (PPO2D, He et al., 2007) and STS01 (PPO2D, Wang et al., 2008).

Results:

1. OD and OD/gram values were highly correlated within each environment. R^2 values were always >0.80.

2. Significant differences in OD and OD/gram values were observed in ANOVA of check varieties and parents for the main effects "environment" and "entry" (Table 1). Environment X entry effect was not significant.

3. PPO levels of the parent PI 117635 did not differ significantly from that of the durum cultivar Ben. PPO levels of Seaspray were higher than both PI 117635 and the low PPO germplasm line IDO580, but were significantly lower than those of Express, Leona and IDO377s (Table 2).

4. Putative DNA markers (Figures 2 and 3) for low PPO activity, amongst the parents and checks, were not very informative. PI 117635 had the same patterns as Express, the check line with the highest PPO levels. Both carry the putative marker for the "high" PPO PPO2D allele. All other checks and Seaspray displayed markers for the "low" allele. At PPO2A, all lines had the putative "low" allele. IDO580 and Seaspray, however, were heterogeneous for a null phenotype – no product was observed in some samples using PPO18 primers (Table 2).

5. The null phenotype was present in a number of progeny lines (Table 3). Lines with the null phenotype had significantly lower PPO than sister lines producing the 876 bp product (Table 3). Many selected progeny lines had 3-year average PPO levels no different from that of the durum wheat Ben, and statistically lower than PPO levels of both parents. These lines have essentially nil levels of PPO activity.

6. PPO activity in the tested lines was highly heritable. This was evidence by the lack of significant environment X entry effects in ANOVA, and by regression analysis. Regression analysis results were:

a. 2007 OD vs 2006 OD: b = 0.80; $r^2 = 0.84$, $p < 0.0001$.

b. 2008 OD vs 2007 OD: b = 0.68; $r^2 = 0.88$, $p < 0.0001$.

c. 2008 OD vs 2006 OD: b = 0.59; $r^2 = 0.88$, $p < 0.0001$.

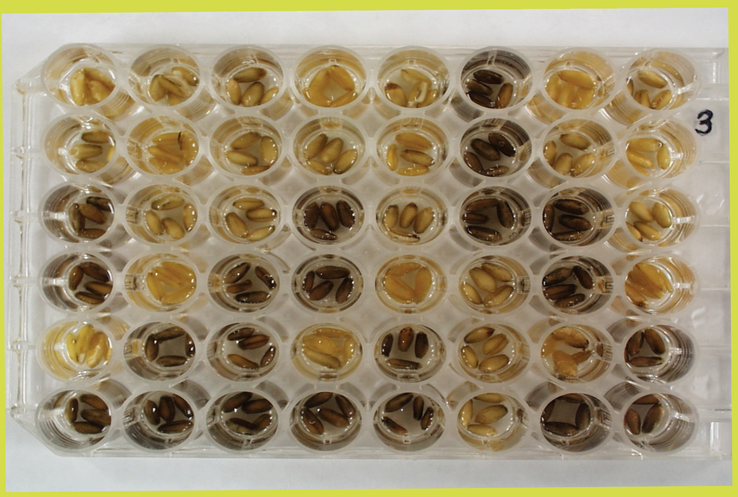

Figure 1: Preliminary screen for low PPO activity using tyrosine as substrate.

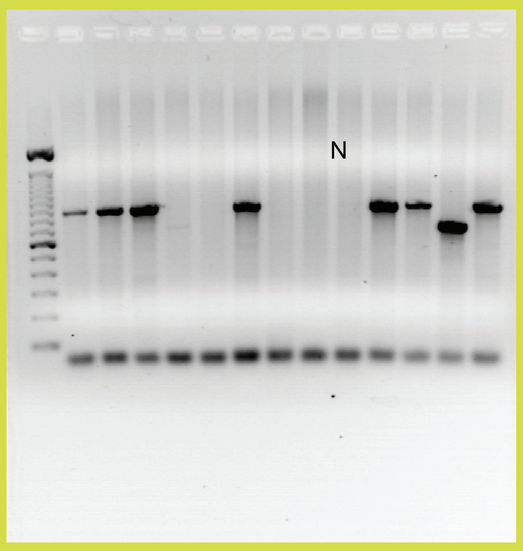

Figure 2: PCR products using primer pair PPO18, PPO2A locus. N = null phenotype

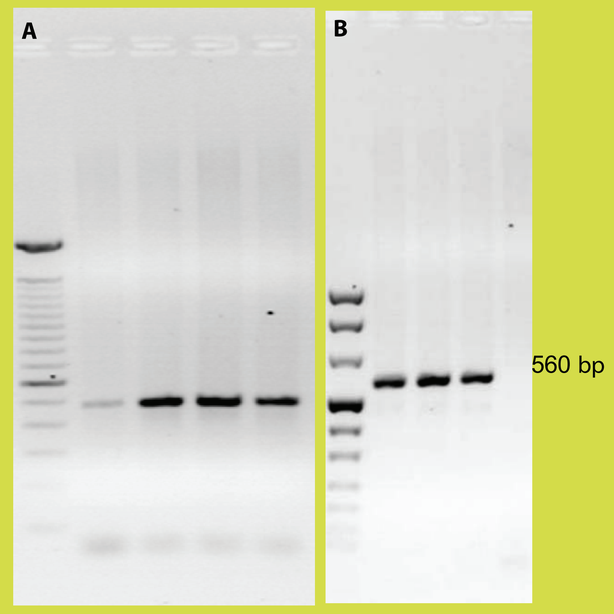

Figure 3: PCR products from complementary primers for alleles at the PPO2D locus. A = PPO29, B = STS01.

Table 1. Analysis of variance, grain PPO levels, check cultivars and parents.

	OD[a]				OD/g			
Source of variation	df	mean square	f	p	df	mean square	f	p
environment	2	0.0755	16.80	<.0001	2	0.1070	3.80	0.027
entry	7	0.2160	48.02	<.0001	7	1.8781	66.74	<.0001
environment*entry	14	0.0065	1.45	0.1555	14	0.0184	0.65	0.8115

[a]OD = optical density, OD/g = optical density per gram seed weight.

Table 2. Mean[a] grain PPO activities, check cultivars and parents

Entry	OD[b]	OD/g	PPO2A[c]	PPO2D[d]	
			PPO18	PPO29	STS01 primers
Express	0.668a	3.460a	876	490	-
Leona	0.419b	2.410b	876	-	560
IDO377s	0.410b	2.585b	876	-	560
Jubilee	0.373bc	2.20b	876	-	560
Seaspray	0.320c	1.624c	876/null[e]	-	560
IDO580	0.202d	1.107d	876/null[e]	-	560
PI117635	0.126de	0.625e	876	490	-
Ben (durum)	0.092e	0.625e	-	-	-

[a]Means followed by the same letter did not differ significantly at p=0.05.
[b]OD = optical density, OD/g = optical density per gram seed weight. [c]PCR products (bp) using primers specific for the PPO2A locus.
[d]PCR products (bp) using primers specific for the PPO2D locus. [e]lines segregating for an apparent null genotype

Table 3. Grain PPO activities of progeny of PI117635/Seaspray, by PPO2D genotypes.

	PPO2A null				PPO2A 876			
	mean	minimum	maximum	std dev.	mean	minimum	maximum	std dev.
OD[a]	0.103	0.075	0.152	0.019	0.122	0.084	0.191	0.023
OD/g	0.524	0.334	0.838	0.121	0.631	0.408	0.937	0.141

[a]OD = optical density, OD/g = optical density per gram seed weight.

Conclusions:

1. Traditional breeding resulted in the generation of selected common wheats with PPO levels significantly lower than those of both parents, and not significantly different from the durum wheat cultivar Ben.

2. As durum wheats are considered to lack PPO, the observed low PPO activity in these lines was designated the "nil" PPO phenotype.

3. Previously published DNA markers for low PPO activity require additional verification.

4. The USDA-ARS group at Lincoln will continue to introgress the nil PPO trait to adapted hard white winter wheats.

References:

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