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Chromosome location of genes affecting polyphenol oxidase activity in seeds of common and durum wheat

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With 3 tables

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Abstract

This study used cytogenetic stocks to investigate the chromosomal location of genes responsible for polyphenol oxidase (PPO) activity in common and durum wheat seeds. Substitution lines of chromosome 2A of hexaploid varieties 'Cheyenne', 'Thatcher' and 'Timstein' in 'Chinese Spring' showed significantly higher PPO activity than all other substitution lines of the same variety, with the exception of substitutions of 'Cheyenne' chromosome 3A and 'Thatcher' chromosome 4B. Substitution lines of chromosome 2A of *Triticum turgidum* var. *dicoccoides* and of chromosome 2D of 'Chinese Spring' into the tetraploid variety 'Langdon' showed a significant increase in PPO activity relative to all other substitution lines in Langdon. The gene(s) responsible for high PPO activity in chromosome 2D from 'Chinese Spring' was mapped on the long arm within a deletion that represents 24% of the distal part of the arm. This study shows that genes located in homoeologous group 2 play a major role in the activity of PPO in wheat.

Key words: *Triticum aestivum* — *Triticum turgidum dicoccoides* — chromosome substitution — end-use quality — gene location — polyphenol oxidase — wheat homoeologous group 2

Consumer preference requires noodles to have a bright and creamy white colour for most classes of noodles. However, noodles produced with flours from many common wheat varieties (*Triticum aestivum* L.) quickly decrease in brightness, which coincides with an increase in grey colour. This darkening process in noodles is associated with polyphenol oxidase (PPO) activity and its corresponding substrates (Miskelly 1984, Kruger et al. 1992, Baik et al. 1995). PPOs catalyse the oxidation of endogenous phenolic acids, resulting in the production of short-chain polymers that decrease noodle brightness. Milling to elevate extraction yield causes the PPO levels to rise dramatically.

A whole-seed PPO assay using a tyrosine substrate is used for breeding selection and differentiation of bread wheat seeds in durum (*Triticum turgidum* L.) samples (Mahoney and Ramsay 1992). Recently, more sensitive tests were developed to quantify variations in PPO activity among hexaploid wheat varieties (Bernier and Howes 1994). A good correlation ($r = 0.77$) was observed between a whole-seed PPO assay and the rate of change in brightness of a noodle sheet (Kruger et al. 1994).

Despite the importance of darkening processes to noodle quality, little is known about the chromosome location of the genes responsible for high PPO activity. Souza et al. (1998) found a significant association between PPO activity and a molecular marker located in homoeologous group 2 in recombinant inbred lines from a cross between NY6432–18 and 'Clark's Cream'. The specific homoeologous chromosome was not identified. In this study, the chromosome location of genes affecting PPO activity was investigated using various tetraploid and hexaploid wheat cytogenetic stocks. Knowledge of the genetic control of PPO activity is important to develop better strategies in breeding programmes to reduce noodle darkening.

Materials and Methods

Cytogenetic stocks of *T. aestivum*: All the cytogenetic stocks of hexaploid wheat (*T. aestivum* L.) used in this study were developed in the genetic background of 'Chinese Spring', which has relatively low levels of PPO activity. The effect of individual chromosomes of 'Cheyenne', 'Timstein' and 'Thatcher' on PPO activity was investigated using complete sets of substitution lines of these varieties in 'Chinese Spring' (Sears 1953, Morris et al. 1966). PPO activity was also evaluated within 'Chinese Spring' using nullisomic–tetrasomic lines of chromosome 2D (N2DT2A, N2DT2B, N2BT2D; Sears 1966) and six 'Chinese Spring' chromosome segment deletion lines of the short and long arm of chromosome 2D (Delaney et al. 1995). These deletion lines can be characterized by the fraction length (FL) values where the breaks occurred (2DS-1, FL = 0.33; 2DS-2, FL = 0; 2 DL-2, FL = 0.10; 2 DL-4, FL = 0.26; 2 DL-8, FL = 0.58; and 2 DL-9, FL = 0.76, Delaney et al. 1995).

J. Dvorak (University of California, Davis, CA, USA) kindly provided the seeds for the nullisomic–tetrasomic lines and 'Cheyenne' substitution lines in 'Chinese Spring'. These seeds were harvested from plants grown at a single experiment at University of California, Davis. Seeds for all other hexaploid cytogenetic stocks were kindly provided by J. Raupp (Wheat Genetics Resource Centre, Kansas State University, Manhattan, KS, USA). Seeds for the Thatcher substitution lines were all grown in 1987 and seeds for the 'Timstein' substitution lines were grown in 1983, except for the substitution of chromosome 6D which was grown in 1987 (J. Raupp pers. comm.). Plants of the deletions of Chinese Spring chromosome 2D where all grown in the greenhouse in Davis in 1998.

Cytogenetic stocks of *T. turgidum*: Two sets of tetraploid substitution lines were used to investigate the chromosomal location of the genes responsible for PPO activity. The first one was a set of 13 disomic substitution lines of *T. turgidum* L. var. *dicoccoides* (Korn. in litt., in Schweinf.) 'Bowden' (accession FA 15–3, from Israel) in the tetraploid *T. turgidum* var. *durum* Desf. variety 'Langdon' (Joppa and Cantrell 1990). Substitution line of chromosome 2B of *T. turgidum* var. *dicoccoides* was not available owing to sterility of the line. Seeds for these 13 substitution lines were harvested from a single increase identified as J88Dic. The second set included 14 disomic substitution lines in which each chromosome of the variety Langdon was replaced by the homo-

eologous D genome chromosome from 'Chinese Spring' (Joppa and Williams 1988). Seeds for these 14 substitution lines were from a single increase identified as J96S. L. R. Joppa (USDA, Fargo, ND, USA), kindly provided seeds for these experiments.

Tyrosine test: The method of Bernier and Howes (1994) was modified for use in detecting differences in PPO activity. One seed per well was placed in 96-well microtiter plates in 0.01 M disodium tyrosinate solution with 0.2% Tween 80 to ensure even wetting. The tyrosine substrate was added to each well and the plates were then incubated at 37°C. For each separate experiment, a preliminary test was done to identify the optimal volume of substrate and time of incubation to maximize differentiation between lines. Hexaploid substitution lines were incubated for 2.5 h in 300 μl of substrate while nullisomic–tetrasomic and deletion stocks, as well as both tetraploid stocks, were incubated for 19 h in 200 μl of substrate. Immediately after removing the seeds, PPO activity was scored using an Alpha Innotech Corp. IS-1000 Digital Imaging System (San Leandro, CA, USA). A plate containing the same volume of substrate used in each experiment was used to zero the instrument.

Experimental designs: Seven different experiments were performed. Seeds used in each experiment were from a single increase at one location (except the 'Timstein' chromosome 6D-substitution line). Seeds from the parental lines grown in the same environments were not available. In each experiment seeds were randomly allocated within the microtiter plate.

Hexaploid substitution lines of 'Cheyenne', 'Timstein', and 'Thatcher' were analysed in three different experiments using seeds as experimental units. Four to eight seeds were measured in each replication. The experiments were replicated twice and replications were analysed as blocks. The 'Cheyenne' substitution lines experiment was performed only once because of seed limitations. Comparisons were made between all possible mean pairs within each set of lines using Tukey's test for least square means.

The two sets of Langdon disomic substitution lines were analysed in different experiments using seeds as experimental units. The experiments were replicated twice and replications were analysed as blocks. Four seeds were measured in each block. Comparisons were made between all possible mean pairs within each set of lines using Tukey's test.

Differences among nullisomic–tetrasomic lines and deletion lines were smaller than among substitution lines and a higher level of replication was used. For each of the nullisomic–tetrasomic stocks, 18 seeds were used in each of the two blocks. For the deletion lines experiment, plants were used as experimental units and seeds as subsamples to increase the precision of the experiment. There were two plants available for 2 DL-2, three plants for 2 DL-4, five plants for 2 DL-8, five plants for 2 DL-9, four plants for 2DS-1, and two plants for 2DS-2. The experiment was replicated twice and the two replications were analysed as repeated measures in a split plot design. Within each replication, the PPO score for each plant was the mean of PPO scores from four seeds. Means were compared using five orthogonal contrasts.

Logarithmic transformations ($\log_{10}$) were performed for the experiments that showed heterogeneity of variances (nullisomic–tetrasomic stocks and 'Cheyenne', 'Thatcher', and tetraploid substitutions). Statistical analyses were made on the transformed scale but means in the tables were calculated from the untransformed data. The average standard error (SE) for each experiment was calculated using the mean square error of the untransformed data (SE = $\sqrt{}$mean square error/n). All statistical analyses were performed using SAS program (SAS Institute 1994).

Results

Hexaploid substitution lines

In substitution lines of 'Cheyenne', the highest PPO activity was found for chromosome 2A, followed by chromosome 3A (Table 1). The difference in PPO activity between these two substitution lines was not significant. The level of PPO activity observed in these two lines was significantly higher than that observed in all other substitution lines of 'Cheyenne' ($P < 0.0001$, Table 1). In substitution lines of 'Thatcher' (Table 1), chromosome 2A also had significantly higher PPO values than all the other substitution lines ($P < 0.0012$), with the exception of chromosome 4B ($P = 0.45$). In substitution lines of 'Timstein' (Table 1), chromosome 2A showed PPO values significantly higher ($P < 0.0001$) than all other substitution lines. The substitution line of chromosome 5D of 'Timstein' showed a similar mean to other substitution lines (mean = 18) but seeds were not well developed and were extremely heterogeneous for PPO scores. This substitution line was excluded from all statistical analysis to have homogeneity of variance.

Table 1: Mean PPO scores for 'Cheyenne', 'Thatcher' and 'Timstein' chromosome substitutions in 'Chinese Spring'. Means with different letters within a column are significantly different ($P < 0.01$)

Substituted chromosome	'Cheyenne'	'Thatcher'	'Timstein'
1A	19.2bcde	15.4bc	23.1bcd
2A	73.1a	29.6a	39.7a
3A	64.1a	18.5bc	22.3bcd
4A	24.0bc	15.9bc	19.3bcde
5A	23.6b	18.1bc	17.5cde
6A	23.4b	15.7bc	26.3b
7A	19.6bcde	16.1bc	21.4bcd
1B	18.5bcde	16.0bc	18.6bcde
2B	12.5bcde	14.2c	16.7de
3B	14.7bcde	15.8bc	23.0bcd
4B	13.7bcde	23.3ab	24.6bc
5B	12.7bcde	14.2c	20.1bcde
6B	16.8bcde	15.4bc	17.7cde
7B	8.8bcde	13.9c	21.1bcd
1D	16.4bcde	13.6c	21.5bcd
2D	11.3cde	13.9c	19.5bcde
3D	10.1de	15.4bc	17.9cde
4D	16.2bcde	14.4c	17.5cde
5D	21.9bcd	13.0c	—
6D	12.5bcde	16.7bc	12.0e
7D	9.5e	15.6bc	20.2bcd
SE	3.6	1.1	1.4

Tetraploid substitution lines

The PPO score of substitution LDN2A(DIC2A) was significantly higher ($P < 0.0001$) than all other substitutions of *T. turgidum* var. *dicoccoides* in Langdon. The PPO score for this line was four- to eight-fold higher than for the other lines. No significant differences were found among the other lines (Table 2).

A significant effect of homoeologous group 2 chromosomes was also found in the set of disomic substitution lines of the D genome in Langdon. Scores for PPO for substitution lines LDN2A(CS2D) and LDN2B(CS2D) were significantly higher than those from all other substitution lines ($P < 0.0079$), but not significantly different from one another ($P = 0.57$). PPO scores for these lines were 2.2- to 10-fold higher than the other lines. There was an almost continuous variation on PPO scores for the rest of the lines, with significant differences only between lines at the extremes of that continuum.

Nullisomic–tetrasomic and deletion lines of 'Chinese Spring'

Polyphenol oxidase tests of the nullisomic–tetrasomics N2DT2A, N2DT2B and N2BT2D confirmed the presence of a

Table 2: Mean PPO scores for tetraploid chromosome substitution lines. Means with different letters within columns are significantly different ($P < 0.01$)

Triticum var *diccocoides* into Langdon Substitution	PPO Score	'Chinese Spring' into Langdon Substitution	PPO Score
LDN1A(DIC1A)	3.6b	LDN1A(CS1D)	3.1bcde
LDN2A(DIC2A)	29.6a	LDN2A(CS2D)	16.7a
LDN3A(DIC3A)	7.2b	LDN3A(CS3D)	3.4bcde
LDN4A(DIC4A)	4.0b	LDN4A(CS4D)	3.2cde
LDN5A(DIC5A)	7.2b	LDN5A(CS5D)	7.5b
LDN6A(DIC6A)	4.7b	LDN6A(CS6D)	6.4bc
LDN7A(DIC7A)	3.7b	LDN7A(CS7D)	2.4e
LDN1B(DIC1B)	4.8b	LDN1B(CS1D)	3.5bcde
LDN2B(DIC2B)	Not available	LDN2B(CS2D)	26.0a
LDN3B(DIC3B)	3.4b	LDN3B(CS3D)	5.2bcd
LDN4B(DIC4B)	4.1b	LDN4B(CS4D)	2.5de
LDN5B(DIC5B)	6.3b	LDN5B(CS5D)	4.3bcde
LDN6B(DIC6B)	7.0b	LDN6B(CS6D)	3.7bcde
LDN7B(DIC7B)	3.5b	LDN7B(CS7D)	2.9cde
SE	1.3		1.0

genetic effect of chromosome 2D on PPO activity. PPO activity in nullisomic–tetrasomic N2BT2D (31.5 ± 1.5 SE) was significantly higher ($P < 0.0001$) than PPO activity in N2DT2A (14.8 ± 0.8 SE) and N2DT2B (12.3 ± 0.7 SE). Differences in PPO activity between N2DT2A and N2DT2B were significant at the 5% probability level, suggesting that 'Chinese Spring' chromosome 2A has a slightly larger effect on PPO activity than chromosome 2B.

Significant differences in PPO activity were found among the deletion lines of chromosome 2D ($P < 0.0001$). Orthogonal contrasts were used to compare the means of the six deletion lines. The first contrast showed that PPO scores for the two lines having deletions in the short arm (complete 2DL arms) were significantly higher than the scores for the group of four lines with deletions in the long arm ($P < 0.0001$). This result indicated that the gene(s) increasing PPO activity is (are) located in the long arm. Three contrasts were used to compare the means of each deletion line for the long arm vs. the proximal deletions. PPO activity for the most distal deletion of the long arm, 2DL-9, was not statistically different ($P = 0.53$) from PPO activity from lines 2 DL-2, 2 DL-4 and 2 DL-8. This result suggests that genetic control of the PPO activity on chromosome 2D is located on the distal 24% of the long arm that is missing in the deletion line 2DL-9. No significant differences were observed in the contrasts between 2DL-8 vs. 2DL-2 and 2DL-4 ($P = 0.55$) and in the contrast between 2DL-4 vs. the most proximal deletion 2DL-2 ($P = 0.62$). Only marginally significant differences were observed between the two deletion lines for the short arm ($P = 0.04$, Table 3). Results were identical in both replications, as indicated by a nonsignificant ($P = 0.35$) interaction between treatments and replication in time (Table 3).

Table 3: Analysis of variance of PPO scores for 'Chinese Spring' deletion lines (DL)

Source of variation	df	Mean square	F	P
Among DL	5	1100.7	12.5	0.00*
Contrasts				
1. Short vs. Long arm	1	3522.7	39.8	0.00*
2. 2DL-9 vs. 2 DL-2, 4, 8	1	36.1	0.4	0.53
3. 2DL-8 vs. 2 DL-2, 4	1	33.0	0.4	0.55
4. 2DL-2 vs. 2 DL-4	1	22.5	0.3	0.62
5. 2DS-1 vs. 2DS-2	1	432.9	4.9	0.04
Mainplot error	15	88.4		
Replication in time	1	2.1	0.3	0.57
DL* Replication in time	5	7.4	1.2	0.35
Error	15	6.1		

*$P < 0.0001$.

Discussion

Substitution lines of 'Cheyenne', 'Timstein' and 'Thatcher' chromosomes in 'Chinese Spring' consistently showed that chromosome 2A is one of the main sources of high PPO activity in these common wheat varieties. Genes located on chromosomes of homoeologous group 2 were also responsible for the higher PPO activity of 'Chinese Spring' (2D) and *T. turgidum* var. *dicoccoides* (2A) relative to *T. turgidum* var. *durum* cv. 'Langdon'. These results indicated that there is a major genetic effect on PPO activity located on the group 2 homoeologous chromosomes. In addition, results from the deletion line experiment indicate that the genetic effect on PPO activity on chromosome 2D is located on the distal 24% of the long arm of chromosome 2D.

Although genes located in homoeologous group 2 have an important effect on the level of PPO activity, other genes may also affect this trait, as exemplified by the relatively high PPO activity in 'Cheyenne' chromosome 3A and 'Thatcher' chromosome 4B. High PPO activity in each of these chromosomes was detected in only one set of substitution lines and was not significantly different from the levels detected in chromosome 2 A of the respective set of substitution lines. Therefore, it is not possible to rule out the possibility that the high PPO activity in substitution lines 'Cheyenne' 3A and 'Thatcher' 4B resulted from the presence of a residual segment of 'Cheyenne' or 'Thatcher' chromosome 2A, respectively, not eliminated during the production of the substitution lines by backcrossing. The presence of these residual segments can be tested by restriction fragment length polymorphism (RFLP) mapping or by classical segregation studies in crosses between the critical substitution lines.

The presence of more than one homoeologous chromosome affecting PPO activity in the substitution lines analysed here paralleled previous mapping results (Udall 1996, Souza et al. 1998). Quantitative trait loci analyses in the cross between NY6432–18 and 'Clark's Cream' showed a major effect on PPO scores associated with an RFLP marker from homoeologous group 2 and smaller effects associated with RFLP markers from homoeologous groups 3 and 5 (Udall 1996). The RFLP marker from homoeologous group 2 associated with high PPO scores (Souza et al. 1998) was mapped in the same chromosome region that is absent in deletion line 2DL-9 (Delaney et al. 1995, Nelson et al. 1995). This result suggests that the gene responsible for the major effect in PPO activity in the NY6432–18/'Clark's Cream' mapping population may be allelic or orthologous to the gene detected in this work in the distal region of chromosome arm 2 DL. It is tempting to speculate that the genes responsible for the major effect on PPO activity located on chromosome 2A of 'Cheyenne', 'Timstein', 'Thatcher' and *T. turgidum* var. *dicoccoides* belong to the same orthologous series. Mapping studies are currently in progress to test this hypothesis.

Differences in PPO activity between chromosome arms 2DS and 2DL in the deletion lines (Table 3) can be explained by two alternative hypotheses: a gene that increases PPO activity in chromosome arm 2DL, or alternatively, a gene that represses PPO activity in chromosome arm 2DS. Two lines of evidence suggest that the first hypothesis is the correct one. Substitutions of Langdon chromosomes 2A or 2B by 'Chinese Spring' chromosome 2D increase PPO activity. Furthermore, nullisomic–tetrasomic line N2BT2D with four doses of chromosome 2D showed higher PPO activity than lines N2DT2A or N2DT2B, which lack chromosome 2D. A similar argument can be used to suggest that the high PPO activity observed in substitution lines of chromosomes 2A of 'Cheyenne', 'Timstein' and 'Thatcher' is determined by a gene that increases PPO activity rather than by a repressor in the substituted chromosome 2A of 'Chinese Spring'. Double dosage of chromosome 2A in N2DT2A compared with N2DT2B resulted in an increase in PPO activity.

The determination of the chromosome location of the genes affecting PPO activity reported here will facilitate more detailed mapping studies in the future.

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