

Variability of polyphenol oxidase (PPO) alleles located on chromosomes 2A and 2D can change the wheat kernel PPO activity

Hongqi Si¹, Chuanxi Ma^{1*}, Xiaobo Wang¹, Xianfang He²

¹School of Agronomy, Anhui Agricultural University; Key Laboratory of wheat Biology and Genetic Breeding in Southern Huanghuai Wheat Region, Ministry of Agriculture; Anhui Key Laboratory of Crop Biology, Hefei 230036, China

²Institute of Crop research, Anhui Academy of Agricultural Sciences

*Corresponding author: chuanxi.ma@yahoo.cn

Abstract

Polyphenol oxidase (PPO) is primarily responsible for the enzymatic browning of Asian noodles and other flour products. In order to investigate how the allelic variability of PPO genes affect the kernel PPO activities, we used the two currently available sequence-tagged-site (STS) markers of PPO genes PPO18 and STS01 located on chromosomes 2A and 2D, for detecting PPO allelic variability in 300 F₄ plants obtained from hybridization of Yangmai 158 × Huaimai 18 and 362 other wheat cultivars. Our results confirmed previous findings that variations in the alleles of PPO genes located on chromosomes 2A and 2D contributed to approximately 50% of the PPO activities. In addition, we divided the average PPO activity of the varieties that contained only one gene by the average PPO activity of all varieties. We found that the contribution of the two PPO genes on chromosome 2A was as much as twice that of the two PPO genes on chromosome 2D. PPO activity was reduced by 23.7% and 8.5% by the allelic genes *PPO-2Ab* and *PPO-2Da*, respectively, and increased by 18.7% and 20.8% by *PPO-2Aa* and *PPO-2Db*. Our study shows that the allelic genes *PPO-2Ab* and *PPO-2Da* are responsible for maintaining the PPO activities at low and medium levels, and *PPO-2Aa*, and *PPO-2Db* at medium high levels. These findings emphasize the selection of the *PPO-2Ab* allelic gene for breeding wheat varieties with low PPO activity.

Keywords: allelic genes; enzymatic browning; molecular markers; polyphenol oxidase (PPO); wheat.

Abbreviations: CAPS - cleaved amplified polymorphic sequence; PPO - polyphenol oxidase; QTL - quantitative trait loci; SNP - single nucleotide polymorphism; STS - sequence-tagged-site.

Introduction

Polyphenol oxidase (PPO) is the key to undesirable enzymatic browning in certain flour products, typically Asian noodles (Baik et al., 1994; Morris et al., 2000). Breeding new varieties of wheat with low PPO activity can solve the browning problem. It has been shown that the main quantitative trait loci (QTLs) responsible for wheat PPO activities are on chromosome 2 (Anderson and Morris, 2001; Anderson et al., 2006; Demeke et al., 2001; Jimenez and Dubcovsky, 1999; Mares and Campbell, 2001; Raman et al., 2005; Udall 1997), whereas the other QTLs are located on the chromosomes 3, 5 and 6B (Demeke et al., 2001). Specifically, current researchers have suggested that PPO activities of the wheat kernel are under the control of two pairs of genes located on chromosomes 2A and 2D (Chang et al., 2007; Ge et al., 2004; Han et al., 2006; He et al., 2007; Wang et al., 2008; Wang et al., 2009). The goal of this study was to analyze in detail how these four allelic genes affect the PPO activity of wheat kernels. Effective molecular markers are the key tools in investigating how these alleles located on chromosomes 2A and 2D can change wheat kernel PPO activity. Simple sequence repeat (SSR) markers linked to QTLs on chromosome 2, Xgwm312, Xgwm294, Xwmc170, Xgwm312, Xgwm294b, and Xwmc198 have been used in previous studies for wheat breeding and selection; however, these markers have limitations: the

complete linkage is dependent on the location of these markers in the PPO genes, and the identification of these markers is both time consuming and expensive (Ge et al., 2003; Raman et al., 2005; Zhang et al., 2005). To solve these problems, an earlier study developed a sequence-tagged-site (STS) co-dominant marker PPO18 located on chromosome 2AL, based on the sequence AY596268 obtained from GenBank (Sun et al., 2005). Functional STS markers on chromosome 2A or 2D have also been developed by a few other studies (He et al., 2007; Wang et al., 2008; Wang et al., 2009); for instance, our group developed STS01, a dominant marker for PPO genes located on chromosome 2D (Wang et al., 2009). In addition, single nucleotide polymorphisms (SNPs) and cleaved amplified polymorphic sequences (CAPS) markers have been identified using expressed sequence tags (EST; GenBank accession number: BQ161439) (Raman et al., 2007). The development and application of these markers for wheat kernel PPO activity will efficiently promote the selection and breeding of new varieties of wheat exhibiting low PPO activity, which will help solve the browning problems of flour products. In the current study, the two STS markers of PPO18 and STS01 were used to detect how the four PPO alleles affect wheat kernel PPO activity. We measured allelic gene variations of PPO activities in 300 F₄ plants obtained from hybridization of Yangmai 158

× Huaimai 18 and 362 other wheat cultivars, as variations in the PPO activity of these wheat varieties are relatively large (Han et al., 2006) and conducted a correlation analysis between the PPO genes and the enzyme activities in these wheat varieties. The alleles *PPO-2Aa* (GenBank: EF070147) and *PPO-2Ab* (GenBank: EF070148) on chromosome 2A were identified using PPO18 (Sun et al., 2005), and *PPO-2Da* (GenBank: EF070149) and *PPO-2Db* (GenBank: EF070150) on chromosome 2D were identified using STS01 (Wang et al., 2009). With these two available PPO markers, we for the first time studied in detail how the four alleles identified by them influence the PPO activity of wheat kernels.

Results

Frequency test of the PPO alleles

Using the dominant STS marker STS01, we did not detect allelic segregation of PPO genes on chromosome 2DL in any of the 300 F₄ plants obtained from the hybridization of Yangmai 158 and Huaimai 18, possibly due to the similar genetic background of this population. However, in this population, the alleles of PPO genes on chromosome 2AL detected by marker PPO 18 were classified into three types: *PPO-2Aa/2Aa*, *PPO-2Aa/2Ab*, and *PPO-2Ab/2Ab*, with gene frequencies of 65%, 15.3%, and 19.7%, respectively. In the other 362 wheat cultivars with different genetic backgrounds, the test frequencies of single alleles *PPO-2Aa*, *PPO-2Ab*, *PPO-2Da*, and *PPO-2Db* were 27.9%, 22.1%, 35.5% and 14.5% respectively. The frequencies of the composite alleles *PPO-2Ab/2Da*, *PPO-2Ab/2Db*, *PPO-2Aa/2Da* and *PPO-2Aa/2Db* were 34.8%, 9.4%, 36.2%, and 19.6%.

ANOVA of kernel PPO activities

We performed ANOVA on the wheat kernel PPO activity with respect to the PPO alleles in the two experimental populations. Next, we calculated the ratio of the average PPO activity of all wheat varieties of certain genotype to the average PPO activity of all wheat varieties used in the experiments. As shown in Table 2, in the 300 F₄ individual plants obtained from the hybridization of Yangmai 158 and Huaimai 18, 66.7% of the changes in kernel PPO activity resulted from the variation in the alleles of PPO genes on chromosome 2A (P<0.01). Segregation of alleles on chromosome 2D was not observed in this population, possibly because of their similar genetic background. On the other hand, for the 362 wheat cultivars of different genetic backgrounds, 48.1% of the differences in kernel PPO activity resulted from variations in the alleles of PPO genes on chromosomes 2A and 2D. By calculating the ratio of the average PPO activity of wheat varieties that contain a single gene to the average PPO activity of all wheat varieties, we determine that the alleles on chromosomes 2A and 2D contributed to 71.9% and 28.1% of the variations in kernel PPO activity, respectively.

Effects of the composite PPO alleles on kernel PPO activities

As shown in Figure 3, more than half of kernel PPO activity was at the medium level among the 362 hexaploid wheat cultivars. Most of the kernel PPO activity of the composite genotype *PPO-2Ab/2Da* was at the low level, whereas most of the activity of *PPO-2Ab/2Db* and *PPO-2Aa/2Da* was at the medium level, and that of *PPO-2Aa/2Db* was above the medium level. The kernel PPO activity in these 362 cultivars increased gradually according to the composite genotypes in

the following order: *PPO-2Ab/2Da*, *PPO-2Ab/2Db*, *PPO-2Aa/2Da*, and *PPO-2Aa/2Db* (corresponding values of activity are 173.11, 230.11, 270.38, and 325.23 AU/min·g, respectively). Compared to the mean PPO activity (243.77 AU/min·g), the PPO activities in cultivars of genotype *PPO-2Ab/2Da* and *PPO-2Ab/2Db* were reduced by 29.0% and 5.6%, respectively, whereas the PPO activities in cultivars of genotype *PPO-2Aa/2Da* and *PPO-2Aa/2Db* were increased by 10.9% and 33.4%, respectively.

Effects of single alleles on kernel PPO activities

Table 4 and Table 5 show that *PPO-2Ab* and *PPO-2Da* reduce PPO activity to different extents; the reduction in PPO activity caused by *PPO-2Ab* was two times higher than that by *PPO-2Da*. The PPO activity caused by *PPO-2Ab* was at the low level, and the PPO activity caused by *PPO-2Da* was at the medium level. *PPO-2Aa* and *PPO-2Db* increased the kernel PPO activities to a relatively similar extent: above the medium level. Therefore, gene *PPO-2Ab* should be chosen for breeding a wheat variety with low PPO activity.

Discussion

This study used molecular markers that have been shown to be involved in the regulation of PPO activity located on chromosomes 2A, 2B, and 2D (Chang et al., 2007; Fuerst et al., 2008; Ge et al., 2004; Han et al., 2006; He et al., 2007; Wang et al., 2008; Wang et al., 2009). A study investigating the chromosome location of the QTLs responsible for changes in the PPO activity of western wheat kernels, it revealed that there was only one main effect QTL in one inbred line which was located on chromosome 2D, two other main effect QTLs located on chromosomes 2A and 2B were identified in another inbred line (Demeke et al., 2001). This suggests that in different genetic backgrounds, the involved main effect QTL may be different. There may have been other less effective genes on chromosomes 3B, 3D and 6B. It has been proposed that in western wheat, there are one or two pairs of main effect genes controlling PPO activity and multiple allelic genes for PPO substrate specialization (Anderson and Morris, 2001). In fact, two pairs of main effect genes that control PPO activities in Chinese wheat have been previously reported (Ge et al., 2004; Han et al., 2006); further research then showed these genes were located on chromosomes 2A and 2D (Zhang et al., 2005).

On the basis of our results, we propose that wheat kernel PPO activity is determined by genes on chromosome 2, and are under the regulation of genes on chromosomes 3 and 5. Chinese wheat has one or two main effect genes related to kernel PPO activity, and these genes are located on chromosomes 2A and 2D. Western wheat has three main effect genes related to kernel PPO activity, which are located on chromosomes 2A, 2B and 2D. The strains ID377s and Klasic both have one main effect gene and four other less effective genes related to kernel PPO activities (Massa et al., 2007). The main effect gene in ID377s is DQ889709 on chromosome 2A, with a 125-bp long intron 2. The main effect gene in Klasic is DQ889708 on chromosome 2B, with a 360-bp long intron 2. On the basis of the hypothesis that the length of intron affects PPO activity (He et al., 2007), the PPO activity of Klasic should be higher than that of ID377s. Indeed, an earlier study has demonstrated higher kernel PPO activity of Klasic than ID377s (Anderson et al., 2001). Therefore, DQ889708 is the more effective main effect gene controlling kernel PPO

Table 1. Classes of wheat kernel polyphenol oxidase (PPO) activity.

Level of activity	Range of activity (AU/min·g)
Low activity	Activity \leq 167
Medium activity	167 < activity < 334
High activity	Activity \geq 334

Table 2. ANOVA on the wheat kernel PPO activities in the 300 F₄ plants resulting from chromosome 2A.

Variation source	DF	SS	F value	SS/SS (%)
Composite allelic genes	2	1109407.4	296.19**	66.7
Error	297	556211.7		
Total variation	299	1665619.1		

Note: Allelic variations on the 2D chromosome are not found.

** indicates significant at 1% probability level. DF means degree of freedom, SS means “sum of squares”.

Table 3. ANOVA on the wheat kernel PPO activities in the 362 wheat cultivars

Variation source	DF	SS	F value	SS/SS (%)
Composite allelic genes	2	1348853.4	166.41**	48.1 ^a
2AL allelic genes	1	969288.1	239.17**	71.9 ^b
2DL allelic genes	1	379565.3	93.66**	28.1
Error	359	1454924.6		
Total variation	361	2803778.0		

^a indicates 1348853.4/2803778.0, ^b indicates 969288.1/1348853.4.

** indicates significant at 1% probability level. DF means degree of freedom, SS means “sum of squares”.

activities, located on chromosome 2B. The allelic genes *PPO-2Ab* and *PPO-2Da* reduce PPO activity, whereas *PPO-2Aa* and *PPO-2Db* increase PPO activity; these findings comply with the results of He et al. (2007). We found that the composite type *PPO-2Ab/2Da*, *PPO-2Ab/2Db*, *PPO-2Aa/2Da*, and *PPO-2Aa/2Db* had increasingly higher PPO activities, consistent with the results of a previous study (Chang et al., 2007). With respect to effect of single gene, the presence of *PPO-2Ab* reduces the PPO activities to below the medium level, and *PPO-2Da* can only reduce the activity to half of that of *PPO-2Ab*. It has been previously pointed out that in the presence of *PPO-2Ab* and absence of the PPO main effect gene on chromosome 2D, the PPO activity reduced to 0 (Chang et al., 2007), suggesting the importance of *PPO-2Ab* for wheat breeding and selection. This has been further confirmed by our results.

The allelic genes on chromosomes 2A and 2D control 48.1% of the variations in kernel PPO activity, suggesting the existence of other main effect genes and other influence factors. In our study to determine the effects of genetic background and its interaction with PPO genes, we found that 50% of the PPO activity was determined by the variations in allelic genes on chromosomes 2A and 2D, whereas 37–50% of the activity was controlled by genetic background and 9% by the interaction between genetic background and PPO genes. This result suggests the existence of main effect genes on chromosome 2B, which have greater effects than those on chromosomes 2A and 2D. In conclusion, our study demonstrated that different PPO alleles affect the PPO activity of wheat kernels differently. As *PPO-2Ab* is responsible for maintaining PPO activities at a low level, it is an ideal target for selection in the breeding of wheat varieties exhibiting low kernel PPO activities and hence tolerant to the undesirable browning of flour products.

Materials and methods

Materials

During the 2005–2006 crop season, 362 hexaploid wheat cultivars (*Triticum aestivum* L., see supplementary data for specifications) were grown in two locations at the experimental station of Anhui Agricultural University, located in Hefei and Fengyang, Anhui Province, China. The cultivation experiment was performed using a randomized complete block design with two replications. In addition, 300 F₄ plants obtained by the hybridization of Yangmai 158 and Huaimai 18 were grown in Hefei experimental station of Anhui Agricultural University during the same crop season. For both cultivation experiments, each plot contained two 2-meter rows spaced 25 cm apart, with 100 plants in each row. We chose the 362 cultivars to study how the four PPO alleles affect PPO activity of wheat kernels from different genetic backgrounds, whereas the segregating population of 300 F₄ plants was used to study the effect of variations in these alleles on the PPO activity of wheat varieties of similar genetic backgrounds.

We also examined how composite genotypes of these alleles affect the PPO activity of wheat kernels. The kernel PPO activities of the experimental varieties were all expressed in AU/min·g. The results showed that in this study all wheat kernel PPO activities represented the normal range, and were between 40 and 500AU/min·g. They have been equally divided into three different levels: low, medium and high, as shown in Table 1. PPO activity less than 167 AU/min·g was considered low.

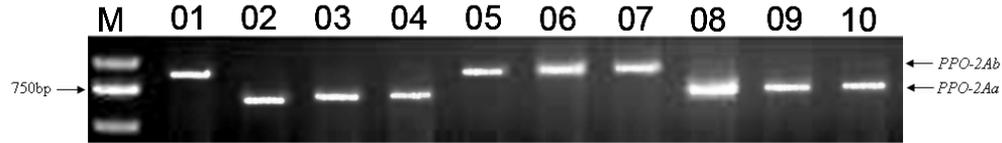


Fig 1. Electrophoresis of PCR fragments amplified by PPO18 that shows 2 alleles (*PPO-2Aa* and *PPO-2Ab*) of the *PPO-2A* gene. The single fragment (>750bp) amplified in the cultivars with low PPO activity indicates *PPO-2Ab* (lane 01, 05, 06 and 07), and another single fragment (<750bp) amplified in the cultivars with high PPO activity indicates *PPO-2Aa*.

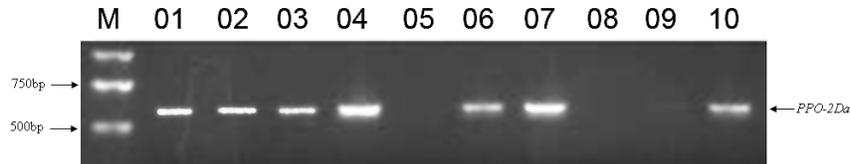


Fig 2. Electrophoresis of PCR fragments amplified by STS01 that shows 2 alleles (*PPO-2Da* and *PPO-2Db*) of the *PPO-2D* gene. The single fragment (>500bp) amplified in the cultivars with low PPO activity indicates *PPO-2Da*, and none fragment amplified in the cultivars with high PPO activity indicates *PPO-2Db* (lane 05, 08, and 09).

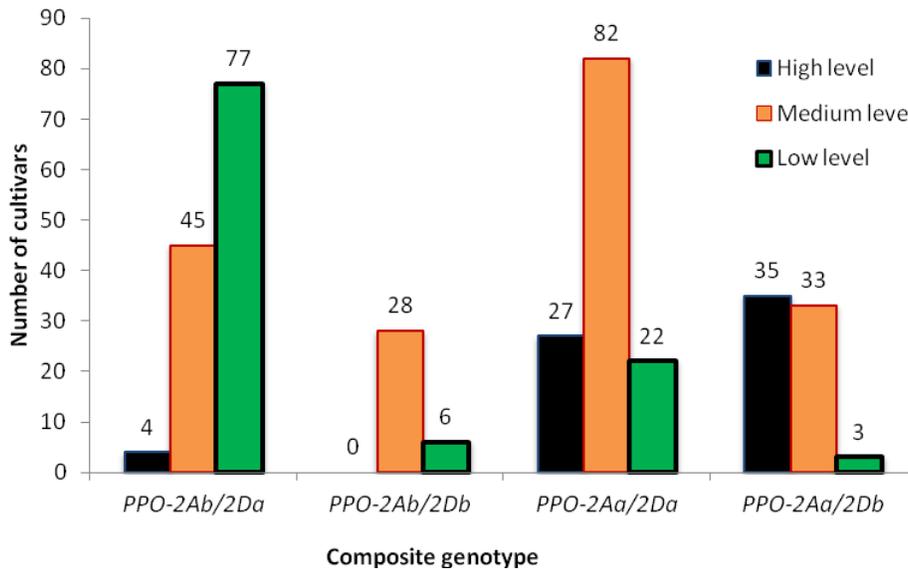


Fig 3. Distribution of the 362 wheat kernel PPO activities in the four composite genotypes.

Methods

Detection of PPO enzyme activity

The PPO activity was measured using the spectrophotometric method first reported by Anderson and Morris and modified by Han (Morris et al., 2000; Han et al., 2006). The modifications included that 0.3 g of whole wheat flour was used as a sample and catechol was used as the substrate for the reaction. In brief, 7.5 ml of freshly prepared 10 mM L-DOPA (3,4-dihydroxyphenylalanine) were added to a 50-ml tube containing 50 mM MOPS (3-(N-morpholino) propane sulfonic acid) buffer (pH 6.5) and 0.3 g whole wheat meal, and the tubes were then incubated in a 37°C water bath and shaken with an orbital shaker at a speed of 100 rpm for 5 min. After

incubation, the tubes were transferred into ice water to slow down the enzyme reaction and the reaction solution was immediately filtered through filter paper. Absorbance (A_{475}) was measured with 0.3 ml of the incubated solution at 475 nm using an Unico UV-4802H spectrophotometer. One unit of PPO activity was defined as the absorbance (A_{475}) value of 1-mg sample through 1-cm path for 1 min at 475 nm. Each reaction was repeated three times.

Identification of allelic genes

Identification of allelic genes was performed according to the protocols used in previous studies (Sun et al., 2005; Wang et al., 2009). The alleles *PPO-2Aa* (GenBank: EF070147) and *PPO-2Ab* (GenBank: EF070148) on chromosome 2A were

Table 4. Relationship between levels of wheat kernel PPO activity and single alleles in 362 cultivars

Level of activity	<i>PPO-2Ab</i>	<i>PPO-2Aa</i>	<i>PPO-2Da</i>	<i>PPO-2Db</i>	Total numbers
High level	4	62	31	35	132
Medium level	73	115	127	61	376
Low level	83	25	99	9	216
Average(AU/min·g)	186.08 ^C	289.46 ^A	223.07 ^B	294.43 ^A	
Effect	-23.7%	+18.7%	-8.5%	+20.8%	

Different letters indicate significance at the 1% probability level. *PPO-2Aa*, *PPO-2Ab*, *PPO-2Da*, and *PPO-2Db* indicate gene sequences with accession numbers EF070147, EF070148, EF070149, and EF070150, respectively. The effect is determined by comparing the averages of single gene activity and total activity. The superscript letters denote significant difference ($t=0.01$) between genotypes.

Table 5. Relationships between levels of wheat kernel PPO activity and genotypes in 300 F4 lines

Level of activity	<i>PPO-2Aa/2Aa</i>	<i>PPO-2Aa/2Ab</i>	<i>PPO-2Ab/2Ab</i>	Total numbers
High level	7	0	0	7
Medium level	180	20	1	201
Low level	8	26	58	92
Average(AU/min·g)	234.62 ^A	163.41 ^B	81.65 ^C	

Different letters indicate significance at the 1% probability level. *PPO-2Aa* and *PPO-2Ab* indicate the gene sequences with accession numbers EF070147 and EF070148, respectively. The superscript letters denote significant difference ($t=0.01$) between genotypes.

identified using the STS marker PPO18 which can amplify a single fragment (>750bp, indicates *PPO-2Ab*) in the cultivars with low PPO activity and another single fragment (<750bp, indicates *PPO-2Aa*) in the cultivars with high PPO activity (Fig.1); the alleles *PPO-2Da* (GenBank: EF070149) and *PPO-2Db* (GenBank: EF070150) on chromosome 2D were identified using the STS marker STS01 which can amplify a single fragment (>500bp, indicates *PPO-2Da*) in the cultivars with low PPO activity and none fragment (indicates *PPO-2Db*) in the cultivars with high PPO activity (Fig.2). All PCR reactions were performed in a MyCycler™ thermal cycler in a total volume of 20 ul containing 20 mM of Tris-HCl (pH 8.4), 20 mM of KCl, 200 uM of each dNTP, 1.5 mM of MgCl₂, 10 nM of each primer, 1.5 units of Taq polymerase, and 100 ng of template DNA. PPO18 PCR amplifications were performed at 94°C for 5 min, followed by 36 cycles of 94°C for 1 min, 60 °C (57.5 °C for STS01) for 1 min, 72 °C for 1 min, with a final extension of 72 °C for 8 min. Amplified PCR fragments were separated on a 1.5% agarose gel and stained with EB and visualized under UV light.

Statistical analysis

The effects of allelic genes and their composite genotypes on kernel PPO activities were analyzed using statistical analysis software SAS (SAS Institute Inc., Cary, NC, USA, www.sas.com), and the differences of mean PPO activities were adjusted with Duncan's Multiple Range Test.

Acknowledgements

Our work was supported by grants from the Provincial Natural Science Key Research Project of Anhui Colleges (KJ2011A113), the National Key Technologies R&D Program (2011BAD35B03), and the National Modern Agro-Industry Technology Research System (nycytx-03).

References

Anderson JV, Morris CF (2001) An improved whole-seed assay for screening wheat germplasm for polyphenol oxidase activity. *Crop Sci* 41:1697-1705

- Anderson JV, Patrick Fuerst E, Hurkman WJ, Vensel WH, Morris CF (2006) Biochemical and genetic characterization of wheat (*Triticum* spp.) kernel polyphenol oxidases. *J Cereal Sci* 44:353-367
- Baik BK, Czuchajowska Z, Pomeranz Y (1994) Comparison of polyphenol oxidase activities in wheats and flours from Australian and US cultivars. *J Cereal Sci* 19:291-296
- Chang C, Zhang HP, Xu J, You MS, Li BY, Liu GT (2007) Variation in two PPO genes associated with polyphenol oxidase activity in seeds of common wheat. *Euphytica* 154:181-193
- Demeke T, Morris CF, Campbell KG, King GE, Anderson JA, Chang HG, USDA A (2001) Wheat polyphenol oxidase: Distribution and genetic mapping in three inbred line populations. *Crop Sci* 41:1750-1757
- Fuerst EP, Xu SS, Beecher B (2008) Genetic characterization of kernel polyphenol oxidases in wheat and related species. *J Cereal Sci* 48: 359-368
- Ge XX, He ZH, Yang J, Zhang QJ (2003) Polyphenol oxidase activities of Chinese winter wheat cultivars and correlations with quality characteristics. *Acta Agron Sin* 4:481-485
- Ge XX, Zhang LP, He ZH, Zhang WM (2004) The mixed inheritance analysis of polyphenol oxidase activities in winter wheat. *Acta Agron Sin* 30:18-20
- Han J, Ma C, Si H, Han L, Chen J (2006) The genetic analysis & variation of PPO activity in the early generations of Yangmai 158x Huaimai 18. *J Triticeae Crops* 26: 38-41
- He X, He Z, Zhang L, Sun D, Morris C, Fuerst E, Xia X (2007) Allelic variation of polyphenol oxidase (PPO) genes located on chromosomes 2A and 2D and development of functional markers for the PPO genes in common wheat. *Theor Appl Genet* 115: 47-58
- Jimenez M, Dubcovsky J (1999) Chromosome location of genes affecting polyphenol oxidase activity in seeds of common and durum wheat. *Plant breeding* 118:395-398
- Mares DJ, Campbell A (2001) Mapping components of flour and noodle colour in Australian wheat. *Aust J Agr Res* 52:1297-1310
- Massa AN, Beecher B, Morris CF (2007) Polyphenol oxidase (PPO) in wheat and wild relatives: Molecular evidence for a multigene family. *Theor Appl Genet* 114:1239-1247

- Morris C, Jeffers H, Engle D (2000) Effect of processing, formula and measurement variables on alkaline noodle color-toward an optimized laboratory system. *Cereal Chem* 77:77-85
- Raman R, Raman H, Johnstone K, Lisle C, Smith A, Matin P, Allen H (2005) Genetic and in silico comparative mapping of the polyphenol oxidase gene in bread wheat (*Triticum aestivum* L.). *Funct Integr genomic* 5:185-200
- Raman R, Raman H, Martin P (2007) Functional gene markers for polyphenol oxidase locus in bread wheat (*Triticum aestivum* L.). *Mol Breeding* 19:315-328
- Sun D, He Z, Xia X, Zhang L, Morris C, Appels R, Ma W, Wang H (2005) A novel STS marker for polyphenol oxidase activity in bread wheat. *Mol Breeding* 16:209-218
- Udall J (1997) Important alleles for noodle quality in winter wheat as identified by molecular markers. M.S. Thesis, University of Idaho, Moscow, ID.
- Wang XB, Ma CX, He KQ, Si HQ, Zhang YL (2008) Development and application of a STS marker for kernel PPO gene located on chromosome 2D in common wheat. *Scientia Agric Sin* 39:1583-1590
- Wang X, Ma C, Si H, Qiao Y, Chang C, He X, Xia Y (2009) Gene markers for kernel polyphenol oxidase activity in common wheat. *Mol Breeding* 23:163-170
- Zhang LP, Ge XX, He ZH, Wang DS, Yan J, Xia XC, Sutherland MW (2005) Mapping QTLs for polyphenol oxidase activity in a DH population from common wheat. *Acta Agron Sin* 31:7-10