Journal of Cereal Science 54 (2011) 387–394

Contents lists available at SciVerse ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Allelic variation of polyphenol oxidase genes impacts on Chinese raw noodle color

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ARTICLE INFO

Article history:
Received 18 May 2011
Received in revised form
28 July 2011
Accepted 1 August 2011

Keywords:
Polyphenol oxidase
Chinese raw noodles
Noodle color
Triticum aestivum

ABSTRACT

Polyphenol oxidase (PPO) activity is a factor in time-dependent discoloration of Asian noodles. At least two major genes *Ppo-A1* and *Ppo-D1* control kernel PPO activity in wheat. The goal was to determine the relative importance of allelic variation for *Ppo-A1* and *Ppo-D1* on Chinese raw noodle color profile and kernel and flour characteristics using a winter wheat recombinant inbred population segregating for *Ppo-A1* and *Ppo-D1*. *Ppo-A1* allelic variation explained 80% and *Ppo-D1* allelic variation explained 2% of the variation in kernel PPO activity. The low PPO allele at each locus gave noodles that were brighter (greater L*), more yellow (greater b*) and more red (greater a*) at 24 h, and noodles with less change in L* but more change in a* and b* with time (0–24 h). The two loci combined explained 27, 35, and 17% of variation in L*, a*, and b* at 24 h, and 51, 55, and 58% of the variation in change in L*, a*, and b* with time, respectively. Allelic variation at *Ppo-D1* explained a larger percentage of variation than did *Ppo-A1* in each case except b* at 24 h where the two loci explained equal amounts.

1. Introduction

The basic noodle ingredients for Asian noodles are flour, salt and water. Noodle properties vary depending on methods of preparation and properties of the flour. The eating quality of Asian noodles is judged by their color and texture. Consumer preferences for noodle texture vary by region and with individual. It is universally accepted that consumers prefer noodles with a stable, bright, creamy color.

A number of studies have shown the concentration of polyphenol oxidase (EC.1.14.18.1) enzyme (PPO) is a factor in time-dependent discoloration of noodles (Baik et al., 1995; Fuerst et al., 2006b; Kruger et al., 1994). The darkening and discoloration is caused by enzymatic oxidation of phenols to produce dark colored products (reviewed in Feillet et al., 2000). This enzyme is most prevalent in the bran (Rani et al., 2001) and more specifically in the aleurone layer of the wheat (*Triticum aestivum* L. subsp. *aestivum*) kernel (Sullivan, 1946). Thus, PPO enzymatic activity within a genotype increases from bran contamination with increasing flour extraction rate (Hatcher and Kruger, 1993).

Genetic studies have pointed to genes on homoeologous group 2 chromosomes as influencing PPO activity. Jimenez and Dubcovsky (1999), using chromosome substitution lines from three cultivars into the land-race Chinese Spring, showed genes affecting PPO activity resided on chromosome 2A. Mapping studies with recombinant inbred populations also detected quantitative trait loci (QTL) for PPO activity on homoeologous group 2 chromosomes for M66/'Opata 85' and NY18/'Clark's Cream' recombinant inbred populations. Individual QTLs explained 18–23% of the variation in PPO activity. In addition, significant QTLs were located to homoeologous group 3 chromosomes for NY18/Clark's Cream and ND2603/'Butte 86' populations (Demeke et al., 2001b). Mares and Campbell (2001) identified a QTL for PPO activity on chromosome 2A in one of three environments, and a QTL on chromosome 2D in all three environments in a 'Sunco'/'Tasman' recombinant inbred population. Raman et al. (2005) identified a major QTL on chromosome 2A that explained more than 80% of the variation in PPO activity in a 'Chara'/WW2449 doubled haploid population. Sadeque and Turner (2010) identified a QTL for PPO activity on chromosome 2AL and QTL with smaller effects on chromosomes 4A and 7B in a doubled haploid population from high and low PPO parents.

Recent developments have led to cloning and complete sequence information for major PPO genes in wheat. Full length sequences for expressed sequence tags (ESTs) from cDNA libraries

Abbreviations: bp, base pair; EBLUP, Empirical best linear unbiased predictors; EST, Expressed sequence tag; L-DOPA, 3,4-dihydroxy-L-phenylalanine; PCR, Polymerase chain reaction; PPO, Polyphenol oxidase; QTL, quantitative trait loci; RIL, Recombinant inbred line; SKCS, Single Kernel Characterization system.

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doi:10.1016/j.jcs.2011.08.003

were used to identify six PPO genes. These clustered into two groups of three genes based on their sequence similarity. One of the two groups was assumed to be expressed in kernels since sequences from it were derived from cDNA from developing kernels (Jukanti et al., 2004). Sun et al. (2005) used published PPO gene sequences from Jukanti et al. (2004) expressed in developing wheat kernels to design a primer pair that amplified a 685 bp fragment from cultivars with high and a 876 bp fragment from cultivars with low PPO activity. The sequence tagged site (STS) marker mapped to chromosome 2AL in a segregating population. He et al. (2007) showed genomic DNA sequences for two PPO genes designated *Ppo-A1* and *Ppo-D1* located on chromosomes 2AL and 2DL, respectively. They demonstrated that the STS marker from Sun et al. (2005) was a functional marker for *Ppo-A1*. They also developed two functional, complimentary STS markers for *Ppo-D1* that mapped to chromosome 2DL. In cases where marker class means for the *Ppo-A1* and *Ppo-D1* were compared, *Ppo-A1* had a larger effect than *Ppo-D1* on kernel PPO activity (He et al., 2007; Wang et al., 2009). Beecher and Skinner (2011) have cloned two additional PPO genes that are expressed in developing wheat kernels, designated *Ppo-A2* and *Ppo-D2*, which were localized to the long arm of chromosomes 2A and 2D, respectively. Based on sequence similarity, the *Ppo-1* genes were determined to be paralogous to the *Ppo-2* genes, meaning the second gene resulted from a duplication of the first gene within a genome.

The time dependent Asian noodle discoloration has a genetic component. Heritability for change in noodle brightness (L*) was 0.90 for white salted and 0.96 for yellow alkaline noodles, respectively (Mares and Campbell, 2001). A QTL for noodle brightness at 24 h and change in brightness with time was identified on chromosome 2D for white salted noodles, and similar results were obtained for yellow alkaline noodles except that an additional QTL was identified on chromosome 2A (Mares and Campbell, 2001). Sadeque and Turner (2010) found a QTL on chromosome 2AL that explained 64% of the phenotypic variation for change in alkaline noodle brightness, but also found additional QTL on chromosomes 1AS, 1B and 7AL. Although PPO activity and noodle color were measured in these studies, effects of allelic variation for *Ppo-A1* and *Ppo-D1* were not measured. Martin et al. (2010) showed that winter wheat lines with *Ppo-A1b* allele had lower kernel PPO activity as well as brighter noodles at 24 h and less change in brightness with time than lines with *Ppo-A1a* allele in two segregating populations. The populations did not segregate for *Ppo-D1*.

Breeders can use the functional markers available for *Ppo-A1* and *Ppo-D1* in marker assisted selection to select genotypes with low levels of kernel PPO activity. In order for the process to be efficient, it is important to know the effect these genes have on noodle color, and kernel and flour characteristics. Our goal was to determine the relative importance of allelic variation for *Ppo-A1* and *Ppo-D1* on the Chinese raw (white salted) noodle color profile and kernel and flour characteristics using a winter wheat recombinant inbred population. The information will be useful to wheat breeders in developing cultivars with improved noodle color characteristics.

2. Materials and methods

2.1. Genetic materials

Hard white winter wheat parents 'Arrowsmith' (PI 633911) (Graybosch et al., 2005) and 'NuSky' (PI 619167) (Berg et al., 2003) were crossed to produce a recombinant inbred population. Single heads were harvested from individual F_2 plants and planted as F_3 head rows. This process was repeated in successive generations through the F_5 generation to produce 301 F_5-derived F_6 recombinant inbred lines (RIL). The RILs were screened with sequence tagged site (STS) markers PPO18, PPO33, PPO16, and PPO29 using primers presented in He et al. (2007). DNA was extracted and pooled from three plants from each line. PPO18 produced 685 and 876 base pair (bp) polymerase chain reaction (PCR) products and PPO33 produced 290 and 481 bp PCR products designating *Ppo-A1a* and *Ppo-A1b*, respectively. Genotypes were screened with complementary STS markers PPO16 and PPO29, where the PPO16 marker produced a 713 bp PCR product, or no product, designated *Ppo-D1a* and *Ppo-D1b*, respectively. Similarly, PPO29 produced a 490 bp PCT product, or no PCR product, designated *Ppo-D1b* and *Ppo-D1a*, respectively. Lines were categorized as homozygous for either allele or mixed, meaning the line derived from a heterozygous F_5 plant, for the *Ppo-A1* and *Ppo-D1* loci. Twenty-five RIL homozygous for each of the four genotype classes were randomly selected.

2.2. Evaluation of recombinant inbred lines

The 100 RILs were grown in an augmented design with four blocks and five check entries randomized in each block. The five check entries were Arrowsmith, 'BigSky' (PI 619166), 'Goldenspike' (PI 614813), NuSky, and 'Rampart' (PI 593889). The experiment was planted at the Arthur H. Post Field Research Laboratory near Bozeman, MT and the Southern Agricultural Research Center near Huntley, MT in Fall 2008. Plots were 3 rows 4 m long at Bozeman and 4 rows 3 m long at Huntley with 30 cm between rows. Each plot was harvested for grain yield. A subsample of grain was used to determine test weight with a Seedburo (Chicago, IL) test weight scale. Grain protein concentration was determined by near-infrared spectroscopy for whole grain using the Infratec 1241 Grain Analyzer (Foss North America, Eden Prairie, MN). Kernel weight and diameter were determined using the Single Kernel Characterization system (SKCS) (Perten Instruments North America Inc., Springfield, IL) from a sample of 300 kernels. Kernel PPO activity was determined following AACC method 22-85 with 10 mM L-DOPA and four kernels for each replication. Kernel PPO activity was expressed as Δ_{475} min^{-1} g^{-1}, since previous work (Demeke et al., 2001a) has shown kernel PPO activity was positively related to kernel weight.

Wheat was milled on a Brabender Automat Quadrumat Junior Mill (South Hackensack, NJ) after a single stage temper to 15.0% moisture. Flour protein was determined with an Infratec 1241 Grain Analyzer with flour NIR attachment (Foss North America, Eden Prairie, MN) and expressed at a 14.0% flour moisture basis. The combustion method was the reference using a LECO FP-528 (LECO Corp., St. Joseph, MI) nitrogen analyzer (AACC Method 46-30).

Chinese raw (white salted) noodles were prepared using 100 g straight grade flour (140 g kg^{-1} moisture basis, fwb) and 29.2 ml salt (NaCl) water solution (4.29% w/v) added to the flour during a 30 s time period. Doughs were mixed in a Finney Special mixer (100 g Micro Dough pin mixer, head speed 102 rev/min (National Manufacturing Co., TMCO, Inc., Lincoln NE)) for 5 min and 45 s. Flour adhering to the inside of the mixing bowl and pins was brushed down and premixed for 15 s prior to adding the salt–water solution. The salt–water solution was slowly added so that the last drop was added 30 s after the initial drop was dispensed to insure uniform hydration and complete incorporation. After 30 s of additional mixing, the mixer was stopped to clean dough off the pins and to break up any large lumps of dough. This step was repeated after an additional 1 min and 30 s of mixing followed by a final 3 min of mixing. The crumbly dough was pressed by hand into a cohesive rectangular block then placed in a plastic bag to rest for 30 min at room temperature. The dough block was