Understanding the molecular basis of Chinese noodle quality

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INTRODUCTION

Improvement of traditional Chinese products such as noodles is very important to meet the domestic market demands in China. There are many types of noodles consumed, and we focus on the dry white Chinese noodles (DWCN), largely due to its popularity and high commercial value. In comparison with pan bread making quality, very little information is available on the genetic basis of DWCN quality although quality of Japanese udon noodle is basically controlled by *Wx-B1* null type (1). Genotype, environment, their interaction, and processing procedure all affect noodle quality. The official standardized procedure for Chinese noodles (SB/T10137-93), has been released, but not widely used in milling industry and breeding programs, largely due to its poor repeatability. Establishment of a standardized laboratory noodle testing procedure is crucial for wheat breeding programs to develop good noodle quality cultivar. Identification of major traits associated with noodle quality, understanding their molecular basis, and development and utilization of molecular markers will definitely improve the efficiency of genetic improvement for noodle quality. The objective of this paper is to review the progress in understanding the environmental effects on quality traits and the molecular basis of DWCN quality by combination of conventional breeding and quality testing, in silico cloning, and molecular marker development and validation.

ENVIRONMENTAL EFFECTS ON QUALITY ATTRIBUTES

For producing high quality end-products, the quality per se and its consistency across locations and years are of primary importance. Most quality traits except for a few traits such as grain hardness, are controlled by at least several genes and are heavily affected by the growing environment. In general, quality attributes such as grain hardness (*Pina*/*Pinb*), dough strength and extensibility (*Glu-1* and *Glu-3*), starch viscosity (waxy), and color associated traits such as polyphenol oxidase (PPO) activity and yellow pigment (YP), are basically controlled by major genes /QTL and are less sensitive to environmental stress although environment also has significant effect, however, protein content and test weight are much more influenced by environment (2, 3). Several environmental factors such as temperature and drought during grain filling stage, rainfall before harvesting, have significant effects on end-use quality. As temperature increases, protein content and dough strength can be improved, however dough quality and end-use products quality will be reduced at temperature above 30^{o}C. High temperature also has negative effect on starch content, thus a reduction on kernel size and milling performance. Flour obtained from sprouted grains loses its viscosity due to starch breakdown, and baked goods from such flour are small in volume and have a compact, sticky crumb structure. A number of genes and QTLs involved in PHS tolerance or seed dormancy have been found and mapped in wheat.

STANDARDIZATION OF LABORATORY TESTING

In order to improve the official method of noodle flour SB/T10137-93, flour extraction rate, optimum water addition, amount of salt addition, mixing time, sheeting, and cooking method were investigated. The recommended composition for laboratory preparation of DWCN is 60% flour extraction rate, 35% water addition including flour moisture, and 1% salt concentration (unpublished data from our lab, 2008). An improved processing method and sensory scoring system were developed (4, 5). The modified scoring system includes six parameters, i.e., color (15), appearance (10), firmness (20), viscoelasticity (30), smoothness (15), taste and flavor (10), with a total score 100 (5). In this scoring system, elasticity and stickiness were combined into viscoelasticity, the weight given to each noodle parameter was modified according to the difference in consumer preference for noodle attributes. Chinese commercial flour Xuehuafen is recommended as a control for sensory evaluation, and testing samples are compared with the control and then a relative score is assigned to each parameter (4). Our experiences indicated that both accuracy and repeatability are greatly improved in comparison with the previous official method. In addition to panel testing, other approaches were also used to measure noodle parameters. The color of cooked noodle was closely associated with measurement from Minolta CR 310, with r=0.73. Hardness of texture profile analysis (TPA) using Texture Analyzer was significantly associated with noodle total score, with r=0.66 (6).

TRAITS AND MOLECULAR MARKERS ASSOCIATED WITH NOODLE QUALITY

Major traits affecting DWCN quality were identified, i.e., gluten strength, starch viscosity, grain hardness, protein content, and flour color associated traits (5, 7, 8). Polyphenol oxidase (PPO), yellow pigment, SDS sedimentation value, and peak viscosity can be used to screen for dry white Chinese noodle quality in the early generations of a wheat breeding program. The association between SDS sedimentation value, Farinograph stability, and Extensograph maximum resistance, and DWCN score fit a quadratic regression model, accounting for 31.0%, 39.0%, and 47.0% of the DWCN score, respectively (7,8). Therefore, medium to strong gluten type with good extensibility is desirable for DWCN quality which are different from Japanese udon noodle. The starch peak viscosity contributed positively to DWCN quality, with r= 0.57, very similar to Japanese udon noodle. Flour ash content and PPO activity had a negative moderate effect on noodle color, while protein content and grain hardness were negatively associated with noodle color, appearance, and smoothness. A very high association was found between flour color grade (FCG) and L* value of flour water slurry (r=-0.95). Strong associations were also established between milling quality index (MQI) and FCG, L* values of dry flour, flour-water slurry, and white salted noodle sheet (9).

More than 200 leading wheat cultivars and advanced lines as well as DH population were used to validate the association between molecular markers from collaborators and our programs and quality traits. Low molecular weight glutenin subunit/allele *Glu-A3d* and *Glu-B3d* (gluten quality) show slightly better noodle quality (10). In addition to its strong negative effect on gluten strength, a major QTL controlling flour yellow pigment was identified on chromosome 1B/1R translocation, indicating its significant role in increasing yellow pigment (11). Two complementary dominant STS markers *PPO16* and *PPO29* were developed for the PPO gene on chromosome 2D (12), and a functional STS marker *PPO18* for the PPO gene on chromosome 2A was also developed (13). A co-dominant marker for phytoene synthase (PSY) gene on chromosome 7A, *YP7A*, was developed to discriminate the alleles *Psy-A1a*/*Psy-A1b* that are highly related to YP content (14). A co-dominant marker for phytoene synthase gene on chromosome 7B, *YP7B-1*, was developed to discriminate *Psy-B1a*/*Psy-B1b* (unpublished data from our lab). Both soft type (*Pina-D1a*/*Pinb-D1a*) and *Pinb-D1b* type are more desirable for milling performance and noodle quality (15). *Wx-7A* controlling starch viscosity is closely associated with good noodle quality (16). As to reduce the costs of marker application, a multiplex PCR assay comprising markers for genes/loci *Ppo-A1*, *Ppo-D1*, and *Wx-B1b* targeting noodle quality improvement was also developed (17). Use of these molecular markers can greatly improve the selection efficiency in early generations and they can also be used to confirm the results from conventional quality testing in more advanced stage.

CHARACTERIZATION OF POLYPHENOL OXIDASE (PPO) GENES

PPO activity is highly related to the undesirable browning of DWCN, and it is mainly conditioned by the PPO genes on homoeologous group 2 chromosomes. Using the method of in silico cloning and PCR validation, the PPO genes on chromosomes 2A, 2B and 2D were cloned and designated *Ppo-A1*, *Ppo-B1* and *Ppo-D1*, respectively. Each of the three PPO genes contains three exons, two introns and an open reading frame (ORF) of 1731 bp (12). Two allelic variants, *Ppo-A1a* and *Ppo-A1b*, were identified at the *Ppo-A1* locus in common wheat, and associated with higher and lower PPO activity, respectively. The main sequence difference between the two alleles is a 191-bp insertion found in the 5' end of the first intron of *Ppo-A1b*, which could cause an alternative splicing, leading to a reduced PPO activity in cultivars with *Ppo-A1b* (12, 13). The functional marker, *PPO18*, could be used to distinguish the two alleles, and co-segregated with the QTL on chromosome 2A in a DH population derived from the cross Zhongyou 9507/CA9632, explaining 28 to 43% of the phenotypic variance across three environments. At the *Ppo-D1* locus, two allelic variants, *Ppo-D1a* and *Ppo-D1b*, associated with lower and higher PPO activity, respectively, were identified in common wheat and could be detected with two dominant markers, *PPO16* and *PPO29*, respectively. The two markers co-localized with a major QTL on chromosome 2D in the population Zhongyou 9507/CA9632, accounting for 9.6 to 24.4% of the phenotypic variance across three environments (12). Furthermore, five new PPO alleles were identified in wheat relatives, i.e. *Ppo-A1c* derived from *T. urartu*, *Ppo-A1d* from *T. boeoticum*, *Ppo-A1e* from *T. monococcum* and *T. durum*, *Ppo-A1f* from *T. dicoccoides*, *Ppo-A1g* from *T. durum*, *Ppo-D1c* from *Ae. tauschii* and *Ppo-D1d* from *Ae. tauschii*. All of the genes except for *Ppo-A1g* and *Ppo-D1d* harboured an ORF of 1731 bp, encoding a polypeptide of 577 residues. The downstream sequence of *Ppo-A1g* was not obtained, and a 73-bp deletion occurred in the third exon of *Ppo-D1d*, resulting in a shorter polypeptide of 466 amino acids. A phylogenetic tree was constructed with the alleles at loci *Ppo-A1*, *Ppo-B1* and *Ppo-D1*, and the topology of the tree showed two distinct genetic lineages at both *Ppo-A1* and *Ppo-D1* loci, implying that more than one *T. dicoccum* and *Ae. tauschii* lines were involved in the origin of common wheat (unpublished data).

CHARACTERIZATION OF PHYTOENE SYNTHASE 1 (PSY1) GENES

High yellow pigment is undesirable for Chinese noodle quality since a bright white color is preferred. Phytoene synthase, a critical enzyme in the carotenoid biosynthetic pathway, demonstrated high association with the yellowness of DWCN. Using the method of in

silico cloning and experimental validation, the *PSY1* genes on chromosomes 7A, 7B and 7D were cloned in common wheat and designated *Psy-A1*, *Psy-B1* and *Psy-D1*, respectively. Each of the three *PSY1* genes has six exons spaced by five introns, and harbours an ORF of 1284 bp, 1263 bp and 1281 bp, respectively (14, and unpublished data). At the *Psy-A1* locus, three allelic variants, *Psy-A1a*, *Psy-A1b* and *Psy-A1c* were found in common wheat, and two in durum wheat, i.e. *Psy-A1d* and *Psy-A1e*. Among the five allelic variants, *Psy-A1a* and *Psy-A1b* were proven to be associated with higher and lower yellow pigment content, respectively. A 37-bp insertion was found at the 5' end of the second intron in *Psy-A1b*, which could cause an alternative intron splicing, resulted in a frame-shift mutation, which subsequently led to a premature translation termination, conferring a lower YP content to the cultivars with *Psy-A1b* (14). A co-dominant marker, *YP7A*, could be used to discriminate *Psy-A1a*/*Psy-A1b* and co-segregated with a major QTL on chromosome 7A in a RIL population PH82-2/Neixing 188, explaining 20.0 to 28.0% of the phenotypic variance across three environments. At the *Psy-B1* locus, five allelic variants were identified in common wheat and designated *Psy-B1a*, *Psy-B1b*, *Psy-B1c*, *Psy-B1d* and *Psy-B1e*, respectively. In Chinese wheat lines, *Psy-B1b* is associated with lower yellow pigment content, whereas *Psy-B1a* and *Psy-B1c* were related with higher yellow pigment content, and a co-dominant marker *YP7B-1* and a dominant marker *YP7B-2* could be used to detect *Psy-B1a*/*Psy-B1b* and *Psy-B1c*, respectively (unpublished data). In CIMMYT spring wheat lines, however, phenotypic differences between lines with various *Psy-B1* alleles were not significant. In durum wheat lines, three allelic variants, *Psy-B1e*, *Psy-B1f* and *Psy-B1g* were found at the *Psy-B1* locus, and *Psy-B1g* is associated with lower YP content, whereas *Psy-B1e* and *Psy-B1f* with higher YP content (unpublished data). A phylogenetic tree was constructed with the alleles at loci *Psy-A1*, *Psy-B1* and *Psy-D1*, and the topology of the tree showed two distinct genetic lineages at both *Psy-A1* and *Psy-B1* loci, implying that more than one *T. dicoccum* lines were involved in the origin of common wheat (unpublished data).

ACKNOWLEDGEMENT

This study was supported by the National Natural Science Foundation of China (30771335), National Basic Research Program (2002CB11300), International Collaboration Project from Ministry of Agriculture (2006-G2), and National 863 Program.

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