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Genetic Analysis of Chromosomal Loci Affecting the Content of Insoluble Glutenin in Common Wheat

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ABSTRACT

In common wheat, insoluble glutenin (IG) is an important fraction of flour glutenin macropolymers, and insoluble glutenin content (IGC) is positively associated with key end-use quality parameters. Here, we present a genetic analysis of the chromosomal loci affecting IGC with the data collected from 90 common wheat varieties cultivated in four environments. Statistical analysis showed that IGC was controlled mainly genetically and influenced by the environment. Among the major genetic components known to affect end-use quality, 1BL/1RS translocation had a significantly negative effect on IGC across all four environments. As to the different alleles of *Glu-A1*, *-B1* and *-D1* loci, *Glu-A1a*, *Glu-B1b* and *Glu-D1d* exhibited relatively strong positive effects on IGC in all environments. To identify new loci affecting IGC, association mapping with 1355 DArT markers was conducted. A total of 133 markers were found associated with IGC in two or more environments ($P < 0.05$), ten of which consistently affected IGC in all four environments. The phenotypic variance explained by the ten markers varied from 4.66% to 8.03%, and their elite alleles performed significantly better than the inferior counterparts in enhancing IGC. Among the ten markers, *wPt-3743* and *wPt-733835* reflected the action of *Glu-D1*, and *wPt-664972* probably indicated the effect of *Glu-A1*. The other seven markers, forming three clusters on 2AL, 3BL or 7BL chromosome arms, represented newly identified genetic determinants of IGC. Our work provided novel insights into the genetic control of IGC, which may facilitate wheat end-use quality improvement through molecular breeding in the future.

KEYWORDS: Common wheat; Insoluble glutenin; *Glu-1*; 1BL/1RS translocation; Association mapping; DArT

INTRODUCTION

Wheat is one of the three major food crops in the world. Compared to rice and maize, wheat is unique in having three families of gluten proteins, high-molecular-weight glutenin subunits (HMW-GSs), low-molecular-weight glutenin subunits (LMW-GSs) and gliadins, in its grains (reviewed in Wrigley et al., 2009). These proteins promote the formation of a gluten complex during flour processing, and confer visco-elastic properties to wheat dough (reviewed in Delcour et al., 2012). Owing to allelic variation, the composition of gluten

Abbreviations: AM, association mapping; FPC, flour protein content; GMP, glutenin macropolymers; GPC, grain protein content; GY, grain yield; HMW-GS, high molecular weight glutenin subunit; IG, insoluble glutenin; IGC, insoluble glutenin content; LMW-GS, low molecular weight glutenin subunit; QTL, quantitative trait locus; SDS-SV, SDS-sedimentation volume; SIG, swelling index of glutenin; UPP, unextractable polymeric proteins; YHRV, Yellow and Huai river valley; Zel-SV, Zeleny sedimentation volume.

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proteins frequently differ among different wheat varieties, leading to differences in dough functional properties and end-use qualities (Payne and Lawrence, 1983; Payne, 1987; Delcour et al., 2012). Consequently, a major objective of current global wheat quality improvement is to optimize the composition of gluten proteins and dough functionality (evaluated mainly by dough stability, elasticity and extensibility) for specific end-use traits (Wrigley et al., 2009).

In common wheat, HMW-GSs are encoded by the *Glu-A1*, *-B1* and *-D1* loci. Within each locus, there are two HMW-GS genes encoding one x-type and one y-type subunits, respectively (Payne, 1987; Delcour et al., 2012). Each locus has many alleles, some of which encode only one, or not all, HMW-GS because of gene silencing. For the expressed subunits, there are usually multiple variants differing in molecular mass. Consequently, common wheat varieties usually express three to five HMW-GSs, which account for 8%−10% of total grain protein (Shewry et al., 1995; Wrigley et al., 2009). LMW-GSs are encoded by *Glu-A3*, *-B3* and *-D3* loci (D'Ovidio and Masci, 2004). Compared to HMW-GSs, the genes encoding LMW-GSs are more numerous and exhibit more complex allelic variations (Dong et al., 2010; Zhang et al., 2013a). To date, the exact number of expressed LMW-GSs has not been determined for any common wheat variety. Nevertheless, it has been estimated that LMW-GSs collectively take up 20%−30% of total grain protein (Branlard et al., 2001; Howitt et al., 2006). Gliadins, specified by six chromosomal loci (*Gli-A1*, *-B1*, *-D1*, *-A2*, *-B2* and *-D2*), are most complex among the three families of gluten proteins in terms of gene member and allelic variation (Qi et al., 2006; Wrigley et al., 2006). They usually account for 40%−50% of the total grain protein, and are highly polymorphic among different common wheat cultivars (Qi et al., 2006).

With respect to the contribution to end-use qualities, HMW-GSs are predominant over LMW-GSs and gliadins (reviewed in Békés, 2012a, 2012b). It has been suggested that changes in HMW-GS composition can explain 40%−75% of the variation in key dough functional properties (Ng and Bushuk, 1988; Shewry et al., 2003; He et al., 2005). Although precise mechanisms underlying the function of the three types of gluten proteins in controlling dough functionality are still unclear, it has been proposed that both the amount and quality of the glutenin macropolymers (GMPs) formed by HMW-GSs and LMW-GSs are vital (Békés, 2012a, 2012b; Delcour et al., 2012). The GMPs in the flour are heterogeneous in size, with only those above 250 kDa being sufficiently large to contribute to dough stability, elasticity and extensibility (Southan and MacRitchie, 1999; Békés, 2012a). HMW-GSs and LMW-GSs interact covalently through disulphide bonds in forming GMPs. Gliadins modulate dough functional properties (and thus end-use qualities) possibly by affecting GMP formation through hydrogen bonding with HMW-GSs and LMW-GSs (Wieser, 2007). In biochemical fractionation of flour proteins, the larger GMPs are SDS insoluble and are thus named as UPP (unextractable polymeric proteins). Flour UPP is usually quantified using size exclusion high performance liquid chromatography (SE-HPLC) (Gupta et al., 1993; Bangur et al., 1997), and has been found to positively correlate with dough functional properties in many studies (Békés, 2012a, 2012b).

In 1990s, Sapirstein and colleagues demonstrated that the content of insoluble glutenin (IG) was positively correlated with dough functionality and breadmaking quality of common wheat (Sapirstein and Johnson, 1996; Sapirstein and Fu, 1998). Later, Hu et al. (2007) suggested that IG may also be used for selecting wheat breeding materials with good noodle processing quality. IG can be effectively separated from the monomeric proteins and soluble glutenin in wheat flour through a two-step fractionation procedure, and is efficiently quantified using a conventional spectrophotometer. SDS-PAGE analysis showed that the major components of IG included HMW-GSs and LMW-GSs (Sapirstein and Johnson, 1996; Sapirstein and Fu, 1998). Thus, it is very likely that IG, like UPP, represents the large-sized and functionally important fraction of GMPs in wheat flour, and is useful as an early screening for the breeding lines with superior end-use qualities. However, to date, IG has only been examined for a few common wheat varieties (less than 25 varieties) cultivated in limited environments (Sapirstein and Johnson, 1996; Sapirstein and Fu, 1998; Hu et al., 2007). No systematic investigations have been conducted to study the genetic loci controlling IG content (IGC). But there have been many quantitative genetic studies on wheat grain and flour components, glutenin content or dough parameters related to IG. For example, a large number of QTLs controlling grain protein content (GPC), flour protein content (FPC), glutenin level, UPP content, dough rheology indicators (e.g., SDS-sedimentation volume, elasticity and extensibility), have been identified (Huang et al., 2006; Nelson et al., 2006; Bordes et al., 2011; Reif et al., 2011; Tsilo et al., 2011; El-Feki et al., 2013; Plessis et al., 2013). These QTLs may aid the study of the genetic control of IGC.

Thus, the major objective of this work was to identify the genetic loci affecting IGC using association mapping (AM) with the data collected from 90 common wheat varieties grown in two locations and for two crop cycles. The major IGC loci identified were assessed for their allelic effects on both IGC and grain yield (GY), and their value for wheat end-use quality improvement was discussed.

RESULTS

Environmental and genetic variation of IGC

According to the ANOVA of IGC data collected from 90 varieties (Table S1) cultivated in four irrigated and fertilized environments, IGC variance was significantly affected by genotype (G), environment (E) and genotype and environment interaction (G × E) (Table S2). The IGC mean varied among different varieties (from 1.47 mg/100 mg flour to 3.93 mg/100 mg flour) and in different environments (from 2.35 mg/100 mg flour to 2.58 mg/100 mg flour), with that of E2 and E3 being the highest and lowest, respectively (Table 1). The genotypic and G × E variance components of IGC were both