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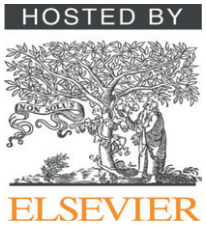

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Characterization and mapping of QTLs on chromosome 2D for grain size and yield traits using a mutant line induced by EMS in wheat

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ABSTRACT

Production of mutants with altered phenotypes is a powerful approach for determining the biological functions of genes in an organism. In this study, a high-grain-weight mutant line M8008 was identified from a library of mutants of the common wheat cultivar YN15 treated with ethylmethane sulfonate (EMS). F_2 and $F_{2:3}$ generations produced from crosses of M8008 × YN15 (MY) and M8008 × SJZ54 (MS) were used for genetic analysis. There were significant differences between M8008 and YN15 in plant height (PH), spike length (SL), fertile spikelet number per spike (FSS), grain width (GW), grain length (GL), GL/GW ratio (GLW), and thousand-grain weight (TGW). Most simple correlation coefficients were significant for the investigated traits, suggesting that the correlative mutations occurred in M8008. Approximately 21% of simple sequence repeat (SSR) markers showed polymorphisms between M8008 and YN15, indicating that EMS can induce a large number of mutated loci. Twelve quantitative trait loci (QTLs) forming QTL clusters (one in MY and two in MS) were detected. The QTL clusters coinciding with (MY population) or near (MS population) the marker *wmc41* were associated mainly with grain-size traits, among which the M8008 locus led to decreases in GW, factor form density (FFD), and TGW and to increases in GLW. The cluster in the *wmc25–barc168* interval in the MS population was associated with yield traits, for which the M8008 locus led to decreased PH, spike number per plant (SN), and SL.

1. Introduction

Common wheat (*Triticum aestivum* L.) provides one-fifth of the calories consumed by humans [1]. With increasing world population, it has been estimated that the global demand for wheat will increase by a further 40% by 2020 [2]. Accordingly, higher yield is the primary objective in wheat breeding programs. Grain yield can be divided into several direct components: spike number per unit area, grain number per spike, and thousand-grain weight (TGW). Mainly because of its effect on yield, increased grain size continues to be a major selection and breeding target in wheat [3,4]. Grain shape and

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size, density, and uniformity are important attributes determining the market value of wheat grain, given that they influence milling performance (flour quality and yield). Theoretical models have predicted that milling yield could be increased by optimizing grain shape and size, with large and spherical grains being the optimum grain morphology [5].

Yield and grain size traits in wheat are complex characters, and are quantitative in nature [6]. Grain-size traits are usually represented in plant breeding practice by TGW, which is determined mainly by grain width, length, and thickness [7,8]. All three aspects are positively correlated with TGW [9,10]. Quantitative trait loci (QTLs) for yield components such as grain weight have been mapped on almost all 21 wheat chromosomes [11]. There have also been QTL studies of grain-size traits [8–10,12–14].

A powerful approach for determining the biological functions of genes in an organism is the production of mutants with altered phenotypes and physiological responses. Among mutagens that have been used, alkylating agents such as ethylmethane sulfonate (EMS) are particularly effective. EMS can form adducts with nucleotides, causing them to mispair with their complementary bases and introducing base changes after replication [15]. EMS-induced mutants have been created in rice [16], maize [17], barley [18], diploid wheat [19,20], and hexaploid wheat [21,22]. Some genes, such as *klu*, regulating seed size [23], and *als3-1*, required by seedlings for growth in aluminum-toxic environments [24], have been isolated using EMS-induced mutations in *Arabidopsis thaliana*. However, genetic studies of EMS-induced mutants in wheat are rare.

M8008 is an EMS-induced common wheat mutant with high grain weight isolated in our laboratory. The main aim of the present study was to determine the chromosomal locations of QTLs for grain size and yield-related traits using two populations derived from M8008 and Chinese winter wheat varieties.

2. Materials and methods

2.1. Plant materials

M8008 was identified from a library of mutants of the wheat cultivar Yannong 15 (YN15) induced by EMS treatment of dry seeds. M8008 had a higher TGW, plant height (PH), and spike length (SL) than YN15. YN15 is a popular cultivar released in 1982 and planted over 6.7 million ha in the past 30 years in Shandong province, China.

For genetic analysis, F_2 populations (200 plants) and a derived $F_{2:3}$ progeny (200 lines) were produced from crosses of M8008 × YN15 (MY population) and M8008 × Shijiazhuang 54 (SJZ54) (MS population). YN15 and SJZ54 have lower TGW than M8008. SJZ54 is a Chinese winter cultivar released in 1964.

2.2. Field trials and trait evaluation

For the F_2 populations, planting rows were 2.5 m in length, spaced 23.3 cm apart and 10.0 cm between plants, in the wheat growing season of 2008–2009. Ten plants per $F_{2:3}$ progeny were planted in a single row with 10 cm between plants and 23.3 cm between rows in the 2009–2010 season. The trials were performed on the experimental farm of Shandong Agricultural University, Tai'an, China. Normal field management was applied during the growth season. The field had loamy soil, and the grain yield was approximately 8000 kg ha^{-1}.

Plant morphological traits, including PH, SL, spike number per plant (SN), fertile spikelet number per spike (FSS), and grain number per spike (GN), were evaluated with three spikes of each plant ten days before harvest. After harvest, grain traits were evaluated: three samples of 20 grains from each plant were lined up lengthwise along a ruler with a precision of 0.1 mm to measure grain length (GL), and then the grains were arranged breadthwise to measure grain width (GW). The GL/GW ratio (GLW) was calculated. Factor form density (FFD), calculated as grain weight/(grain length × grain width), describes differences in grain density and the deviation of a shape from a cylindrical form [25]. TGW was evaluated by weighing two samples of 100 grains from each plant.

For $F_{2:3}$ progeny, all investigated traits were described by the mean values of five plants for the corresponding line from each F_2 individual.

2.3. Simple sequence repeat (SSR) analysis

DNA was extracted from fresh young leaves of each plant in the F_2 by the SDS method [26]. PCR amplification was performed in a 20 μL mixture containing 1× PCR buffer, 100 ng DNA, 3 mmol $MgCl_2$, 1.5 mmol dNTP, 1 pmol of each primer, and 1 U *Taq* DNA polymerase. Amplifications were performed under the following conditions: 5 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 50–60 °C and 30 s at 72 °C; and a final extension step of 10 min at 72 °C. PCR products were separated in 6% denaturing polyacrylamide gels. Bands amplified from M8008 and individuals containing its alleles were termed as "A", those from YN15 or SJZ54 were termed as "B", and individuals from whom both "A" and "B" could be amplified were termed as "H".

A total of 903 SSR markers distributed evenly on the genetic map [27], and 166 expressed sequence tag (EST)-SSR markers developed by our laboratory [28], were used for a polymorphism survey. Primer sequences for SSR markers were obtained from GrainGenes 2.0 (http://wheat.pw.usda.gov/GG2/). Markers were initially analyzed in the parents and a preferred small group (PSG) [29] comprising five typical low- and five high-TGW plants in the F_2 of the MY population. The polymorphic SSRs were further used to assay the F_2 populations. In a preliminary analysis to determine whether the grain size traits were controlled by loci on chromosome 2D, the SSR and EST-SSR markers on 2D were used to analyze the MY and MS F_2 populations.

2.4. Genetic mapping and QTL analysis

The linkage map was constructed with Mapmaker 3.0 [30]. CentiMorgan units (cM) were calculated using the Kosambi mapping function [31]. QTLs were analyzed using a mixed linear model in QTLNetwork 2.0 [32]. Composite interval analysis was performed using forward-backward stepwise multiple linear regressions with a probability of 0.05 for adding and removing markers from the model, and a window size of 10 cM. Significance thresholds for QTL detection were calculated for each dataset using 1000 permutations and genomewide error rates of 0.10 (suggestive) and 0.05 (significant).