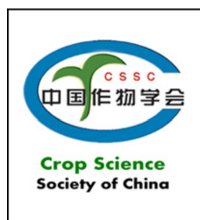

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Sequence-based genetic mapping of *Ds*-tagged insertions to characterize malting-related traits in barley

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ABSTRACT

Among various functional genomics tools used to characterize genes in plants, transposon-based mutagenesis approaches offer great potential, especially in barley and wheat, which possess large genomes and in which genetic transformation is not routine. Two *Ds* transposon flanking sequences (TNPs), TNP-29 (27.4 cM (centiMorgan)) and TNP-79 (70.3 cM), were mapped in the vicinity of a malting quality QTL located on chromosome 4H of barley. Reactivation of the *Ds* transposon sequence from these TNP lines led to the identification of genes in the malting QTL regions. Several *Ds* (dissociation) lines were generated by crossing TNP-29 and TNP-79 with an *AcTPase* (activator) expressing line (25-B), and F_2 progenies were subsequently screened for *Ds* insertions at new locations. To further characterize these *Ds* mutants, we mapped the new *Ds* flanking sequences on a barley genetic map and found that 29% of *Ds* were located in regions associated with the malting QTL located on chromosome 4H and in close proximity to other important malting-associated QTL across the barley chromosome. Using a sequence based approach, a linkage map was generated that confirmed the position of *Ds* loci in the barley genome map. Locating these *Ds* loci on the barley map opens avenues to dissect important malting QTL for facilitating identification of candidate malting genes.

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1. Introduction

The barley (*Hordeum vulgare*) genome is of approximately 5.1 Gb in size [1], and more than 80% of the genome consists of repetitive DNA sequences [2] making gene cloning studies cumbersome. Owing to collaborative international efforts, impressive progress has been made in establishing freely available, public resources in barley genomics — an advantage not realized in many other major crop species [3]. These resources include the development of extensive genetic maps, most of which are curated and available on GrainGenes (http://wheat.pw.usda.gov/GG2/maps.shtml#barley). Genetic linkage maps have been useful in identifying QTL [4] and are essential for mapping of unknown genes sequences, most of which still have no assigned function. Integration of these mapping resources with T-DNA and transposon insertions allows the development of efficient and robust gene cloning strategies [5].

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The use of the maize *Ac/Ds* transposon system is an effective approach for gene identification and cloning in heterologous species. Using this system, single-copy *Ds* insertion lines (TNPs) were generated in barley to identify, tag, and characterize genes [6,7]. These genetic populations were developed by hybridizing *Ds* insertion lines (TNPs) with *AcTPase*-expressing sources developed previously using different plant transformation approaches [5].

These genetic populations can be used to target QTL linked to important but difficult traits and characterize the genes affecting them. For instance, malting quality is a complex and low-heritability phenotype controlled by QTL that have been mapped throughout the barley chromosomes. Various QTL affecting malting quality traits have been identified in barley germplasm [4,8–17]. In addition, recent studies have identified QTL for major malting traits such as malt extract (chromosome 1H, 2H), wort protein (chromosome 1H, 5H, 6H), soluble/total protein (S/T) (chromosome 6H), diastatic power (DP) (chromosomes 2H, 6H, 7H), alpha-amylase (chromosome 4H), and beta-glucan (BG) (chromosomes 5H, 6H, 7H) [18,19].

Two major malting QTL are located on chromosome 4H: QTL2 located in the telomeric region of 0–30 cM (fine-mapped to 0–15 cM), and another QTL located in the 72.6–85.2 cM region. Phenotypic variation of 18% to 25% for major malting traits like malt extract, diastatic power and alpha amylase was observed before [4]. Further investigation into these QTL is required to characterize the gene/genes affecting malting traits.

In the present study we reactivated *Ds* insertion lines with an insertion site mapped in the vicinity of two malting QTLs on chromosome 4H to generated new lines, the sequence of new lines were subsequently mapped across barley chromosomes. The map location of *Ds* loci may provide information leading to the identification and effective dissection of malting QTL. Mapping of *Ds* loci was achieved by identification of polymorphisms in the sequences flanking *Ds* using iPCR, TAIL (thermal asymmetric interlaced) PCR, and adapter ligation techniques. A set of *Ds* insertions described in Singh et al. [5] has been constructed in an old malting variety, Golden Promise, in which successful transformation of barley was first accomplished [20]. However, Golden Promise is not a parent of the well-characterized mapping population currently available (http://wheat.pw.usda.gov/ggpages/map_summary.html). We accordingly used the other highly polymorphic mapping population Oregon Wolfe Barley dominant (OWB-D) × Recessive (OWB-R), Steptoe × Morex, and Dicktoo × Morex. Among all mapping techniques, a sequence-based approach has proven successful, as used earlier to map 19 *Ds* loci on the barley linkage map [21]. Restriction digest-based assay of PCR products, generally known as CAPS (cleaved amplified polymorphic sequence) is also a preferred method for performing SNP assays in mapping populations and has been used earlier [21]. A more recently created barley sequence database (IPK: http://webblast.ipk-gatersleben.de/barley/) also provided information about the physical and genetic map locations of *Ds* loci [1].

The overall aim of this study was to identify *Ds* loci mapping close to malting QTL for future identification of genes involved in malting quality. We mapped the *Ds* flanking sequences generated previously [5] and in the present study, to a barley linkage map. *Ds* flanking sequences mapped proximal, and into the malting QTL regions may act as candidates for identification of genes involved in the malting process through functional characterization.

2. Material and methods

2.1. Generating *Ds* flanking sequences

TNP-29 and TNP-79 single-*Ds* insertion lines and a 25-B line expressing *AcTPase* were generated by Copper et al. [21]. TNP-29 and TNP-79 were mapped near malting quality QTL on chromosome 4H to map positions 27.4 and 70.4 cM respectively. Seeds of these lines were obtained from TNP repository at USDA (Aberdeen, ID, USA) and grown under appropriate greenhouse conditions. TNP-29 and TNP-79 plants were crossed with *AcTPase*-expressing plants and F_2 seeds were grown and screened for new *Ds* transpositions. DNA isolation and PCR were performed as described in Singh et al. [5]. Sequences of *Ds* flanking regions from new transpositions were obtained by HE-TAIL PCR [22], iPCR [5], and adapter ligation [23]. The HE-TAIL PCR was performed in four separate runs using genomic DNA, TaKaRa Ex*Taq* DNA polymerase, 10× PCR buffer containing 10 mmol L^{-1} $MgCl_2$, and 2.5 mmol L^{-1} dNTPs (TaKaRa-Bio, China). These PCR runs included preamplification and primary, secondary, and tertiary amplification, respectively, following Tan and Singh [22], using a GeneAmp PCR System 9700 (Applied Biosystems, USA).

For iPCR, genomic DNA was digested with either *Nco* I or *Nhe* I (both enzymes have a restriction site in the *Ds* element), followed by heat inactivation and phenol:chloroform purification of the digested DNA. The digested DNA was self-ligated and the product was used for subsequent PCR amplification. The cocktail for the first cycle of the iPCR was prepared with 1.0 μL of 0.1 μg of purified and ligated DNA, 2.5 μL of 10× buffer, 2.5 μL of 2.5 mmol L^{-1} dNTP mixture, 0.5 μL of 10 μmol L^{-1} of the first set of nested primers for the 5′ end, JIPR3 and JIPF4 and the 3′ end JIPR9 and JIPF2, and 0.25 μL of 1 U of Ex*Taq* DNA polymerase (Invitrogen, USA) in a total reaction volume of 25 μL. PCR products from the first round were diluted to 1:20 for the 5′ flanking end of *Ds* insertion and 1:50 for the 3′ end with nuclease-free water and used as template for the second round of PCR. Specific PCR products were generated in the second round of PCR amplification with nested primers (JIPR5 and JIPF7 at the 5′ end and JIPR6 and JIPF1 at the 3′ end). The PCR products obtained from the second iPCR were gel-purified using a gel extraction kit (QIAquick Gel extraction kit, Qiagen, USA).

In the adapter ligation (AL-PCR) method, adapters with appropriate overhangs were designed that contained a specific primer binding site (AC946R), used with four set of restriction enzymes (*Ase* I, *Hinf* I, *Ava* II, and *Hha* I). These enzymes were selected based on the position of restriction sites in the modified sequence (*Ds*-bar, consisting of minimal 5′ and 3′ *Ds* termini flanking an ubiquitin-driven bar cassette). Adapters were ligated to the restricted genomic DNA, and three rounds of PCR with three sequentially nested primers specific to 3′ and 5′ termini of known sequences were performed.

2.2. Bioinformatic analysis

Bioinformatic analysis of the flanking sequences was performed using BLAST (Basic Local Alignment Search Tool) query against databases HarvEST (http://harvest.ucr.edu/), NCBI (http://www.ncbi.nlm.nih.gov/) and Gramene (http://www.gramene.org/).

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