Microcolinearity between the grain protein content QTL region in wheat chromosome arm 6BS and rice chromosome 2

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ABSTRACT

The conservation of the linear order (colinearity) of genetic markers along large chromosome segments in wheat and rice is well established, but less is known at the molecular level (microcolinearity). Previous comparative maps between these two species revealed conserved linkage between rice chromosome 2 and wheat chromosome 6. As part of our efforts to identify a locus affecting Grain Protein Content (GPC) using a positional cloning approach, we established the microcolinearity between a region on wheat chromosome 6BS, including the GPC locus, and 20.4 cM on rice chromosome 2. Blast analysis of the rice BAC sequences covering the colinear GPC region against the Triticeae EST database revealed several sequences that were used as markers in the wheat genetic map. Using this approach we mapped eleven colinear genes between wheat and rice in this region. The closest markers flanking the GPC locus were mapped 0.9 cM apart in wheat and 100-kb apart in rice. Further work is underway to complete the mapping of additional wheat ESTs identified within the 100-kb rice sequence and to construct a physical map using a tetraploid wheat BAC library that contains a *T. dicoccoides* 6BS chromosome segment carrying the high GPC locus (http://agronomy.ucdavis.edu/Dubcovsky/BAC-library/BAC_Langdon.htm). These results demonstrated a high level of microcolinearity between rice and wheat in the GPC region.

INTRODUCTION

High Grain Protein Content (GPC) is an important economic trait in wheat because it determines its nutritional value and quality. One approach to increase the GPC is to utilize the high grain protein alleles from wild relatives of wheat. Wild emmer (*Triticum turgidum* ssp. d*icoccoides*, DIC hereafter) accession FA15-3 from Israel is a potential source of high GPC alleles (Avivi, 1978). Recombinant substitution lines of *T*, *dicoccoides* FA15-3 chromosome 6B in the cultivar 'Langdon' (*Triticum turgidum* var *durum*, LDN hereafter) showed that a QTL for high GPC was present between RFLP markers *Xpsr8* and *Xpsr113* on chromosome arm 6BS (Fig. 1, Joppa et al, 1997). These markers were mapped also on rice chromosome 2 and therefore define a 30 cM colinear region between the two species (Fig. 1).

Comparative mapping in plants has provided evidence for conservation of markers and gene order (colinearity) between related genomes. Rice is a particularly valuable reference for the grass family because most of its small diploid genome has been sequenced. We used

information from the rice genomic sequence to saturate with markers the wheat chromosome 6B region of the QTL for high grain protein content.

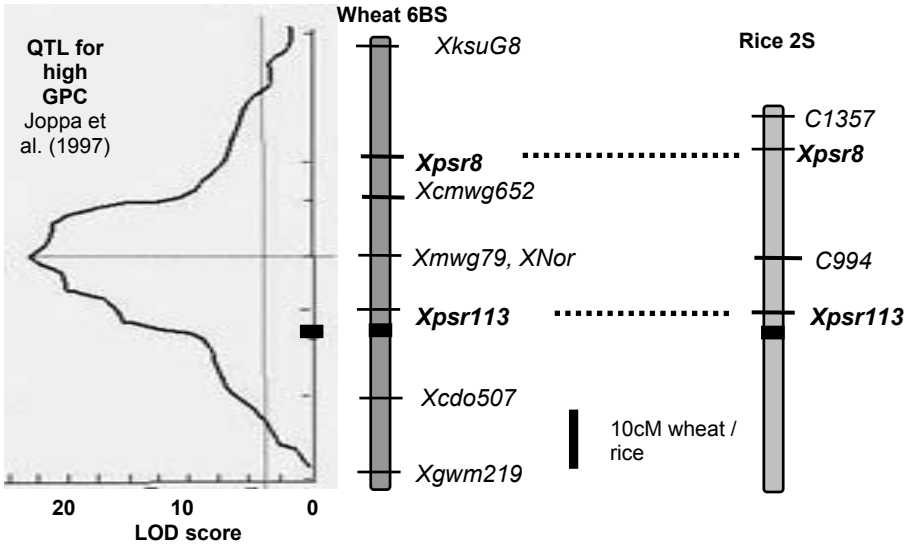

Fig. 1: Wheat-rice colinearity: Left; QTL for high GPC on wheat chromosome 6B. Right; Genetic map of rice chromosome 2S. Centromeres are indicated with a black rectangle.

MATERIAL AND METHODS

The mapping population included homozygous recombinant substitution lines (RSLs) developed by L.R. Joppa from the cross LDN(DIC-6B) x LDN, and new RSLs generated at UC Davis from crosses between RSL65 x LDN (Olmos et al. 2003). Plants were characterized for GPC in three field experiments with 10 replications organized in a Randomized Complete Block Design (Olmos et al. 2003).

Rice genes present in the *Xpsr8* - *Xpse113* interval were used to search the wheat and barley EST databases. RFLP probes and PCR markers were developed from those ESTs that showed significant similarity to the rice genes. Specific pairs of primers were designed for each EST based on the gene structure predicted by the alignment of the Triticeae ESTs with the rice genomic sequence. The specific primers were used to amplify PCR products, which were purified and used as a probe for hybridization or were sequenced to develop PCR markers. New loci mapped with polymorphic EST probes were assigned UCW numbers.

RESULTS AND DISCUSSION

Approximately 40 pairs of EST specific primers were designed but only 20 PCR products were successfully amplified by PCR and used as probes. However, only eleven of these probes showed polymorphism between the parental lines and were further used for mapping. The conservation of gene order (micro-colinearity) between wheat and rice in the GPC region was well established. All eleven probes originated from a 20.4-cM in rice were mapped flanking the GPC locus and their order was conserved in both species. The GPC gene was mapped into a 0.9-cM interval defined by flanking loci *Xucw70* and *Xucw73*. The wheat region between *Xucw70* and *Xucw73* corresponds to a 100-Kb segment in rice located within a single BAC (Fig. 2). Probes *Xucw66*, *Xucw68 and Xucw76* were also colinear between wheat and rice but they were not included in the figure because they were 11.2, 16.1 and 20.4 cM from the markers in Fig. 2.

To define more precisely the location of the GPC locus, we have initiated new field experiments to measure the GPC of the lines with critical recombinants within this region. Probes from the *Xucw70, Xucw71, Xucw72,* and *Xucw73* genes were used to screen the tetraploid wheat BAC library that includes the *T. dicoccoides* 6BS chromosome segment carrying the high GPC gene (Cenci et al, 2003). Fingerprintings of the BACs identified with these genes determined separate contigs for the A and B genomes, confirming the large differentiation between genomes reported before (Cenci et al, 2003). Loci *Xucw72* and *Xucw73* were detected within the same wheat BAC, but were not connected to the contigs detected by probes *Xucw70* and *Xucw71*. This was expected based on previous determinations of the ratios between genetic and physical distances in a similar region in wheat chromosome 5 (Yan et al. 2003).

These results demonstrated an excellent microcolinearity between wheat and rice in the GPC region, suggesting that the rice genomic sequence will be a valuable tool for the fine mapping and positional cloning of this wheat gene. Further work is underway to complete the mapping of additional wheat ESTs identified within this 100-kb rice sequence and to construct a wheat physical map of the region.

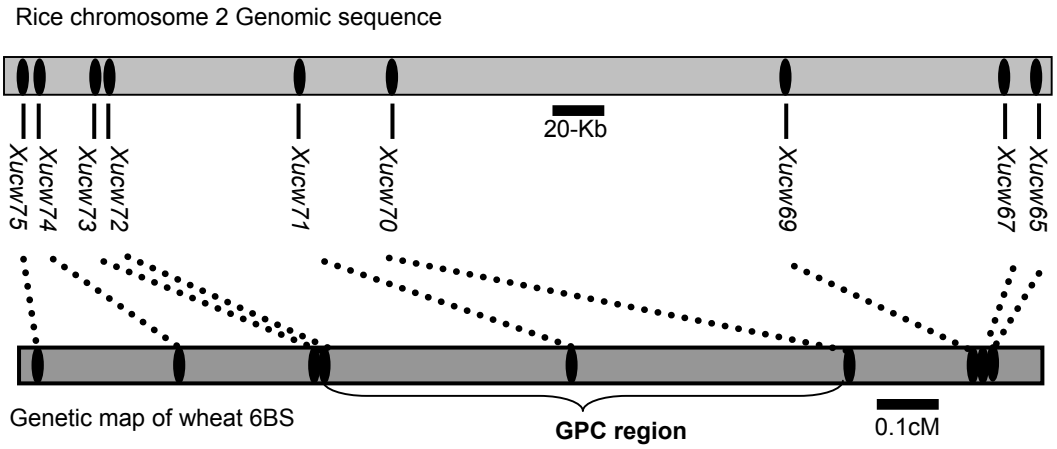

Fig. 2: colinearity between the genetic map of wheat GPC region and the rice chromosome 2. Mapped genes are indicated by black ovals and the GPC gene region is indicated below the genetic map.

ACKNOWLEDGEMENTS

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