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Development of PCR-Based Markers for a High Grain Protein Content Gene from *Triticum turgidum* ssp. *dicoccoides* Transferred to Bread Wheat

I. A. Khan, J. D. Procnier, D. G. Humphreys, G. Tranquilli, A. R. Schlatter, S. Marcucci-Poltri, R. Froberg, and J. Dubcovsky*

ABSTRACT

Grain Protein Content (GPC) of wheat (*Triticum aestivum* L. and *T. turgidum* L.) is important for improved nutritional value and is also one of the major factors affecting breadmaking and pasta quality. A quantitative trait locus (QTL) for high GPC was detected a few years ago in the short arm of chromosome 6B from accession FA15-3 of *Triticum turgidum* L. var. *dicoccoides*. New molecular markers are presented here to facilitate the transfer of this high GPC gene into tetraploid and hexaploid wheat cultivars. Two sets of PCR (polymerase chain reaction) primers were designed to amplify regions of the non-transcribed spacer of the *XNor-B2* locus. This locus was selected because it mapped on the peak of the QTL for GPC. The first pair of allele-specific primers produced an amplification product only when the *T. turgidum* var. *dicoccoides* *XNor-B2* allele was present. The second pair of primers amplified fragment(s) of similar length in the different genotypes that after digestion with the restriction enzyme *Bam*HI allowed differentiation of the *T. turgidum* var. *dicoccoides* allele. Four microsatellites markers were mapped on the short arm of chromosome 6B at both sides of the QTL peak and two on the long arm. Five additional amplified fragment length polymorphism (AFLP) markers were mapped into the QTL region on 6BS. These PCR markers together with 10 restriction fragment length polymorphism (RFLP) markers showed that the hexaploid cultivar Glupro, selected for high GPC, carries a distal segment of chromosome 6BL and a proximal segment of 6BS from *dicoccoides* accession FA15-3 encompassing the segment with highest LOD score for the GPC QTL.

GRAIN PROTEIN CONTENT of wheat is important for improved nutritional value and is also one of the major factors affecting breadmaking and pasta quality (Dick and Youngs, 1988; Finney et al., 1987). In spite of its importance, progress in breeding for high GPC has been slow and difficult. The first limitation is that genetic variation for protein content is small compared with variation due to differences in growing environments. The second limitation is that there is a strong negative correlation between GPC and grain yield; cultivars with high GPC tend to be low yielders. There are,

however, exceptional genotypes that combine excellent yield potential and high GPC, probably by a more efficient relocation of nitrogen from senescing tissues to grain, or by a more efficient uptake of nitrate and ammonia from the soil (Blackman and Payne, 1987).

A promising source of high GPC was detected years ago in a survey of wild populations of tetraploid *Triticum turgidum* var. *dicoccoides* (accession FA15-3 from Israel; Avivi, 1978), referred to as *dicoccoides* hereafter. Substitution lines of the chromosomes of this *dicoccoides* accession in the cultivated durum cultivar Langdon (*Triticum turgidum* var. *durum*) showed that a gene for high protein content was present on chromosome 6B (Joppa and Cantrell, 1990). Recombinant substitution lines (RSLs) from a cross between the LDN(DIC 6B) substitution line and Langdon were developed by Joppa et al. (1997). These lines were used to map a QTL for GPC on the proximal region of the short arm of chromosome 6B that accounted for 66% of the variation in GPC present in that particular cross (Joppa et al., 1997).

Other researchers crossed the same *dicoccoides* 6B-substitution line with high yielding durum cultivars from North Dakota (Chee et al., 1998) and Canada (Kovacs et al., 1998) to introgress the high-GPC gene. Results from the North Dakota recombinant lines showed a single gene effect and no interaction between the increase in GPC content and the genetic background or growing environments. The effect of this gene was independent of protein quality, plant height, heading date, and yield (Chee et al., 1998). Results from the Canadian recombinant lines showed a positive effect on protein and quality (Kovacs et al., 1998). The incorporation of the high-GPC gene from *dicoccoides* into the Canadian lines resulted in increased protein content and a positive effect on pasta cooking quality. Kovacs et al. (1998) concluded that this gene would be a valuable resource to increase the protein level for durum wheat breeding programs.

The high-GPC gene from *dicoccoides* has also been transferred to hexaploid wheat. Hexaploid cultivar Glu-

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Abbreviations: AFLP, amplified fragment length polymorphism; ASA, allele specific amplification; BC, backcross; CAPS, cleavage amplified polymorphic sequence; cM, centimorgan; DIC, *Triticum turgidum* var. *dicoccoides*; GPC, grain protein content; LDN, Langdon; NOR, nucleolar organizing region; PCR, polymerase chain reaction; QTL, quantitative trait loci; RFLP, restriction fragment length polymorphism; RSL, recombinant substitution line.

pro was developed by Dr. R. Froberg from a three-way cross between two bread wheat cultivars and the same *dicoccoides* accession used to develop the substitution line LDN(DIC6B). Mesfin et al. (1999) showed that Glupro and *dicoccoides* accession FA15-3 shared five RFLP markers from a 15-cM proximal region of chromosome 6B. The positive effect of this segment on grain protein content in a bread wheat genetic background was recently demonstrated in double haploid lines (Humphreys et al., 1998) and recombinant inbred lines (Mesfin et al., 1999) developed from crosses between Glupro and bread wheat cultivars from Canada and USA.

The objectives of this work were to (i) develop PCR-based markers to facilitate the manipulation of the gene for high GPC in marker assisted selection programs, (ii) detect additional molecular markers in the GPC gene region, and (iii) extend the molecular comparison between Glupro and *dicoccoides* accession FA15-3 to determine the size of the *dicoccoides* chromosome 6B segment transferred to Glupro.

MATERIALS AND METHODS

Dr. L. Joppa provided the RSLs of chromosome 6B from *dicoccoides* and chromosome 6B from Langdon in a Langdon genetic background. In this study, only the RSLs showing at least one recombination event between RFLP markers *Xcmwg652* and *Xcdo534* were included (Joppa et al., 1997). The 25 selected RSLs (5, 8, 11, 14, 19, 28, 30, 32, 36, 41, 44, 47, 48, 50, 54, 56, 58, 59, 60, 63, 65, 67, 68, 77, and 78) have additional crossovers in other regions of chromosome 6B. Hexaploid wheat cultivar Glupro ('Columbus'/*T. turgidum* var. *dicoccoides*/'Len') and its progenitor cultivars Len and Columbus were tested with the different molecular markers developed in this study. Tetraploid cultivars and breeding lines ('Kronos', 'Kofa', 'Westbred Turbo', UC908, UC1112, UC1113, and UC1114) and hexaploid cultivars and breeding lines ('Express', 'RS15', 'Anza', 'Yecora Rojo', UC1036, UC1037, and UC1041) were tested only with probe pTA250.15.

Probes and RFLP Procedures

DNA was extracted from the previous cultivars and RSLs by the method described by Dvorak et al. (1988). A small scale DNA isolation protocol was used for the marker assisted backcrossing program (Weining and Langridge, 1991). Procedures for Southern blots and hybridization were as described by Dubcovsky et al. (1994). Clones used in this study were kindly provided by J. Dvorak (University of California, Davis), M. Gale (John Innes Centre, Norwich, UK), A. Graner (Institute for Plant Genetics & Crop Plant Research, Gatersleben, Germany), and M. Sorrells (Cornell University, Ithaca, NY). Probe pTA250.15 is a 750-bp *HhaI* fragment from the spacer region of the wheat rDNA and was used to detect the *XNor-B2* locus (Appels and Dvorak, 1982).

PCR Procedures

The sequence of the non-transcribed spacer of the *XNor-B2* locus (Barker et al., 1988) was used to design two sets of PCR primers with the computer program PRIMER (Version 0.5, Lincoln and Daly, 1991). One primer set (NTS#2F: 5'-ATG ATG GTC AAC AAA CGG TGC-3' and 18S#2R: 5'-TTT ATT GTC ACT ACC TCC CCG-3') amplified a region

of the non-transcribed spacer having the highly polymorphic *BamHI* site (Kim et al., 1992). This primer set is designated as a cleavage amplified polymorphic sequence (CAPS, Jarvis et al., 1994). PCR cycling conditions were 94°C 3 min; (94°C 1 min, 58°C 1 min, 72°C 2 min) 35 cycles; 72°C 10 min in a volume of 25 μ L. The final concentration of the different products used in the PCR reaction were: 1 \times *Taq* polymerase buffer, 2.5 U of *Taq* polymerase, 1.5 mM MgCl₂, 1 ng/ μ L of each primer, 250 μ M each dNTP, and 50 to 100 ng of genomic DNA. Fifteen microliters of the amplification product were then digested with 10 U of the restriction enzyme *BamHI* in a 1 \times final concentration of the commercial buffer provided with the enzyme. The product was then loaded in a 0.8% (w/v) agarose gel, run for 4 h to permit good separation of the bands and the gel was stained with ethidium bromide.

The second pair of primers amplified a sequence specific to the *XNor-B2* non-transcribed spacer of *dicoccoides*. These are designated as allele specific amplification (ASA) primers and their sequences are ASA#2R: 5'-CTA CCA TCG AAA GTT GAT AGG GA-3' and ASA#1F: 5'-TTC ACA AAC TAA GGG GAG GGA-3'. Cycling conditions and final concentrations in the PCR reaction were similar to those for the first primer set except for the annealing temperature of 57°C. The PCR product was visualized as mentioned above.

Seven microsatellites (*Xgwm193*, *Xgwm219*, *Xgwm361*, *Xgwm508*, *Xgwm518*, *Xgwm626*, and *Xgwm644*) were used in this study. Primer sequences and PCR conditions for these microsatellites are published (Röder et al., 1998). PCR reactions were performed in a volume of 25 μ L and amplification products were separated on denaturing 6% (w/v) polyacrylamide gels at 45 W for 3 h; the products were visualized by silver staining. Size of each band was estimated by a molecular weight standard (25-bp ladder, Gibco-BRL, Rockville, MD).

AFLP assays were performed by a modified version of the method described by Milbourne et al. (1997). Briefly, 500 ng of wheat genomic DNA were subject to restriction-ligation in a single step during 6 h in a 30- μ L reaction mix (10 mM Tris-acetate pH 7.5, 10 mM Mg-acetate, 50 mM K-acetate, 5 mM dithiothreitol, 50 ng/mL BSA, 5U *PstI*, 5U *MseI*, 1U T4 DNA ligase (Gibco), 5 pmol *PstI* adaptors, 50 pmol *MseI* adaptors, and 12 pmol ATP). Five microliters of each adaptor-ligated template DNA were preamplified in a 25- μ L PCR reaction containing 75 ng of both P01 and M01 AFLP primers (5'-GAC TGC GTA CAT GCA GA-3' and 5'-GAT GAG TCC TGA GTA AA-3' respectively), 0.2 mM dNTPs, 1 \times PCR buffer (1.5 mM MgCl₂) and 1U of Perkin Elmer Amplitaq LD (PE Corporation, Norwalk, CT). Selective amplifications were performed with 1 μ L of non-diluted preamplification product and 30 ng of each selective non-labeled + 3 primer and the cycling conditions described by Vos et al. (1995). Ten microliters of formamide dye were added to the 20- μ L PCR reactions and amplification products were separated in 6% (w/v) denaturing polyacrylamide gels and silver stained (Promega Sequencing Silver staining kit; Promega, Madison, WI). Selective primer combinations assayed were P36=ACC, P37=ACG, P40=AGC, P41=AGG against M31=AAA, M37=ACG, M38=ACT, M39=AGA, M40=AGC, M41=AGG, M42=AGT, M43=ATA, M44=ATC, M45=ATG.

Mapping Procedures

The 25 RSLs with at least one recombination event between RFLP markers *Xcmwg652* and *Xcdo534* were scored for the polymorphic markers and the data was incorporated in the data matrix used by Joppa et al. (1997) kindly provided by G. Hart (Texas A&M University). Linkage maps were con-

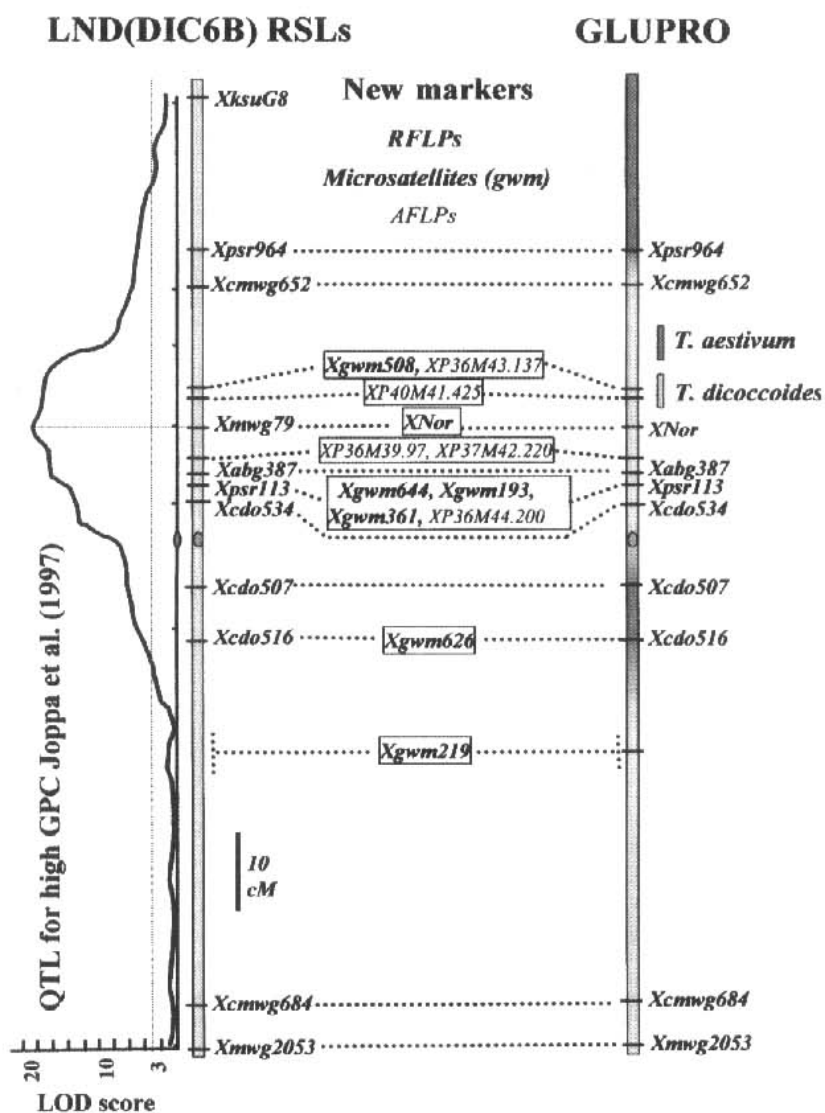


Fig. 1. Left: 6B map developed by Joppa et al. (1997) with QTL analysis for high GPC. Center: molecular markers added in this work. Right: inferred distribution of *dicoccoides* and bread wheat chromosome segments in hexaploid cultivar Glupro based on the same molecular markers. RFLP markers indicated as present in *dicoccoides* 6B substitution line and Glupro are also absent in Columbus and Len, the hexaploid progenitors of Glupro. Genetic distances are inferred from the map on the left. Distances between markers are proportional to genetic distances. Centromeres are indicated with a circle.

structed with the aid of the computer program Mapmaker/EXP 3.0 (Lander et al., 1987) using the Kosambi function.

RESULTS

Development of a PCR Marker for the *XNor-B2* Locus

The *XNor-B2* locus was mapped in the same population of RSLs of chromosome 6B used by Joppa et al. (1997). In these RSLs, *XNor-B2* was completely linked to *Xmwg79*, the locus with highest LOD score for the QTL for high GPC (Joppa et al., 1997) (Fig. 1). When probe pTa250.15 was hybridized with wheat genomic DNA digested with *TaqI*, LDN(DIC 6B) substitution line showed a 2.9-kb restriction fragment that differed from the two restriction fragments of 2.7 and 3.1 kb

present in the original Langdon DNA (Fig. 2). The 2.9-kb band was a useful marker to differentiate the *dicoccoides* segment from most of the tetraploid and hexaploid cultivars tested. However, a few cultivars like tetraploid Kronos or hexaploid Columbus showed restriction fragments of similar mobility to the *dicoccoides* 2.9-kb band. In those cases, autoradiographs were overexposed and the LDN(DIC6B) allele was traced by the presence of two additional 7- and 14-kb restriction fragments that cosegregate with the 2.9-kb restriction fragment. These two additional restriction fragments were not detected in any of the tetraploid or hexaploid cultivars tested in our marker assisted selection program (data not shown).

To facilitate the use of this marker in breeding programs, PCR-based markers were developed for the *XNor-B2* locus of *dicoccoides*. PCR with the CAPS

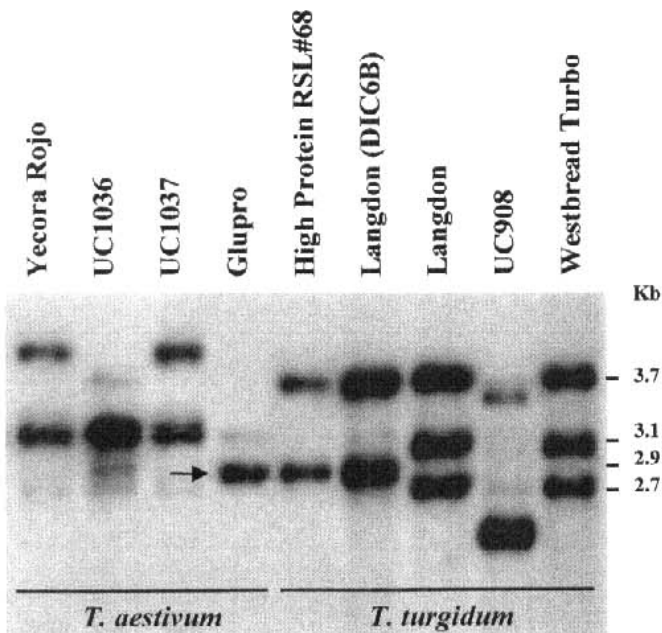


Fig. 2. Hybridization patterns of clone pTa250.15 (segment of the *XNor-B2* locus) with *TaqI* digested DNAs from hexaploid and tetraploid wheat DNAs. Left side: First three lanes are *T. aestivum* cultivars and breeding lines (Yecora Rojo, UC1036, and UC1037) without the high GPC gene followed by cultivar Glupro carrying the high GPC gene. Right side: *T. turgidum* RSL #68 and substitution line of *dicoccoides* complete chromosome 6B in Langdon both carrying the high GPC gene followed by three lanes of durum cultivars and breeding lines (Langdon, UC908, Westbread Turbo) without the high GPC gene.

primers yielded a single band for all genotypes that after cutting with *Bam*HI gave a doublet when the *dicoccoides* allele was present and a singlet when this allele was absent (Fig. 3).

A second pair of allele specific amplification primers (ASA) was designed to differentiate the *dicoccoides* allele without further digestion with a restriction enzyme. The ASA primers amplified a band only when the *dicoccoides* allele was present. No PCR product was observed when the *dicoccoides* allele was absent (Fig. 3). However, this PCR reaction was less robust than the reaction using the CAPS primers; occasional false positives (a light band present) were detected.

Microsatellite Markers

All microsatellite markers tested, with the exception of *Xgwm518*, showed polymorphism between LDN(DIC6B) and LDN (Fig. 4). The molecular weights of the amplified fragments on LDN(DIC6B) and LDN were 136 and 138 bp for *Xgwm508* (Fig. 4), 194 and 174 bp for *Xgwm193* (Fig. 4), 138 and 142 bp for *Xgwm361*, 152 and 150 bp for *Xgwm644*, 135 and 105 bp for *Xgwm626*, and 150 and 154 bp for *Xgwm219* (Fig. 4).

Polymorphic microsatellite markers were mapped in the population of RSLs developed by Joppa et al. (1997) (Fig. 1). Microsatellite markers *Xgwm193*, *Xgwm361*, and *Xgwm644* were completely linked with each other and with RFLP marker *Xpsr113* (Fig. 1). The LOD score for the QTL for GPC at this locus was approximately four units lower than the LOD score at

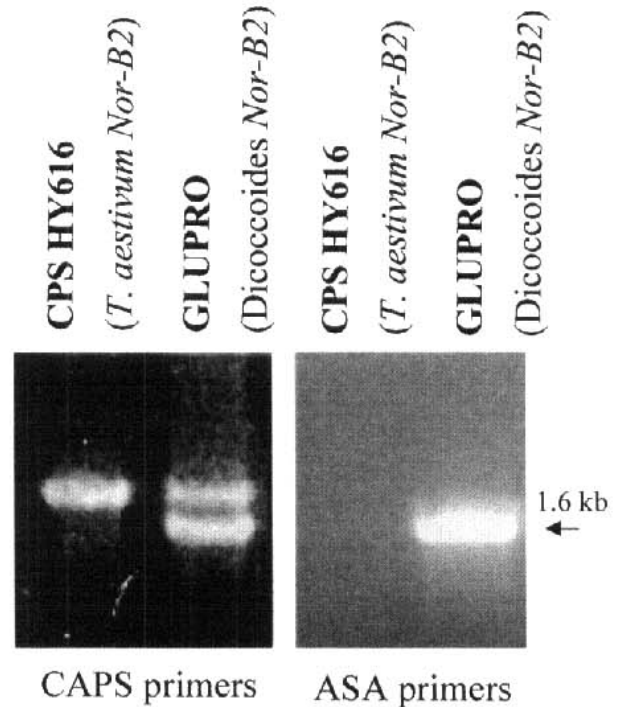


Fig. 3. PCR markers for the *XNor-B2* locus. In both panels the first lane is bread wheat Canadian Prairie Spring line HY616 (*T. aestivum* allele at the *XNor-B2* locus) and the second lane is Glupro (*T. turgidum* var. *dicoccoides* allele at the *XNor-B2* locus). Left panel: PCR reaction using cleavage amplification polymorphic sequence (CAPS) primers followed by digestion with *Bam*HI. A doublet is observed when the *dicoccoides* allele is present. Right panel: PCR reaction using allele specific amplification (ASA) primers. A PCR product is observed only when the *dicoccoides* allele is present.

the *XNor-B2* locus (Fig. 1). Microsatellite *Xgwm508* was 4.8 cM distal to the *XNor-B2* locus and its LOD score was approximately 2 units lower than the LOD score at the *XNor-B2* locus. These results indicated that there is a large probability that the gene for high GPC is located within the interval delimited by these microsatellite markers. Two microsatellite markers were mapped on the long arm of chromosome 6B. Microsatellite *Xgwm626* was completely linked to RFLP *Xcdo516* and microsatellite *Xgwm219* was mapped within the *Xcmwg669-Xglk762* interval (Joppa et al., 1997).

Microsatellite markers detected non-parental alleles in a few RSLs. RSL#30 and RSL#32 showed alleles different from Langdon and *dicoccoides* for microsatellite loci *Xgwm193*, *Xgwm361*, *Xgwm644*, and *Xgwm219*. The non-parental allele found with microsatellite *Xgwm219* in RSLs #30 and #32 was also present in RSL#60.

AFLP Markers

Forty *Pst*I-*Mse*I primer combinations differing in the three 3' end nucleotides were tested for polymorphism between Langdon and RSL#68 that carries a 30-cM *dicoccoides* segment between *Xcmwg652* and *Xcdo534*. Five of the 2400 AFLP fragments detected with the selected primers were mapped in this region.

AFLP locus *XP36M43.137* was completely linked to

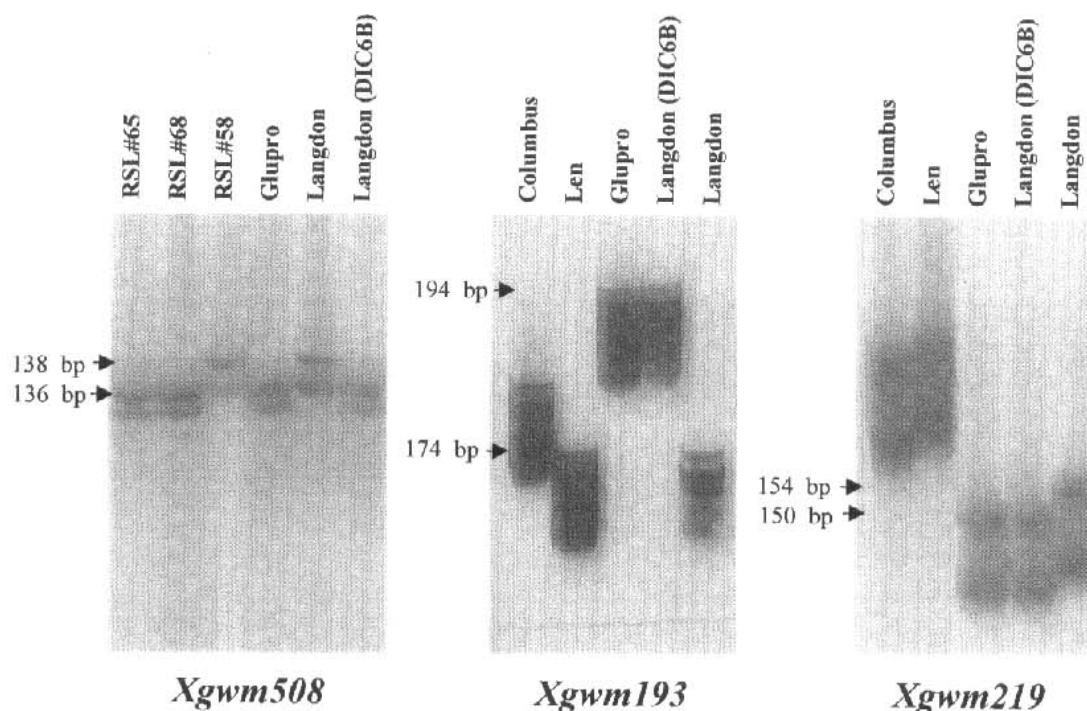


Fig. 4. Silver stained polyacrylamide gels containing PCR amplification products of microsatellite primers for loci *Xgwm508* (left) and *Xgwm193* (center) on the short arm of chromosome 6B, and *Xgwm219* (right) on the long arm of the same chromosome.

microsatellite marker *Xgwm508* (Fig. 1). The bands for the two alleles of this locus were one base pair apart and this marker was scored as codominant. Locus *XP40M41.425* was mapped in repulsion with the *dicoccoides* allele within the *Xgwm508*–*XNor-B2* interval (Fig. 1). Two AFLP loci were mapped in the *XNor-B2*–*Xabg387* interval, *XP36M39.97* in repulsion with the *dicoccoides* allele and *XP37M42.220* in coupling. Finally, locus *XP36M44.200* was mapped in coupling with the *dicoccoides* allele completely linked to RFLP marker *Xpsr113* and microsatellite markers *Xgwm193*, *Xgwm361*, and *Xgwm644*.

One unexpected result was the detection of 28 segregating AFLP fragments that did not map in the targeted region of chromosome 6B. Seven of these markers were polymorphic between Langdon and RSL#68 and 21 were not polymorphic between these lines. These results suggest that LDN(DIC 6B) and LDN have genetic differences in chromosomes other than chromosome 6B.

Characterization of the *dicoccoides* segment transferred to Glupro using RFLP

All previous polymorphic microsatellite and AFLP markers and 14 RFLP markers previously mapped on homoeologous group 6 chromosomes of the Triticeae (Joppa et al., 1997; Graner et al., 1991; Marino et al., 1996; Dubcovsky et al., 1996) were tested on DNA samples from Glupro, Len, Columbus, LDN(DIC 6B), and LDN. The presence of common polymorphism in LDN(DIC 6B) and Glupro, and absence in Len and Columbus were used as criteria to establish the boundaries of the *dicoccoides* segment present in Glupro.

Five AFLP, five microsatellite and seven RFLP markers showed the same polymorphism in LDN(DIC 6B)

and Glupro. The only marker tested for the short arm that did not show a *dicoccoides* allele was *Xpsr946*, indicating that a recombination event took place in the *Xpsr964*–*Xcmwg652* interval during the development of Glupro (Fig. 1). Two additional recombination events were detected in intervals *Xcdo534*–*Xcdo507* and *Xgwm626*–*Xgwm219* (Fig. 1). These data indicate that two separate chromosome segments from *dicoccoides* were transferred to Glupro chromosome 6B. One in the distal region of the long arm and the other one in the proximal region of the short arm encompassing the 6BS segment with highest LOD score for the GPC QTL (Fig. 1). RFLP locus *Xcdo507* and microsatellite locus *Xgwm626* showed identical polymorphisms in Glupro and Len that were absent in Columbus and LDN(DIC 6B).

DISCUSSION

The *XNor-B2* locus is a useful RFLP marker to trace the gene for high GPC not only because of its tight linkage with the peak of the QTL but also because thousands of copies of the rRNA gene (2C) are present in this locus (Flavell and O'Dell, 1979). The large number of copies reduces the amount of DNA that needs to be digested with restriction enzymes to 2 or 3 μg /lane, and the exposure time of the autoradiographs to less than 1 h. Though this RFLP is relatively easy to use, RFLP technology is more expensive and cumbersome than PCR technology. Consequently, the RFLP marker was converted into a PCR marker to facilitate its use in breeding programs.

The ASA primer pair was used to generate a dominant marker in coupling with the *dicoccoides* high GPC

gene. However, since the specificity of this marker was based on differences of a few base pairs, occasional false positives were amplified when the PCR conditions were not perfectly optimized. The CAPS primers combined with digestion of the PCR product with restriction enzyme *Bam*HI provided a more reliable marker. A possible strategy to minimize costs is to use the ASA marker for the initial testing and then use the CAPS marker to confirm the genotype of the critical plants.

These PCR-based markers have already provided a valuable tool for the detection of the high GPC gene from *dicoccoides*. Double haploid lines from BC₂F₁ from crosses between Glupro and two high yielding Canadian Prairie semi-dwarf advanced breeding lines were selected using these PCR markers. Double haploid lines carrying the PCR marker for the *dicoccoides* allele showed a significantly higher protein content than lines without the *dicoccoides* allele ($P < 0.001$, Humphreys et al., 1998).

Though the *XNor-B2* locus is closely linked to the peak of the QTL, recombination between this marker and the high-GPC gene may result in the loss of the targeted gene during the marker assisted selection process. The distal microsatellite marker *Xgwm508* can be used in combination with any of the three tightly linked proximal microsatellites *Xgwm193*, *Xgwm361*, and *Xgwm644* to monitor the transfer of a segment of *dicoccoides* chromatin (Fig. 1) that has a high probability of including the high-GPC gene. To reduce the linkage drag of *dicoccoides* chromatin of the long arm during the backcrossing process, long arm microsatellites markers can be used to select BC plants carrying the high-GPC gene on the short arm and a reduced *dicoccoides* chromosome segment on the 6BL arm. By this procedure, short-stature, hard-red-spring cultivars carrying the high-GPC gene and reduced *dicoccoides* 6BL arm are being developed as an alternative source of the high-GPC gene for new marker assisted selection programs.

An alternative source of the same GPC gene in hexaploid wheat may be line ND683 [*Stoa sib*'ND645 (Columbus/*T. dicoccoides* var. *dicoccoides*/'Coteau')] developed by R. Froberg (Mesfin et al., 1999). The small size of the *dicoccoides* chromosome segment present in this line was indicated by the fact that none of the RFLP or PCR markers analyzed in this study showed the characteristic *dicoccoides* alleles in ND683. Mesfin et al. (1999) indicated only one RFLP locus, *Xcdo365*, showing a *dicoccoides* allele in ND683. Though ND683 has the advantage of a reduced linkage drag that may eliminate potentially undesirable linked traits, it also has the disadvantage that none of the PCR markers developed here can be used to trace the GPC genes in marker assisted selection programs.

At the tetraploid level, RSL#68 can be used as a starting material to transfer the high-GPC gene to tetraploid cultivars. This line has a 6BS *dicoccoides* segment including RFLP markers *Xcmwg652* and *Xcdo534* (Joppa et al., 1997) that can be traced with the PCR markers presented in this study. However, RSL#68 is in a Langdon genetic background and has poor agronomic and quality characteristics. By means of the molecular

markers developed in this work, short-stature durum cultivars carrying the high-GPC gene and reduced *dicoccoides* 6BL arm are being developed as alternative sources for the high-GPC gene.

A final objective of this work was to explore the possibility of targeting additional markers to the GPC gene region by AFLPs. Of the 2400 AFLP fragments detected by 40 primer combinations, five were mapped in the targeted region. This result suggests that selection of polymorphic AFLPs between Langdon and RSL#68 is a viable strategy to saturate this region with additional molecular markers, providing a preliminary step towards the positional cloning of the high-GPC gene.

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Increased Chromosomal Variation in Transgenic versus Nontransgenic Barley (*Hordeum vulgare* L.) Plants

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ABSTRACT

Plants from in vitro culture can exhibit somaclonal variation, two characteristics of which are structural rearrangements and variation in chromosome number. These characteristics were studied in barley (*Hordeum vulgare* L. cv. Golden Promise) callus and plants derived from nontransgenic and transgenic callus of approximately the same age; chromosomes were studied in cells from callus and root tips from plants. Analysis of these data revealed greater variation in ploidy in transgenic compared with nontransgenic plants. Of 59 independent transgenic lines, only 32 (54%) had normal diploid complements of $2n = 2x = 14$, while 27 (46%) were tetraploid ($2n = 4x = 28$) or aneuploid around the tetraploid level (i.e., 26, 27, 29 and 30 chromosomes); no aneuploidy around the diploid number was observed. Nontransgenic plants regenerated after in vitro culture alone had a much lower percentage of tetraploids (0–4.3%). Most diploid plants had normal gross morphology, while tetraploid plants had abnormal morphological features. Ploidy determinations were made on randomly selected cells from callus of immature embryos cultured for 0 to 14 d. The number of tetraploid cells in 1-d- to 7-d-old callus was around 2 to 4%; in callus comparable in age to that used to regenerate both the transgenic and the nontransgenic sets of plants, 23% of the cells were tetraploid. This percentage is lower than the percentage (46%) of tetraploid plants from the transgenic lines; however, it is considerably higher than the percentage (0–4.3%) of tetraploid plants from nontransgenic callus. Therefore, although chromosomal variation and abnormalities occur in callus and nontransgenic plants, the extent of ploidy changes in transgenic plants is exacerbated, perhaps due to the additional stresses that occur during transformation.

CONSIDERABLE EFFORT has recently been focused on improving crops through transformation and molecular breeding. For transformation success in plants, DNA must be introduced into single, totipotent cells that are then proliferated in vitro during the selection process and regenerated to give rise to transformed plants. The passage of many plant tissues through an in vitro-culture phase frequently causes chromosomal instability (Bayliss, 1980; Constantin, 1981). The gross genetic changes, which occur as a result of the instability, are characterized as both structural rearrangements and numerical variation in the chromosomes. Although previously utilized as a tool for crop improvement, the cytogenetic and phenotypic variation arising from in vitro culture, termed somaclonal variation (Larkin and Scowcroft, 1981), leads mostly to undesirable changes (Karp, 1988; Lee and Phillips, 1988; Karp, 1991). Numerous reports have characterized the chromosomal variation in cultured tissues and regenerated plants of crops such as rice (*Oryza sativa* L.) (Nish and Mitsuoka, 1969), wheat (*Triticum aestivum* L.) (Karp and Maddock, 1984), maize (*Zea mays* L.) (McCoy and Phillips, 1982), oat (*Avena sativa* L.) (McCoy et al., 1982), Italian ryegrass (*Lolium multiflorum* L.) (Jackson and Dale, 1988), triticale (\times *Triticosecale* Wittmack) (Armstrong et al., 1983; Brettel et al., 1986), pearl millet (*Pennisetum*

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