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Linkage between RFLP markers and genes affecting kernel hardness in wheat

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Abstract A molecular-marker linkage map of wheat (Triticum aestivum L. em. Thell) provides a powerful tool for identifying genomic regions influencing breadmaking quality. A variance analysis for kernel hardness was conducted using 114 recombinant inbred lines (F7) from a cross between a synthetic and a cultivated wheat. The major gene involved in kernel hardness, ha (hard), known to be on chromosome arm 5DS, was found to be closely linked with the locus Xmta9 corresponding to the gene of puroindoline-a. This locus explained around 63% of the phenotypic variability but there was no evidence that puroindoline-a is the product of Ha (soft). Four additional regions located on chromosomes 2A, 2D, 5B, and 6D were shown to have single-factor effects on hardness, while three others situated on chromosomes 5A, 6D and 7A had interaction effects. Positive alleles were contributed by both parents. A three-marker model explains about 75 % of the variation for this trait.

Key words Kernel hardness · Wheat - RFLP · QTL · Puroindoline

Introduction

Among the aims for the improvement of cultivated wheat is greater breadmaking quality. Changes in kernel hardness affect many factors important to quality, including milling conditions, granularity, and amount of starch damage (Pomeranz and Williams 1990).

The genetic basis of kernel hardness is relatively well established. Using the particle size index, Worzella (1942) concluded that granularity (a standard measure of hardness) was inherited as a quantitative character but that relatively few genes were concerned. Symes (1965) showed that one major locus was involved. He was able to produce isogenic soft and hard lines for kernel hardness, using the Australian wheat cultivars Falcon and Heron (Symes 1969). Law et al. (1978), studying the genetic control of kernel protein amounts by chromosome 5D, showed that one gene affecting this trait was located close to the major locus influencing hardness, named ha (for hardness). This gene was assigned to the short arm of chromosome 5D (Mattern et al. 1973; Law et al. 1978), but chromosome arms 5AS and 5DL may also carry a minor locus (Morrison et al. 1989). Heritability of the trait is very high, ranging between 0.7 and 0.9 (Williams and Sobering 1984).

The biochemical basis of kernel hardness remains largely unknown. However, because neither storage protein nor starch granule differences could account for differences in hardness between wheat varieties (Barlow et al. 1973), Simmonds et al. (1973) suggested that hardness is due to a cementing agent between starch and proteins. Hardness was also found to involve the continuity of the protein matrix and the strength with which it physically entrapped starch granules (Stenvert and Kingswood 1977).

More recently, increased amounts of free polar lipids were shown to be strongly correlated with kernel softness (Morrison et al. 1984, 1989). The Mr 15 000 polypeptide, referred to as either “friabilin” (Greenwell and Schofield 1989) or “grain softness protein” (GSP; Jolly et al. 1990, 1993), was found to be a marker of kernel softness. Examination of near-isogenic lines differing in hardness indicated that GSP was associated with Ha, suggesting that GSP may be the product of Ha and thus the major factor determining the milling characteristics of bread wheats.

Recently, Rahman et al. (1994) showed that GSP is a mixture of different puroindoline-like polypeptides. Puroindolines are basic cysteine-rich proteins (CRP) isolated...
from *Triticum aestivum* (Blochet et al. 1993) which are characterized by a unique trypophan-rich domain. Characterization of cDNAs showed that puroindolines are synthesized in the form of pre-proproteins and that at least two proteins (puroindoline-a and -b), which are 60% identical, are members of the CRP family (Gautier et al. 1994). Currently no evidence directly implicates puroindolines in softness.

Strategies using either restriction fragment length polymorphism (RFLP) or random amplified polymorphic DNA (RAPD) have already been successfully used to map such wheat genes as *Vrn1* and *Frl* (Galiba et al. 1995) or the leaf rust resistance gene *Lr24* (Schachermayr et al. 1995). In an RFLP mapping study in wheat (Nelson et al. 1995a, b, c) using the population on which the present study is based, we previously identified chromosome arm 5DS markers linked with the kernel-softness gene *Ha*. Here we report the linkage of kernel softness with the puroindoline-a gene, as well as with markers in other regions of the wheat genome.

### Materials and methods

#### Plant material

The mapping population consisted of 114 SSD lines (F2 generation) derived from the cross between W-7984 (Synthetic: synthetic amphiploid wheat derived from a cross between *T. tauschii* and Altar 84 durum) and Opata 85 (Opata), a hard red spring bread wheat from CIMMYT (Van Deynze et al. 1993) provided by M. Sorrells. A single row of each entry was sown in the nursery at the INRA station in Clermont-Ferrand, France in 1993 and harvested and analysed in 1994. Plants were grown under normal field conditions with fungicide application to control rusts and powdery mildew. Only 86 of the 114 recombinant inbred lines (RILs) provided enough grain (15 g) for hardness evaluation. Five seeds were also sown in the greenhouse to provide fresh tissue for DNA extraction.

#### RFLP analysis and mapping

The procedures for RFLP analysis at INRA were performed with digoxigenin as described in Lu et al. (1994) except that alkali-labile DIG-11-dUTP was used to avoid the carryover of signals from probes previously hybridized on the same membrane. RFLP mapping at Cornell was done with radio-labelled probes as described in Nelson et al. (1995b). The restriction enzymes EcoRI, EcoRV, HindIII, and Dral (Boehringer Mannheim) were used for DNA digestion and hybridization following the manufacturer’s instructions.

More than 1100 loci were mapped in the population (Nelson et al. 1995a, b, c). Some of the cDNA and genomic DNA probes were described in Van Deynze et al. (1995). We also developed our own library of 200–2000-bp genomic fragments from etiolated seedlings of the varieties Courtot (pTaFBA library) or Chinese Spring (pTaFBB library), cloned in the *PstI* site of pBluescript vector.

The marker cloned in Gautier et al. (1994) corresponds to the puroindoline-a cDNA obtained from a *T. aestivum* cDNA library constructed with poly(A)+ RNA isolated from immature kernels (23 days after flowering). This clone is 679 bp long and contains a 23-bp 5'-untranslated sequence followed by an uninterrupted reading frame of 444 bp and a 3'-untranslated sequence of 196 bp before the poly(A) tail (16 bp). The primers used for puroindoline-a amplification were 5' ATGAAGGCTCTTCTCTCA-3' (position 43–61 on the sequence) and 5' TCACCAGTAAATAGCCAATTAGT-3' (position 470–449 on the sequence).

Mapping data were analysed with MAPMAKER version 3.0 (Lander et al. 1987). Loci whose order was established at a LOD of 3.0 were assigned exact positions on the map and the remainder were placed in the intervals in which they best fit using the “place” command. A partial map of chromosome arm 5DS including locus *Xmta9* is given in Figure 1. Complete chromosome maps appear in Van Deynze et al. (1995), Nelson et al. (1995a, b, c), and Marino et al. (1996).

#### Evaluation of kernel hardness

Kernel hardness was evaluated by near-infrared reflectance spectroscopy (NIR) using an Inframatic 8620 system (Scantec). The analysis followed the methods of AACC (1989) using a Cyclotec lab mill (Tecator) for wholemeal production. Reflectance was measured at 1680 and 2230 nm and hardness was computed with the formula:

hardness = 1475 × log (1/R2230) − 1099 × log (1/R1680).

The NIR instrument is standardized with a batch of five hard samples and five soft samples, with the mean of hard samples made to equal 75 and that of soft samples 25. Owing to genotype/environment interactions, some lines may have scores greater than 100.

#### Statistical analysis

The associations between markers and kernel hardness were evaluated by a one-way ANOVA using Splus software (Becker et al. 1992). Normality of the residuals was checked with the Pearson test of fit (Dagnelie 1975). Estimates of the locations of the QTLs and effects of each QTL were estimated via either the least squares estimates (LSmeans) of the general linear model (GLM) procedure SAS (SAS Institute Inc. 1991) or marker regression. Interaction effects between loci were also evaluated with the GLM procedure. Interaction parameters between markers M1 and M2 for their respective allelic forms i or j (θij) are computed from the LS means estimates as:

\[ \theta_{ij} = \text{LSmean}_ij - m - \text{a}_i - \text{a}_j \]