

ISOLATION OF CANDIDATE GENES INVOLVED IN BARLEY DEVELOPMENT

L. ROSSINI*, L. NICOLOSO*, A. VECCHIETTI**, F. GERVASONI*, S. FRANZAGO***,
F. SALAMINI***, C. POZZI**

*) Dipartimento di Produzione Vegetale, Università di Milano, Via Celoria 2, 20133 Milano

**) Fondazione Parco Tecnologico Padano, CERSA, Via Einstein, 26900 Lodi

***) Dipartimento di Biologia, Università di Milano, Via Celoria 26, 20133 Milano

candidate gene, synteny, barley, SNP, development

A synteny approach between the genomes of rice and barley was adopted to individuate candidate genes (CGs) for the barley developmental mutants previously mapped by our group. Map positions were used to select sequenced RFLPs linked with the mutant loci. *In silico* mapping of these markers by BLAST searches against the rice genome sequence allowed the identification of syntenic chromosomal regions in rice. CGs of particular interest include the rice ortholog of maize *Liguleless1* (*Lgl*), which represents a candidate for the barley *liguleless* (*lig*) locus, and the rice *FRIZZY PANICLE* (*FZP*) gene, which represents a candidate for the barley *branched1* (*brc1*) locus.

A fragment of the barley *Lgl* gene was isolated by PCR with degenerate primers and mapped *via* SNP mapping on chromosome 2H, in the region hosting the *lig* locus, providing preliminary evidence of the correlation between CG and mutant phenotype. Cloning of the entire gene sequence and cosegregation analysis with the mutant phenotype are underway.

The *brc1* locus (previously *brc-5*, Franckowiak and Lundqvist 2002) was mapped on barley chromosome 2H, sublinkage group 17 (Castiglioni et al. 1998). A syntenic chromosomal region was defined on rice chromosome 7. Annotation of this region resulted in the identification of *FRIZZY PANICLE* (*FZP*), a rice gene involved in inflorescence architecture and orthologous to maize *Branched Silkless1* (*BD1*). The barley orthologue was isolated and is being SNP-mapped on the high-density molecular map developed in our lab, to corroborate the correlation between the locus and the mutant phenotype.

A strong CG was also found for the *calcaroides* b19 and *calcaroides* C15 loci located on barley chromosome 5H. The rice syntenous region for these loci hosts a predicted gene exhibiting high sequence similarity to *rough sheath2*, a MYB gene implicated in the negative regulation of *knox* activity in maize (Timmermans et al. 1999; Tsiantis et al. 1999). The barley *rough sheath2* (*Brs2*) homologue was isolated in our group and its expression characterized by *in situ* hybridization on barley vegetative apices and immature inflorescences. The gene shows a strong expression in a ring-shaped domain at the lemma basis. Two experimental evidences are contrasting with a possible functional role of *Brs2* in the *calcaroides* phenotype: a. differences in gene expression between the wild type and *calcaroides* mutant were not evident from reverse transcription experiments; b. *Brs2* was mapped on chromosome 7H, in proximity to the *short awn* (*lks2*) locus. Cosegregation between the MYB gene and the *lks2* locus is under investigation.

Castiglioni P, Pozzi C, Heun M, Terzi V, Muller Kj, Rohde W, Salamini F (1998) An AFLP-based procedure for the efficient mapping of mutations and DNA probes in barley. *Genetics* 149: 2039-2056.

Franckowiak JD and Lundqvist U (2002) BGN 32:114.

Timmermans MC, Hudson A, Becraft PW, Nelson T (1999) ROUGH SHEATH2: a Myb protein that represses knox homeobox genes in maize lateral organ primordia. Science Apr 2;284(5411):151-3.

Tsiantis M, Schneeberger R, Golz JF, Freeling M, Langdale JA (1999) The maize rough sheath2 gene and leaf development programs in monocot and dicot plants. Science Apr 2;284(5411):154-6.