Barley Genetics Newsletter 36:12-16 (2006)

SSR Linkages to Eight Additional Morphological Marker Traits

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Introduction

More than 1000 morphological markers have been identified in barley (Franckowiak and Lundqvist 2002). These phenotypic traits were observed as spontaneous or artificially generated mutants in a wide range of cultivars over decades of research. Traits identified with alternate alleles include plant height, spike morphology, and seed size, among others. Approximately 275 of these markers have been placed on the morphological marker linkage map of the seven barley chromosomes (Franckowiak unpublished 2002) and/or on the consensus molecular marker linkage map (Kleinhofs 2002); approximately 150 to 200 additional markers have been placed on other maps but the rest have not been mapped.

Simple sequence repeat (SSR) markers are PCR-amplified regions of two or three base DNA repeats. Primers were designed to anneal to DNA on either side of each repeated segment, so size differences in amplification products are caused by different numbers of repeats in different genotypes. PCR reactions are easy to set up, do not use any hazardous chemicals, and only take a few hours for amplification. Products can be separated on agarose or acrylamide gels and results can be obtained in one day, which make these molecular markers ideal for short-term projects. We have worked with high school students in the North Dakota Governor's School to map morphological traits over the last several summers. This report describes the location of eight additional morphological markers through linkage to SSR markers.

Materials and Methods

Mapping population development A set of mutant lines were selected from various barley collections based on phenotype and backcrossed four to six times to 'Bowman' (Table 1). Homozygous backcrossed lines, BC_nF_2 populations, and BC_nF_2-derived F_3 lines were developed for mapping with molecular markers. Fifty BC_nF_2 seeds were sown for each mutant line in a greenhouse, along with the homozygous mutant parent and Bowman. The greenhouse was maintained at 21-26^oC with a 16 h day/8 h night cycle supplied by sodium halide lights. Each plant was scored as either normal or mutant. Plants were grown to maturity and harvested. Twelve BC_nF_3 seed were sown for each BC_nF_2 plant to identify heterozygous lines. Parent and BC_nF_2 seed were sown one per 15 cm clay pot, in a soil-less potting mix supplemented with a slow release fertilizer (14-14-14). The BC_nF_3 seed were sown with six seed per 15 cm clay pot and scored for the mutant trait at the appropriate time. Plants were treated with Marathon (Imidaclorprid) systemic insecticide at approximately the 2-3 leaf stage.

Trait Analysis Backcross-derived near-isogenic lines (NILs) and Bowman were planted in a field near Christchurch (Leeston), New Zealand and near Aberdeen, ID in 2002 and 2003 for agronomic analysis. Height (cm) and lodging (1-9 scale) were measured at Christchurch only in both years at the hard dough stage. Peduncle (cm) and awn length (cm), and the number of kernels per spike were measured in all four environments. Leaf length (cm) and width (mm) were measured on the penultimate leaf blade in all environments except 2002 Christchurch.

Rachis internode length (mm) was measured in all environments except 2003 Aberdeen. Kernel weight (mg per kernel based on 100 kernels) was measured in all environments except 2002 Aberdeen. Yield (g/m) was measured at Christchurch in 2002 and Aberdeen in 2003. Heading date was measured at Aberdeen in 2002. Lines also were grown in Dundee, Scotland in 2005, measuring heading date, height, peduncle and awn length, rachis internode length and number of kernels per spike. Plots were sown in an augmented block design with Bowman repeated across blocks. Each plot was a 2-m row with rows spaced 60 cm apart. The seedling rate was approximately 20 seeds per meter of row. Data were analyzed by Analysis of Variance using SAS (SAS Institute Inc. 2002) and least squares means were compared using the General Linear Model procedure. Trait means of the near-isogenic lines were compared to Bowman means using Least Significant Difference values.

Molecular marker testing Leaf tissue was harvested from young parent and BC_nF_2 plants grown in the greenhouse and the DNA extracted using the method of Dahleen et al. (2003). The DNA was then resuspended in 200 µL of modified TE (10 mM Tris-Cl, pH 7.4 and 0.1 mM EDTA). Simple sequence repeat (SSR) markers (Ramsay et al. 2000) were screened against the mutant near-isogenic lines and Bowman to identify polymorphisms. The PCR methods used were described in Dahleen et al. (2003). Amplified fragments were separated by gel electrophoresis using 4% Super Fine Resolution (SFR) agarose (Amresco, Solon, OH) in1 X TAE (40 mM Tris-acetate and 1 mM EDTA). Alternatively, acrylamide gel electrophoresis was used as described by Wang et al. (2003). The gels were stained with ethidium bromide, and photographed under UV light. Markers that detected polymorphisms between Bowman and a mutant near isogenic line were tested on the corresponding BC_nF_2 population. SSR and mutant trait segregation data were entered into MAPMAKER software (Lander et al. 1987; Lincoln et al. 1992) to test linkage between the markers and the mutant trait.

Table 1. Morphological markers, Barley Genetic Stock number, their chromosome locations, pedigrees of the mapping populations and the number of backcrosses to Bowman to develop the near-isogenic lines

Gene	BGS No.	Chromosome	Pedigree	Backcrosses
cer-zt.389	BGS437	2H	cer-zt.389/5*Bowman	4
dsp.ah		7H	DWS1180 Mut4841/6*Bowman	5
dsp.at		3H	Bowman*5/DWS1220 7117	4
dsp.ba		7H	DWS1357 UT1713/6*Bowman	5
int-k.47	BGS546	7H	int-k.47/7*Bowman	6
nec.50 (*pmr2*)	BGS634	7H	Bowman*5/nec.50	4
nec.54 (*pmr2*)	BGS634	7H	Bowman*5/nec.54	4
pyr.ai		3H	Bowman*6/DWS1018	5

Results and Discussion

Linkage between SSR markers and the morphological traits was identified for each of the eight backcrossed morphological marker lines. All F_2 populations segregated 3:1 normal:mutant as expected for single recessive traits. There were no significant differences between Bowman and near-isogenic lines for lodging, leaf width or heading date.

The eceriferum mutant *cer-zt*.389 (BGS 437) was located approximately in bin 1 of chromosome 2H, linked to Bmac0134 (Fig. 1). This NIL only differed from Bowman for kernels per spike, with a significant increase in *cer-zt*. The intermedium spike mutant *int-k*.47 (BGS 546) was located in the centromeric region of chromosome 7H, closely linked to markers Bmag0217 and Bmac0162 in bins 6 to 7. The *int-k*.47 NIL had significantly reduced height, peduncle length, awn length, kernels/spike, leaf length, kernel weight, and yield. The two necroticans mutants (*nec.50* and *nec.54*) are alleles at the premature ripe 2 (*pmr2*) locus (BGS 634). Analysis of individual populations indicated both were linked to the same markers on chromosome 7H (Fig. 1). Because they are alleles, a combined linkage analysis was conducted, which showed tight linkage to markers in bin 5 of 7H (Fig. 2). The *nec.54* NIL was analyzed in the field and showed reduced yield compared to Bowman. The pyramidatum mutant *pyr.ai* was linked between markers on chromosome 3H in bins 5 to 7 (Fig. 1). The NIL was shorter than Bowman, had shorter rachis internodes, and showed a significant increase in kernels per spike.

Three additional dense spike mutants were mapped, *dsp.ah*, *dsp.at*, and *dsp.ba*. The first two were linked to markers on chromosome 7H near the centromere while *dsp.ba* was located near the centromere on the short arm of chromosome 3H (Fig. 1). Previously located dense spike loci include *dsp1* on chromosome 7HS, *dsp9* on chromosome 6HL, and *dsp10* on chromosome 3HS. Agronomic trait performance of these three lines was similar to Bowman for most traits. The *dsp.ah* mutant showed significantly reduced height, peduncle length and rachis internode length, and lower yields but showed a significant increase in the number of kernels per spike. The *dsp.at* mutant was shorter and had reduced peduncle and rachis internode lengths, while *dsp.ba* only showed a decrease in rachis internode length compared to Bowman. These differences in the *dsp* mutants indicate it is unlikely they are alleles, and they are unlikely to be alleles of *dsp1*, *ert-a, ert-d* or *ert-m*, mutants with similar dense spikes.

In summary, we have placed eight additional morphological mutants on the barley molecular linkage map. One (*cer-zt*) mapped to chromosome 2H, two (*dsp.ba* and *pyr.ai*) mapped to chromosome 3H, and five (*dsp.ah*, *dsp.at*, *int-k*.47, *nec*.50 and *nec*.54) mapped to chromosome 7H.

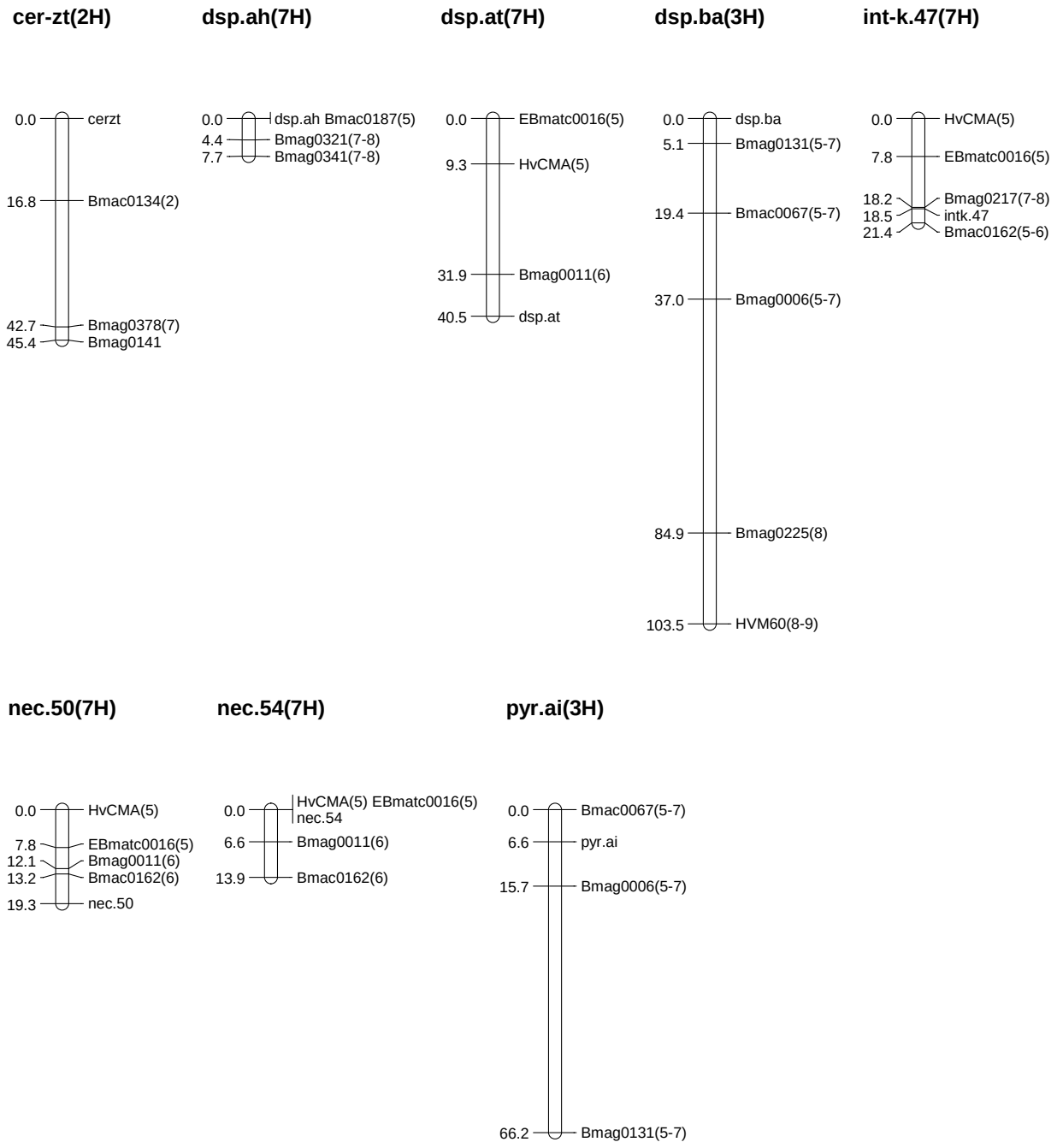

Figure 1. Linkage maps showing positions of morphological markers in relation to SSR markers mapped in segregating populations of 50 F_2 plants for each trait. Cumulative linkage distances are on the left of the vertical bar and marker names are on the right. Approximate chromosomal bin locations for the SSRs are in parentheses.

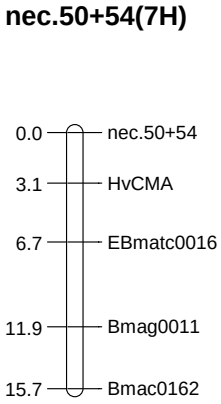

Figure 2. Linkage map showing the position of the *nec*.50 and *nec*.54 when the trait and marker data were combined for these alleles at the *pmr2* locus (BGS 634).

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