

## Molecular and physical mapping of genes affecting awning in wheat

P. SOURDILLE<sup>1</sup>, T. CADALEN<sup>1</sup>, G. GAY<sup>1</sup>, B. GILL<sup>2</sup> and M. BERNARD<sup>1,3</sup>

<sup>1</sup> INRA, Station d'Amélioration des Plantes, Domaine de Crouelle, 234 Avenue du Brézet, F-63039 Clermont-Ferrand Cedex 2, France; <sup>2</sup> Wheat Genetics Resource Center, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5501, USA; <sup>3</sup> Corresponding author. E-mail: Michel.Bernard@clermont.inra.fr

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### Abstract

Quantitative trait loci (QTL) for three traits related to awning (awn length at the base, the middle and the top of the ear) in wheat were mapped in a doubled-haploid line (DH) population derived from the cross between the cultivars 'Courtot' (awned) and 'Chinese Spring' (awnless) and grown in Clermont-Ferrand, France, under natural field conditions. A molecular marker linkage map of this cross that was previously constructed based on 187 DH lines and 550 markers was used for the QTL mapping. The genome was well covered (more than 95%) and a set of anchor loci regularly spaced (one marker every 20.8 cM) was chosen for marker regression analysis. For each trait, only two consistent QTL were identified with individual effects ranging from 8.5 to 45.9% of the total phenotypic variation. These two QTL cosegregated with the genes *Hd* on chromosome 4A and *B2* on chromosome 6B, which are known to inhibit awning. The results were confirmed using 'Chinese Spring' deletion lines of these two chromosomes, which have awned spikes, while 'Chinese Spring' is usually awnless. No quantitative trait locus was detected on chromosome 5A where the *B1* awn-inhibitor gene is located, suggesting that both 'Courtot' and 'Chinese Spring' have the same allelic constitution at this locus. The occurrence of awned speltoid spikes on the deletion lines of this chromosome suggests that 'Chinese Spring' and 'Courtot' have the dominant *B1* allele, indicating that *B1* alone has insufficient effect to induce complete awn inhibition.

**Key words:** *Triticum aestivum* — awn inhibition — deletion lines — molecular markers — physical mapping — QTL

Inheritance of 'awnedness' in wheat, *Triticum aestivum*, has been well studied since the beginning of the twentieth century. As well known in barley, awns may play a major role in the elaboration of the yield in wheat, especially under drought conditions. The total surface area of the awns can sometimes equal that of the ground surface, and can exceed that of the flag leaf blade in *Triticum durum* (McDonough and Gauch 1959). In addition, awns are ideally placed for light interception and CO<sub>2</sub> uptake, the pathway for assimilate movement from awns to the kernel being minimal (Evans et al. 1972). Moreover, they have less tendency to senesce than the flag leaves since they develop later. In wheat, the presence of awns can double the rate of net ear photosynthesis (Evans and Rawson 1970). This is much significant under drier conditions, where awned cultivars surpass awnless or de-awned wheats in yield (Miller et al. 1944, Vervelde 1953, Grundbacher 1963), although awns have only a limited effect on yield in wet climates (Vervelde 1953).

The genetic control of this trait was generally found to be simple, and only a few genes were involved in the differences

between awned and awnless varieties. Three dominant inhibitors are described (McIntosh et al. 1998): *Hd* (Hooded), *B1* and *B2* (tipped 1 and 2). In the hooded mutant type (*hd*), awn length of the main tillers is considerably reduced and the awns are often bent at the base in a characteristic way. In addition, glumes are inflated and the awns of the late tillers are either reduced to a hook or bent round on themselves to form a close spiral. The tipped-1 mutants (*b1*) have very short awn tips at the base and the middle of the ear but then increase in length in the apical quarter and may sometimes reach 1 cm. These awn tips are usually straight and not bent at the base. The other tip-awned wheat mutants (*b2*) are quite different. Generally, the length of the tips does not vary all along the ear, occasionally close to the centre of the spike but not at the apex. Length rarely exceeds 6 mm and if awns are curved, they are never curved around themselves as hooded wheats (Watkins and Ellerton 1940). Wheat genotypes carrying the three recessive *hd*, *b1* and *b2* alleles in homozygous conditions are fully awned, while those with either *Hd B2* (such as 'Chinese Spring') or *B1 B2* (such as 'Federation') are awnless.

Assignment of these three genes to chromosomes was achieved by using aneuploid lines: *Hd* was located on the short arm of chromosome 4A (Sears 1954, Rao 1981); *B1* was located on the long arm of chromosome 5A (Sears 1954); and *B2* was located on the long arm of chromosome 6B (Sears 1954, 1966). Development of molecular marker linkage maps in wheat (Liu and Tsunewaki 1991, Gale et al. 1995, Nelson et al. 1995a,b,c) allows location of these genes on a precise genetic map. In addition, their physical location can be estimated using aneuploid stocks such as deletion lines (Endo and Gill 1996).

In this study, a genetic linkage map developed using a doubled haploid population from a cross between the fully-bearded cultivar 'Courtot' and the awnless cultivar 'Chinese Spring' (Cadalen et al. 1997) was used to locate the genes that may segregate in this cross. Genetic mapping results were confirmed using phenotypic observations together with a molecular characterization of 'Chinese Spring' deletion lines.

### Materials and Methods

**Plant materials and awning evaluation:** A population of 138 doubled-haploid (DH) lines was produced through anther culture from 'Courtot' (Ct) × 'Chinese Spring' (CS) F<sub>1</sub> hybrids (Félix et al. 1996, Cadalen et al. 1997). A total of 106 lines were genotyped for all the markers, the others being genotyped only for anchor loci. The lines,

together with the parents, were sown at Clermont-Ferrand in the autumn 1997. Three-row plots (1.5 m) were grown in a nursery with two replications under normal field conditions with fungicide application to control rusts and powdery mildew. At flowering, awn length (in mm) was measured for each line at the base, the middle and the top of three main spikes and the means of all these data were computed.

'Chinese Spring' deletion lines were sown into 10 cm pots and placed in a vernalization cabinet at 6–8°C with an 8-h day for 6 weeks. The plants were then moved into a glasshouse until flowering. Awning of the spikes was then observed.

**Molecular analysis:** The probes and the techniques for DNA extraction, digestion, electrophoresis, blotting and hybridization were described by Cadalen et al. (1997). The protocol using non-radioactive probes as detailed in Lu et al. (1994) and Sourdille et al. (1996). Several microsatellite loci (Devos et al. 1995, Röder et al. 1995, 1998, Plaschke et al. 1995, 1996, Guyomarc'h et al. 2002) were also mapped in this population. Protocols for polymerase chain reaction (PCR) reactions are detailed in Tixier et al. (1998). The detection of microsatellites using a non-radioactive silver nitrate staining method was described by Tixier et al. (1997). Some amplified fragment length polymorphisms (AFLPs) markers obtained following the procedure described in Bert et al. (1999) were also added to the map.

**Statistical analysis:** The map obtained from the DH population was constructed with 550 markers using MAPMAKER/EXP version 3.06 (Lander et al. 1987), as described by Sourdille et al. (2002). The map covered 3634 cM (more than 95% of the genome) with one marker every 6.6 cM, on average. The whole genome was well covered except for chromosomes of the homoeologous group 4 as well as chromosomes 5D and 7D on which a gap slightly larger than 50 cM remained. A set of anchor loci regularly spaced all over the genome (one marker every 20.8 cM) was chosen for marker regression analysis.

The associations between markers and awning were evaluated by one-way ANOVA with  $P = 0.001$  to keep the overall type I error risk below 0.05, in order to detect those markers having the main effects. Estimates of the location of the QTL, origin of the positive alleles and additive value of the QTL were then assessed for each linkage group using the marker regression method according to Kearsey and Hynes (1994), the most significant markers from the other groups being used as covariates as proposed by Jansen and Stam (1994).

## Results

The two parents of the DH population 'Courtot' and 'Chinese Spring' differed widely for awning, with average awn lengths (MA) of 22 mm (awning at the base of the spike (AB) = 5 mm; awning at the middle of the spike (AM) = 35 mm; awning at the top of the spike (AT) = 25 mm) for 'Courtot' and 2 mm (AB = 2 mm; AM = 3 mm; AT = 2 mm) for 'Chinese Spring'. 'Chinese Spring' carries the awn inhibitors *Hd* and *B2* (McIntosh et al. 1998), while the genotype of fully-bearded 'Courtot' is probably *hd b1 b2*. Thus, segregation should be observed at least at the level of the *Hd* and *B2* loci. The four traits (AB, AM, AT and MA) were not normally distributed (Fig. 1). Distributions were largely skewed towards the awnless or short awn phenotypes. Few transgressive lines were observed, suggesting that only a few genes were involved and that 'Courtot' carried awning alleles of these genes. The three traits were correlated with correlation coefficients ranging between 0.59 between AB and AT and 0.70 between AM and AT.

Only two major QTL were detected (Table 1) and both were related to awn length, at the base, the middle or the top of the spike. The first was located on chromosome arm 4AS, close to the locus *Xfba78*. This quantitative trait locus explained

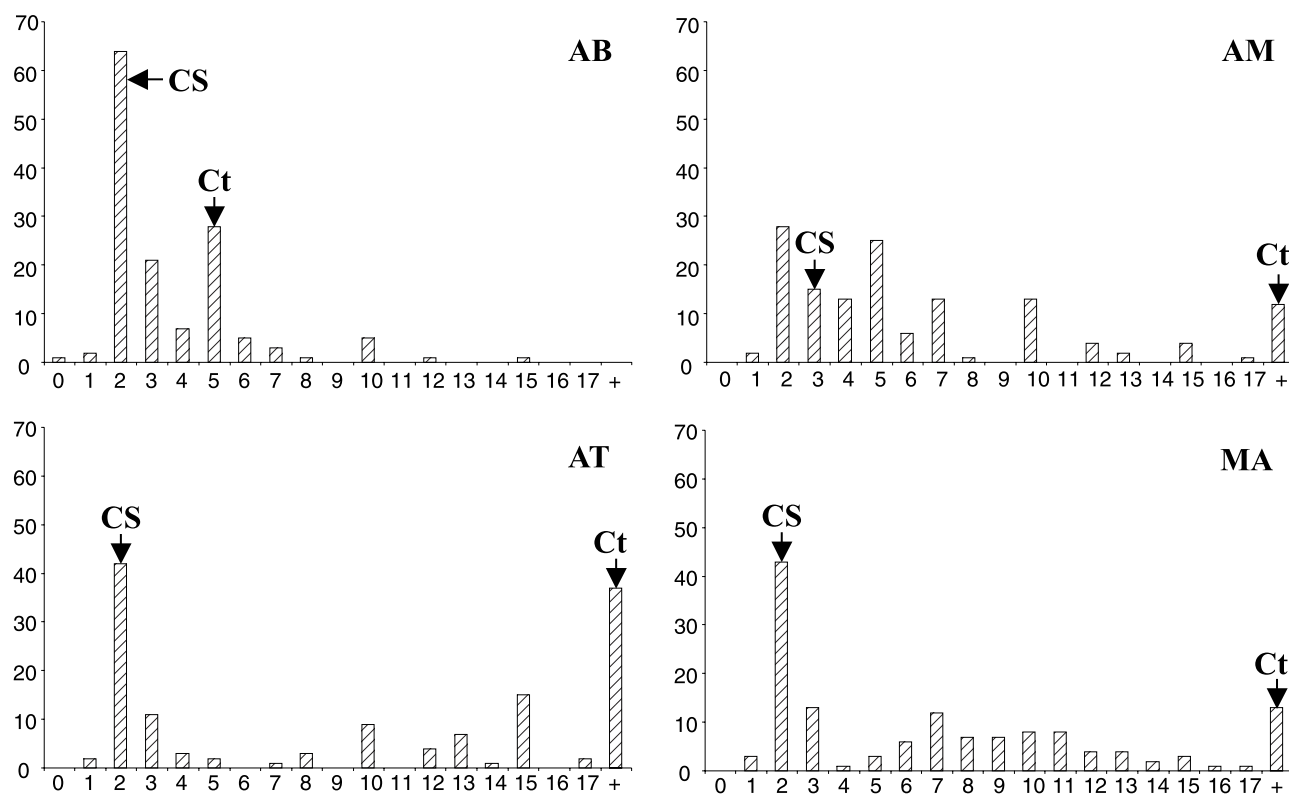


Fig. 1: Frequency distribution of the four traits. AB, awning at the base of the spike; AM, awning in the middle of the spike; AT, awning at the top of the spike; MA, mean of awning along the spike. Parental values for 'Courtot' (Ct) and 'Chinese Spring' (CS) are indicated by arrows

Trait	Loci	$r^2$	df	F-value	P	Allele <sup>2</sup>
AB	<i>Xfba78-4A</i>	27.1	131	48.73	$1.32 \times 10^{-10}$	Ct
	<i>Xwmc182-6B</i>	9.9	131	14.42	$2.23 \times 10^{-4}$	Ct
AM	<i>Xfba78-4A</i>	16.7	131	26.34	$1.01 \times 10^{-6}$	Ct
	<i>Xwmc182-6B</i>	37.6	131	78.83	$4.44 \times 10^{-15}$	Ct
AT	<i>Xfba78-4A</i>	45.6	131	109.88	$6.52 \times 10^{-20}$	Ct
	<i>Xwmc182-6B</i>	8.5	131	12.16	$6.66 \times 10^{-4}$	Ct
MA	<i>Xfba78-4A</i>	39.7	131	86.09	$4.44 \times 10^{-16}$	Ct
	<i>Xwmc182-6B</i>	20.8	131	34.41	$3.44 \times 10^{-8}$	Ct

Table 1: Markers significantly associated with awning at  $P = 0.001$ <sup>1</sup>

<sup>1</sup> AB, awning at the base of the spike; AM, awning at the middle of the spike; AT, awning at the top of the spike; MA, mean awning value.

<sup>2</sup> Allele: allele at the quantitative trait locus favouring awning; Ct, 'Courtot'.

between 16.7% and 45.6% of the variability (mean 39.7%). The second quantitative trait locus was located on chromosome arm 6BL, close to the locus *Xwmc182*. The locus explained between 8.5% and 37.6% (mean 20.8%) of variation for the trait. In both cases, alleles favouring awning were introduced by 'Courtot', the awned genotype. The quantitative trait locus on chromosome arm 4AS exhibited higher  $r^2$  values for traits concerning the awns located at both ends of the spike (AB and AT) while the effect of the quantitative trait locus on chromosome arm 6BL was stronger for the awns located in the middle of the spike (AM).

Neither significant effects on chromosome 5A carrying the *B1* gene nor significant interaction between the markers were detected.

The results obtained by this QTL analysis were confirmed by using deletion lines (Endo and Gill 1996). 'Chinese Spring' deletion lines 4AS-1, 4AS-3 and 4AS-4, lacking segments of the short arm of chromosome 4A, or deletion lines 6BL-5 and 6BL-6, lacking segments of the long arm of chromosome 6B, exhibited an awned phenotype, while the standard variety 'Chinese Spring' is awnless (Fig. 2). The microsatellite WMC182 gives several amplification products, three loci being assigned to the chromosomes from the homoeologous group 7, and one locus on chromosome 6B. This latter locus, which is linked to the *B2* locus, was not detected for deletion lines 6BL-5 and 6BL-6 (data not shown). This confirmed that the loci *Xwmc182-6B* and *B2* were deleted in these two lines.

A comparison between genetic and physical maps in this region (Fig. 3) was made. Consequently, *Hd* was located on chromosome 4A between the deletion 4AS-3 and the telomere while *B2* was on chromosome 6B between deletion 6BL-6 and the telomere.

No quantitative trait locus was detected on chromosome arm 5AL carrying the *B1* awn inhibitor, even when the threshold was set to 0.01 (data not shown). However, using the deletion lines 5AL-10 and 5AL-17 (Fig. 2), it was noted that both had a classical speltoid phenotype due to the lack of the gene *Q*, located in the same region of this chromosome arm. Furthermore, line 5AL-10 was slightly bearded, indicating deletion of the inhibitor *B1*. In addition, two microsatellites, WMC173 and WMC182, which were found to be linked to *Hd* and *B2*, respectively, were tested on four 5AL deletion lines that do or do not exhibit awning. These two microsatellites were clearly deleted on the deletion lines of chromosomes 4A (WMC173) and 6B (WMC182) but were present in the 5AL deletion lines. This confirmed that these lines have no interstitial deletions in the regions carrying *Hd* and *B2* on chromosomes 4A and 6B, respectively, which may have explained the occurrence of awning.

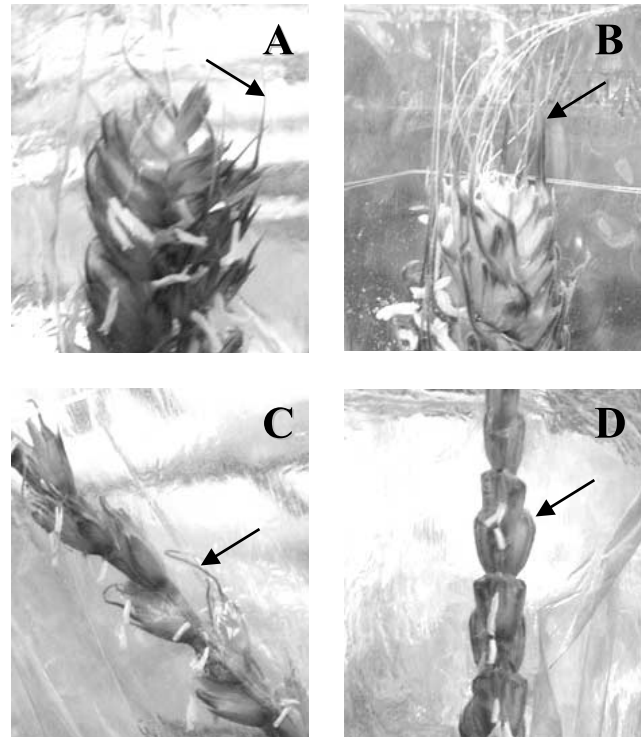


Fig. 2: Difference in spike morphology between 'Chinese Spring' deletion lines. A. 4AS-4; B. 6BL-5; C. 5AL-10 (awned speltoid phenotype); D. 5AL-17 (awnless speltoid phenotype)

## Discussion

Distributions of the traits were largely skewed towards the awnless or short awn phenotypes. This was not expected since DH lines involving no dominance effects were used. A bias was also observed for some markers on the genetic map, which showed severe segregation distortion. On chromosome 6B, which is known to carry the *B2* gene inhibiting the awns, most of the molecular markers were biased towards an excess of 'Chinese Spring' alleles (from 58 to 96% of CS alleles; Cadalen et al. 1997). A possible explanation for this is the occurrence of the *Ki* allele (pollen killer) located on chromosome 6B of 'Chinese Spring'. The three traits were correlated, with correlation coefficients ranging between 0.59 between AB and AT and 0.70 between AM and AT. This rather strong correlation could be explained by the fact that each awn inhibitor naturally inhibits awning, but because these correlations were not higher these genes may act differently on the different parts of the spike, as has been recorded in the literature.

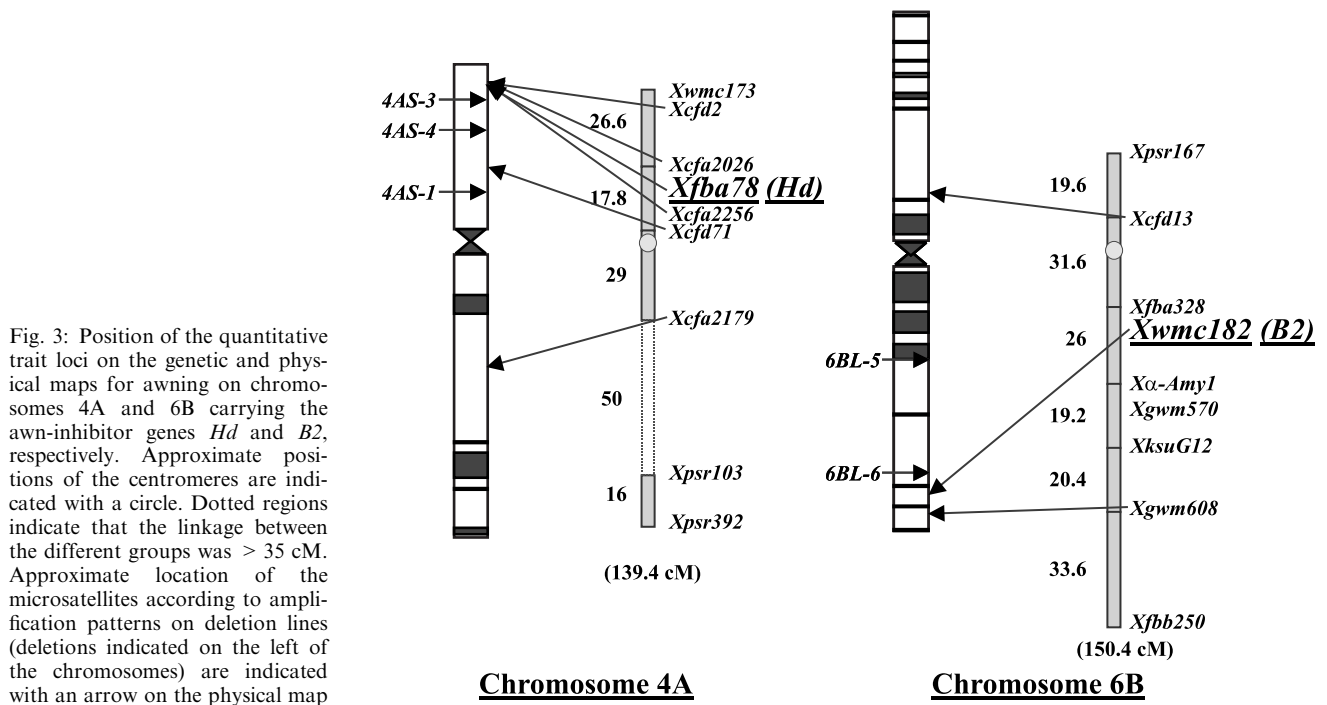


Fig. 3: Position of the quantitative trait loci on the genetic and physical maps for awning on chromosomes 4A and 6B carrying the awn-inhibitor genes *Hd* and *B2*, respectively. Approximate positions of the centromeres are indicated with a circle. Dotted regions indicate that the linkage between the different groups was > 35 cM. Approximate location of the microsatellites according to amplification patterns on deletion lines (deletions indicated on the left of the chromosomes) are indicated with an arrow on the physical map

Only two major QTL were detected. The first was located on chromosome arm 4AS and corresponded to the *Hd* gene mapped in the same region by the use of aneuploid stocks (Sears 1954, Rao 1981). The second locus was located on chromosome arm 6BL and the awning difference was probably due to the *B2* gene previously mapped on this chromosome arm (Sears 1954, 1966). The effects of these two genes depended upon the individual trait: *Hd* exhibited higher  $r^2$  values for traits involving the awns located at both ends of the spike (AB and AT) while the effect of *B2* was stronger for the awns located in the middle of the spike (AM). This was in accordance with the results of Watkins and Ellerton (1940), who noted that in *b2* tip-awned wheat mutants the length of the tips generally does not vary along the ear, but perhaps close to the centre of the spike but never at the apex. The absence of significant effect detected on chromosome 5A carrying the *B1* gene, suggests that both 'Courtot' and 'Chinese Spring' have the same allelic constitution at this locus. In the same way, a lack of significant interaction detected between the markers suggests the absence of epistatic effects in this cross. Such effects between *B1* and *B2* or between *B1* or *B2* and various promoter genes have been mentioned in the literature (McIntosh et al. 1998). Interactions between *B2* and other regions of the genome may be undetectable because of the strong bias existing at this locus, which results in a weakening of strength for some genotypic classes.

The 6BL-6 deletion displays deleted DNA markers that were present in the other 6BL deletions (Gill et al. 1993). Therefore, Endo and Gill (1996) suggested that awning of the 6BL-6 line was explained by an interstitial deletion that includes the locus of the *B2* awn-inhibitor gene.

No quantitative trait locus was detected on chromosome arm 5AL carrying the *B1* awn inhibitor, while the deletion line 5AL-10 was slightly bearded, indicating the deletion of *B1*. Evidence showed that this latter line had no interstitial deletions in the regions carrying *Hd* and *B2* on chromosomes

4A and 6B, respectively, which might have explained awning occurrence. Therefore, the absence of quantitative trait locus detection suggests that both 'Courtot' and 'Chinese Spring' have the same allelic constitution at this locus. The occurrence of awns in the 'Chinese Spring' deletion lines indicates that this allele is probably *B1*, since a deletion of this inhibitor induced short awns to appear. Thus, 'Courtot' has the following allelic constitution *hd B1 b2*, resulting in a fully-bearded type, while 'Chinese Spring' is *Hd B1 B2*, leading to an awnless phenotype. This result also indicates that *B1* alone is unable to induce complete awn inhibition since 'Courtot', which probably carries this allele, is fully awned. It was also assumed that *Hd B1* genotypes were awnless (McIntosh et al. 1998). If 'Chinese Spring' is *Hd B1 B2*, these results suggest that *Hd B1* genotypes are not awnless, since deletion of *B2* only in 'Chinese Spring', which then becomes *Hd B1-*, induced awns to appear.

These results showed the advantages of integrating molecular and physical genetic maps to achieve the cloning of many agronomic genes. Further studies will be carried out to identify bacterial artificial chromosome (BAC) clones bearing the closest markers to reach this goal.

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