

Genetics and Mapping of Barley Stripe Mosaic Virus Resistance in Barley

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ABSTRACT

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The inheritance of resistance to barley stripe mosaic virus (BSMV) in barley was investigated in the Steptoe/Morex doubled haploid population developed by the North American Barley Genome Mapping Project. The number of resistant (66) and susceptible (84) progeny approximated a 1:1 ratio, indicating that a single gene was involved in conferring resistance to BSMV strain CV42 in Morex barley. This resistance gene was mapped to the centromeric region of the plus (short) arm of chromosome

1, based on its linkage to molecular markers on the Steptoe/Morex map, and was found to cosegregate with restriction fragment length polymorphism (RFLP) marker *ABC455*. Marker *ABG011* was located 6.1 centimorgans (cM) distal to the resistance locus. The nearest opposite flanking marker mapped was *Amy2*, located at a distance of 8.1 cM and on the other side of the centromere. The identification of a cosegregating marker may facilitate both the selection for BSMV resistance in barley breeding programs and the high resolution mapping of the centromeric region in the vicinity of this locus.

Additional keywords: disease resistance, *Hordeum vulgare*.

Barley stripe mosaic has been reported from nearly all barley producing regions worldwide (8,14). The causal agent of this disease, barley stripe mosaic virus (BSMV), is a tripartite RNA *Hordeivirus* dependent on seed transmission for its survival in nature (16). Barley (*Hordeum vulgare* L.) is the principal natural host, although strains exist which incite disease in other cereals such as wheat (*Triticum aestivum* L.) and oat (*Avena sativa* L.). The inheritance of BSMV resistance in barley has been the subject of several studies since McKinney first discovered the viral etiology of the disease in 1951 (9). A single recessive gene was reported to control resistance to the California "E" isolate of BSMV in seedling tests of the barley genotypes Modjo (CI 3212) and Modjo-1 (CI 14048) (13,19), although the same authors suggested that a second gene was involved at later stages of plant growth (13). A single recessive gene also was reported to determine the reaction to BSMV strain ND1 in Traill (CI 9538), Modjo-1, and Moreval (CI 5724) barley, while a multiple allelic series was suggested to control the reaction to the type strain (17). More recently, Timian and Franckowiak (18) identified a single recessive gene for BSMV strain CV42 resistance that was linked to the *Lk2* locus (controlling awn length) on chromosome 1 in Modjo-1, Moreval, and CI 4197.

Because of the differences in virus strains and barley genotypes used in the above studies, definitive relationships among the identified genes could not be established. The usefulness and value of such genetic data can be greatly enhanced if linkage relationships can be established with known molecular markers on the barley genome. A highly saturated molecular map of the barley genome has been developed by the North American Barley

Genome Mapping Project based on the Steptoe/Morex doubled haploid (DH) population (4,5). In a preliminary experiment, we found that Morex was resistant to BSMV strain CV42, whereas Steptoe was susceptible. The purpose of this investigation was to determine the number and chromosomal location of genes conferring BSMV resistance in Morex barley.

MATERIALS AND METHODS

Plant and virus sources. One hundred and fifty DH progeny from the Steptoe/Morex mapping population (4,5) were evaluated for their reaction to BSMV. Seed of parents and progeny was originally obtained from P. M. Hayes (Oregon State University, Corvallis) and subsequently increased at Fargo, ND. BSMV strain CV42 was originally isolated from oat and has been well-characterized (17,21,22). Although pathogenic to oat and barley, this BSMV strain is unable to infect certain resistant barley genotypes, such as Modjo, Modjo 1, and Moreval (16). A culture of CV42 which had been preserved in Black Hullless (CI 666) barley tissue in liquid nitrogen since 1988 was used for these experiments. After removal from storage, the isolate was propagated in Black Hullless barley in a 27°C greenhouse with a 16-h photoperiod.

Evaluation of plants for reaction to BSMV. Twelve seeds of each progeny line were planted in a peat moss/perlite (3:1) potting mixture in a greenhouse with the photoperiod extended to 16 h using metal halide lamps generating 295 μmol of photon $\text{m}^{-2} \text{s}^{-1}$ (29.5 W m^{-2}) at plant height. Temperatures in the greenhouse normally ranged from 22 to 29°C, although, in the second experiment, temperatures reached 30 to 31°C for several hours just after inoculation. All plants were grown simultaneously in the same greenhouse to ensure that each would be evaluated under identical environmental conditions.

Six days after planting, seedlings were sprayed with inoculum comprised of sap from BSMV-infected barley plants (strain CV42, diluted 1/10 with water) and carborundum (400 grit, 0.1 g/ml) using an artist's airbrush and a nozzle pressure of 414 kPa.

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A single inoculum preparation was used on all seedlings in a given experiment. The uniformity of inoculation and inoculum quality were monitored by inoculating one set of check plants for every group of 25 DH progeny. Check plants consisted of Rodney oat (CI 6661) and Steptoe (CI 15229), Morex (CI 15773), and Black Hulless barley (total of six sets of each per experiment). Rodney oat and Black Hulless barley were chosen as additional checks, because both are readily infected by CV42 and both provided an additional verification of inoculum quality and infection phenotype. Plants were assessed visually for signs of viral infection beginning at 6-days postinoculation; observations continued to be recorded for 2-weeks postinoculation. Enzyme-linked immunosorbent assay (ELISA) was used to verify the absence of infection in apparently healthy plants (1). A random sample of twenty symptomless progeny lines were sampled at 2-weeks postinoculation, subjected to ELISA, and found to be BSMV negative (data not shown). The experiment was repeated twice, although a few lines were tested again in a third trial (referred to in Results).

Progeny were classified as resistant or susceptible if the percent infection with BSMV was within one standard deviation of the mean percent infection of either the resistant (Morex) or susceptible (Steptoe) parent. The time required for symptom onset also was considered. Segregation of the DH population for reaction to BSMV was subjected to chi-square analysis. Linkage analysis was performed and map position of the resistance gene determined using the MAPMAKER computer program (7). Recombination data were converted to centimorgans (cM) using the Kosambi mapping function (6).

RESULTS

The Steptoe/Morex population was well-suited for studying the genetics of resistance to BSMV. Steptoe was quite susceptible, with infection apparent after 5 days in many plants and an average of 84.1% of inoculated plants developing a prominent stripe mosaic symptom within 1 week after inoculation. In contrast, 95% of Morex plants exhibited no signs of infection 7 days after inoculation. Some Morex plants eventually developed symptoms, with an average of 22.2% visibly infected at 2-weeks postinoculation (versus 88.5% for Steptoe). No further increase in the number of infected plants was observed. Inoculation of other check plants resulted in 100% infection of Black Hulless barley and 97% infection of Rodney oat plants. The high infection rates found for the Black Hulless and Rodney checks indicated that inoculation technique and inoculum quality were not major factors affecting the percentage of plants infected.

The percent infection in individual progeny lines was very similar between the first and second experiments; therefore, the classification of progeny as resistant or susceptible was based on pooled data. Nearly all of the 150 progeny were readily classifiable as one or the other of the parental types. The majority (144) were within one standard deviation of the mean percent infection of either Morex or Steptoe at 7- or 14-days postinoculation (dpi) (Morex_{7dpi} = 5% ± 9%; Morex_{14dpi} = 22.2% ± 18.4%; Steptoe_{7dpi} = 84.1% ± 15.4%; Steptoe_{14dpi} = 88.5% ± 12.5%). The six remaining progeny were well within two standard deviations of the mean percent infection of the respective parents (Fig. 1). Phenotypes of these progeny were verified in a third experiment. In all cases, the original phenotype classification was reconfirmed by considering the time required for symptom onset as well as the percentage of plants infected. Of the 150 progeny lines evaluated, 84 were of the Steptoe phenotype and 66 were of the Morex phenotype. A calculated chi-square value of 2.16 and associated probability of 0.14 supported the expected 1:1 ratio, indicating that a single major gene controlled the reaction to BSMV in Morex. Linkage analysis with MAPMAKER placed this BSMV resistance locus on the plus (short) arm of chromosome 1, near the centromere

(Fig. 2). The resistance gene was found to cosegregate with restriction fragment length polymorphism (RFLP) marker *ABC455*. Marker *ABG011* was 6.1 cM distal to the resistance locus. The nearest opposite flanking marker mapped was *Amy2*, located at a distance of 8.1 cM and on the other side of the centromere.

DISCUSSION

The lack of 100% infection of 'susceptible' genotypes (Steptoe and progeny) and the infrequent infection of 'resistant' plants (Morex and progeny) was expected, but is not completely understood. Varying levels of BSMV infection of barley have been observed to occur depending on the virus strain/host genotype combination (10,16; M. C. Edwards, *unpublished data*). Although the possible influence of temperature or the existence of genes with minor effects cannot be excluded, this variation could be due to the nature of the host response. Unfortunately, very little is known about the nature of this resistance or its mechanism. BSMV resistance is functional in protoplasts derived from a number of different barley lines, suggesting that resistance may be due to inhibition of virus replication (22).

The identification of a single gene for BSMV resistance in Morex barley complements existing knowledge of BSMV pathogenicity determinants. Using pseudorecombinants constructed

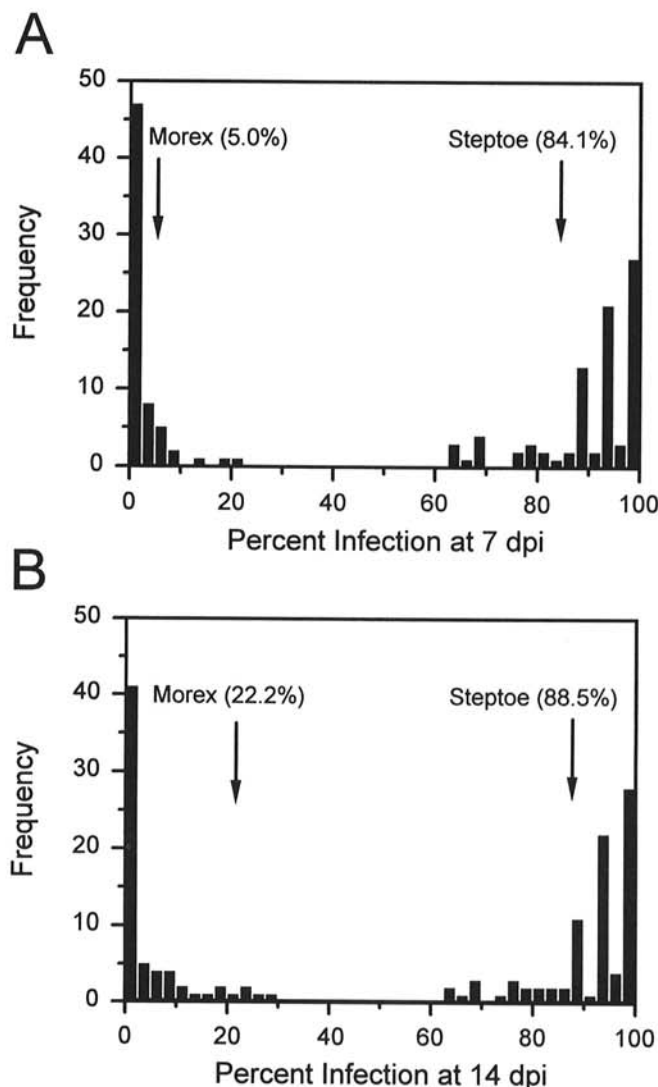


Fig. 1. Frequency distribution for percent barley stripe mosaic virus (strain CV42) infection of 150 doubled haploid progeny derived from the cross Steptoe/Morex. Percent infection was determined at A, 7-days postinoculation, and B, 14-days postinoculation.

with both native viral RNAs and transcripts from full-length cDNA clones, determinants of BSMV pathogenicity to barley have been shown to be located on RNA α (2; M. C. Edwards, unpublished data). This RNA encodes only a single open reading frame (the α gene) and is required for viral replication (12). Its gene product is thought to be an essential component of the BSMV replicase because it has sequence similarities to analogous proteins known to be involved in RNA replication (3). Thus, the CV42 resistance of Morex could possibly result from a gene-for-gene interaction. Such gene-for-gene interactions have been identified in other host-virus systems. For example, mutations in the analogous replicase genes of tobacco mosaic virus have been shown to overcome *Tm-1* gene resistance in tomato (11).

Studies on the inheritance of BSMV resistance in barley by Sisler and Timian (13) and Vazquez et al. (20) resulted in the identification of five possible resistance gene loci. These loci were listed as *rsm1* to 5 by Sjøgaard and von Wettstein-Knowles (15), but none were assigned to a chromosome. A single recessive gene for resistance to BSMV in Modjo-1, Moreval, and CI 4197 was also identified in a preliminary report by Timian and Franckowiak (18; R. G. Timian, personal communication). Because the F₂ progeny from a diallel cross among these barley genotypes were all resistant to strain CV42, Timian and Franckowiak (18) concluded that the same resistance gene was present in all three genotypes. Although the studies of Sisler and Timian (13) and Vazquez et al. (20) involved BSMV strains other than CV42, Modjo-1 and Moreval barley were among the genotypes included. It is possible, therefore, that fewer than five resistance loci actually have been identified.

Timian and Franckowiak (18; R. G. Timian, personal communication) also concluded that the resistance gene identified in their study, which they designated *rsm*, was linked to the *n* locus for naked caryopsis and to the *Lk2* locus for awn length, both located on the minus (long) arm of chromosome 1. Their results are consistent with a proximal location of *rsm* to these loci (i.e., near the centromeric region). It is therefore possible that the gene identified in this study is the same as that found by Timian and Franckowiak (18). Unfortunately, this relationship cannot be determined with certainty until allelism tests are conducted. Until

this is done, the BSMV resistance gene from Morex will be temporarily designated as *RsmMx*. The use of an upper case 'R' commonly denotes dominant gene action for resistance; however, this designation is temporarily being used until the dominant or recessive nature of the resistance allele can be determined.

A number of other markers (*Adh7*, *ABR329*, *WG719*, *ABG476*, *BCD340C*, *ABC322A*, and *ABC254*) (4,5) have been mapped to the region near marker *ABC455* and are, therefore, in relative proximity to the BSMV-resistance gene. Because this is a region of very low recombination frequency, any attempt to determine the precise order of these markers relative to the BSMV-resistance locus would be of limited value without evaluation of a much greater number of DH progeny. Still, the identification of molecular markers close to the BSMV-resistance gene provides a starting point for the development of a higher resolution map of the centromeric region in the vicinity of this locus. Research leading to the construction of such a map is in progress.

A small genetic distance between two markers in the centromeric region of a barley chromosome may represent a rather large physical distance. However, the number of markers in this region may yet allow the identification of those useful for the positional cloning of the BSMV-resistance gene. The cloning and subsequent characterization of this gene would significantly improve our understanding of the mechanisms of virus resistance in plants. Although barley stripe mosaic can be readily controlled through clean seed programs, the identification of closely linked markers also may facilitate the incorporation of BSMV resistance into barley cultivars via molecular marker-assisted selection. In this regard, a polymerase chain reaction-based marker (sequence-tagged site) has been developed for the RFLP marker *ABC455* (T. K. Blake, personal communication).

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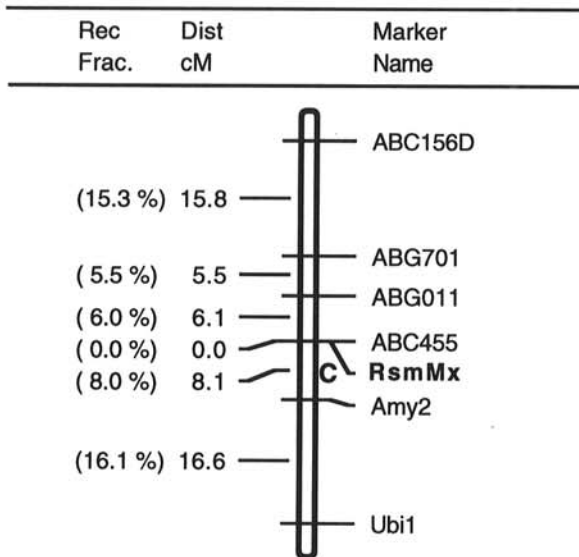


Fig. 2. Linkage map of the centromeric region of barley chromosome 1 in a 52.1-centimorgan (cM) span between markers *ABC156D* and *Ubi1*, showing the location of the barley stripe mosaic virus-resistance locus (*RsmMx*) relative to neighboring markers. The linkage map is based on 150 doubled haploid progeny from the cross Steptoe/Morex. The letter 'C' indicates the approximate location of the centromere. The relative distance between markers is given in centimorgans. Recombination fraction is given as a percentage in parenthesis at the left.

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