A genetic linkage map for hexaploid, cultivated oat (*Avena sativa* L.)
based on an intraspecific cross ‘Ogle/MAM17-5’

**Abstract** Genetic research and breeding of oat (*Avena sativa* L.) would be aided by development of a genetic linkage map for a breeding population. Such a map could be used for localization of qualitative and quantitative trait loci, marker-assisted selection and other genetic analysis in an adapted, agronomically useful background. The objectives of this research were to develop a genetic linkage map of hexaploid cultivated oat, to identify homoeologous relationships of linkage groups, and to compare homologous linkage groups between this map and the previously published hexaploid oat map from the cross ‘Kanota/Ogle’ (KO). A total of 510 markers, including 172 restriction fragment length polymorphisms (RFLP), 324 amplified fragment length polymorphisms (AFLP) and 14 simple sequence repeats (SSR), were assessed on a recombinant inbred population of 152 F5:6 lines derived from the cross, ‘Ogle/MAM17-5’ (OM). Twenty eight linkage groups of 5 cM or longer were formed using 476 of the markers, while 34 markers remained either unlinked or in small fragments less than 5 cM. The 28 linkage groups contained from 3 to 33 markers, and varied in size from 5.2 to 123.0 cM, representing a total map length of 1,396.7 cM. Three putative homoeologous groups (OM7, OM8 and OM18; OM2 and OM23; OM13 and OM16) were identified. Comparison with the published KO map indicated that nine OM linkage groups could be determined to be homologous to linkage groups in the KO map. Further comparison of the homologous linkage groups revealed that residual differences in genomic rearrangements existed between the two hexaploid oat populations. Some linkage groups were significantly extended compared with the KO map. Since the OM mapping population is segregating for a number of agronomically important traits, this genetic map will provide a useful tool for identification of qualitative and quantitative loci for these traits.

**Keywords** *Avena sativa* · Genetic map · Molecular markers · Homology

**Introduction**

Oat (*Avena sativa* L.) is an important cereal crop used for both human consumption and animal feed. Cultivated oat is an allohexaploid with 21 pairs of chromosomes and a basic chromosome number of *x* = 7 (Rajhathy 1963). Genetic study and breeding of oat would be aided by development of a saturated genetic linkage map for an elite breeding population. Such a map could be used for localization of qualitative and quantitative trait loci, map-based gene cloning, marker-assisted selection and other genetic analyses ( Tanksley et al. 1989). Linkage maps have already been developed for several important cereal crop species such as rice (Harushima et al. 1998), wheat (Messmer et al. 1999), maize (Davis et al. 1999) and barley (Heun et al. 1991; Ramsay et al. 2000). To understand and manipulate the oat genome, molecular linkage maps also have been developed for diploid (O’Donoughue et al. 1992; Kremer et al. 2001) and hexaploid (O’Donoughue et al. 1995; Portyanko et al. 2001) oat, primarily by using RFLP and AFLP markers.

The genetic map of ‘Kanota/Ogle’ (KO) (O’Donoughue et al. 1995) is currently the most complete hexaploid oat map. It has been used to detect quantitative trait loci (QTLs) for agronomic traits (Siripoonwiwat et al. 1996), crown rust resistance (Bush and Wise 1996) and vernalization response (Holland et al. 1997) in oat. However, the KO map was based on a wide, interspecific cross and only 71 recombinant inbred lines (O’Donoughue et al.
1995), a population which is too small to be used for accurate QTL mapping. Because a single mapping population can not be segregating for loci controlling all traits, it is necessary to derive different populations to map specific traits. Mapping populations derived from intraspecific crosses made with two breeding parents are particularly useful, because once markers are associated with QTLs or genes controlling agronomically important traits, they could be directly used in practical breeding programs.

The QTL identified for a trait could be validated through comparison of QTLs detected for different populations. Homologous and homoeologous relationships of linkage groups between the maps for the two different populations need to be identified before a comparison of QTLs is made. If one QTL for a trait is detected on a linkage group in a population, and one QTL for the same trait is identified on a homologous or homoeologous linkage group in another population, the reliability for the QTL detected would be greatly increased. Comparison of the linkage maps between two hexaploid mapping populations has been rare in oat, however. In addition, previous comparisons of the linkage maps between two mapping populations were exclusively focused on comparing linkage groups with homoeologous or unknown relationships. Limited synteny was revealed by comparing linkage maps of diploid (A) and hexaploid (A, C and D) oats (O'Donoughue et al. 1995; Kremer et al. 2001; Portyanko et al. 2001). Moreover, the three hexaploid oat genomes were defined as only 'segmentally' homoeologous (Kianian et al. 1997). Low colinearity among homoeologous linkage groups should not be surprising, however, since chromosomes within each genome have probably undergone rearrangement during the process of evolution prior to the three genomes combining at the hexaploid level. High colinearity between two homologous linkage groups for different hexaploid oat populations is expected if chromosome rearrangements are relatively fixed in hexaploid oat populations (Stebbins 1971). Relatively recent genomic rearrangements between two hexaploid oat populations could, therefore, be revealed by comparing homologous linkage groups between the two populations.

The objectives of this study were to develop a linkage map of hexaploid cultivated oat based on 152 F$_{5.6}$ recombinant inbred lines (RILs) derived from a breeding cross 'Ogle/MAM17-5' (OM), to identify homoeologous relationships based on multi-locus polymorphic markers, and to compare homologous linkage groups between this map and the previously published hexaploid oat KO map. The linkage map developed in this study is a useful supplement to, and an improvement on, other hexaploid oat maps, since some linkage groups have been significantly extended compared with the previously published hexaploid oat KO map. In addition, the OM linkage map has recently been used for QTL detection of crown rust resistance, and other important traits, in an agronomically improved genetic background (Zhu and Kaeppler 2002).

Materials and methods

Plant materials

Two hexaploid, cultivated oat (A. sativa L.) genotypes, 'Ogle' (CI9401) and MAM17-5, with contrasting responses to the crown rust pathogen (Puccinia coronata) were used as parents to produce a mapping population. MAM17-5 was selected in the spring oat breeding program at the University of Wisconsin-Madison (Moustafa et al. 1992) and has a complex pedigree. Ogle was developed in the spring oat breeding program at the University of Illinois (Brown and Jedliński 1983). Ogle was also one of the two parents of the KO population, on which a relatively complete RFLP map was developed (O'Donoughue et al. 1994). Ogle differs from MAM17-5 in many agriculturally important traits such as crown rust resistance, plant height, days to heading, barley dwarf virus resistance, groat oil content, groat protein content and other quality traits. The 152 F$_{5.6}$ RILs used in this mapping experiment were derived using the single-seed descent method.

RFLP analysis

The majority of RFLP probes used in this study were selected based on the KO map, such that markers should be located approximately one every 20 cm to uniformly cover the hexaploid oat RFLP map. Additional molecular markers identified in publications as linked to crown rust and stem rust resistance genes or QTLs in oat were also included as probes. A total of 195 RFLP probes were screened; however, only 135 of them showed polymorphism between the parents and were used to characterize the RILs (Table 1). This included 17 markers putatively associated with crown rust and stem rust resistance genes or QTLs in oat (Table 2).

Clones from the BCD, CDO and WG libraries (Heun et al. 1991) were developed at Cornell University, Ithaca, N.Y., and the ISU library (Rayapati et al. 1994) were developed at Iowa State University, Ames, Iowa. These clones were distributed by the Western Regional Research Center, USDA-ARS, Albany, Calif. Clones from the UMN library (Kianian et al. 1997) were kindly provided by Dr. Ronald Phillips, University of Minnesota, St. Paul, Minn. Clones from the OG (Goffreda et al. 1992) and RZ (Causse et al. 1994) libraries were generously provided by Dr. Mark Sorrells and Dr. Susan McCouch, respectively, Cornell University, Ithaca, N.Y. (Table 1).

Leaf tissue samples of the parents, Ogle and MAM17-5, and of the 152 RILs (15 plants per line) were collected from greenhouse-grown plants at Zadors Growth Stage 31 (Zadoks et al. 1974). Samples were lyophilized, and ground through a 1-mm mesh with a cyclone sample mill (UDY Co., Fort Collins, Colo.). A modified hexadecyl trimethylammonium bromide (CTAB) procedure (Saghai-Maroof et al. 1988) was used for DNA extractions. Restriction digests were made of extracted genomic DNA with one of the four enzymes Dral, EcoRI, EcoRV, and HindIII (Promega, Madison, Wis.) using standard restriction digestion procedures recommended by the manufacturer. Twenty microgram samples of restricted DNA were loaded onto 0.8% agarose gels, electrophoresed for approximately 16 h at 35 V, and transferred onto either an Immobilon-S (Millipore Co., Bedford, Mass.) or Hybond-N$^+$ (Amersham Pharmacia, U.K.) nylon membrane with 5 × SSC. Probes were labeled with [32P] by random priming (Feinberg and Vogelstein 1983). Hybridizations were done in capped glass tubes in a roller oven overnight at 65 °C. After hybridization, membranes were first rinsed 2 × 10 min at 65 °C with 2 × SSC and 0.1% SDS in the tubes. The membranes were then washed for 2 × 20 min at 65 °C with 0.2 × SSC and 0.1% SDS in plastic tubs. BioMax films (Eastman Kodak Co., Rochester, N.Y.) were exposed at –80 °C for 2–15 days.