GENETIC AND PHYSIOLOGICAL RELATIONSHIPS BETWEEN OAT GRAIN QUALITY COMPONENTS

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ABSTRACT

The use of oats for human consumption is increasing every day due to the health benefits of oat products. With the objective to study relationships among factors affecting oat grain quality, two Recombinant Inbred Lines (RIL) mapping populations ('ND030299' x 'ND991151' and 'ND030299' x 'Souris') have been used in this study. The two populations with their parents and three check cultivars were evaluated in a square lattice design in 2008 and 2009 at two North Dakota locations. Data were recorded on the following agronomic traits: grain yield, test weight, 1000 kernel weight, thin kernels, heading date, and plant height. Chemical and grain physical analysis were performed for β-glucan, oil, and groat percentage. A total of 4975 SNP markers were assessed on the two populations using a 32-bead chip platform developed by Illumina. QTLs for agronomic and grain physical traits were mapped and characterized in the two populations using Windows QTL Cartographer. Grain yield was positively correlated with test weight, thin kernels, plant height, β-glucan content, and associated negatively with 1000 kernel weight. Thirty linkage groups using 1168 polymorphic markers were formed for population 05021, whereas population 05026 comprised 33 linkage groups using 1024 polymorphic markers. The 30 linkage groups of population 05021 contained from 3 to 62 markers, and varied in size from 15.8 to 225.3 cM for a total map size of 2601.7 cM. The 33 linkage groups of population 05026 comprised from 2 to 42 markers, and varied in size from 2.3 to 143.2 cM for a total map size of 1174.2 cM. Nineteen genomic regions on 14 linkage groups were significantly associated with agronomic and grain chemical traits in the population 05021. Fourteen genomic regions on 12 linkage groups were identified for agronomic traits in the population 05026. The same genomic region on LG 05021-16 was associated with thin kernels, test weight, 1000 kernel weight, and oil content. LG 05026-19 loci, from position 23.7 to 47 cM, had strong effects on

heading date, plant height, and grain yield. The QTLs consistently detected across environments and between the two populations could serve as starting points for marker-assisted selection.

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DISSERTATION ORGANIZATION

The present dissertation contains five chapters including a general introduction (Chapter 1), three body chapters (Chapters 2 – 4), and a general conclusion. Each of the 3 body chapters is a separate manuscript that will be submitted for publication. Chapter 1 includes a general introduction along with a literature review portion and the objectives of the dissertation research. Chapter 2 addresses objective 1 of the dissertation and consists essentially in the evaluation of genotypic and phenotypic relationship among agronomic and grain chemical traits affecting oat grain quality. Chapter 3 covers objective 2 of the dissertation and presents the construction of genetic linkage maps from two spring oat populations followed by the comparison of homologous linkage groups between the two populations and the recently published oat consensus map. Chapter 4 identifies genomic regions associated with genes underlying the expression of agronomic and grain chemical traits in two oat populations by addressing objective 3 of the dissertation. Chapter 5 is the general conclusion and gives a brief outline of the research.

CHAPTER 1

1.1. General introduction

North Dakota is one of the states that leads grain production in the North Central plains region and is a considerable oat (*Avena sativa L.*) producer. During the past decade, North Dakota has been consistently among the five states leading oat production in the United States with acreage of about 176,000 ha. In 2012, the amount of oat produced in North Dakota was 100,000 tonnes (t) and the planted area was 80,937 ha. The average price for oat in 2012 was approximately US \$46.5 kg^{-1} and oat production represents a value of \$US 21.9 million in the global economy of the state (USDA-NASS, 2013).

Oat has many uses worldwide: food industry and animal feed. The use of oats for human consumption is increasing every day due to the health benefits of oat products. Oat products reduce cholesterol and arthrosclerosis, decrease the risk of diabetes, and provide antioxidant protection and supply a number of important nutrients (Schrickel, 1986; Murphy and Hoffman, 1992). Oat is a good source of calcium (0.25 - 0.32% dry weight) (Broadley et al., 2003; Tiwari et al., 2006), iron (300 mg kg^{-1}), magnesium (0.6% DW) and potassium (2% DW) (Peterson et al., 1974) and it is rich in total dietary fiber and high in essential fatty acids. The protein content of oat is high relative to other cereals and of excellent quality for human and monogastric animal nutrition (Casey, 2008).

During the past 4 years in ND, a reduction in the oat production has been observed. For example, the production in 2012 was 60% smaller than 2009 and the estimated yield (2.2 t/ha) was down 0.2 t/ha (USDA, 2013). The principal cause of this decrease could be disease pressure, shifting farm practices, and bad weather conditions including rotation of drought and flood conditions. Oat is subject to a large number of diseases that can cause severe damage to quality

and reduce yield. The most common oat diseases, crown rust caused by *Puccinia coronata* Corda var. *avenae* W.P. Fraser & Ledingham, stem rust caused by *Puccinia graminis* Pers:Pers. f. sp. *avenae* and barley yellow dwarf virus (BYDV) can reduce significantly the grain quality. Milling markets require high quality standards such as high test weight, bright color, high groat percentage, low oil content, high protein and high β-glucan content (Ransom et al., 2007). Development of new oat varieties with enhanced grain quality and resistance to diseases would be desirable for the future of oat production in North Dakota.

Cultivated oats are an allohexaploid species having 21 pairs of chromosomes with basic chromosome number of 7 ($2n = 6x = 42$) (Zhu and Kaeppler, 2003) and a relatively large genome of 11315 Mbp (Pal et al., 2002). Molecular mapping research in oat was initiated in the 1990s with the publication of the first molecular map of hexaploid oat based primarily on restriction fragment length polymorphism (RFLP) markers (O'Donoughue et al., 1995). Other mapping studies based on amplified fragment length polymorphism (AFLP) markers, simple sequence repeat (SSR) markers, and diversity array technology (DArT) markers were also reported (Jin et al., 2000; Portyanko et al., 2001; Zhu and Kaeppler, 2003; Tinker et al., 2009). An important advance, in the study of oat genome, was the recent publication of a linkage map containing 21 linkage groups, anchoring the 21 oat chromosomes, using mainly single nucleotide polymorphism (SNP) markers (Oliver et al., 2013). Nevertheless despite more than 20 years of genomic research, oat has lagged behind and has not kept pace with other small grains such as wheat and barley. As a result, quantitative trait loci (QTL) analysis and marker assisted selection has not been used at a large scale in oat cultivar development.

Today the world population is increasing quickly at a rate ever observed, and by the year 2050 the world population will reach approximately 12 billion people. At such rate of increase,

the world will need to produce more than twice the amount of food we are producing now. As a consequence, the continuous development of genotypes with improved qualities is of great importance (Todorovska et al., 2005). Detection of important QTLs and marker-assisted selection (MAS) are potential tools with the objective to increase efficiency by allowing earlier selection and reducing plant population size used during selection. The presence of tight linkage, less than 10 cM, between an important economic trait and a genetic marker can be useful in MAS to increase gain from selection (Todorovska et al., 2005).

1.2. Objectives

The objectives of this research are:

- Evaluate through a two year multi-environment experiment, genotypic and phenotypic relationships among factors such as grain yield, test weight, 1000 kernel weight, thin kernels, and β-glucan content affecting oat grain quality
- Develop a genetic linkage map in two oat populations using SNP and DArT markers.
- Identify quantitative trait loci (QTLs) associated with agronomic traits in two oat populations.

1.3. Literature review

Oat is an important cereal crop used for both human consumption and animal feed (Zhu and Kaeppler, 2003; Peterson et al., 2005) and has long been recognized as a high-quality food and feed (Rines et al., 2006). The beneficial effects and the positive impact of the total dietary fiber on some of the risk factors of cardiovascular diseases have stimulated great interest in oat (Redaelli et al., 2009). The objective of oat breeders is to improve yield while maintaining good grain quality. Some traits important for growers and millers and related to oat grain quality

include agronomic traits (yield, plant height, and lodging, heading date and pest resistance), grain physical traits (groat and kernel weight, test weight, groat percentage) and grain chemical composition traits (protein, oil, β-glucan) (Peterson et al., 2005). Doehlert et al., 2001 indicated that test weight, groat percentage, groat weight and groat composition were the characteristics most commonly used to describe oat quality. When considering the widespread use of oat in human food and animal feed, modern oat breeders utilize biochemical traits to measure grain quality and thus protein content, oil content, and β-glucan content constitute the major groat compositional characteristics related to oat grain quality (Doehlert et al., 2001). Oat grain quality also is related to grain yield production and conditions that lead to production of high yields generally also lead to improved grain quality.

1.3.1. Physical traits affecting oat grain quality

1.3.1.1. Test weight

Among the physical traits that affect grain quality, test weight has been the traditional way to measure oat grain quality and high test weight in oat generally is associated with high grain quality (Forsberg and Reeves, 1992; Doehlert and McMullen, 2008). Test weight, also known as bulk density, can be defined as the weight of grain that fits into a specific volume (Forsberg and Reeves, 1992) and represents the bulk density of oat grain i.e. the measure of the density of oat grain as they are packed into a given volume (Doehlert et al., 2001). Test weight is a volumetric measure and it is commonly expressed in kg m^{-3} or g L^{-1}. Forsberg and Reeves (1992) reported that the standard test weight of oat grain is 412 kg m^{-3} and modern oat cultivars have test weight values ranging from 463 to 515 kg m^{-3}. Several factors influence oat test weight such as kernel size and shape, groat density, groat percentage, presence of awns and disease (Forsberg and Reeves, 1992; Doehlert et al., 2001). Doehlert et al., 2006 found a negative relationship between

test weight and grain length and a positive relationship between test weight and grain width. They concluded that long kernels with unstable hulls or awns have more space between them and pack less well than shorter kernels. Jianzhong, 2005 concluded that high test weight is dominant over low test weight by crossing two lines, one with low test weight (496 kg m^{-3}) and the other with high test weight (553 kg m^{-3}), with all the progeny exhibiting high test weight ranging from 534 to 577 kg m^{-3} with a mean value of 555 kg m^{-3}.

1.3.1.2 Groat percentage

The groat or caryopsis refers to the kernel after the hulls (lemma and palea) have been removed (Ransom et al., 2007) and is a primary determining factor in grain quality, whether for feed or milling purposes (Rines et al., 2006). The groat percentage, also known as groat proportion and caryopsis percentage, is the measure of the proportion of the whole oat that is recovered as groat after dehulling (Doehlert et al., 2001) and represents the economic yield that a given lot of oat grain can produce (Doehlert et al., 1999). Depending on genotype and growth conditions, groat percentage in oat grain varied from 70 to 75% in non-stress environments, but can be much lower in environments under biotic and abiotic stress (Rines et al., 2006). Some oat cultivars are hulless (naked oat), but grain with the hull present is often preferred for production and processing. The principal reason is that naked oats, due to the soft texture of the kernels, are more susceptible to weathering and discoloration, saprophytic fungal invasion, and harvest damage (Rines et al., 2006). Bartley and Weiss (1951) indicated that groat percentage was highly influenced by environmental effects whereas Doehlert et al., 2001 found that groat percentage was equally affected by environment and genotype. Ronald et al., 1999 and Wesenberg and Shands, 1973 found that groat percentage was a quantitatively inherited trait with a broad sense heritability of 0.36 to 0.92.

1.3.2. Biochemical traits affecting oat grain quality

1.3.2.1. Oil content

Oat kernels contain a high concentration of lipid which is uniformly distributed throughout the grain and a favorable ratio of unsaturated to saturated fatty acids (Forsberg and Reeves, 1992; Casey, 2008). The major content of oil in oat grain is found in the endosperm (Peterson, 1992) and range between 40 and 110 g kg^{-1} (Rines et al., 2006). However, Holland et al., 2001 reported oil content of 180 g kg^{-1} in an experimental line developed by recurrent selection. High oil content is advantageous for animal feeding because of its high content of energy, but in food applications, high oil concentration is deleterious because of its potential to produce enzymatic rancidity reactions that give rise to bitter, grassy or other undesirable flavors (Kianian et al., 1999; Doehlert et al., 2001). Rines et al., 2006 indicated that shelf life of oat products can be severely affected by high oil content with high proportion of unsaturated fatty acids. Youngs et al., 1982 in a survey including studies conducted in various countries reported lipid concentration on oat groat ranging from 20 to 118 g kg^{-1}. Luby and Stuthman, 1983 reported lipid concentration in *A. sativa* ranged from 56 to 76 g kg^{-1}. In a study involving 12 genotypes of oat grown at four different locations in ND, Doehlert et al., 2001 found that groat lipid concentration varied from 46.4 to 78.1 g kg^{-1} with a mean of 62.9 g kg^{-1}.

Youngs and Foster, 1979 and Gullord, 1986 showed that fatty acid composition for oat genotypes is relatively stable over a wide range of environments, which indicated that environment does not affect fatty acid composition. Doehlert et al., 2001 demonstrated also the high stability of lipid concentration in oat by reporting a mean square 10 fold greater for genotype than for environment. Broad sense heritability estimated and reported by Karow and Forsberg, 1984 for the different components of oat oil have been high: 0.63 to 0.91 for palmitic

acid, 0.66 to 0.99 for oleic acid and 0.64 to 0.94 for linoleic acid. Because of the stability of oil in oat kernel and the fact that it is highly heritable, breeders can select for both high and low oil with little confounding from environment.

1.3.2.2. β-glucan content

Oat is particularly high in dietary fiber which can be defined as plant polysaccharides and lignin that are resistant to hydrolysis by human digestive enzymes (Peterson, 1992; Casey, 2008). Depending on genotype and environmental growth conditions, β-glucan content in oat varied from 30 to 60 g kg^{-1} (Rines et al., 2006). However, an experimental line with 71 g kg^{-1} of β-glucan had been reported by Cervantes-Martinez et al., 2001. The dietary fiber of oat is a mixture of soluble and insoluble fractions. The presence of a high concentration of [(1→3), (1→4) -β-D-glucans] known, as β-glucan, in the soluble fraction is high relative to other cereals (Peterson, 1992). β-glucan resides in the cell walls of the subaleurone regions throughout the bran and the endosperm (Bacic and Stone, 1981); it is also present in the inner region of the aleurone cell walls (Peterson, 1992). β-glucan lowers the serum cholesterol levels of blood, balances the glucose and insulin content of serum after meals and reduces the risk of cardiovascular diseases (Anderson and Chen, 1986). On the other hand, feed rations high in β-glucan can be detrimental to the weight gain of animals due to the energy differential in metabolism (Anderson et al., 1978). High β-glucan and low oil content are suitable in the food market whereas low β-glucan and high oil-content are more favorable for the feed industry. Doehlert et al., 2001 found that β-glucan content for genotypes adapted to oat production in ND varied from 43.3 to 57.6 g kg^{-1} with a mean of 50.3 g kg^{-1}. Kianan et al., 2000 evaluated recombinant inbred lines from two populations: Kanota x Ogle and Kanota x Marion. They found that the β-glucan content in the population Kanota x Ogle varied from 37.2 to 57.6 g kg^{-1} with a mean of 45.3 g kg^{-1} while in the

population Kanota x Marion the β-glucan content ranged from 42.8 to 59.6 g kg^{-1} with an average of 50.6 g kg^{-1}. Lee et al., 1997, in a study conducted in ND with 10 oats cultivars grown at two locations, reported values of total β-glucan content ranged from 44.4 to 60.5 g kg^{-1}. Levels of β-glucan in oat groat are influenced by both genetic and environmental factors (Doehlert et al., 2001; Lee et al., 1997). Holthaus et al., 1996 and Kianian et al., 2000 reported that β-glucan content in oat is controlled by multiple genetic loci with primarily additive effects and its broad sense heritability is estimated at 0.55. Kianian et al., 2000 using Restriction Fragment Length Polymorphism (RFLP) analysis in 137 Recombinant Inbred Lines (RIL) developed from the cross Kanota x Ogle showed that regions on linkage groups 3, 6, 11, 13, 14, 17, 20 contributed significantly to the β-glucan content. Kanota contributed three alleles and Ogle contributed four alleles for the loci that had a positive influence on this trait.

1.3.3. Cytogenetics of oats

Oats belong to the family Gramineae (Poaceae) and to the genus *Avena*, which consist of diploid (2n = 2x = 14), tetraploid (2n = 4x = 28) and hexaploid (2n = 6x = 42) species (Rines et al., 2006). The diploid species contain A or C genome (AA or CC), tetraploid species have either AB (AABB) or AC (AACC) genomes, and the hexaploid such as cultivated species *A. sativa* and *A. byzantina* have ACD (AACCDD) genomes (Drossou et al., 2004; Morikawa and Nishihara, 2009). It has been proposed that the evolution of cultivated oats from diploid and tetraploid species occurred in two steps (Li et al., 2000; Nikoloudakis et al., 2008). First, an interspecific hybridization of two diploid species occurred to produce a tetraploid (AACC) after chromosome doubling. After which, this tetraploid species intercrossed with a diploid to form the hexaploid, *A. sativa*, by doubling of the chromosomes. The diploid and tetraploid progenitors of the cultivated oats have been subject of many discussions.

Initially, *A. strigosa* was considered the A genome donor of hexaploids (Rajhathy and Thomas, 1974). However, southern hybridization of a satellite DNA sequence isolated from *A. strigosa* showed dissimilarities among the As and the A/D genome chromosomes (Linares et al., 1998). Li et al. 2000 using satellite DNA sequence reported that Ac genome of *A. canariensis* is the progenitor and A genome donor of hexaploids. Recent molecular study of Nikoloudakis et al., 2008 using restriction fragment length polymorphism (RFLP) analysis to screen 54 accessions containing 35 diploid, 12 tetraploid and seven hexaploid suggested that the diploid *A. longiglumis* is the donor of the A genome. In contrast, Fu and Williams, 2008 found out that *A. maroccana* (AACC) is the tetraploid progenitor of the hexaploid oats, *A. wiestii* is the diploid donor of the A genome, and the diploid *A. eriantha* appears to be the C genome donor. Sequence Characterized Amplified Region (SCAR) markers analysis among 29 accessions done by Nikoloudakis and Katsiotis, 2008 provided molecular evidence that the diploid *A. ventricosa* is the C genome donor. Moreover, they observed major dissimilarities to *A. eriantha* and *A. clauda* species (CpCp) lacking support of their possible participation in the *Avena* allopolyploid formation. A previous report confirmed that *A. ventricosa* is the C genome donor for both tetraploid and hexaploid species (Loskutov, 2001). For the D genome donor, the molecular study of Linares et al., 1998 revealed that some chromosome pairs in the A genome have been identified in the D genome, suggesting that the A and the D genomes are closely related. Leggett, 1996 proposed that the D genome did not exist and could be derived from the A genome. Li et al., 2000 used satellite DNA sequence from a microsatellite library of *A. sativa* to sequence 12 wild oat species. They suggested that the wild hexaploid, *A. fatua*, is the direct ancestor of the cultivated hexaploids, *A. sativa* and *A. byzantina*. Based on complex studies, the

identification of the two basic genomes (A and C) was confirmed, however B and D genomes seemed to be derivatives of the A genome (Loskutov, 2008).

1.3.4. Development of molecular linkage maps in oat

1.3.4.1. Mapping in diploid species

Cultivated oats are allohexaploid species having 21 pairs of chromosomes with basic chromosome number of 7 (2n = 6x = 42) (Zhu and Kaeppler, 2003) with a relatively large genome of 11315 Mbp (Pal et al., 2002). Genomic research in oats began in 1992 by the group of O'Donoughue. In order to reduce the complexity of map construction relative to the hexaploid cultivated oats, and to facilitate the future construction of hexaploid maps, the first linkage maps for the oat genome were constructed using diploid species. O'Donoughue et al., 1992 were able to publish the first molecular map for diploid species based on 44 F_2 families derived from a cross between two nondomesticated species *A. atlantica* x *A. hirtula* using RFLP markers. According to Moore et al., 1995, and Devros and Gale, 2000 this map turned into a pillar in comparative mapping among grasses because the same RFLP markers were mapped in several other grass species. The second diploid map was constructed by Rayapati et al., 1994 using also F_2 families from the domesticated diploid oat *A. strigosa* and nondomesticated *A. wiestii*. That map was corrected and enhanced later by Yu and Wise, 2000 using AFLP markers, and by Kremer et al., 2001 using RFLP markers.

1.3.4.2. Mapping in hexaploid oats

In 1995, O'Donoughue published the first hexaploid map (KO map) based on 71 recombinant inbred lines (RILs) from two parents *A. byzantina* cv 'Kanota' and *A. sativa* cv 'Ogle' using RFLP markers. They reported 38 linkage groups that covered genetic distance of

1482 cM with estimated the complete map size of oats to be 2932 cM (O'Donoughue et al., 1995). The KO map is considered as the primary base map in cultivated oat.

Others researchers such as Jin et al., 2000, and Portyanko et al., 2001, independently worked on the enhancement of the original map using AFLP markers. The original map coverage was expanded from 1482 cM to 2049 cM (Portyanko et al., 2001) and 2351 cM (Jin et al., 2000), respectively. However, some problems were encountered such as the number of linkage groups. The map had 38 linkage groups which represented an excess of 17 on the basis of the expected 21chromosome of hexaploid oats. The failure to assign the 38 linkage groups with the 21 oat chromosomes, despite the use of a complete set of 21 monosomics lines from Kanota (Morikawa, 1975), has been associated to the relative small population (71 RILs) used (Rines et al., 2006). The presence of translocations and other chromosomal rearrangements (Jellen et al., 1993) is another difficulty pointed out in mapping in the KO population. These translocations can cause segregation distortions and make it difficult the ordering of markers within the linkage groups.

In 2003, Zhu and Kaeppler developed a new map using more RILs (152 instead of 71) with combination of RFLP, AFLP and SSR markers to address the problem on linkage groups. They used two hexaploid cultivated genotypes: 'MAM17-5' from the spring oat breeding program of the University of Wisconsin-Madison and 'Ogle' from the oat breeding program of the University of Illinois. These two cultivars showed different characteristics relative to crown rust resistance, plant height, days to heading, barley yellow dwarf virus resistance, groat oil content, and groat protein content. The 15l RILs were produced by the single-seed descent method. They also produced 159 RFLP probes and a combination of 64 AFLP primers by

digesting DNA with four restriction enzymes: DraI, EcoRI, EcoRV, and HindIII. The final map contained 476 marker loci, 28 linkage groups of 5 cM or longer and represented 1396.7 cM.

Tinker et al. 2009 developed a new map using the new Diversity Array Technology (DArT). DArT is based on microarray technology that enables whole-genome profiling even without a need for sequence information (Wenzl et al., 2004). The markers developed are biallelic, which can either be dominant or co-dominant (Kilian et al., 2005; Gupta et al. 2008). DArT array as discussed by Kilian et al. 2005 is an assembly of genomic DNA from a pool of individuals representing the genetic diversity of a species. The DNA mixture is then subjected to complexity reduction to lower the level of repetitive DNA. Complexity reduction involves combination restriction enzymes, adapter ligation, and amplification of adapter-ligated fragments (Wenzl et al., 2004; Kilian et al., 2005). The genomic fragments, known as genomic representation, are amplified and cloned to a vector into *Escherichia coli* to construct a library. Gupta et al. 2008 described that the labeled genomic representations are hybridized. Those polymorphic clones referred as DArT markers are assembled into a genotyping array for routine assay. DArTsoft software is used for analysis of hybridization intensities. Tinker el al., 2009 used a population of 80 recombinant inbred lines (RILs) from a cross between the genotypes 'Kanota' x 'Ogle' (KxO) including most of the 71 RILs from the map developed by O'Donoughue et al. 1995 to develop the map. A clone library was constructed from 60 cultivated and 14 non-cultivated accessions using *PstI/TaqI* method. The molecular mapping was done using 1010 DArT markers and 287 markers from previous map. The final map had 30 linkage groups for 2028 cM.

The discovery of single nucleotide polymorphism (SNP) markers was an important step in genetic studies. SNP markers are the most common type of DNA-based markers, represent the

smallest unit of genetic variation, and can provide a rich source of useful molecular markers (Cho et al., 1999). For example, 90% of the human genome are SNPs (Kwok and Gu, 1999). Tenaillon et al., 2001 reported 1 SNP every 104 base pair (bp) in the maize genome. Among 15 soybean genotypes, Van et al., 2005 found out that SNPs occurred at a frequency of 1 per 2038 bp in 16302 bp of coding sequence, and 1 per 191 bp in 16960 bp of noncoding regions. As a result, SNPs are useful in the construction of high-density genetic maps because they can be analyzed using high-throughput systems (Van et al., 2005).

The most recent map of hexaploid oat which conciliates the number of linkage groups with the number of chromosomes was developed by Oliver et al., 2013 using a new chromosome anchoring strategy and SNP markers. They developed six mapping populations and derived monosomics lines from the cultivars 'Kanota' and 'Sun II' to anchor the linkage groups to the 21 oat chromosomes. The molecular map was constructed using 1054 SNP markers and contained 21 linkage groups, anchored to the 21 chromosomes, with a total length of 1838 cM. Based on an iterative mapping approach to remove problematic loci and multiple crossovers, the total genetic length of the oat genome is estimated to be closer to 2000 cM (Oliver et al., 2013).

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CHAPTER 2. PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF TWO RECOMBINANT INBRED OAT POPULATIONS FOR GRAIN QUALITY

2.1. Abstract

The use of oats for human consumption is increasing every day due to the health benefits of oat products. Oat products reduce cholesterol and arthrosclerosis, decrease the risk of diabetes, and provide antioxidant protection and supply a number of important nutrients. With the objective to study relationships among factors affecting oat grain quality, two $F_{4:6}$ Recombinant Inbred Lines (RIL) mapping populations developed by Single Seed Descent (SSD) have been used in this study. The first population (05021) consisted in 97 RILs derived from the cross 'ND030299 x ND991151' and the second population (05026) with 93 RILs was derived from the cross 'ND030299 x ND961161'. The 190 RILs from the two populations with the three parents and three check cultivars (HIFI, HIFI-9, and YOUNGS) were evaluated in a square lattice design (14x14) with two replicates in 2008 and 2009 at two North Dakota locations. Data were recorded on a plot basis for all experiments on the following agronomic traits: grain yield, test weight, 1000 kernel weight, thin kernels, heading date, and plant height. Chemical analysis and grain physical analysis were performed for β-glucan, oil content, groat percentage and dehulling efficiency. Broad-sense heritability estimates combined across environments were calculated for each trait. Using covariance parameters, genotypic and phenotypic correlations between traits were also estimated. After looking for normality and homogeneity of variance, a combined ANOVA was performed, using the MIXED procedure from SAS, where environments were considered random and genotypes were considered fixed. Combined across years, grain yield varied from 942 kg/ha to 7744 kg/ha (population 05021), and from 824 kg/ha to 7741 kg/ha

(population 05026). Test weight ranged from 398 kg/m^3 to 514 kg/m^3 for genotypes of the population 05021, and from 443 kg/m^3 to 524 kg/m^3 for genotypes of the population 05026. In general, plants were taller in population 05021 ranging from 93 to 125 cm than in population 05026 (89 to 111 cm). Population 05026 had more thin kernels (11%) than population 05021 (7%). Most of the variance observed in both populations was associated with the genotype main effect. Grain yield was positively correlated with test weight, thin kernels, plant height, β-glucan content, and associated negatively with 1000 kernel weight. The wide variation observed is an indication of the capacity to improve both populations with respect to grain quality.

2.2. Introduction

Oat is an important cereal crop and has value either as food for humans and feed for livestock (Zhu and Kaeppler, 2003; Peterson et al., 2005; Ozbas et al., 2009). The quality of oat grains can be, in many aspects, superior to that of other cereals. The beneficial effects and the positive impacts of the total dietary fiber on some of the risk factors of cardiovascular diseases have stimulated great interest in oat (Redaelli et al., 2009). The improvement of oat grain quality for human consumption and animal feed has been a primary objective for oat breeders. Important oat agronomic traits include grain yield, plant height, resistance to lodging, heading date; of grain physical traits, the most important are test weight, 1000 kernel weight, groat percentage; and grain chemical composition traits: protein, oil, β-glucan (Peterson et al., 2005). Test weight is an index of grain quality for farmers and millers, and high test weight is generally associated with high grain quality (Forsberg and Reeves, 1992; Klein et al., 1993; Doehlert and McMullen, 2008). Frey and Wiggans, 1956 reported that high test weight is related with high percentage of germination, good seedling stand, and high milling yield. Standard test weight for modern oat cultivars with values ranging between 463 to 515 kg/m^3 has been reported by Forsberg and

Reeves, 1992. Test weight can be influenced by several factors including kernel size and shape, groat density, groat percentage, presence of awns and diseases (Doehlert et al., 2001). Groat percentage, also known as groat proportion and caryopsis percentage, represents the economic yield that a given lot of oat grain can produce (Doehlert et al., 1999). One of the major objectives of oat breeders is the reduction of plant height in order to minimize substantially lodging problems that affect grain quality and quantity (Milach et al., 1997). High oil content is advantageous for animal feeding due of its high content of energy, but in food applications high oil content is deleterious because of its potential to produce enzymatic rancidity reactions that give rise to bitter, grassy or other undesirable flavors (Kianian et al., 1999; Doehlert et al., 2001). The presence of a high concentration of β-glucan in the cell walls of the groat (Peterson, 1992) lowers the serum cholesterol levels of blood, balances the glucose and insulin content of serum after meals and reduces the risk of cardiovascular diseases (Anderson and Chen, 1986). Genotypic and phenotypic correlations had been reported between test weight and groat percentage (Doehlert at al., 2004; Peterson et al., 2005), oil and groat percentage (Doehlert et al., 2001; Lyrene and Shands, 1975), yield and test weight (Holland and Munkvold, 2001), heading date and test weight (Pixley and Frey, 1992), and 1000 kernel weight and test weight (Holland and Munkvold, 2001). Genotypic and phenotypic correlations among traits can help in the improvement of selection (direct or indirect), and in the calculation of multiple trait selection indices (Falconer and Mackay, 1996). The objective of this chapter is to evaluate, through a two-year multi-environment field experiment, genotypic and phenotypic relationships among factors such as grain yield, test weight, kernel weight, heading date, plant height, thin kernels, oil, and β-glucan affecting oat grain quality.

2.3. Materials and methods

Two $F_{4:6}$ Recombinant Inbred Lines (RIL) mapping populations developed by Single Seed Descent (SSD) have been used in this study. The first population (05021) consisted in 97 RILs derived from the cross 'ND030299' x 'ND991151' and the second population (05026) with 93 RILs is derived from the cross 'ND030299' x 'Souris'. The two populations had a common parent (ND030299) with the objective to provide a degree of biological replication. 'ND030299' and 'ND991151" are two experimental lines from the oat breeding program of North Dakota State University (NDSU) with high content of β-glucan (6%) and low content of β-glucan (4.3%), respectively. 'Souris' is an oat line released in 2006 by the oat breeding program of NDSU with intermediate content of β-glucan (5.2%). Souris is stable for stem rust resistance and crown rust resistance because it possesses the crown rust resistance gene *Pc-91* and the stem rust race *NA27* resistance conferred by gene *Pg-13*. 'ND030299' was derived from a cross between 'HIFI' and 'IAN979-5-1-22'. The pedigree of 'HIFI' included 'ND90141' and 'ND900118'. 'ND90141' was derived from 'R801441', a synthetic hexaploid derived from an *A. magna*/*A. longiglumis* hybrid, and 'RL3038'; a breeding line received from R. McKenzie (Winnipeg, Canada) and possessed genes *Pc-38*, *Pc-39*, *Pg-2*, and *Pg-13*. 'Souris' is a 'HIFI' sister selection. 'ND991151' was derived from a cross between 'Youngs' and 'ND931318' (McMullen, communication personal).

The 190 RILs from the two populations with the three parents and three check cultivars (HIFI, HIFI-9, and YOUNGS) were evaluated in a square lattice design (14x14) with two replicates in 2008 and 2009 at two North Dakota locations: Fargo and Casselton. The randomization within blocks was done by population with the objective that each population stays together in the field. Fargo is located at $46^{o}52'$ latitude north, and $96^{o}54'$ longitude west

with an elevation of 275 m above sea level. The average precipitation during the growing season (May to October) is 348 mm and the mean air temperature is 17.9^{o}C. The soil type where the experiments have been conducted is a silty clay (fine smectitic frigid Typic Epiaquert). Casselton is located at a latitude of 46°55' N and a longitude of 97°13' W. The average precipitation in the growing season is 364 mm and the mean temperature is 17.6^{o}C. The soil type is a silty clay loam (fine silty mixed frigid Typic Haploquolls). In 2008, experiments at Fargo and at Casselton were planted on April 22 and April 29, respectively whereas in 2009 experiments were planted on May 11 and May 20, respectively.

A seeding rate of 2.47 x 10^{6} kernels/ha was used for all experiments. Experimental units consisted of four rows spaced 0.3 m apart and 2.4 m long. Data were recorded on a plot basis for all experiments on the following agronomic traits: grain yield (kg/ha), test weight (kg/m^{3}), 1000 kernel weight (g), thin kernels (%), heading date (days after 31st may), and plant height (cm). Heading date, corresponding to the days after May 31st on which the first nodes on 50% of the plants in the plot had emerged completely above the flag leaf, and plant height at maturity, from ground level to the tips of the panicles, were measured on each plot. The two center rows in each plot were harvested with a two-row binder and the plants were bundled together and dried at ambient temperature for 1 week, after which the plants were threshed with a plot thresher and grain yield was measured. Test weight was calculated by weighing a known volume of whole oat and converting to a Winchester bushel. Thin kernels also known as shrunken or damaged kernels are the weight of all matter that passes through a 0.064" x 3/8' oblong-hole sieve after sieving. Percentage of thin kernels was calculated as follows for each plot:

$$Thin\ kernels\ (\%) = \frac{thin\ kernels\ (kg/ha)x100}{\text{Grain yield}\ (kg/ha)}$$

After removing foreign material and broken kernels, 300 kernels were counted and weighted to obtain 1000 kernel weight as follow (measured only in 2008):

$$1000\ kernel\ weight = \frac{300\ kernels\ weight\ (g) x 1000}{300}$$

Chemical analysis and grain physical analysis were performed for each plot, only in 2008 planting season, for β-glucan content, oil content, groat percentage, and dehulling efficiency. Before performing chemical analysis the oat samples were treated in a vegetable steamer for 20 minutes in order to inactivate enzymes. After, the sample was dehulled with a Codema Laboratory Oat Huller (Codema Inc., Eden Prairie, MN) and milled in a Retsch Model ZM-1 Centrifugal Mill with a 0.5 mm collar screen (Brinkmann Instruments, West-Bury, NY). The milled samples were dried in a convection oven at 130°C for 2h to eliminate interference of the water with the oil signal. The oil analysis was performed using an Oxford 4000 NMR (Abingdon, England). β-glucan concentration was determined by the method of McCleary and Glennie-Holmes, 1985. Groat percentage and dehulling efficiency were assessed using the impact dehuller (Ganssmann and Vorwerck, 1995).

Broad-sense heritability estimates were calculated for the agronomic traits as:

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2/_e + \sigma_e^2/_{er}}$$

Where, h^2 = heritability, σ_G^2 = Genotypic variance, σ_{GE}^2 = Genotype x environment interaction, σ_e^2 = error variance, e= number of environments, and r = replications.

Using genotypic variances and covariance parameters estimates from SAS PROC MIXED, genotypic and phenotypic correlations between traits were estimated as:

$$r_{gij} = \frac{\sigma_{Gij}}{\sigma_{Gi}\sigma_{Gj}}$$

$$r_{pij} = \frac{\sigma_{Pij}}{\sigma_{Pi}\sigma_{Pj}}$$

Where, r_{gij} = genotypic correlation between traits i and j, σ_{Gij} = genotypic covariance between traits i and j, σ_{Gi} = genotypic standard deviation for trait i, σ_{Gj} = genotypic standard deviation for trait j.

r_{pij} = phenotypic correlation between traits i and j, σ_{Pij} = phenotypic covariance between traits i and j, σ_{Pi} = phenotypic standard deviation for trait i, σ_{Pj} = phenotypic standard deviation for trait j.

Genotypic and phenotypic coefficient of variation were calculated according to Singh and Chaudhary, 1977:

$$GCV = \frac{\sqrt{\sigma_G^2}}{\bar{X}} x100$$

$$PCV = \frac{\sqrt{\sigma_P^2}}{\bar{X}} x100$$

Where, GCV=genotypic coefficient of variation, PCV=phenotypic coefficient of variation, σ^2G=genotypic variance, σ^2P=phenotypic variance, $\bar{X}$ = general mean

Transgressive segregation was also estimated for all traits. According to Vega and Frey, 1980 a transgressive genotype had to exceed the parental mean by one "least significant difference (LSD)". Due to the lack of repetitions, Pearson correlations were calculated among biochemical traits, and between biochemical and agronomic traits.

Each environment (a combination of year and location) was analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC, 2009). Normality of distribution of the traits analyzed was estimated using Shapiro-Wilk test. Homogeneity of variances among environments

was determined by the ratio of the largest to the smallest variances followed by Levene's test and Welch's ANOVA. Except for thin kernels, variances were found to be normal and homogeneous for all variables. In order to perform analysis of variance (ANOVA), the variable thin kernels was log transformed to normalize the variance. After looking for normality and homogeneity of variance, a combined ANOVA by year for all environments was performed, using the MIXED procedure from SAS (SAS Institute, Cary, NC, 2009), where environments were considered random and genotypes were considered fixed.

For the purposes of estimating variance and covariance components, heritability, genotypic and phenotypic correlations, parental and check cultivars were removed from the data set. Variance and covariance parameters were obtained using the COVTEST and ASYCOV options from PROC MIXED of SAS, assuming environments and genotypes to be random. Heritability estimates, genotypic and phenotypic correlations were calculated by multivariate restricted maximum likelihood (REML) implemented in PROC MIXED of SAS as described by Holland et al., 2003 and Holland, 2006. Approximate standard errors for heritability, genotypic and phenotypic correlations were estimated using the delta method (Lynch and Walsh, 1997). Matrix computations necessary to estimate the standard errors were calculated using SAS PROC IML.

2.4. Results

Analysis of variance showed significant ($p<0.01$) genotype-by-environment interaction for all traits except for 1000 kernel weight. Genotype main effect was highly significant ($p<0.01$) for all traits. Environment main effect was significant for all traits ($p<0.05$) but not for grain yield (Table 2.1). 'ND991151' and 'ND030299' showed statistically the same grain yield. 'ND991151' had higher test weight, 1000 kernel weight, plant height, and less percentage of thin

kernels than 'ND030299'. 'ND030299' headed later than 'ND991151' (Table 2.3). For population 05026, 'Souris' yielded significantly higher than 'ND030299'. Both parents showed similar 1000 kernel weight, test weight, plant height, and percentage of broken kernels. 'ND030299' headed later than 'Souris' (Table 2.3).

Combined across years and locations, grain yield varied from 942 kg/ha to 7744 kg/ha with an average of 6144 kg/ha (population 05021), and from 824 kg/ha to 7741 kg/ha with an average of 6664 kg/ha (population 05026). Test weight ranged from 398 kg/m^3 to 514 kg/m^3 (average 488 kg/m^3) for genotypes of the population 05021, and from 443 kg/m^3 to 524 kg/m^3 for genotypes of the population 05026 (average 495 kg/m^3). For population 05021, 1000 kernel weight averaged 32 g and ranged from 23 to 40 g, whereas for population 05026, 1000 kernel weight ranged from 25 to 40 g (average 30 g). Heading date ranged from 31 to 41 days after 31st May (population 05021) and from 33 to 45 days after 31st May (population 05026). In general, plant height was taller in population 05021, ranging from 93 to 125 cm (average 109 cm), than in population 05026, ranging from 89 to 111 cm (average 103 cm). Population 05026 had greater percentage of thins kernels, average 11%, than population 05021, average 7% (Table 2.3).

Transgressive genotypes at least in one direction were observed for all traits. In the population 05021, more transgressive segregates were observed for low grain yield, only one genotype yielded significantly more than the high-yielding parent. Transgressive segregation for low 1000 kernel weight, low test weight, and high percentage of thins kernels was observed. Transgressive segregation for heading date occurred more for lateness than for earliness. For plant height, transgressive segregates for tall and short plants occurred at the same frequency (Table 2.3). For the population 05026, no transgressive segregates were observed for high grain yield. Transgressive segregation for high 1000 kernel weight, short plants, high test weight, early

heading, and high percentage of thin kernels were observed (Table 2.3). In conclusion, more transgressive segregants were observed for high 1000 kernel weight, high test weight, earliness, and tall plants in the population 05026. At the other end, more transgressive segregants were observed for high grain yield in the population 05021.

Most of the variance observed in both populations was associated with the genotype main effect, except for heading date that had a large variance associated with the environment main effect due mainly to the difference in planting date between the two locations. The variance associated with the genotype-by-environment interaction was less than 10% of the total variance for all traits (Table 2.2). The variances associated with environment main effect and genotype-by-environment interaction were negligible for 1000 kernel weight in the population 05021 (Table 2.2).

Broad-sense heritability estimates were high for all traits, ranging from 0.93 for grain yield to 0.98 for heading date and percentage of thin kernels. Generally, heritability estimates were slightly higher in population 05021 than in population 05026 for grain yield, test weight, plant height, and percentage of broken kernels. Heritability estimates were statistically similar for heading date for both populations (Table 2.5).

Phenotypic and genotypic coefficients of variation (GCV and PCV) were similar for both populations. For population 05021, GCV and PCV ranged from 3.5% for test weight to 39.5 and 40%, respectively for broken kernels, whereas for population 05026, GCV and PCV ranged from 2.9 and 3.0% for test weight to 20.0 and 20.5% for thin kernels. The highest GCV and PCV were observed for thin kernels followed by grain yield, heading date, plant height, and finally test weight (Table 2.2).

Table 2.1. Combined Analysis of Variance (ANOVA) for agronomic traits of two oat populations grown at two ND locations (Fargo, Casselton) and two years (2008, 2009).

	Grain yield			1000 kernel weight			Test weight			Heading date			Height			Thin kernels		
SOV	Df	MS		Df	MS		Df	MS		Df	MS		Df	MS		Df	MS	
Environment	3	10,482,253	ns	1	128.5	*	3	35,460.0	*	3	10,314.0	*	3	10,793.0	*	3	29.306	**
Rep(environment)	4	9,066,216		2	2.3		4	3,319.7		4	10.1		4	854.2		4	0.947	
Block(rep*environment)	104	1,454,209		52	9.6		104	163.6		104	1.6		104	77.4		104	0.252	
Genotypes	198	3,569,407	**	194	31.3	**	196	1,708.4	**	198	45.4	**	198	313.5	**	198	3.071	**
Genotypes*environment	580	313,646	**	193	1.6	ns	577	111.0	**	582	1.4	**	581	15.5	**	576	0.094	**
Error	644	168,514		327	1.4		653	60.4		671	0.7		659	9.8		651	0.038	

Df=degrees of freedom MS=mean square *significant at 5% **significant at 1%

Table 2.2. Estimates of variance component for two oat populations grown at two ND locations (Fargo, Casselton) and two years (2008, 2009).

Covariance parameter	Grain yield	1000 kernel weight	Test weight	Heading date	Heig ht	Broken kernels
			Population 05021			
Environment	20,011	0.000	122.3	27.11	35.02	0.072
Rep(environment)	35,136	0.142	25.4	0.12	3.53	0.003
Block(rep*env)	215,764	1.224	9.9	0.17	12.28	0.023
Genotypes	795,093	11.455	285.8	5.26	45.96	0.559
Genotypes*env	102,278	0.000	18.1	0.36	4.14	0.035
Error	172,198	1.562	66.4	0.73	8.70	0.044
CV	6.8	4.0	1.7	2.2	2.7	11.1
GCV	14.5	10.7	3.5	5.8	6.2	39.5
PCV	14.9	10.9	3.5	5.9	6.4	40.0
			Population 05026			
Environment	0	0.950	51.3	26.38	17.82	0.078
Rep(environment)	48,616	0.339	10.1	0.01	4.52	0.020
Block(rep*env)	138,426	0.188	9.2	0.04	4.58	0.011
Genotypes	451,084	4.275	207.7	6.98	26.71	0.224
Genotypes*env	63,255	0.324	30.3	0.36	2.77	0.024
Error	159,417	1.113	58.0	0.63	10.25	0.033
CV	6.0	3.5	1.5	2.0	3.1	7.7
GCV	10.1	6.9	2.9	6.8	5.0	20.0
PCV	10.5	7.2	3.0	6.9	5.2	20.5
			Combined			
Environment	3,272	0.300	83.9	26.75	26.18	0.075
Rep(environment)	32,469	0.000	16.0	0.04	3.47	0.002
Block(rep*env)	192,662	1.228	13.6	0.15	10.01	0.029
Genotypes	689,849	8.656	248.4	6.19	42.79	0.449
Genotypes*env	82,445	0.148	26.4	0.37	3.32	0.030
Error	166,403	1.324	62.3	0.67	9.56	0.038
CV	6.4	3.7	1.6	2.1	2.9	9.2
GCV	13.0	9.6	3.2	6.4	6.2	31.6
PCV	13.4	9.8	3.3	6.5	6.3	32.0

CV=coefficient of variation, GCV=genotypic coefficient of variation, PCV=phenotypic coefficient of variation

The association among agronomic traits is shown by the genotypic and phenotypic correlations (Table 2.5). Genotypic and phenotypic correlation coefficients were very similar for both populations. Grain yield was genotypically and phenotypically correlated positively with

test weight and thin kernels, and associated negatively with 1000 kernel weight. A significant positive phenotypic correlation was observed between grain yield and plant height, but genotypically that correlation was not significant. A highly significant negative relationship was observed between 1000 kernel weight and thin kernels. Test weight was negatively correlated to heading date.

Relationships between biochemical, grain physical and agronomic traits are shown in Table 2.6 and Table 2.7. Groat percentage was positively correlated ($r=0.57$, $p<0.01$) to dehulling efficiency. Dehulling efficiency was negatively correlated ($r=-0.20$, $p<0.05$) with oil content, and a positive correlation ($r=0.38$, $p<0.01$) was observed between oil and β-glucan content. Grain yield was positively correlated to dehulling efficiency ($r=0.40$, $p<0.01$), β-glucan content ($r=0.23$, $p<0.05$), and lodging ($r=0.25$, $p<0.05$). Percentage of thin kernels was correlated positively with β-glucan ($r=0.21$, $p<0.05$) and oil content ($r=0.38$, $p<0.01$), whereas 1000 kernel weight was negatively correlated with both as expected. A positive relationship was observed between test weight and groat percentage ($r=0.26$, $p<0.01$), and dehulling efficiency ($r=0.40$, $p<0.01$). Heading date was negatively correlated with groat percentage ($r=-0.38$, $p<0.01$), and plant height positively correlated to lodging ($r=0.26$, $p<0.01$) as expected.

2.5. Discussion

2.5.1. ANOVA, means and components of variance

The two populations used in this study consisted of genotypes that are adapted to the conditions of eastern North Dakota, which are characterized by warm and dry summers. For all the traits studied, genotype main effect was highly significant and showed considerable amount of variation (Table 2.1).

Table 2.3. Overall means and descriptive statistics of agronomic traits for oat population 05021 (ND991151 x ND030299) and population 05026 (Souris x ND030299) grown at two locations (Fargo, Casselton) and two years (2008, 2009).

Population	Parents		Population statistics				Transgressive segregation			
	ND991151 Souris	ND030299	Mean	SD	Min	Max	$X<P_1-SD$		$X>P_2+SD$	
							%	n	%	n
				Grain yield (kg/ha)						
05021	5998.1 a	6476.4 a	6143.6	953.0	941.9	7743.8	7.2	7	1.0	1
05026	7439.4 a	6476.4 b	6664.1	740.2	823.8	7741.4	2.2	2	0.0	0
				1000 kernel weight (g)						
05021	37.4 a	30.1 b	31.6	3.5	23.0	39.6	7.2	7	0.0	0
05026	29.4 a	30.1 a	30.0	2.3	24.6	40.0	4.3	4	14.0	13
				Test weight (kg/m^3)						
05021	521.2 a	488.0 b	488.2	17.8	398.5	514.1	13.4	13	0.0	0
05026	500.3 a	488.0 a	495.0	14.7	442.5	523.9	5.4	5	7.6	7
				Heading date (June)						
05021	37 b	41 a	39.0	2.3	33.7	44.5	1.0	1	6.2	6
05026	37 b	41 a	38.8	2.7	33.4	45.2	1.1	1	2.2	2
				Height (cm)						
05021	116.8 a	104.2 b	108.7	7.0	93.0	125.0	4.1	4	2.1	2
05026	99.7 a	104.2 a	103.3	5.4	89.3	110.7	6.5	6	11.9	11
				Broken kernels (%)						
05021	2.1 b	8.3 a	6.6	0.77	1.4	55.4	0.0	0	12.4	12
05026	8.8 a	8.3 a	10.6	0.48	3.4	33.9	7.6	7	29.2	27

Table 2.4. Genotypic and phenotypic correlations of two oat populations combined across two ND locations (Fargo, Casselton) and two years (2008, 2009) – genotypic correlation in the upper diagonal, phenotypic correlation in the lower diagonal, standard errors between parenthesis.

	Grain yield	Kernel weight	Test weight	Heading date	Height	Broken kernels
			Population 05021			
Grain yield	-	0.01 (0.11)	0.47 (0.10)	0.04 (0.11)	0.24 (0.11)	0.33 (0.10)
Kernel weight	0.00 (0.08)	-	0.16 (0.11)	-0.18 (0.10)	0.07 (0.11)	-0.84 (0.03)
Test weight	0.38 (0.07)	0.23 (0.08)	-	-0.34 (0.10)	0.07 (0.11)	-0.04 (0.11)
Heading date	0.05 (0.08)	-0.15 (0.09)	-0.30 (0.07)	-	-0.01 (0.11)	0.10 (0.10)
Height	0.26 (0.07)	0.01 (0.08)	0.05 (0.08)	0.04 (0.08)	-	-0.05 (0.11)
Broken kernels	0.19 (0.08)	-0.79 (0.03)	-0.11 (0.08)	0.10 (0.09)	-0.03 (0.08)	-
			Population 05026			
Grain yield	-	-0.30 (0.11)	0.07 (0.19)	0.08 (0.11)	0.22 (0.11)	0.22 (0.11)
Kernel weight	-0.14 (0.07)	-	0.20 (0.12)	-0.09 (0.11)	0.14 (0.12)	-0.76 (0.05)
Test weight	0.06 (0.12)	0.23 (0.08)	-	-0.45 (0.09)	-0.11 (0.11)	-0.26 (0.11)
Heading date	0.06 (0.08)	-0.10 (0.09)	-0.36 (0.07)	-	0.11 (0.11)	0.03 (0.11)
Height	0.27 (0.07)	0.11 (0.08)	-0.05 (0.07)	0.11 (0.08)	-	0.07 (0.11)
Broken kernels	0.12 (0.08)	-0.68 (0.04)	-0.26 (0.07)	0.04 (0.09)	0.02 (0.08)	-
			Combined across populations			
Grain yield	-	-0.17 (0.08)	0.43 (0.08)	-0.01(0.08)	0.10 (0.08)	0.37 (0.07)
Kernel weight	-0.14 (0.05)	-	0.09 (0.08)	-0.09 (0.08)	0.20 (0.08)	-0.84 (0.02)
Test weight	0.33 (0.06)	0.17 (0.06)	-	-0.42 (0.06)	-0.04 (0.08)	0.03 (0.08)
Heading date	-0.01 (0.08)	-0.10 (0.06)	-0.34 (0.05)	-	0.07 (0.07)	0.02 (0.07)
Height	0.19 (0.05)	0.08 (0.06)	-0.02 (0.05)	0.09 (0.06)	-	-0.15 (0.07)
Broken kernels	0.23 (0.05)	-0.77 (0.02)	-0.10 (0.06)	0.03 (0.06)	-0.12 (0.06)	-

Cells in color mean significant at 5% and 1%

Table 2.5. Broad-sense heritability estimates and standard error of two oat populations combined across 2 ND locations (Fargo, Casselton) and 2 years (2008, 2009).

Traits	Population 05021		05026		Combined	
	H^2	SE	H^2	SE	H^2	SE
Grain yield (kg/ha)	0.94	0.01	0.93	0.01	0.94	0.01
Test weight (kg/m^3)	0.96	0.01	0.93	0.01	0.95	0.02
Heading date (June)	0.97	0.01	0.98	0.00	0.97	0.00
Height (cm)	0.96	0.01	0.93	0.01	0.95	0.01
Broken kernels (%)	0.98	0.00	0.96	0.01	0.97	0.00

H^2=heritability estimate SE=standard error

Table 2.6. Pearson correlations between biochemical traits and grain physical traits for oat population 05021 grown in 2008.

Traits	Groat %	Dehulling efficiency	β-glucan	Oil	Lodge
Groat		0.57**	0.007	-0.13	-0.003
Dehulling			-0.06	-0.20*	0.18
β-glucan				0.38**	0.10
Oil					-0.09

*significant at 5% **significant at 1%

Table 2.7. Pearson correlations between biochemical, agronomic, and grain physical traits for oat population 05021 grown in 2008.

Traits	Groat %	Dehulling efficiency	β-glucan	Oil	Lodge
Grain yield	0.17	0.40**	0.23*	0.04	0.25*
Broken kernels	0.07	0.003	0.21*	0.38**	0.02
Kernel weight	0.06	0.12	-0.22*	-0.35**	-0.05
Test weight	0.26**	0.40**	-0.07	-0.13	0.16
Heading	-0.38**	-0.12	0.08	0.12	-0.06
Height	-0.17	-0.04	-0.14	-0.05	0.26**

*significant at 5% **significant at 1%

Such wide variation is an indication of the capacity to improve both populations with respect to grain quality. Genotype x environment interaction was also observed for all traits measured, except 1000 kernel weight, although the magnitude of the interactions mean squares were relatively small in comparison to environment main effect and genotype main effect. The most likely important factor contributing to the significant G x E interaction for grain yield, test

weight, height, and thin kernels can be attributed to the differences in weather conditions especially rainfall. For the 2008 growing season, a total of 671.2 mm of rainfall was reported, whereas during the 2009 growing season only 392.3 mm of rainfall was registered. Similar behavior was reported by Doehlert et al., 2001 for grain yield, and test weight, only the factor contributing to the significant GxE interaction was due to the differential level of crown rust infection among the cultivars. The absence of significant genotype-by-environment interaction for 1000 kernel weight suggests that this trait will remain constant over a range of environmental conditions, and selection for high 1000 kernel weight should be practical.

The magnitude of variance components in both populations suggested that all the traits evaluated were more strongly influenced by genotype than environment. Doehlert et al., 2001 reported that grain yield was more influenced by environment, whereas test weight was equally affected by environment and genotype.

Because the highest yielding parents (Souris and ND030299), with also high percentage of thin kernels were in the population 05026, the genotypes in that population had, on average, higher grain yield, higher test weight, and higher percentage of thin kernels than the genotypes in the population 05021. For the other traits, the genotypes in the population 05021 had higher 1000 kernel weight and plant height than the genotypes in the population 05026. Heading date was similar for genotypes in both populations (Table 2.3 and Table 2.4).

Lower yield than those reported in this experiment, ranging from 3140 kg/ha to 4110 kg/ha (average 3590 kg/ha) for 12 oat cultivars, and from 1510 kg/ha to 2750 kg/ha (average 2010 kg/ha) was reported by Doehlert et al., 2001, and Holland and Munkvold, 2001. In a study conducted at 3 locations in Idaho during 3 consecutive years, Peterson et al., 2005 reported grain yield ranging from 2900 to 8660 kg/ha with an average of 5264 kg/ha.

Values of test weight reported for oat cultivars adapted to the conditions of North Dakota (ND) are very similar to those found in this study. Doehlert et al., 2001 found that the test weight of 12 genotypes grown at four locations in ND varied from 433 to 601 kg m^{-3} with a mean value of 490 kg m^{-3}. In a study conducted at five locations in ND over 3 years and 10 oat cultivars, Doehlert et al., 2004 reported test weight across genotypes ranging from 418 to 517 kg m^{-3} with a mean value of 467 kg m^{-3}. Doehlert et al., 2006, analyzed oat grain and groat size from 10 genotypes grown in 10 environments in ND and reported test weight values ranging from 413 to 529 kg m^{-3} with a mean value of 468 kg m^{-3}. In another study, Doehlert and McMullen, 2008 reported values of test weight, from six oat cultivars grown at three locations in ND, ranging from 513 to 556 kg m^{-3}. Higher test weight ranging from 517 to 585 kg/m^3 was reported by Peterson et al., 2005. Lower test weight from 379 kg/m^3 to 453 kg/m^3 was reported by Holland and Munkvold, 2001. Redaelli et al., 2008 reported values for test weight grown in Italy ranging from 417 to 468 kg/m^3.

Values of 1000 kernel weight between 20 and 29 g (average 24 g) were reported by Holland and Munkvold, 2001. Nersting et al., 2006 analyzing Nordic oat material comprising landraces and recent cultivars reported 1000 kernel weight for landraces ranging from 19 to 41 g (average 32 g), and for recent cultivars ranging from 24 to 46 g (average 34 g). Redaelli et al., 2008 found out that 1000 kernel weight for oat cultivated in Italy varied from 30 g to 35 g with an average of 30 g.

A good numbers of RILs, in the population 05026, were identified with superior performance for test weight and plant height that headed early with low percentage of thin kernels. Those transgressive genotypes suggested that those traits are quantitative in nature and

the favorable additive alleles are brought by both parents as indicated by Ayele, 2011 and Vega and Frey, 1980.

2.5.2. Genotypic and phenotypic correlations among traits

According to Falconer and Mackay, 1996 genotypic correlation among traits is important in the determination of direction and magnitude of response to selection, and the relative efficiency of indirect selection. Genotypic correlation is also a key factor in the calculation of multiple trait selection indices. The absence of strong correlations among traits, except 1000 kernel weight and thin kernels, indicated that improvement of grain yield, test weight, heading date, and plant height should not be difficult in the two populations under study. Grain yield was positively correlated with test weight, plant height, β-glucan content, and dehulling efficiency. This suggests that when quality traits are improved higher yields can be achieved.

Many of the correlations found on this study had been reported previously. Doehlert et al., 2004 ($r=0.723$), Peterson et al., 2005 ($r=0.552$) and Doehlert and McMullen, 2008 ($r=0.694$) found that test weight was positively correlated with groat percentage. Forsberg and Reeves, 1992 concluded that high test weight and high groat percentage generally are associated with well-filled kernels. Peterson et al., 2005 reported a negative relationship between oil and kernel weight ($r=-0.38$, $p<0.05$). A negative correlation between oil and groat percentage was reported by Peterson et al., 2005 ($r=-0.39$, $p<0.05$), and Doehlert et al., 2001 ($r=-0.59$, $p<0.01$). We also found a negative correlation between oil and groat percentage but that correlation was not significant. Negative correlation between groat percentage and groat oil content ($r=-0.387$) has been reported by Lyrene and Shands, 1975 and Peterson et al., 2005 respectively.

Holland and Munkvold, 2001 reported a positive phenotypic and genotypic correlation between grain yield and test weight ($r_G=0.58\pm0.10$, $r_P=0.47\pm0.08$), and between 1000 kernel

weight and test weight (r_G=0.38±0.09, r_P=0.34±0.09). We also found a positive phenotypic and genotypic correlation between grain yield and test weight, but a negative genotypic correlation between 1000 kernel weight and test weight, significant only for population 05026. Doehlert and McMullen, 2000 showed that test weight and grain yield was negatively correlated (r=-0.46, p<0.01). Doehlert and McMullen, 2000 reported a positive correlation between β-glucan, oil (r=0.37, p<0.05), and grain yield (r=0.80, p<0.01).

Kernel weight was negatively correlated with oil content; this result is congruent with Holland et al., 2001 that indicated higher oil oats tended to have thinner kernels. Heading date tended to be negatively correlated with test weight (Pixley and Frey, 1992) and groat percentage (Tanhuanpaa et al., 2012).

2.5.3. Heritabilities

Heritability estimates are important parameters used to determine the role of heredity in the expression of a trait (Allard, 1960). When genotypic effects are substantial, and environmental effects are small, which indicates high heritability, effective selection can be achieved. The high heritabilities observed (Table 2.6) suggested that all the traits measured should respond well to selection on a family-mean basis. Holland and Munkvold, 2001 reported also high values of heritability in a F_6 oat population for grain yield (0.62±0.06), 1000 kernel weight (0.89±0.02), and test weight (0.80±0.04). In a population of 132 oat recombinant inbred lines, heritability estimates reported by Holland, 2006 was high for heading date (0.92±0.01), and moderate for plant height (0.54±0.07). Pixley and Frey, 1992 in 13 oat crosses evaluated at $F_{2:3}$ generation, reported moderate to high heritabilities for grain yield (0.60 to 0.82), test weight (0.63 to 0.91), kernel weight (0.77 to 0.95), plant height (0.61 to 0.87), and heading date (0.57 to 0.95). The lower heritabilities observed on a plot-basis (data not shown) indicated the importance

of replication and multiple environment testing to evaluate grain yield, 1000 kernel weight, test weight, heading date, and plant height.

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CHAPTER 3. GENETIC LINKAGE MAPS FOR HEXAPLOID CULTIVATED OAT (*AVENA SATIVA L.*) FROM TWO SPRING OAT POPULATIONS

3.1. Abstract

The development of linkage maps from diverse breeding populations can be useful in the genetic research of cultivated oat. The objectives of this chapter were to develop genetic linkage maps from two spring oat populations using SNP markers, identify and compare homologous linkage groups between the two populations and the recently published oat consensus map. A total of 4975 SNP markers were assessed on two recombinant inbred populations derived from the crosses 'ND991151/ND030299' (population 05021) and 'Souris/ND030299' (population 05026), using a 32-bead chip platform developed by Illumina. Individual linkage maps were constructed for the two populations using CarthaGene software with a LOD score of 9 and a maximum recombination fraction of 0.30. Thirty linkage groups using 1168 polymorphic markers were formed for population 05021, whereas population 05026 comprised 33 linkage groups using 1024 polymorphic markers. The 30 linkage groups of population 05021 contained from 3 to 62 markers, and varied in size from 15.8 to 225.3 cM for a total map size of 2601.7 cM. The 33 linkage groups of population 05026 comprised from 2 to 42 markers, and varied in size from 2.3 to 143.2 cM for a total map size of 1174.2 cM. Comparison with the recently published oat consensus map indicated that 26 of the 30 linkage groups from population 05021 can be placed on 19 of the 21 oat chromosomes, and that 31 of the 33 linkage groups from population 05026 showed homology with 20 of the 21 oat chromosomes. Further comparison of the homologous regions revealed differences in the ordering of markers between the two populations and the oat consensus map, and are an indication that genomic rearrangements and

intervarietal chromosome interchanges exist in the genome of cultivated oat. Some linkage groups, mostly in the population 05021, were significantly extended compared with the oat consensus map. Since those two linkage maps are from breeding cultivars and provide good coverage of the oat genome, they would be useful tool for identification of qualitative and quantitative trait loci.

3.2. Introduction

Cultivated oats belong to the genus *Avena* and are an allohexaploid species having 21 pairs of chromosomes with a basic chromosome number of 7 (2n=6x=42) (Zhu and Kaeppler, 2003) and a relatively large genome of 11315 Mbp (Pal et al., 2002). Genomic research in oats began in 1992 with the publication of the first molecular map for diploid species based on a cross between *A. atlantica* x *A. hirtula* (O'Donoughue et al., 1992). In 1995, the first hexaploid map based on 71 Recombinant Inbred Lines from *A. byzantina* cv 'Kanota' and *A. sativa* cv 'Ogle' (KO) was published (O'Donoughue et al., 1995). That map contained 561 RFLP markers covering a distance of 1482 cM. The KO map, over the years, had been enhanced and expanded using AFLP and DArT markers (Jin et al., 2000; Wight et al., 2003; Tinker et al., 2009). Subsequently, maps have been published for several hexaploid oat populations including 'OT207/Kanota' (Milach et al., 1997), 'Clintland64/IL86-5698' (Jin et al., 2000), 'Ogle/TAM O-301' (Portyanko et al., 2001), 'Kanota/Marion' (Groh et al., 2001), 'Ogle/MAM17-5' (Zhu and Kaeppler, 2003), 'Terra/Marion' (Dekoeyer et al., 2004), 'MN841801-1/Noble-2' (Portyanko et al., 2005), 'Dal/Exeter' (Hizbai et al., 2012), and 'Aslak/Matilda' (Tanhuanpaa et al., 2012).

The most recent map of hexaploid oat, which conciliates the number of linkage groups with the number of chromosomes, was developed by Oliver et al., 2013 using a new chromosome anchoring strategy and SNP markers. They developed six mapping populations and

derived monosomics lines from the cultivars ‘Kanota’ and ‘Sun II’ to anchor the linkage groups to the 21 oat chromosomes. The molecular map was constructed using 1054 SNP markers and contained 21 linkage groups, anchored to the 21 chromosomes, with a total length of 1838 cM.

However, the oat consensus map is a useful tool that cannot be used directly in the identification of QTLs affecting important traits in cultivated oats because it is a combination of 6 mapping populations. Specific mapping populations derived from two breeding parents are particularly useful to target genes controlling agronomically important traits in oats.

The oat genome is also subject to several cytogenetics abnormalities such as inversions, translocations, and chromosomal rearrangements that make mapping difficult. If we assume that chromosomal rearrangements are relatively fixed in hexaploid oat populations, high collinearity between two homologous linkage groups for different populations is expected (Wight et al., 2003). Therefore, the oat consensus map can be used as a guide to facilitate mapping and reveal recent genomic rearrangements in the oat genome between two hexaploid oat populations.

The objectives of this chapter were to develop genetic linkage maps from two spring oat populations, identify and compare homologous linkage groups between the two populations and the recently published oat consensus map.

3.3. Materials and methods

3.3.1. DNA extraction and genotyping

F_9 seed from the two populations described earlier were sown in the fall 2012 greenhouse, for 15 days, to provide leaf tissue for DNA extraction. The leaf samples were collected in two 96-deep-well plates filled with silica gel. The use of silica gel eliminates the need for liquid nitrogen or freeze drying. Leaf fragment of 2” was cut and inserted in each well, after that the two plates were sealed and sent to Dr. Shioman Chao’s USDA-ARS laboratory in Fargo for DNA

extraction and genotyping analysis. The DNA was extracted following the procedure of Slotta et al., 2008. Briefly, a preheated (65°C) extraction buffer (600 µL 0.1M Tris-HCl, pH 7.5, 0.05 M EDTA, pH 8.0, 1.25% SDS) was added to each well. The plates were then heated at 65°C for 15 min and placed at 4°C to cool before the addition of 300 µL chilled 6M ammonium acetate. In order to pellet proteins and cell debris, the plates were centrifuged for 20 min at 2250 g at 4°C. The supernatant was then transferred to new plates containing 400µL 100% isopropanol per well. DNA was pelleted by centrifugation for 20 min at 4°C, and then washed twice with 500 µL 70% ethanol followed by 10 min centrifugation at 2250g. The quality of the DNA obtained was estimated by gel electrophoresis and quantified using a NanoDrop spectrophotometer.

After DNA extraction, the two populations were genotyped using the oat 6K SNP chip containing 4975 SNPs on an Illumina BeadStation using a 32-bead chip platform developed by Illumina (San Diego, CA). Initially, the oat 6K SNP chip contains 5743 SNPs including 3847 EST markers, 1162 DArT markers, and 734 GBS markers. At the manufacturing stage 768 SNPs failed, and at the end a total of 4975 SNPs can be assayed (Chioman, communication personal). GenomeStudio V.3 software was used to perform allele calls. To avoid mistakes due to the occurrence of cluster compression each allele call was manually edited. After validation of allele calls, the genotype data was exported from GenomeStudio to Excel for linkage mapping analysis.

3.3.2. Map construction

Individual linkage maps were constructed for the two populations using CarthaGene software (www.inra.fr/bia/T/carthagene). Segregation ratios of 1:1 corresponding to a recombinant Inbred Line (RIL) population of all segregating markers on the 190 individuals were checked using a χ^2 test. Markers with highly-distorted segregation ratios (χ^2>20) and ≥10% missing genotypes were

removed from the analysis. Due to the large number of markers available, the large size of the oat genome, and some cytogenetic abnormalities such as inversions, translocations, to achieve a robust result and avoid the effects of pseudolinkage, linkage groups were first obtained by the group command using two-point analysis with a LOD (Logarithm of Odds) score of 9 and a maximum recombination fraction of 0.30. The grouping threshold was then relaxed to a LOD score of 6 to validate the joining of groups based on the oat consensus map (Oliver et al., 2013) and other sources of information (Tinker et al., 2009). The ordering of linked markers was determined by maximum likelihood and simulated annealing using the commands build 20, greedy, flip and annealing of the CarthaGene software, with a LOD threshold of 3 and a marker mapping distance no greater than 25 cM. The method of simulated annealing estimates the shortest linear map by simulating different loci orders and keeping only the shortest orders. The Kosambi mapping function was used to convert recombination fractions into map distances in centimorgans (cM). Linkage groups between the two populations studied and the oat consensus map were declared to be homologous if they shared four or more markers as indicated by Hizbai et al., 2012. Graphic presentation of the LGs, QTLs, and homology between Population and oat consensus map was obtained using MapChart version 2.2 (Voorrips, 2002).

3.4. Results

3.4.1. Population 05021

A total of 1284 markers loci were polymorphic on the 97 RILs of the population 05021, 1168 of them can be placed on linkage groups, 51 were discarded for severe distortion, and 65 remained unlinked. Upon analysis, 1168 loci formed 30 linkage groups (LG) of 3 or more markers, 523 of them (46%) cosegregate with mapped loci (Table 3.2), then the final framework map contained 640 markers loci (Appendix 1). Based on homology with the oat consensus map

(Oliver et al., 2013), 26 of the 30 linkage groups (87%) can be placed on 19 of the 21 oat chromosomes (Table 3.1). No polymorphism can be detected for chromosome 10D, and 21D. The remaining 13% (4 LG) cannot be placed on any of the oat chromosomes (Data not shown). The 26 LG, homologs to the oat chromosomes, contained from 3 to 62 markers (average 24 markers) for a total of 627 markers, and varied in size from 15.8 to 225.3 cM for a total map size of 2601.7 cM (Table 3.1). The average distance between markers was 4.1 cM when omitting the cosegregant markers, and 2.2 cM when considering all the markers. A total of 401 markers (64%) of the 627 were Expressed-Sequenced Tags (EST) markers, 159 (25%) were Diversity Array Technology (DArT) markers and the remaining 67 (11%) were Genotyping-By-Sequencing (GBS) markers (Table 3.3). The 4 LG that did not share any markers with the oat chromosomes contained a total of 18 markers ranging from 3 to 6 markers, and varied in size from 8.2 to 15.9 cM, for a total map size of 49.6 cM. Total length and marker density differed between genomes. The C genome had more length (1169.2 cM) and marker density (276) than the A and the D genome. The A genome (759.7 cM – 188 markers) and the D genome (672.7 cM – 163 markers) had approximately the same length and marker density (Table 3.1).

156 marker loci (25%) deviated significantly ($\chi^2>10$, $p<0.001$) from the expected 1:1 segregation ratio as determined by χ^2 analysis, 81 of them were extremely distorted ($\chi^2>15$). All the distorted markers were skewed towards the 'ND030299' allele. Five linkage groups showed regions with high segregation distortion and 4 linkage groups had regions moderately distorted. Seventy-seven percent of LG 05021-1.1 and 51% of LG 05021-6 contained essentially distorted markers (Table 3.5).

After comparative mapping, 26 LG were found to be homologous with 19 of the 21 oat chromosomes. All the chromosomes of the C and A genomes are present in the population.

Chromosome 10D and 21D are missing for the D genome. A total of 178 markers, covering 1028.7 cM in the population 05021 and 773.2 cM in the oat consensus map, were shared between the two. The number of shared markers ranged from 2 (LG 05021-9 and oat chromosome 9D) to 23 (LG 05021-16 and oat chromosome 16A) (Table 3.4).

3.4.2. Population 05026

Analysis of the segregation of the SNP markers in the 93 05026 RILs population identified a total of 1054 polymorphic markers, 1024 of them can be placed into linkage groups, 16 were discarded for severe segregation distortion, and 14 remained unlinked. More than 60% of the polymorphic markers (622) cosegregate with mapped loci (Table 3.7). The final framework map contained 398 markers and formed 33 linkage groups (Appendix 2). Thirty-one (31) of the 33 LG (94%) showed homology with 20 of the 21 oat chromosomes (Oliver et al., 2013). No polymorphism can be detected for oat chromosome 21D. The 31 LG comprised from 2 to 42 markers (average 13) for a total of 392 markers, and varied in size from 2.3 cM to 143.2 cM for a total map size of 1174.2 cM (Table 3.6). When all the markers were taken into account, the average distance between markers was 1.2 cM, and when only the framework markers were considered the average distance was 3.0 cm (Table 3.8). Of the 392 markers of the framework map, a total of 285 markers (72%) were EST markers, 45 (11%) were DArT markers and the remaining 62 (16%) were GBS markers (Table 3.8). As for population 05021, total length and marker density differed between genomes. The C genome, with 211 markers and 638.0 cM, had more length and more density, followed by the A genome with 119 markers and 351.3 cM. Finally the D genome with 62 markers and 184.9 cM had less length and less density (Table 3.6).

Table 3.1. Summary of the molecular linkage map from 'ND991151' x 'ND030299' F_6 recombinant inbred oat population (Population 05021) with length, average marker interval length, number of markers on each linkage group, and corresponding chromosome to the oat consensus map.

Linkage group	Chromosome	Length	No cosegregating markers		Including cosegregating markers	
		cM	Total markers	Average length	Total markers	Average length
			C genome			
05021-1.1	1C	71.2	23	3.1	55	1.3
05021-1.2	1C	63.4	23	2.8	37	1.7
05021-2	2C	141.6	28	5.1	59	2.4
05021-3	3C	155.3	31	5.0	41	3.8
05021-4	4C	118.1	28	4.2	48	2.5
05021-5	5C	171.7	42	4.1	71	2.4
05021-6	6C	151.1	34	4.4	69	2.2
05021-7.1	7C-17A	168.1	41	4.1	119	1.4
05021-7.2	7C-17A	53.6	12	4.5	17	3.2
05021-7.3	7C-17A	16.4	4	4.1	5	3.3
05021-7.4	7C-17A	34.6	7	4.9	8	4.3
05021-7.5	7C-17A	24.1	3	8.0	4	6.0
12		**1169.2**	**276**	**4.2**	**533**	**2.2**
			A genome			
05021-8	8A	171.9	51	3.4	98	1.8
05021-11	11A	83.8	18	4.7	18	4.7
05021-13	13A	121.5	21	5.8	31	3.9
05021-15	15A	28.0	3	9.3	3	9.3
05021-16	16A	219.5	62	3.5	125	1.8
05021-19	19A	135.0	33	4.1	72	1.9
6		**759.7**	**188**	**4.0**	**347**	**2.2**
			D genome			
05021-9	9D	59.7	17	3.5	23	2.6
05021-12	12D	225.3	54	4.2	88	2.6
05021-14.1	14D	90.0	23	3.9	55	1.6
05021-14.2	14D	36.3	9	4.0	13	2.8
05021-14.3	14D	38.7	7	5.5	9	4.3
05021-14.4	14D	15.8	7	2.3	9	1.8
05021-18	18D	146.6	30	4.9	42	3.5
05021-20	20D	60.3	16	3.8	31	1.9
8		**672.7**	**163**	**4.1**	**270**	**2.5**
26		**2601.6**	**627**	**4.1**	**1150**	**2.3**

Table 3.2. Cosegregation by genome observed in the linkage map from 'ND991151' x 'ND030299' F_6 recombinant inbred oat population (Population 05021).

Linkage group	Chromosome	Cosegregate markers	% of cosegregation
		C genome	
05021-1.1	1C	32	58
05021-1.2	1C	14	38
05021-2	2C	31	53
05021-3	3C	10	24
05021-4	4C	20	42
05021-5	5C	29	41
05021-6	6C	35	51
05021-7.1	7C-17A	78	66
05021-7.2	7C-17A	5	29
05021-7.3	7C-17A	1	20
05021-7.4	7C-17A	1	13
05021-7.5	7C-17A	1	25
		257	**48**
		A genome	
05021-8	8A	47	48
05021-11	11A	0	0
05021-13	13A	10	32
05021-15	15A	0	0
05021-16	16A	39	54
05021-19	19A	63	50
		159	**46**
		D genome	
05021-9	9D	6	26
05021-12	12D	34	37
05021-14.1	14D	32	58
05021-14.2	14D	4	31
05021-14.3	14D	2	22
05021-14.4	14D	2	22
05021-18	18D	12	29
05021-20	20D	15	48
		107	**40**
		523	**46**

Table 3.3. Summary of marker type for the molecular linkage map from 'ND991151' x 'ND030299' F_6 recombinant inbred oat population (Population 05021).

Linkage group	Including cosegregating markers				No cosegregating markers			
	cDNA	DArT	GBS	Total	cDNA	DarT	GBS	Total
				C genome				
05021-1.1	36	10	9	55	11	10	2	23
05021-1.2	23	9	5	37	13	8	2	23
05021-2	43	9	7	59	13	9	6	28
05021-3	24	10	7	41	19	7	5	31
05021-4	33	12	3	48	17	9	2	28
05021-5	57	8	6	71	34	5	3	42
05021-6	46	13	10	69	25	6	3	34
05021-7.1	72	21	26	119	24	12	5	41
05021-7.2	9	6	2	17	5	5	2	12
05021-7.3	4	1	0	5	3	1	0	4
05021-7.4	5	2	1	8	4	2	1	7
05021-7.5	3	0	1	4	2	0	1	3
	355	**101**	**77**	**533**	**170**	**74**	**32**	**276**
				A genome				
05021-8	69	25	4	98	31	17	3	51
05021-11	13	4	1	18	13	4	1	18
05021-13	17	7	7	31	14	3	4	21
05021-15	3	0	0	3	3	0	0	3
05021-16	97	17	11	125	42	13	7	62
05021-19	55	9	8	72	23	8	2	33
	254	**62**	**31**	**347**	**126**	**45**	**17**	**188**
				D genome				
05021-9	13	7	3	23	8	6	3	17
05021-12	59	19	10	88	35	16	3	54
05021-14.1	40	6	9	55	15	5	3	23
05021-14.2	4	6	3	13	3	4	2	9
05021-14.3	9	0	0	9	7	0	0	7
05021-14.4	5	2	2	9	3	2	2	7
05021-18	31	4	7	42	23	4	3	30
05021-20	24	3	4	31	11	3	2	16
	185	**47**	**38**	**270**	**105**	**40**	**18**	**163**
	794	**210**	**146**	**1150**	**401**	**159**	**67**	**627**

GBS = Genotyping-By-Sequencing markers

Table 3.4. Homologous segments between the molecular linkage map from population 05021 and oat consensus map.

Population 05021				Oat consensus map		
LG	Homologous segments	Length (cM)	Markers shared	Chromosome	Homologous segments	Length (cM)
05021-1.1	4.3-31.6	27.3	14	1C	24.5-43.8	19.3
					71.5-74.8	3.3
05021-1.2	17.0-27.7	10.7	5	1C	47.2-63.2	16.0
05021-2	22.2-63.2	41.0	11	2C	3.0-39.9	36.9
05021-3	7.2-21.2	14.0	6	3C	11.5-15.7	4.2
	77.1-131.1	54.0			49.5-86.6	37.1
05021-4	4.3-11.4	7.1	6	4C	75.5-90.5	15.0
	100.1-104.3	4.2				
05021-5	1.1-51.2	50.1	17	5C	17.4-76.4	59.0
	144.7-153.2	8.5			0.0-7.1	7.1
05021-6	23.7-109.3	85.6	18	6C	15.4-77.9	62.5
05021-7.1	69.0-167.0	98.0	13	7C-17A	0.0-78.4	78.4
05021-8	30.2-59.0	28.8	12	8A	17.3-62.0	44.7
	137.3-139.5	2.2				
05021-9	5.5-47.1	41.6	2	9D	64.2-91.0	26.8
05021-12	38.6-85.3	46.7	14	12D	25.7-115.0	89.3
	126.5-225.3	98.8				
05021-13	22.8-61.4	38.6	5	13A	60.1-91.4	31.3
05021-14.1	52.4-76.0	23.6	6	14D	77.1-102.3	22.0
05021-14.3	22.9-38.7	15.8	2	14D	0.0-22.0	22.0
05021-16	1.1-41.3	40.2	23	16A	10.8-80.8	70.0
	85.7-205.6	119.9				
05021-18	5.3-33.8	28.5	8	18D	0.0-39.9	39.9
	93.4-146.1	52.7				
05021-19	0.0-3.8	3.8	11	19A	28.6-97.5	68.9
	68.8-135.0	66.2				
05021-20	24.7-45.5	20.8	5	20D	56.3-75.8	19.5
Total		**1028.7**	**178**			**773.2**

Table 3.5. Region of segregation distortion for the molecular linkage map from 'ND991151' x 'ND030299' F_6 recombinant inbred oat population (Population 05021).

Linkage group	Region (cM)	Length (cM)	% of linkage group	Comment
05021-1.1	15.4-70.0	54.6	77	Highly distorted (χ^2>15)
05021-2	44.9-77.7	32.8	23	Highly distorted (χ^2>15)
05021-8	29.7-84.5	54.8	32	Highly distorted (χ^2>15)
05021-16	27.0-63.6	36.6	17	Highly distorted (χ^2>15)
05021-18	50.7-110.6	59.9	41	Highly distorted (χ^2>15)
05021-5	43.4-100.1	56.7	33	Moderately distorted (χ^2>10)
05021-6	42.6-119.9	77.3	51	Moderately distorted (χ^2>10)
05021-14.3	22.4-38.7	16.3	42	Moderately distorted (χ^2>10)
05021-19	37.6-85.9	48.3	36	Moderately distorted (χ^2>10)

Less segregation distortion, compared to population 05021 was observed. Only 35 markers (9%) showed distortion and deviated significantly (χ^2>10, p<0.001) from the expected 1:1 segregation ratio, 24 of them were extremely distorted (χ^2>15). Twenty-five (25) of the distorted markers were skewed towards the 'ND030299' allele, the remaining 10 markers were skewed towards the 'Souris' allele. Linkage group 05026-12.2, 05026-18, 05026-13.1 from position 0 to 13.9 cM, and 05026-11 from position 0 to 0.3 contained essentially extremely distorted markers. Linkage groups 05026-13.1, 05026-12.2, and 05026-18 were all skewed towards to 'ND030299' allele whereas linkage group 05026-11 was skewed towards the 'Souris' allele.

647.3 cM, representing 171 markers, in the population 05026, showed homology with 557.5 cM in the oat consensus map. The number of shared markers varied from 2 (LG 05026-14.2 and OC 14D) to 28 (LG 05026-2 and OC 2C) (Table 3.9). 396 markers, covering 886.7 cM in the population 05021 and 540.9 cM in the population 05026, were shared between the two populations. Homologous markers ranged from 3 (LG 05021-13 and LG 05026-13.2) to 53 (LG 05021-16 and LG 05026-16.1) (Table 3.10).

Table 3.6. Summary of the molecular linkage map from 'Souris' x 'ND030299' F_6 recombinant inbred oat population (Population 05026) with length, average marker interval length, number of markers on each linkage group, and corresponding chromosome to the oat consensus map.

Linkage group	Chromo-some	Length	Including cosegregating markers		No cosegregating markers	
		cM	Total markers	Average length	Total markers	Average length
			C genome			
05026-1.1	1C	36.8	34	1.1	17	2.2
05026-1.2	1C	24.3	28	0.9	11	2.2
05026-2	2C	71.1	87	0.8	20	3.6
05026-3	3C	143.2	81	1.8	38	3.8
05026-4	4C	23.5	43	0.5	21	1.1
05026-5.1	5C	128.7	109	1.2	42	3.1
05026-5.2	5C	45.3	47	1.0	16	2.8
05026-6	6C	75.0	46	1.6	17	4.4
05026-7.1	7C-17A	71.5	27	2.6	15	4.8
05026-7.2	7C-17A	18.0	18	1.0	12	1.5
05026-7.3	7C-17A	0.6	3	0.2	2	0.3
11		**638.0**	**523**	**1.2**	**211**	**3.0**
			A genome			
05026-8.1	8A	24.1	79	0.3	14	1.7
05026-8.2	8A	22.3	8	2.8	3	7.4
05026-11	11A	35.0	34	1.0	15	2.3
05026-13.1	13A	38.1	46	0.8	14	2.7
05026-13.2	13A	26.7	12	2.2	5	5.3
05026-15	15A	74.4	22	3.4	15	5.0
05026-16.1	16A	60.4	74	0.8	28	2.2
05026-16.2	16A	17.0	14	1.2	5	3.4
05026-17	17A-7C	4.5	14	0.3	5	0.9
05026-19	19A	48.8	49	1.0	15	3.3
10		**351.3**	**352**	**1.0**	**119**	**3.0**
			D genome			
05026-9	9D	2.3	5	0.5	2	1.2
05026-10	10D-F-1	26.3	19	1.4	11	2.4
05026-12.1	12D	53.3	20	2.7	8	6.7
05026-12.2	12D	2.3	4	0.6	2	1.2
05026-14.1	14D	29.0	20	1.5	8	3.6
05026-14.2	14D	5.2	8	0.7	4	1.3
05026-14.3	14D	4.3	3	1.4	3	1.4

Table 3.6. Summary of the molecular linkage map from 'Souris' x 'ND030299' F_6 recombinant inbred oat population (Population 05026) with length, average marker interval length, number of markers on each linkage group, and corresponding chromosome to the oat consensus map (Continued).

Linkage group	Chromosome	Length	Including cosegregating markers		No cosegregating markers	
		cM	Total markers	Average length	Total markers	Average length
05026-18	18D	47.2	30	1.6	10	4.7
05026-20.1	20D	7.5	18	0.4	7	1.1
05026-20.2	20D	7.5	12	0.6	7	1.1
10		**184.9**	**139**	**1.3**	**62**	**3.0**
31		**1174.2**	**1014**	**1.2**	**392**	**3.0**

3.5. Discussion

3.5.1. Comparative mapping

3.5.1.1. Population 05021 and oat consensus map

178 markers were shared between population 05021 and oat consensus map representing 40% of the former (1028.7 cM) and 42% of the latter (773.2 cM). The order of markers was very well conserved on linkage groups 05021-3, 05021-7.1, 05021-13, 05021-14, and 05021-20 comparing to the corresponding oat chromosomes 3C, 7C-17A, 13A, 14D, and 20D.

Minor rearrangements and one or several inversions of markers were observed for linkage groups (LG) 05021-1.1, 05021-1.2, 05021-5, 05021-6, 05021-8, 05021-12, 05021-16, and 05021-19. On LG 05021-1.1, markers DS_cc9481_218, ES_cc13854_225, ES15_c16679_330, and ES_cc11019_290 (Figure 3.2, red) mapped at position 23.6 to 29 cM were interchanged with a group of 4 cosegregating markers mapped at position 17 cM and marker ES02_c6368_605 mapped at position 4.3 (Figure 3.1, green). Comparing to oat chromosome 1C, markers ES02_c12621_204, ES02_lrc13446_346, and ES01_c3447_952 located at position 25.4 to 27.7 cM (Figure 3.1, violet) on LG 05021-1.2 switched order with markers ES01_C9472_428 and ES02_lrc13446_328 mapped at position 17 to 18.1 cM (Figure

3.1, dark blue). Marker ES15_C7706_583 positioned at the end of LG 05021-2 (112.2 cM) had been mapped at the beginning of oat chromosome 2C (3 cM) (Figure 3.2).

A group of 4 markers (Figure 3.2, red) placed at the end of LG 05021-5 from position 106.8 to 153.2 cM had been mapped at the very beginning of oat chromosome 5C from 0 to 7.1 cM. Another group of 5 markers (Figure 3.3, green) located at the middle of LG 05021-5 from position 36.3 to 43.9 cM had been mapped at the end of oat chromosome 5C from position 73.2 to 76.4 cM. Two small rearrangements were observed in LG 05021-6. Marker ES01_c5633_477 changed order with marker ES02_c247_241 (Figure 3.4, red and green). Markers ES02_c15952_348 and ES02_c28827_474 that cosegregated at position 53.9 cM had been mapped respectively at position 15.4 to 17.5 cM in oat chromosome 6C (Figure 3.4, violet). Cosegregating markers ES02_c15926_519 and ES17_c11418_547 placed at the beginning of LG 05021-8 at position 30.2 cM, had been mapped at the opposite end of oat chromosome 8A at position 61.6 and 62 cM, respectively (Figure 3.5, red). Two group of markers were inverted in LG 05021-12 comparing to oat chromosome 12D. A first group of 6 markers (Figure 3.6, red) placed at position 126.5 to 225.3 cM had been mapped in the oat chromosome 12D at position 25.6 to 67.9 cM. The second group comprised 8 markers (Figure 3.6, green) placed at position 38.6 to 85.3 cM had been mapped at position 91.3 to 115 cM in oat chromosome 12D. Two groups of markers were also inverted in LG 05021-16. The first group consisted of 9 markers (Figure 3.7, red) had been mapped, in the same order in comparison with oat chromosome 16A, at position 85.7 to 197.3 cM. The second group of 9 markers also (Figure 3.7, green) had been mapped, in reverse order relative to oat chromosome 16A, at position 19.1 to 1.1 cM. Some markers were inverted in LG 05021-18 comparing to oat chromosome 18D. Markers ES15_c10680_325 and ES02_c4268_604, ES17_c3693_421 and ES15_c13674_329, ES17_c2976_706 and ES02_c13415_701 switched order (Figure 3.8).

Table 3.7. Cosegregation by genome observed in the linkage map from 'Souris' x 'ND030299' F_6 recombinant inbred oat population (Population 05026).

Linkage group	Chromosome	Cosegregate markers	% of cosegregation
		C genome	
05026-1.1	1C	17	50
05026-1.2	1C	17	61
05026-2	2C	67	77
05026-3	3C	43	55
05026-4	4C	22	51
05026-5.1	5C	67	61
05026-5.2	5C	31	66
05026-6	6C	29	63
05026-7.1	7C-17A	12	44
05026-7.2	7C-17A	6	33
05026-7.3	7C-17A	1	33
		312	**60**
		A genome	
05026-8.1	8A	65	82
05026-8.2	8A	5	63
05026-11	11A	19	56
05026-13.1	13A	32	70
05026-13.2	13A	7	58
05026-15	15A	7	32
05026-16.1	16A	46	62
05026-16.2	16A	9	64
05026-17	17A-7C	9	64
05026-19	19A	34	69
		233	**66**
		D genome	
05026-9	9D	3	60
05026-10	10D-F-1	8	42
05026-12.1	12D	12	60
05026-12.2	12D	2	50
05026-14.1	14D	12	60
05026-14.2	14D	4	50
05026-14.3	14D	0	0
05026-18	18D	20	67
05026-20.1	20D	11	61
05026-20.2	20D	5	42
		77	**55**
		622	**61**

Table 3.8. Summary of marker type for the molecular linkage map from 'Souris' x 'ND030299' F_6 recombinant inbred oat population (Population 05026).

Linkage group	Including cosegregating markers				No cosegregating markers			
	cDNA	DArT	GBS	Total	cDNA	DArT	GBS	Total
				C genome				
05026-1.1	27	1	6	34	11	0	6	17
05026-1.2	25	3	0	28	9	2	0	11
05026-2	67	10	10	87	14	3	3	20
05026-3	53	14	14	81	24	7	7	38
05026-4	31	5	7	43	16	3	2	21
05026-5.1	86	6	17	109	33	2	7	42
05026-5.2	38	7	2	47	13	2	1	16
05026-6	30	8	8	46	13	3	1	17
05026-7.1	16	7	4	27	9	4	2	15
05026-7.2	6	5	7	18	3	4	5	12
05026-7.3	3	0	0	3	2	0	0	2
	382	**66**	**75**	**523**	**147**	**30**	**34**	**211**
				A genome				
05026-8.1	62	15	2	79	11	3	0	14
05026-8.2	8	0	0	8	3	0	0	3
05026-11	29	1	4	34	14	0	1	15
05026-13.1	36	7	3	46	10	3	1	14
05026-13.2	5	4	3	12	2	1	2	5
05026-15	16	2	4	22	11	1	3	15
05026-16.1	59	6	9	74	21	2	5	28
05026-16.2	13	0	1	14	5	0	0	5
05026-17	14	0	0	14	5	0	0	5
05026-19	37	5	7	49	12	1	2	15
	279	**40**	**33**	**352**	**94**	**11**	**14**	**119**
				D genome				
05026-9	3	1	1	5	0	1	1	2
05026-10	14	2	3	19	9	1	1	11
05026-12.1	14	6	0	20	7	1	0	8
05026-12.2	3	0	1	4	1	0	1	2
05026-14.1	13	1	6	20	4	1	3	8
05026-14.2	5	0	3	8	3	0	1	4
05026-14.3	3	0	0	3	3	0	0	3

Table 3.8. Summary of marker type for the molecular linkage map from 'Souris' x 'ND030299' F_6 recombinant inbred oat population (Population 05026) (Continued).

Linkage group	Including cosegregating markers				No cosegregating markers			
	cDNA	DArT	GBS	Total	cDNA	DArT	GBS	Total
05026-20.1	16	1	1	18	6	0	1	7
05026-20.2	8	0	4	12	5	0	2	7
	101	**12**	**26**	**139**	**44**	**4**	**14**	**62**
	762	**118**	**134**	**1014**	**285**	**45**	**62**	**392**

Table 3.9. Homologous segments between the molecular linkage map from population 05026 and oat consensus map.

Population 05026				Oat consensus map		
LG	Homologous segments	Length (cM)	Markers shared	Chromo some	Homologous segments	Length (cM)
05026-1.1	0.0-15.8	15.8	3	1C	47.2-63.2	16.0
05026-1.2	0.0-20.3	20.3	4		68.7-74.8	6.1
05026-2	0.0-61.3	61.3	28	2C	3.0-44.3	41.3
05026-3	26.9-143.2	116.3	19	3C	11.5-86.6	75.1
05026-4	5.8-22.4	16.6	6	4C	60.8-90.5	29.7
05026-5.1	66.5-101.0	34.5	17	5C	5.6-30.3	24.7
05026-5.2	0.0-44.7	44.7	16	5C	60.1-108.4	48.3
05026-6	6.6-56.5	49.9	7	6C	26.6-63.9	37.3
05026-8.1	3.5-7.6	4.1	10	8A	17.3-20.6	3.3
05026-10	9.4-26.3	16.9	2	10D-F-1	0.0-4.8	4.8
05026-11	0.0-35.0	35.0	7	11A	11.1-36.0	24.9
05026-12.1	0.6-53.3	52.7	3	12D	43.9-104.7	60.8
05026-13.1	0.0-36.4	36.4	9	13A	83.7-111.2	27.5
05026-14.1	17.6-29.0	11.4	4	14D	96.2-105.8	9.6
05026-14.2	0.0-5.2	5.2	2	14D	22.0-25.5	3.5
05026-15	0.0-48.5	48.5	4	15A	0.0-48.2	48.2
05026-16.1	36.4-60.4	24.0	11	16A	10.8-36.6	25.8
05026-18	37.3-42.1	4.8	2	18D	31.4-41.4	10.0
05026-19	2.2-48.8	46.6	8	19A	0.0-51.9	51.9
05026-20.1	1.7-4.0	2.3	7	20D	72.6-75.8	3.2
Total		**647.3**	**171**			**552.0**

Table 3.10. Homologous segments between the molecular linkage map from population 05026 and population 05021.

Population 05021				Population 05026		
LG	Homologous segments	Length (cM)	Markers shared	LG	Homologous segments	Length (cM)
05021-1.1	0.0	0.0	9	05026-1.2	14.5-15.1	0.6
05021-1.2	17.0-63.4	46.4	18	05026-1.1	0.0-29.9	29.9
05021-2	22.2-35.2	13.0	38	05026-2	0.0-71.1	71.1
	60.0-141.6	81.6				
05021-3	0-131.1	131.1	27	05026-3	20.7-143.2	122.5
05021-4	2.7-17.5	14.8	28	05026-4	4.6-22.4	17.8
	35.9-39.3	3.4				
	100.1-104.3	4.2				
05021-5	0.0-4.9	4.9	23	05026-5.1	66.5-122.4	55.9
	73.0-171.7	98.7				
05021-5	35.8-51.2	15.4	10	05026-5.2	24.3-36.9	12.6
05021-6	0.0-6.0	6.0	33	05026-6	73.2-75.0	1.8
	24.2-30.2	6.0			52.5-56.0	3.5
	63.7-64.8	1.1			34.3-36.0	1.7
	114.4-144.4	30.0			0.6-7.2	6.6
05021-7.2	0.0-16.6	16.6	10	05026-7.1	36.2-44.6	8.4
05021-7.3	0.0-14.8	14.8	3	05026-7.3	0.0-0.6	0.6
05021-7.1	0.0	0.0	3	05026-7.2	2.9-12.4	9.5
05021-8	53.5-84.5	31.0	73	05026-8.1	0.0-24.1	24.1
	120.0-138.4	18.4				
05021-9	47.1-49.2	2.1	4	05026-9	0.0-2.3	2.3
05021-11	0.0-2.2	2.2	10	05026-11	19.2-21.6	2.4
	40.4-41.1	0.7			31.6-33.3	1.7
05021-12	38.6-59.6	21.0	18	05026-12.1	0-53.3	53.3
	119.5-126.5	7.0				
	208.6-225.3	16.7				
05021-12	168.0-168.6	0.6	4	05026-12.2	0-2.3	2.3
05021-13	61.4-96.5	35.1	5	05026-13.1	8.9-38.1	29.2
05021-13	0.0-13.0	13.0	8	05026-13.2	23.3-26.7	3.4
05021-14.1	62.8-90.0	27.2	12	05026-14.1	4.0-29.0	25.0
05021-14.3	0.0-10.8	10.8	3	05026-14.3	0.0-4.3	4.3
05021-14.3	37.1-38.7	1.6	3	05026-14.2	0.0-0.7	0.7
05021-14.4	0.8-7.0	6.2	5	05026-14.1	0.0-0.1	0.1
05021-16	98.2-122.0	23.8	65	05026-16.1	0.0-60.4	60.4
	146.3-151.4	5.1				
	188.5-219.5	31.0				
05021-7.1	120.6-124.3	3.7	12	05026-17	2.8-4.5	1.7
05021-18	136.5-146.6	10.1	7	05026-18	42.1-47.2	5.1
05021-19	68.8-135.0	66.2	35	05026-19	16.6-48.8	32.2
05021-20	43.4-58.7	15.3	11	05026-20.1	3.4-7.5	4.1
05021-20	8.6-22.0	13.4	10	05026-20.2	0.0-4.6	4.6
Total		**850.2**	**487**			**599.4**

An inversion between two groups of markers was observed in LG 05021-19 in comparison with oat chromosome 19A. A group of 4 markers (Figure 3.9, red) mapped at position 68.8 to 135 cM were inverted with another group of 7 markers (Figure 3.9, green) mapped at position 0 to 3.8 cM.

The longer map observed in population 05021 comparing to oat consensus map might be due to the expansion of some regions of population 05021. Markers ES17_c3418_95, DS_cc8468_91, ES_cc11290_204, DS_cc4033_368 found at position 22.2 to 29 cM on LG 05021-2, and markers ES_cc4978_509, ES01_c8470_599, ES01_c1635_353, ES_cc8700_285, ES01_c24681_389 located at position 60 to 63.2 cM had been mapped together in oat chromosome 2C (Figure 3.4, green and violet). Two groups of markers located at a distance of 90 cM in LG 05021-4 had been mapped together in oat chromosome 4C over 15 cM (Figure 3.10). Two markers (ES_02_c17364_288 and ES02_c13236_178) located respectively at position 1.1 and 4.9 cM in LG 05021-5 and 3 markers (ES17_c3625_404, ES01_c16767_69, ES02_c16344_73) found at position 59 to 60.1 cM had been mapped together in oat chromosome 5C (Figure 3.3, blue and violet). Marker ES02_c16344_816 located at position 59 cM and a group of 9 cosegregating at position 138.4 cM in LG 05021-8 had been mapped together in oat chromosome 8A (Figure 3.5, blue).

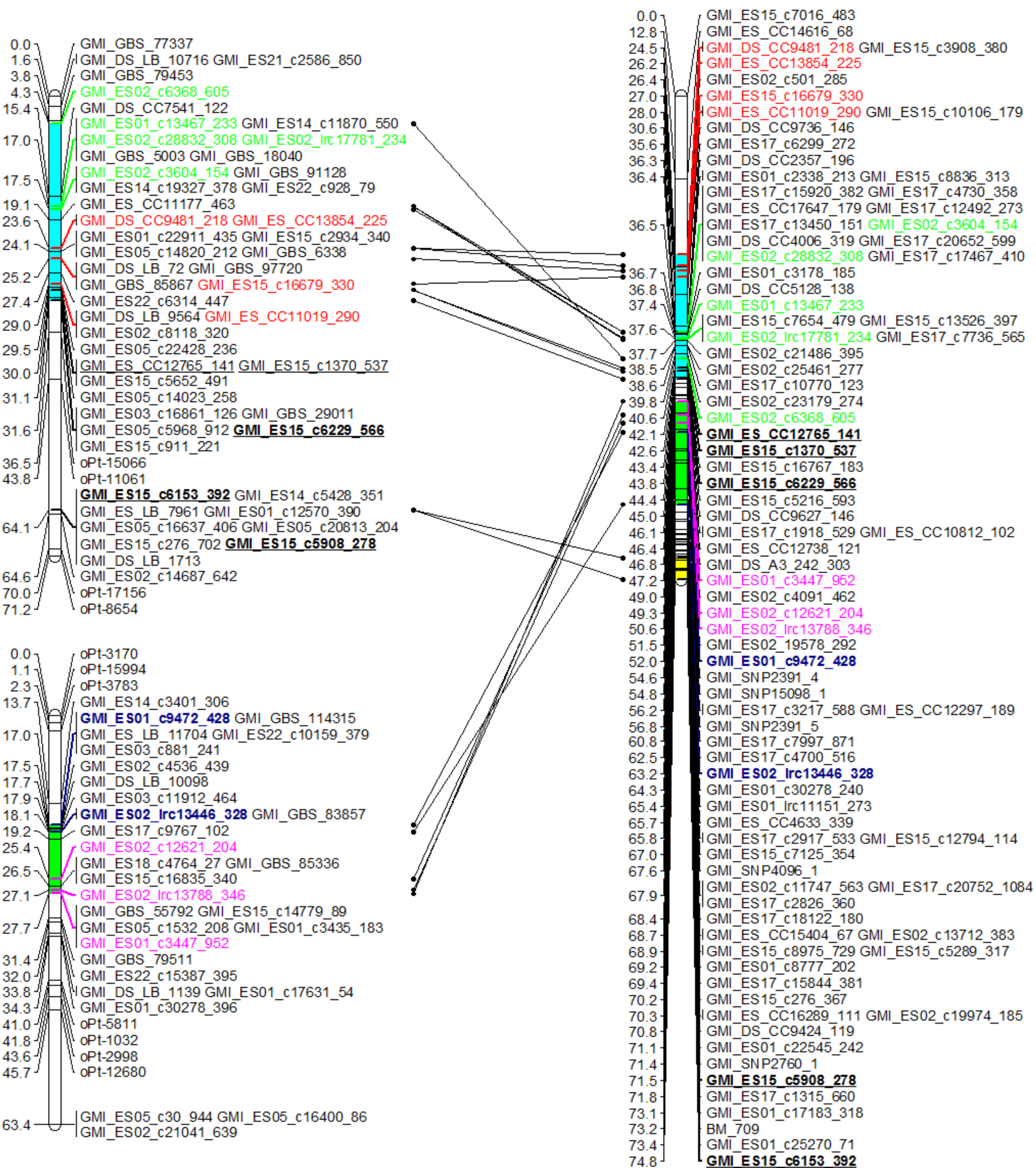

Figure 3.1. Conserved segments between LG 05021-1.1, 05021-1.2, and oat chromosome 1C.

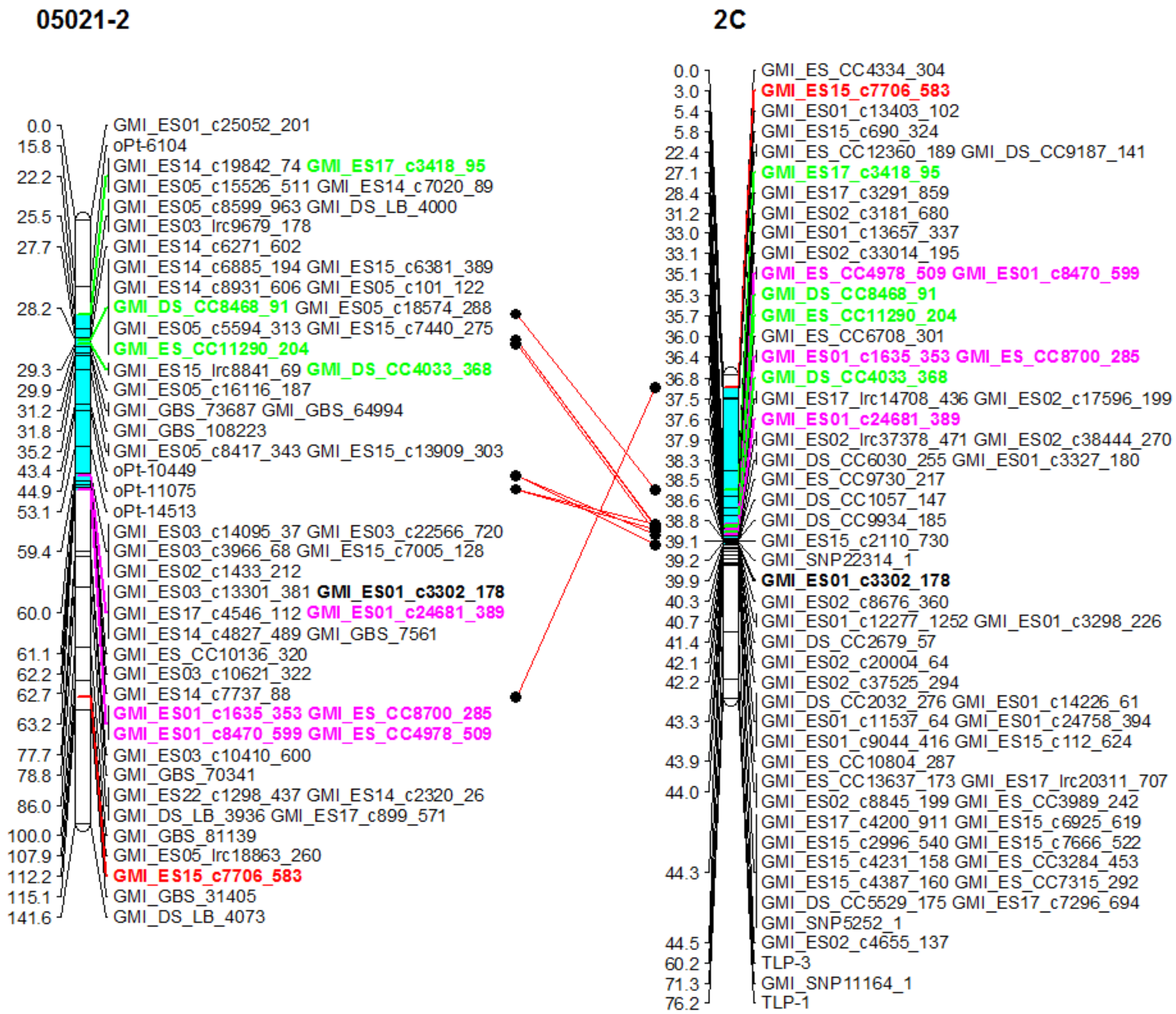

Figure 3.2. Conserved segments between LG 05021-2, and oat chromosome 2C.

Figure 3.3. Conserved segments between LG 05021-5, and oat chromosome 5C.

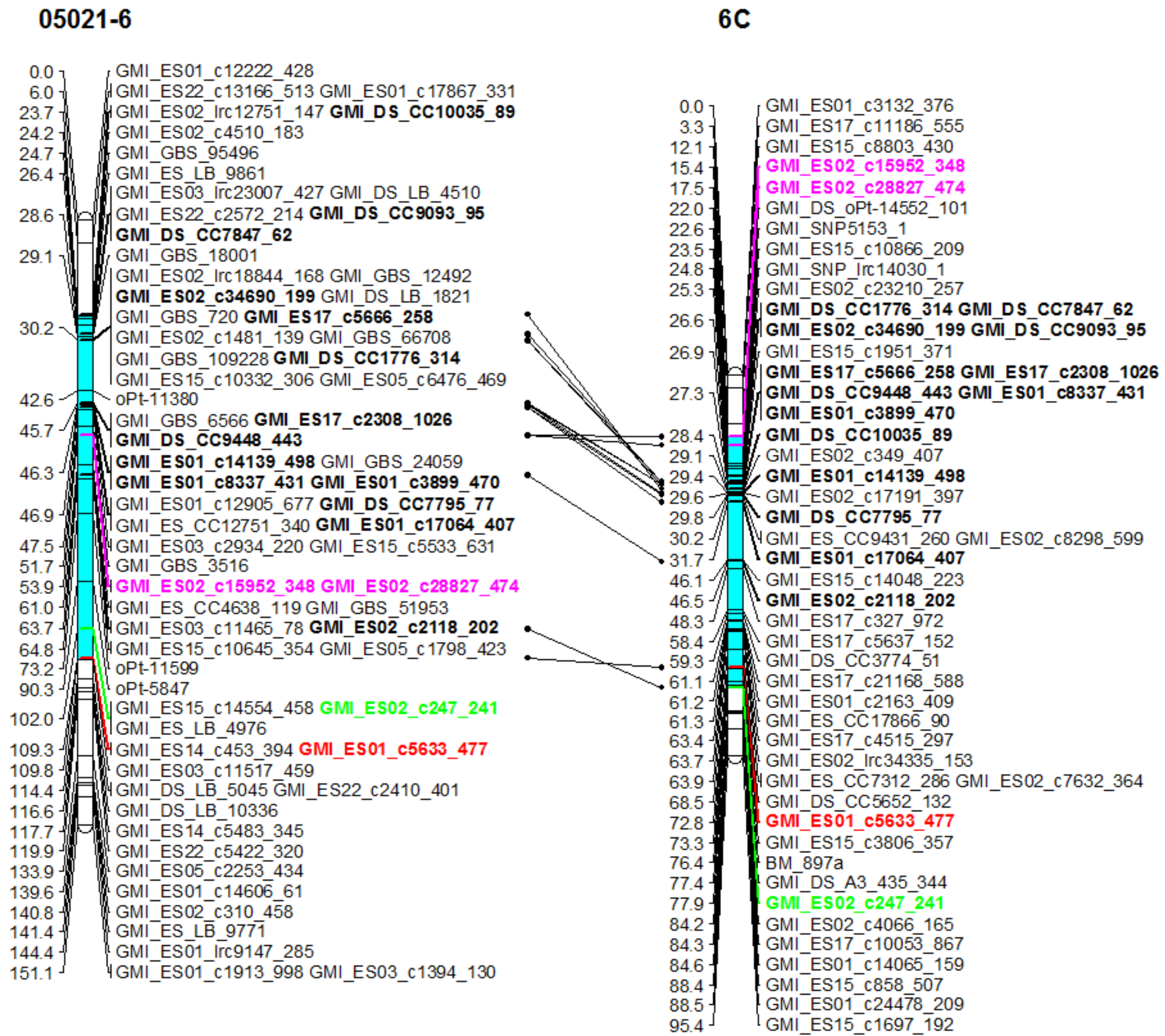

Figure 3.4. Conserved segments between LG 05021-6, and oat chromosome 6C.

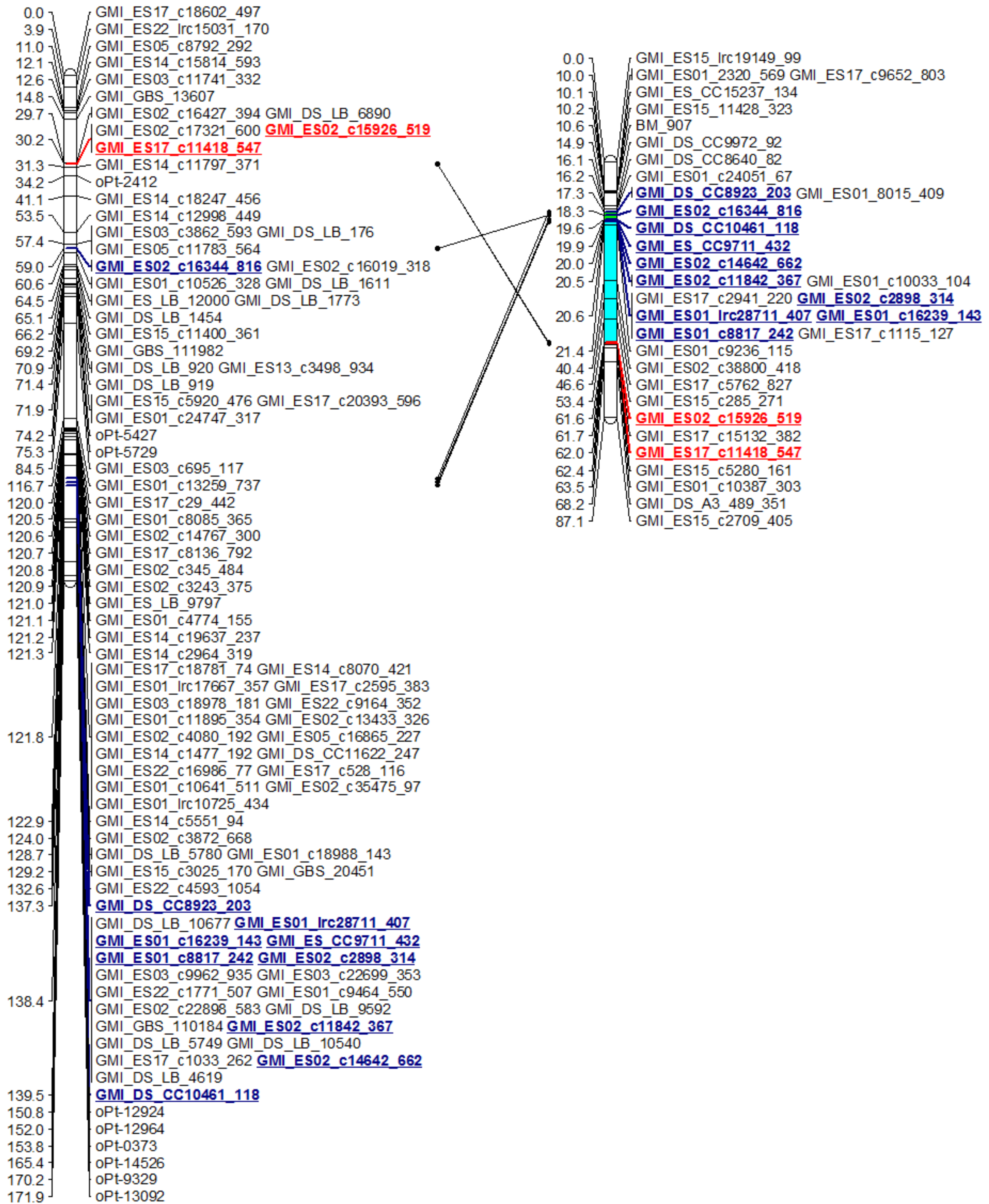

Figure 3.5. Conserved segments between LG 05021-8, and oat chromosome 8A.

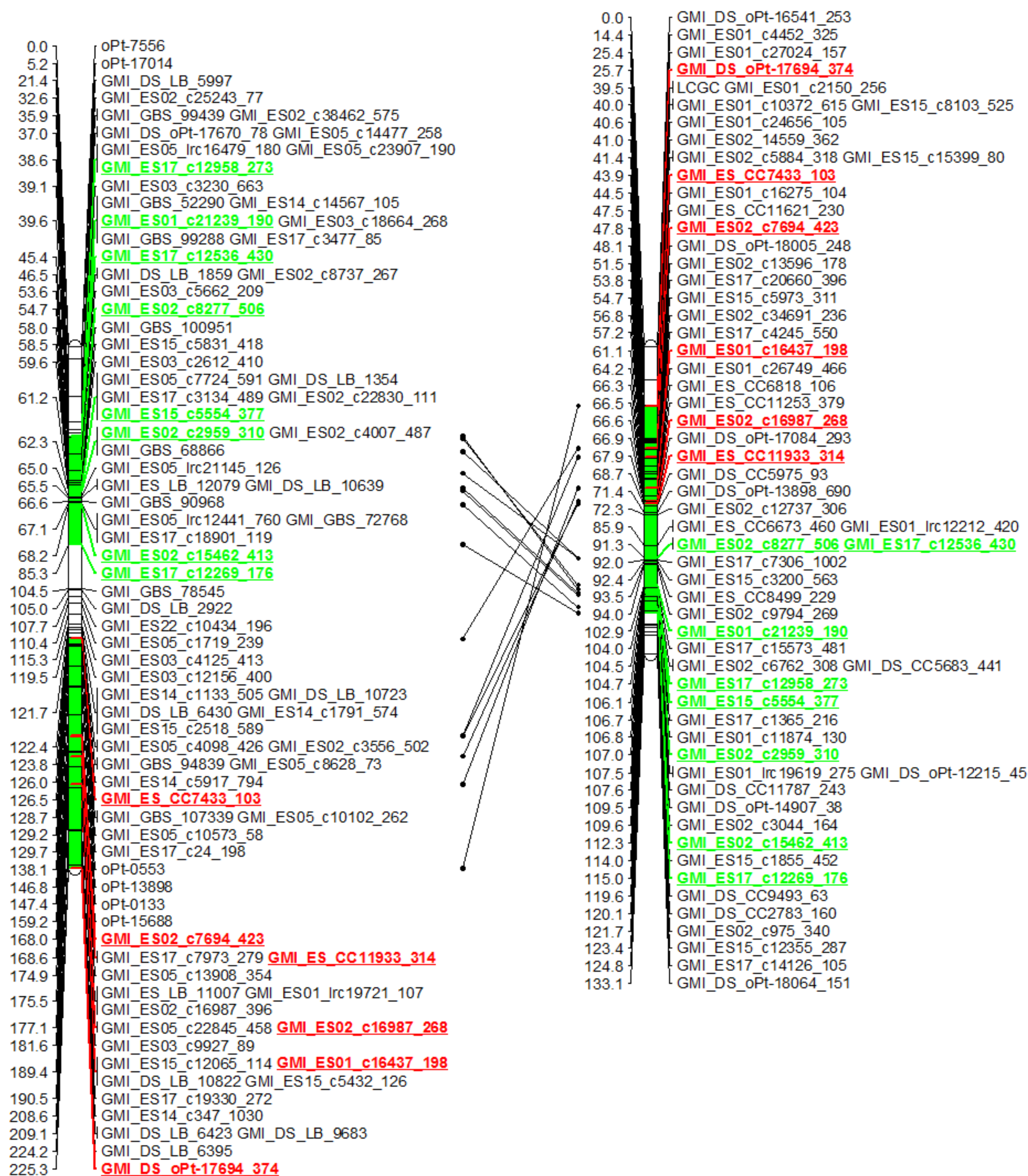

Figure 3.6. Conserved segments between LG 05021-12, and oat chromosome 12D.

Figure 3.7. Conserved segments between LG 05021-16, and oat chromosome 16A.

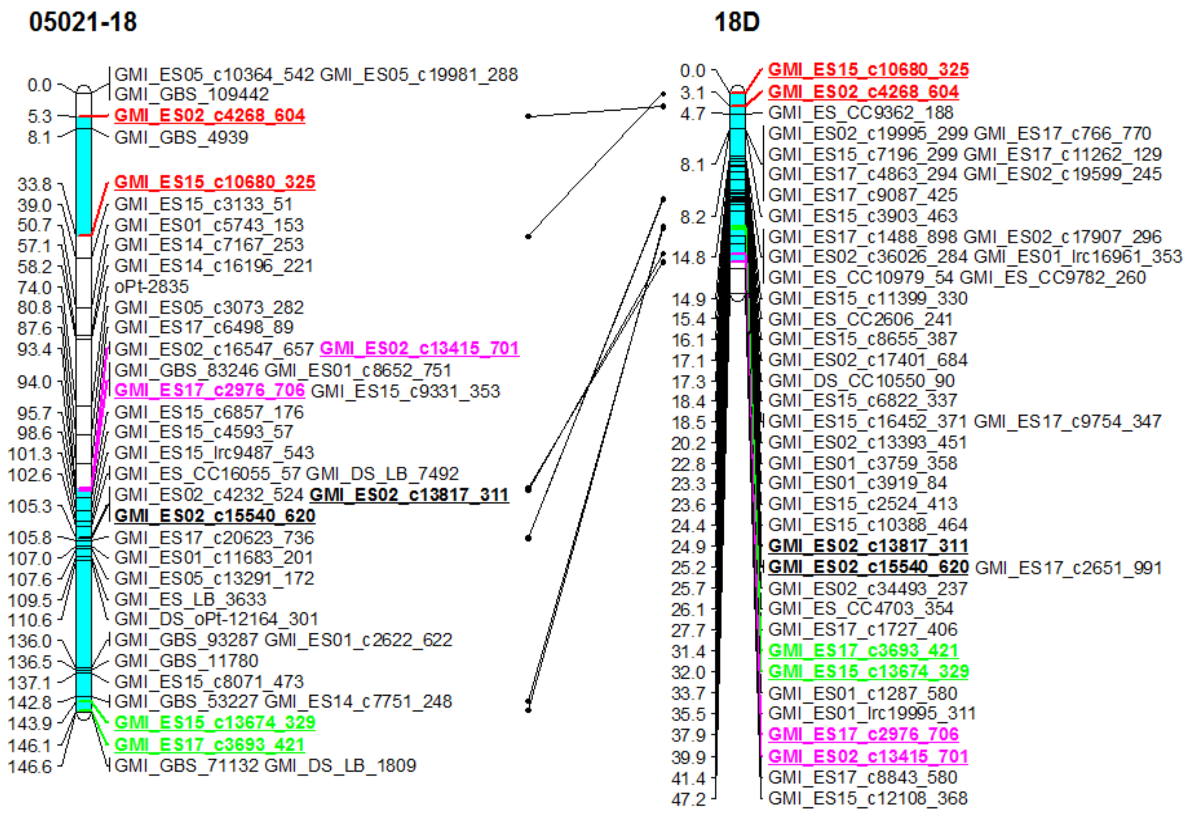

Figure 3.8. Conserved segments between LG 05021-18, and oat chromosome 18D.

05021-19

0.0 GMI_ES02_lrc13785_433 GMI_ES_LB_11028
GMI_ES_LB_11026
1.1 GMI_ES05_c6960_544 GMI_ES02_c2449_552
GMI_ES02_lrc38264_316
2.2 GMI_ES_LB_8506
GMI_ES05_c2225_383 GMI_ES05_c16739_323
GMI_ES02_lrc38280_700 GMI_ES15_c6243_310
3.3 GMI_ES02_c14349_258 GMI_ES03_c8567_90
GMI_ES01_c12979_488 GMI_ES05_c9412_222
GMI_ES17_c10189_970 GMI_ES17_c5870_387
GMI_ES01_c677_223
3.8 GMI_ES_LB_11401 GMI_ES01_c13113_119
GMI_ES15_c794_171 GMI_GBS_28206
14.6 GMI_ES22_c10247_408
19.3 oPt-16024
37.6 GMI_ES01_c1793_450
54.0 oPt-15971
55.2 oPt-3224
57.0 oPt-16979
62.9 oPt-11532
64.1 oPt-11426
68.8 GMI_ES22_c11596_73 GMI_ES15_c12694_287
72.1 GMI_ES22_c9203_288 GMI_ES03_c10715_468
74.8 GMI_ES02_c20190_105
76.4 GMI_ES01_c1107_182
76.9 GMI_ES01_c19628_354
78.3 GMI_ES22_c16946_366
80.5 GMI_ES05_c4438_302 GMI_GBS_25081
GMI_ES17_c3050_316
81.2 GMI_GBS_92025
GMI_ES01_c2436_405 GMI_ES13_c14470_87
81.7 GMI_ES21_c6485_431 GMI_GBS_10457
GMI_ES15_c8569_380
82.2 GMI_ES16_c20195_287 GMI_GBS_100319
GMI_ES15_c17597_267 GMI_ES17_c7039_546
GMI_DS_LB_9310 GMI_ES_LB_11958
83.3 GMI_ES_LB_11974 GMI_ES02_c1833_408
GMI_ES05_c14901_270 GMI_DS_LB_6019
83.8 GMI_ES22_c18838_446 GMI_ES_CC15240_111
84.3 GMI_ES03_c9122_309 GMI_DS_LB_950
84.8 GMI_ES02_c16381_584
85.9 GMI_ES_LB_11633 GMI_GBS_96826
GMI_ES02_c3016_666
93.3 GMI_ES14_c118_502
109.1 GMI_ES05_c2066_503 GMI_ES22_c4463_275
114.3 GMI_GBS_3525
134.5 GMI_ES17_c7110_570
135.0 GMI_ES01_c27869_512 GMI_GBS_80511

19A

0.0 GMI_DS_oPt-13151_665
2.0 GMI_ES02_c14927_478
7.5 GMI_ES01_c14349_357
13.1 GMI_ES01_c19245_178
19.3 GMI_ES01_c507_760
28.6 GMI_ES15_c12694_287
30.0 GMI_ES17_c2454_883
39.5 GMI_SNP5469_1
39.7 GMI_ES15_c6155_291
40.8 GMI_ES17_c1912_929 GMI_ES01_c13342_301
43.4 GMI_ES01_c19628_354
43.7 GMI_ES01_lrc12590_96
44.2 GMI_ES17_c3195_668
46.7 GMI_ES17_c19385_93
47.2 GMI_ES_CC11076_204
47.6 GMI_ES01_c18839_132
49.4 GMI_DS_CC10481_156
49.5 GMI_ES02_c7768_318
49.7 GMI_ES01_c27869_512
49.9 GMI_ES_CC15389_69
50.7 GMI_ES02_lrc34335_153
51.1 GMI_ES02_lrc12182_165
51.3 BM_130b
51.4 GMI_ES01_c12378_433
51.5 GMI_ES15_c18828_128
51.7 GMI_ES15_c12274_189
51.8 GMI_ES01_c796_180
51.9 GMI_ES_CC15240_111
52.6 GMI_ES01_c10310_366
53.9 GMI_ES15_c8191_415
54.2 GMI_ES02_c22225_492
54.8 AM87-2
55.2 GMI_ES_CC13848_210
55.5 GMI_DS_oPt-15595_189 GMI_ES01_c1511_1015
56.0 BM_874
60.1 AB_STS_24
67.3 BM_219
68.9 GMI_DS_CC6556_81
69.8 GMI_ES15_c15790_334
70.7 GMI_ES15_c6102_442 GMI_ES17_c8412_398
70.9 GMI_ES15_c11012_143
72.8 BM_692a
73.5 GMI_ES01_c25884_181 GMI_ES02_c6784_277
75.2 GMI_DS_CC11093_89
75.8 GMI_ES02_c4352_145
81.4 GMI_ES02_c26937_168
82.3 GMI_DS_CC885_419
84.5 GMI_ES02_c6122_167
87.7 GMI_DS_oPt-12991_114
88.5 GMI_ES02_lrc13785_433
91.6 GMI_ES02_c2449_552
97.4 GMI_DS_CC6354_109
GMI_ES02_c14349_258 GMI_DS_CC12000_73
97.5 GMI_ES01_c677_223 GMI_ES01_c13113_119
GMI_ES15_c5819_415 GMI_ES17_c5870_387
GMI_ES15_c794_171
97.7 GMI_ES17_c2598_325 GMI_ES15_c9521_561
GMI_ES15_c2046_330
98.0 GMI_ES02_c12488_534
98.8 GMI_ES_CC15133_247
99.6 GMI_ES17_c8066_237
100.7 BM_74
104.2 GMI_ES02_c631_591
106.3 GMI_DS_CC3275_96
108.8 GMI_ES02_c15682_295 GMI_ES02_c7238_473
109.2 GMI_ES02_c36828_363
109.4 GMI_ES02_lrc18911_190
115.4 GMI_ES_CC11271_65
115.5 GMI_ES02_c12761_183

Figure 3.9. Conserved segments between LG 05021-19, and oat chromosome 19A.

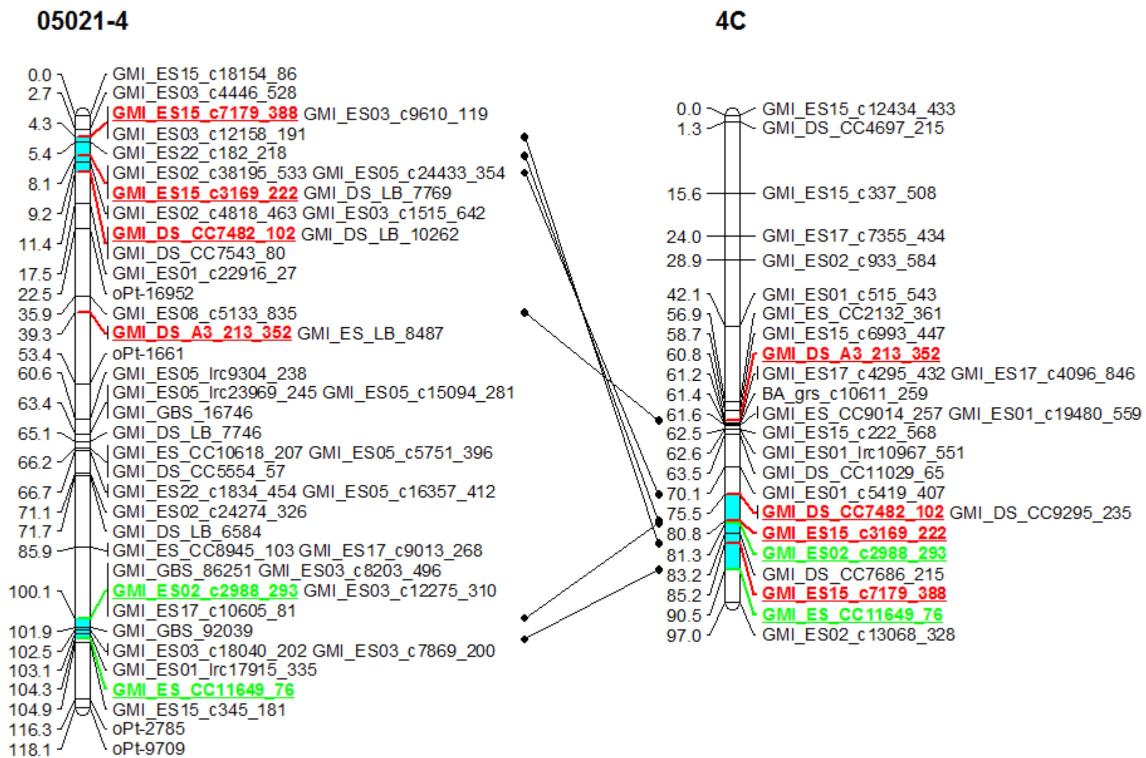

Figure 3.10. Conserved segments between LG 05021-4, and oat chromosome 4C.

3.5.1.2. Population 05026 and oat consensus map

171 markers were shared covering 647.3 cM in population 05026 (55% of the total map), and 552 cM in the oat consensus map (30% of the map). Marker order within the syntenic segments was well conserved on linkage groups 05026-3, 05026-4, 05026-5.2, 05026-11, 05026-10, 05026-14.2, 05026-19, and 05026-20.1. The same order was observed, but in reverse, for linkage groups 05026-1.1, 05026-5.1, 05026-6, 05026-12.1, 05026-14.1, and 05026-15. On LG 05026-1.2, marker ES15_c5908_278 switched order with marker ES15_c6153_392 (Figure 3.11, red). Three small inversions were observed in LG 05026-2. Marker ES15_c7706_583 located at the end of LG 05026-2 (position 61.3 cM) had been mapped at the beginning of oat chromosome 2C (Figure 3.12, red). Cosegregating markers DS_CC8468_91and ES_CC11290_204 changed

order with other cosegregating markers ES01_c1635_353 and ES_CC8700_285 at position 5.8 cM for the former and 6.4 cM for the latter (Figure 3.12). Cosegregating markers ES17_lrc20311_707 and ES_CC3989_242 (Figure 14, blue) at position 0 cM changed order with a group of 10 cosegregating markers at position 0.6 cM (Figure 3.12, violet). Marker ES17_c5784_752 (Figure 3.15, blue) positioned at 45.3 cM in LG 05026-3 changed order with a group of 5 cosegregating markers at position 46.5 cM (Figure 3.13, red). Marker ES02_lrc16798_330 located at the beginning of LG 05026-13.1 had been mapped at the end of oat chromosome 13A (Figure 3.14, red). According to Singh and Kolb, 1991 the differences noted in the ordering of markers between the two populations and the oat consensus map are an indication that genomic rearrangements and intervarietal chromosome interchanges exist in the genome of cultivated oat.

3.5.2. Segregation distortion

Twenty five percent of the markers tested for population 05021, and 9% for population 05026 showed significant distortion from the expected segregation ratio. Similar results to those obtained for population 05026 (Zhu and Kaeppler, 2003; Portyanko et al., 2001; O'Donoughue et al., 1995) and for population 05021 (Hizbai et al., 2012; O'Donoughue et al., 1992) had been previously reported in other mapping studies. Zhu and Kaeppler, 2003 reported 9% of segregation distortion in the Ogle/MAM 17-5 mapping population, Portyanko et al., 2001 reported 13% in the Ogle/TAM O-301 population, and O'Donoughue et al., 1995 reported 8% of segregation distortion in the Kanota/Ogle population. Hizbai et al., 2012 found out that 27% of the markers in the Dal/Exeter mapping population exhibited segregation distortion, and O'Donoughue et al., 1992 reported 19% of distortion in the diploid map *A. atlantica* x *A. hirtula*.

Figure 3.11. Conserved segments between LG 05026-1.1, 05026-1.2, and oat chromosome 1C.

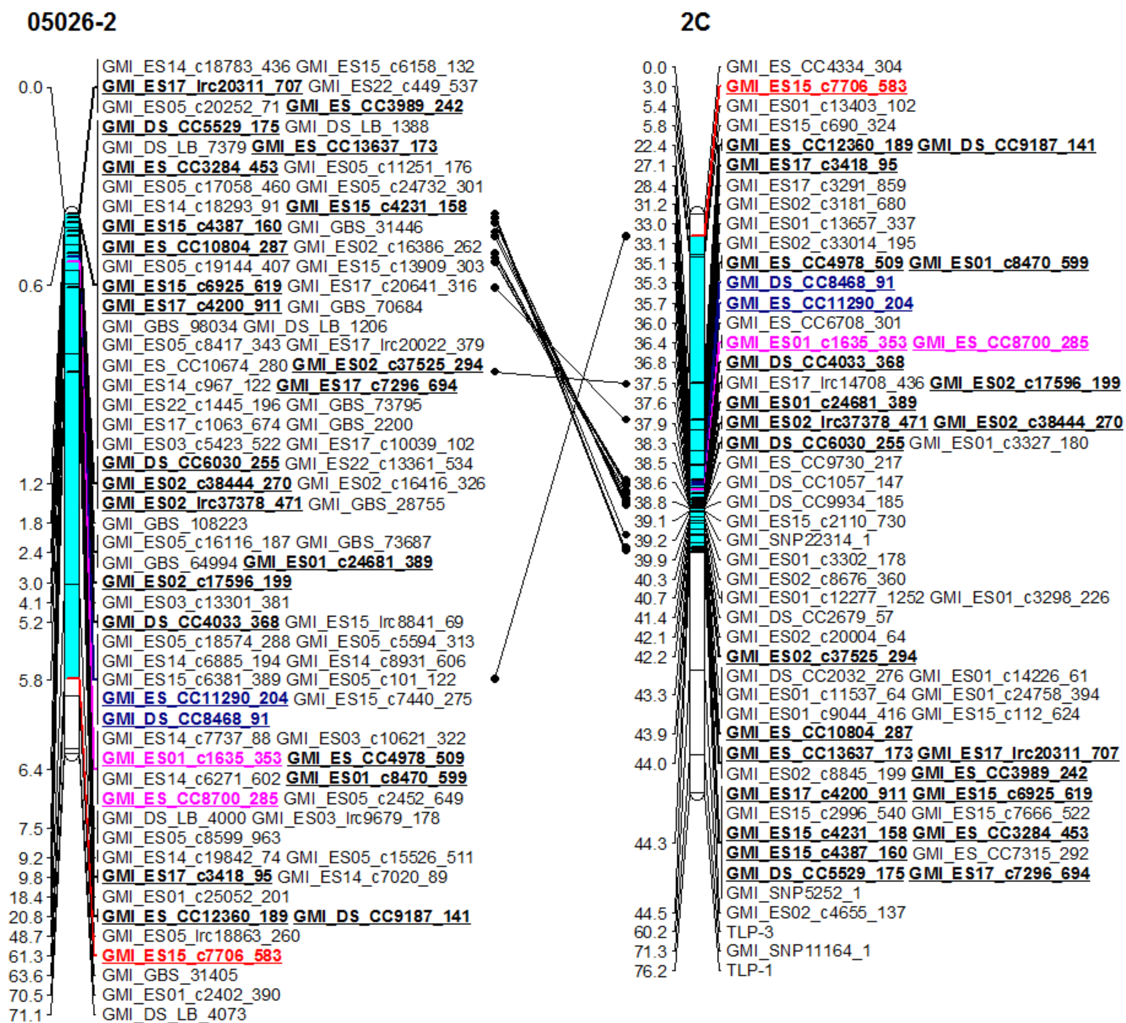

Figure 3.12. Conserved segments between LG 05026-2 and oat chromosome 2C.

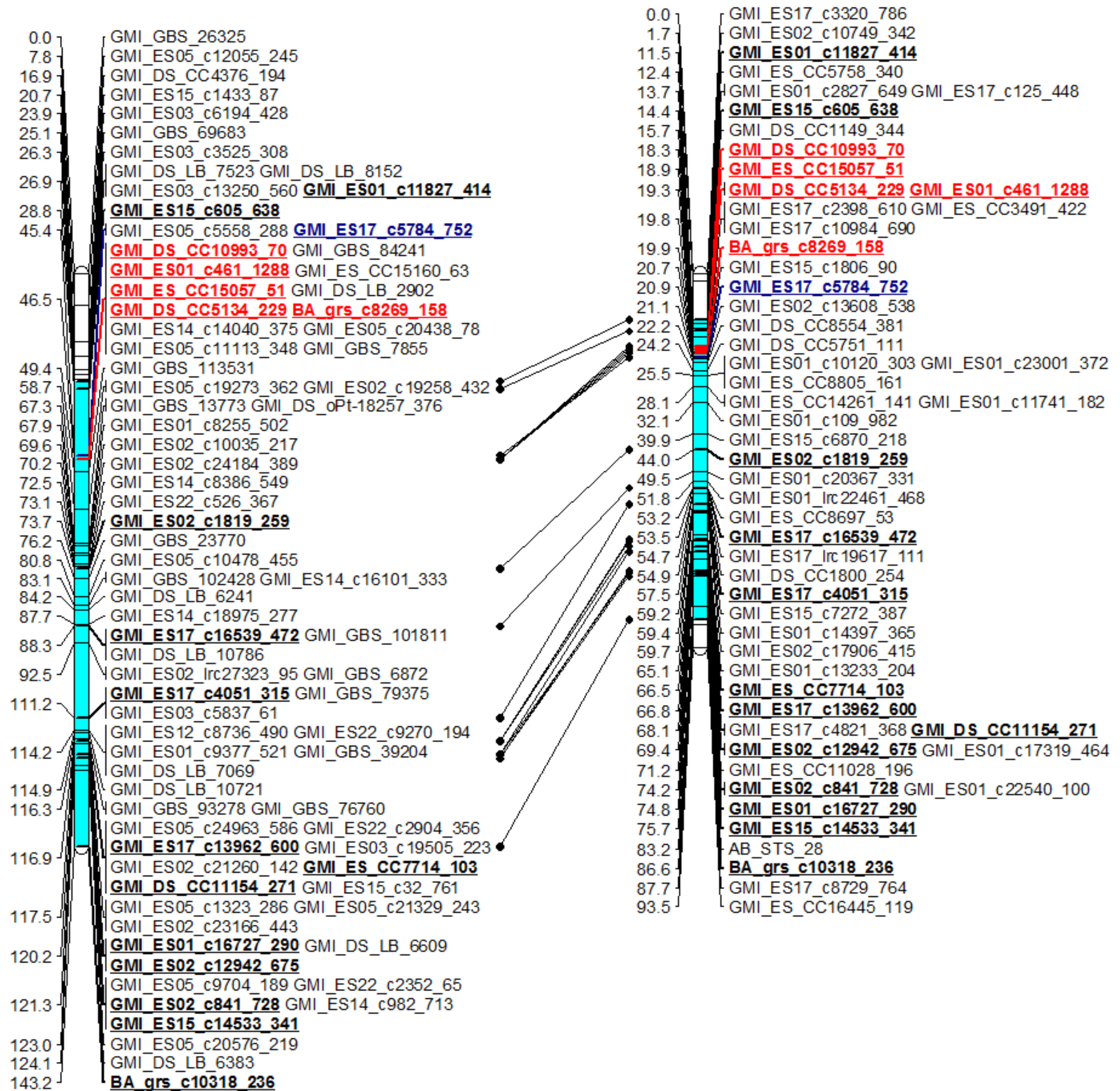

Figure 3.13. Conserved segments between LG 05026-3 and oat chromosome 3C.

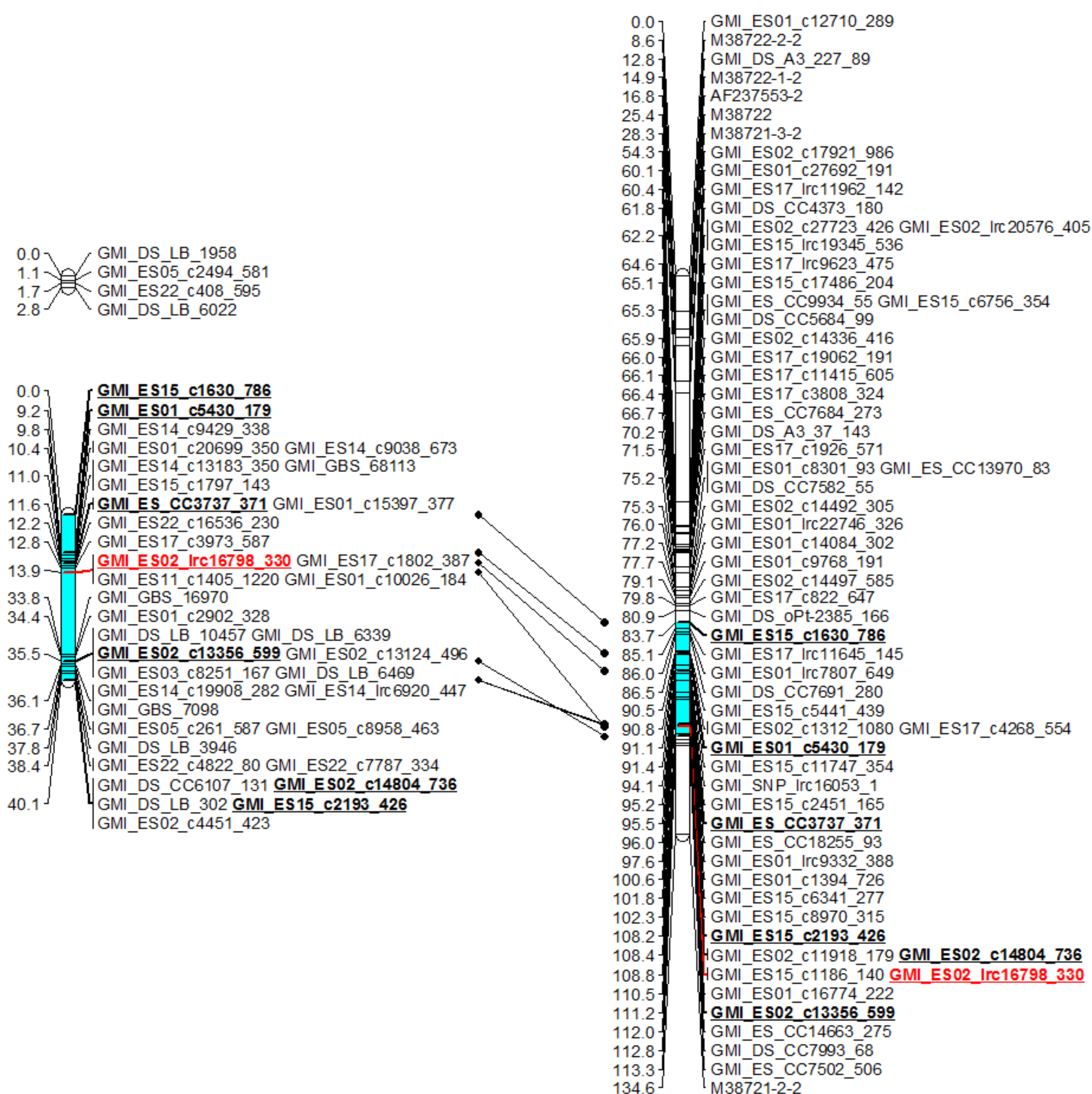

Figure 3.14. Conserved segments between LG 05026-13.1 and oat chromosome 13C.

Specific regions of segregation distortion had been reported in the Kanota/Ogle population KO 6, KO 11, KO 24 (O'Donoughue et al., 1995), the Ogle/MAM 17-5 population OM 1, OM 3 (Zhu and Kaeppler, 2003), and the Dal/Exeter population (Hizbai et al., 2012).

Those linkage groups showed homology with distorted regions reported in population 05021 and population 05026. KO 6 is homologous to 05026-13; KO 11, OM 3, and DE 7 are homologous to 05021-1.1 and 05021-16; KO 24 is homologous to LG 05021-16, and OM 1 is homologous to 05021-19. The presence of segregation distortion in the same region across different mapping populations supports the evidence of genes affecting distortion on those genomic regions. All the distorted markers in population 05021 were skewed toward only one parent. One possible cause could be inadvertent selection due to small population size during the development of the population 05021 (Kianian et al., 2001). Liu et al., 2010 reported that in RIL populations, a high proportion of segregation distortion may be due to artificial sampling and natural selection of many generations. Chromosomal microrearrangements related to introgression of alien segments carrying desirable genes from wild germplasm (Portyanko et al., 2001) and chromosome translocation (Liu et al., 2010) can be also the cause of segregation distortion. A major translocation, between chromosome 7C and 17, in oat affected mapping in other populations (Jellen and Beard, 2000). As spring-type oats (*A. sativa*), the parents used in this study are expected to have the same allele of this translocation, and consequently no extreme distortion was found on LGs 05021-7.1, 05021-7.2, 05021-7.3, 05021-7.4 homologous to oat chromosome 7C-17A. It is possible then, that other chromosomal rearrangements and minor translocations have influenced segregation distortion in population 05021. The distorted markers, in population 05026, were skewed toward both parents. The apparent preferential transmission of one parental genotype indicated that the regions in question may contain genes that affect gamete and/or hybrid viability (O’Donoughue et al, 1995; Zhu and Kaeppler, 2003).

3.5.3. Map size and cosegregation

The map developed for population 05021 was significantly longer than the one developed for population 05026. The parents used to develop population 05026 are more closely related than those used to develop population 05021. One of the parents of population 05026 'Souris' is a sister line of 'HIFI' and the other parent 'ND030299' has 'HIFI' as a parent (McMullen, communication personal). The shorter map observed in population 05026 may be the result of inadequate polymorphism in some regions with similar ancestry (Hizbai et al., 2012). 487 markers polymorphic in population 05021 were also mapped in population 05026. The 1.5 fold reduction in recombination for regions detected by these markers is quite similar to the two-fold decrease in total length for the two maps. Similar ancestry in some regions, combined with reduced recombination is the best explanation for the differences between the two maps. The map size of the oat consensus map (1838 cM) was in between the two populations (Oliver et al., 2013). Similar map size had been reported for population 05021 (Jin et al., 2000) and also for population 05026 (Zhu and Kaeppler, 2003). Zhu and Kaeppler, 2003 and Kianian et al., 2001 indicated also that map size can be affected by mapping strategy and reduced recombination frequencies.

In general, 45% of the markers in population 05021 and 61% in population 05026 cosegregated with other mapped markers. This amount of cosegregation is much more than those reported in other oat map studies (Zhu and Kaeppler, 2003; Jin et al., 2001; O'Donoughue et al., 1995). The principal reason is that many of the EST markers turned out to be closely linked, because initially those markers were designed for wheat and barley (Howarth et al., 2013).

3.6. Conclusion

In conclusion, DNA markers are useful in the construction of genetic maps of different cereal species (Heun et al. 1991; Messmer et al. 1999; Ramsay et al. 2000). Mapping populations obtained as a result of crossing two different homozygous parents are particularly useful in the process of creating these maps. Molecular breeding in oats has until recently been limited due to lack of available markers. The use of next-generation sequencing (NGS) for genotyping and comparative genomics has revolutionized the field of genetics by the introduction of sequence-based SNP markers. Recently a consensus map for hexaploid oats has been constructed using six mapping populations. Many of the markers placed in the two maps, reported in this study, are not in the consensus map. It is expected that some of the information provided here can be applied to improve the oat consensus map. The constructed maps can also be used in the mapping of quantitative trait loci for important agronomic traits in oats. Future studies should focus in the construction of a physical map for the hexaploid oat, and would constitute the first step in sequencing the oat genome.

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CHAPTER 4. GENETIC ANALYSIS OF QUANTITATIVE TRAIT LOCI AFFECTING AGRONOMIC AND GRAIN QUALITY TRAITS IN TWO SPRING OAT POPULATIONS

4.1. Abstract

Grain yield, test weight, 1000 kernel weight, heading date, and plant height are major agronomic traits in cultivated oat (*Avena sativa L.*). On the other hand, β-glucan content, oil content, groat percentage and dehulling efficiency are major quality and grain physical traits. Information regarding quantitative trait loci affecting those traits would facilitate the development of oat cultivars with desirable quality. QTLs for agronomic and grain physical traits were mapped and characterized in two spring oat populations derived from the crosses 'ND991151/ND030299' (population 05021 – 97 RILs) and 'Souris/ND030299' (population 05026 – 93 RILs). The two populations were evaluated for 4 years at Fargo, ND and 2 years at Casselton, ND. Composite Interval Mapping (CIM) from Windows QTL Cartographer was used for QTL analysis with two framework maps consisting of 640 molecular markers for population 05021, and 398 molecular markers for population 05026, respectively. Nineteen genomic regions on 14 linkage groups were significantly associated with agronomic and grain chemical traits in the population 05021. Fourteen genomic regions on 12 linkage groups were identified for agronomic traits in the population 05026. The same genomic region on LG 05021-16, from 19.4 to 45.8 cM, was associated with thin kernels, test weight, 1000 kernel weight, and oil content. Two QTLs affecting grain yield and test weight had been mapped at the exact same position on LG 05021-34. LG 05026-19 loci, from position 23.7 to 47 cM, and had strong effects on heading date, plant height, and grain yield. These correlated responses could be due to linkage of the

underlying QTLs or to pleiotropy. The QTLs consistently detected across environments and between the two populations could serve as starting points for marker-assisted selection.

4.2. Introduction

Plant breeding consists essentially of the selection of specific plants with desirable traits with the objective to assemble more desirable combinations of genes in new varieties (Collard and Mackill, 2008). Grain yield, test weight, 1000 kernel weight, heading date, and plant height are major agronomic traits in cultivated oat (*Avena sativa L.*). On the other hand, β-glucan content, oil content, groat percentage and dehulling efficiency are major quality and grain physical traits. Identification of genes determining important functional agronomic traits had been done in oats over the last 15 years (Okon and Kowalczyk, 2012). One of the most important mapping populations of cultivated oats was generated from the cross Kanota/Ogle (KO) containing 561 genetic markers making up 38 linkage groups (O'Donoughue et al., 1995). Restriction Fragment Length Polymorphism (RFLP) markers from the KO mapping population has been useful in identifying QTLs for grain yield, test weight, groat percentage, days to heading, and plant height (Siripoonwiwat et al., 1996). RFLP markers were also used to look for genomic regions linked to dwarf genes in the OT207/Kanota mapping population (Milach et al., 1997). Major QTLs controlling crown rust resistance and resistance to barley yellow dwarf virus had been reported in the KO population by Bush and Wise, 1996, and Barbosa-Neto et al., 2000, respectively. Vernalization and photoperiod response to heading date and plant height had been detected in the KO population (Holland et al., 1997) and in the Ogle/TAMO-301 (OT) population (Holland et al., 2002). Three Sequence-Characterized Amplified Region (SCAR) markers and one Cleaved Amplified Polymorphic Sequence (CAPS) marker had been identified to be linked with β-glucan content and oil content (Orr and Molnar, 2008). Major QTLs controlling resistance to *Puccinia*

coronata in the field had been detected in the OT mapping population mostly based on Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR) markers (Jackson et al., 2010). Diversity Array Technology (DArT) markers had been used to target genomic regions associated with oil content (Hizbai et al., 2012).

The discovery of single nucleotide polymorphism (SNP) markers was an important step in genetic studies. SNP markers are the most common type of DNA-based markers, represent the smallest unit of genetic variation, and can provide a rich source of useful molecular markers (Cho et al., 1999). For example, 90% of the human genome contains SNPs (Kwok and Gu, 1999). Tenaillon et al., 2001 reported 1 SNP every 104 base pair (bp) in the maize genome. Among 15 soybean genotypes, Van et al., 2005 found out that SNPs occurred at a frequency of 1 per 2038 bp in 16302 bp of coding sequence, and 1 per 191 bp in 16960 bp of noncoding regions. As a result, SNPs are useful in the construction of high-density genetic maps and QTL detection because they can be analyzed using high-throughput systems (Van et al., 2005).

The purpose of this chapter was to identify, using SNP and DArT markers, genomic regions associated with genes underlying the expression of several agronomically important traits in two recombinant inbred oat mapping populations.

4.3. Materials and methods

Genotypes from population 05021 and population 05026, described in chapter 2, were grown at two ND locations: Fargo and Casselton. Field experiments were conducted for 4 years at Fargo and during 2 years at Casselton for a total of 6 environments (years and locations combined). A square lattice design was used at Fargo and Casselton during 2008 and 2009 planting seasons as explained in chapter 2, whereas an augmented design was used at Fargo during 2011 and 2012 planting seasons (Table 4.1). The following agronomic traits: grain yield,

test weight, heading date, plant height, and thin kernels were evaluated in all the environments. 1000 kernel weight, oil content, β-glucan content, groat percentage, lodging, and dehulling efficiency were assessed only during the 2008 planting season. Genotypic means were obtained using the MIXED procedure from SAS (SAS Institute, Cary, NC) and JMP genomics 6.1 software (SAS Institute, Cary, NC). For the purpose of QTL mapping, and because significant genotype-by-environment interaction were observed for all traits (See chapter 2), combined genotypic means by year and by location were also obtained for a total of 6 combinations (Table 4.2).

Table 4.1. Description of environments used to evaluate two recombinant inbred oat populations for grain quality.

Environment	Location	Year	code	Design	Traits evaluated
1	Fargo	2008	Far08	Lattice	Yld, kwt, twt, head, height, brk
2	Fargo	2009	Far09	Lattice	Yld, twt, head, height, brk
3	Casselton	2008	Cass08	Lattice	Yld, kwt, twt, head, height, brk,
4	Casselton	2009	Cass09	Lattice	Yld, twt, head, height, brk,
5	Fargo	2011	Far11	Augmented	Yld, kwt, twt, head, height, brk, lodge
6	Fargo	2012	Far12	Augmented	Yld, kwt, twt, head, height, brk,

Table 4.2. Description of combined environments used to evaluate two recombinant inbred oat populations for grain quality.

Environment	Name	Description	Code	Traits evaluated
1	Combined 2008	Fargo 2008 + Casselton 2008	Comb08	Yld, kwt, twt, head, height, brk,
2	Combined 2009	Fargo 2009 + Casselton 2009	Comb09	Yld, twt, head, height, brk,
3	Combined Fargo	Fargo 2008 + Fargo 2009	Combfar	Yld, twt, head, height, brk
4	Combined Casselton	Casselton 2008 + Casselton 2009	Combcass	Yld, twt, head, height, brk,
5	Combined 08/09	Fargo 2008 + Casselton 2008 + Fargo 2009 + Casselton 2009	Comb0809	Yld, twt, head, height, brk,
6	Combined 11/12	Fargo 2011 + Fargo 2012	Comb1112	Yld, twt, head, height, brk,

Yld=grain yield, kwt=1000 kernel weight, twt=test weight, head=heading date, height=plant height, brk=percentage of broken kernels

Agronomic and chemical traits were quantitatively mapped by Composite Interval Mapping (CIM) using Windows QTL Cartographer version 2.5 (Wang et al., 2007). CIM method is a combination of interval mapping and multiple regression. Interval mapping fit a linear model at every position in the genome whereas multiple regression fit covariates to control linked and unlinked QTL effects and reduce the model residual (Silva et al., 2012). The general CIM statistical model is as follow:

$$y_i = \mu + Z_i B + \sum_{r=1}^{m} X_{ir}\beta_r + e_i$$

Where,

y_i = phenotypic trait value of genotype i

μ = overall mean

Z_i = predictor variables corresponding to the effects of a putative QTL

B = Effects of a putative QTL which depends on the mating design

X_{ir} = Predictor variables corresponding to the rth cofactor marker

β_r = Coefficient associated with rth cofactor marker

e_i = random error.

A forward stepwise regression and backward elimination with the standard CIM model with the following parameters, a probability of 0.1 to enter and leave the model, a window size of 5 cM, and a walk speed of 1 cM, was used to search for the main QTLs. A LOD threshold setting for significant QTLs was determined based on 1000 permutation tests at a significance level of 0.05. QTLs detected using this procedure were considered valid if they were observed at the same position in the genome in at least 50% of the environments and combined environments evaluated, when the signs of the additive effects were consistent across environments (Portyanko et al., 2005). Quantitative trait loci (QTLs) with overlapping support intervals for the same trait

were considered as a single QTL. If the overlapping support intervals represented different traits, the QTLs were assumed to be linked or pleiotropic.

4.4. Results

4.4.1. Grain yield

A total of 5 QTLs, associated with grain yield, were identified in the population 05021on 3 linkage groups (LG). Linkage group 05021-7.1 corresponding to oat chromosome 7C-17A had 3 QTLs whereas one QTL was observed on each of LG 05021-8 (oat chromosome 8A) and 05021-34. The QTL with the largest effect, which accounts for an average of 21% of the phenotypic variation in grain yield, was located on LG 05021-34 in a region flanked by the DArT markers oPt-15309 and oPt-15736 (Figure 4.1 – 05021-34). This QTL has been consistently observed on 7 of the 12 environments evaluated and the positive allele was contributed by the lower yielding parent 'ND991151' with and additive effect of 247 kg/ha (Table 4.3-I). The second major QTL, accounting for an average of 15% of the phenotypic variation, was mapped in LG 05021-8 in a region containing 3 EST markers (ES15_c11400_361, ES13_c3498_934, and ES03_c695_117), the GBS marker GBS_111982, and the DArT marker oPt-5729 (Figure 4.1 – 05021-8). This QTL was observed in 5 of the 12 environments evaluated and the positive allele was contributed by the higher yielding parent 'ND030299' with an additive effect of 300 kg/ha (Table 4.3-I). Three smaller QTLs, explaining only 11% of the phenotypic variation, were detected in 10 environments at 3 positions along LG 05021-7.1 (Table 4.3-I and Figure 4.1 – 05021-7.1).

On the basis of composite interval mapping (CIM), 3 minor QTL significantly associated with grain yield were identified in the population 05026. All alleles for grain yield were derived from 'Souris', the higher yielding parent. The QTL on LG 05026-5.1, homologous to oat

chromosome 5C, was located in a region containing 9 EST markers and 1 GBS marker tightly linked (Figure 4.2 – 05026-5.1), explained 12% of the total phenotypic variation, and was consistently detected in 8 of 12 environments. The second QTL, observed in 6 environments and accounting for 10% of the phenotypic variation, was mapped on LG 05026-16.1, homologous to oat chromosome 16A, in a region of 10 cM involving 8 EST markers, 2 GBS markers, and 1 DArT marker (Figure 4.2 – 05026-16.1). Located in a region of 10.5 cM, involving 6 EST markers and 2 GBS markers (Figure 4.2 – 05026-19), the third QTL was identified on LG 05026-19 and explained only 11% of the phenotypic variation (Table 4.5-I).

4.4.2. 1000 kernel weight

A total of 3 QTLs, associated with 1000 kernel weight and explaining together 49% of the total phenotypic variation among the progenies, were identified on 3 LGs of population 05021 in all the 3 environments evaluated. All the positive alleles were contributed by 'ND991151', the parent with higher 1000 kernel weight. The QTL with the largest effect, which explains 34% of the phenotypic variation with an additive effect of 2.3 g, was located on LG 05021-16 homologous to oat chromosome 16A at position 62.8 cM and tightly linked to the EST marker ES02_c12776_148 (Figure 4.1 – 05021-16). The two remaining QTLs, with minor effects accounting for 8% and 7% with an additive effect of 1 g, were identified on LG 05021-14.1 (oat chromosome 14D) and LG 05021-18 (oat chromosome 18D), respectively (Table 4.3-II and Figure 4.1 – 05021-14.1 and 05021-18).

A total of 3 minor QTL for 1000 kernel weight, explaining 27% of the total phenotypic variation, were discovered in the population 05026 on linkage groups LG 05026-1.1, LG 05026-5.1, LG 05026-5.2, and were observed in all the environments evaluated. Both parents

contributed alleles for higher 1000 kernel weight. ‘Souris’ provided two alleles and ‘ND030299’ provided one allele (Table 4.5-II and Figure 4.2 – 05026-1.1, 05026-5.1, 05026-5.2).

4.4.3. Test weight

A total of 3 QTLs, associated with test weight, were identified in the population 05021 on LG 05021-14.1 (oat chromosome 14D), LG 05021-16 (oat chromosome 16A), and LG 05021-34. These QTLs, all with minor effects, explained together 34% of the total phenotypic variation among the progenies. Positive alleles on LG 05021-16 and LG 05021-34 were contributed by the lower test weight parent ‘ND030299’ with an additive effect of 9 kg/m^3 and 6 kg/m^3, respectively. A positive allele on LG 05021-14.1 was contributed by ‘ND991151’, the parent with higher test weight with an average additive effect of 12 kg/m^3 (Table 4.3-III). The region on LG 05021-14.1 explained 13% of the phenotypic variation and involved 4 EST markers, 1 DArT marker, and 1 GBS marker (Figure 4.1 – 05021-14.1). A region on LG 05021-16, also associated with 1000 kernel weight, explained 9% of the phenotypic test weight variation and comprised a group of 7 EST markers and 2 DArT markers (Figure 4.1 – 05021-16). A group of 4 DArT markers are involved in the region of LG 05021-34 and explained 12% of the phenotypic variation (Figure 4.1 – 05021-34). This genomic region is also associated with grain yield.

A total of 3 QTLs were found to affect test weight in the population 05026 on linkage groups LG 05026-1.1, LG 05026-4, and LG 05026-5.1. All the positive alleles were contributed by ‘Souris’ the parent with higher test weight. Together, the 3 QTL explained 39% of the phenotypic variation. The QTL with the largest effect, accounting for 16% of the phenotypic variation and detected in 7 environments, was located on LG 05026-5.1, homologous to oat chromosome 5C, in a region flanked by the EST markers ES17_c18602_497, and ES05_c8792_292 (Figure 4.2 – 05026-5.1). The QTL on LG 05026-4 had the second highest

effect (explaining alone 12%) and had been identified in a region involving 6 EST markers and 2 DArT markers (Table 4.5-III and Figure 4.2 - 05026-4).

4.4.4. Thin kernels

Two QTLs, associated with thin kernels, were identified in the population 05021on genomic regions belonging to LG 05021-16 and LG 05021-31. The two positive alleles were contributed by ‘ND030299’, the parent with higher percentage of thin kernels. The QTL with the largest effects, which accounted for 14% of the phenotypic variation, was mapped on LG 05021-16 homologous to oat chromosome 16A at position 19.4 to 22.9 cM and was flanked by two EST markers: ES02_c15898_126 and ES02_c12776_148 (Figure 4.1 – 05021-16). This QTL was consistently detected in all the environments evaluated. The other QTL with minor effects explained only 9% of the phenotypic variation and was identified in 5 environments on LG 05021-31 on a region including the DArT marker oPt-8936 (Table 4.3-IV and Figure 4.1 - 05021-31).

A total of 3 QTLs significantly associated with thin kernels were detected in the population 05026 on linkage groups LG 05026-1.1, LG 05026-4, and LG 05026-8.1. Both parents contributed alleles for greater percentage of thin kernels with most contributed by the high thin kernels parent ‘ND030299’. Together, the 3 QTL explained 43% of the phenotypic variation associated with thin kernels. The QTL with the largest effect, explaining 23% of the phenotypic variation, was located on LG 05026-8.1, Homologous to oat chromosome 8A, in a genomic region involving 3 EST markers and 3 DArT markers (Figure 4.2 – 05026-8.1). The two other QTL with minor effects were identified on LG 05026-1.1 (oat chromosome 1C), and LG 05026-4 (oat chromosome 4C) (Table 4.5-IV).

4.4.5. Plant height

Two QTLs, found to affect plant height, were identified in the population 05021 on LG 05021-19 and LG 05021-6, and together explained 31% of the total phenotypic variation. The QTL with the largest effect (17% of the phenotypic variation) was located on LG 05021-19 homologous to oat chromosome 19A in a distorted region involving the EST marker ES01_c1793_450 and a group of 4 DArT markers: oPt-15971, oPt-3224, oPt-16979, and oPt-11532 (Figure 4.1 – 05021-19). This QTL was detected on all the environments evaluated and the positive allele was contributed by the taller parent 'ND991151' with an increase on plant height of 3.6 cm. The second QTL which accounted for 14% of the phenotypic variation was identified on LG 05021-6 (oat chromosome 6C) within also a distorted region flanking by the EST marker ES05_c1798_423 and the DArT marker oPt-11599 (Figure 4.1 – 05021-6). The positive allele of this QTL, observed only in 3 environments, was contributed by the shorter parent 'ND030299' with a reduction on plant height of 3.5 cm (Table 4.3-V).

Two QTL, associated with plant height and explaining 32% of the total phenotypic variation, were identified in the population 05026. Souris-derived alleles detected on LG 05026-19, in a region involving 4 EST markers and 2 GBS markers (Figure 4.2 – 05026-19), were associated with shorter plants, reduced plant height by 2.6 cm, and explained 21% of the phenotypic variation. On the other hand, the ND030299 alleles on LG 05026-6 explained 11% of the phenotypic variation and were associated with taller plants (Table 4.5-V).

4.4.6. Heading date

Three major QTLs, accounting for 54% of the total phenotypic variation observed for heading date, were discovered in the population 05021 on 3 LGs. The first major QTL and the most important one, detected in 11 of 12 environments, was located on LG 05021-1.2 (oat

chromosome 1C) at a peak position of 31.4 to 46.7 cM involving 3 EST markers, 4 DArT markers, and 1 GBS marker (Figure 4.1 – 05021-1.2). The positive allele for this QTL, explaining 19% of the phenotypic variation, was contributed by the earlier parent 'ND991151' and reduced heading date by 1.1 day. The second major QTL was mapped on LG 05021-14.1 (oat chromosome 14D) in a region flanking by two EST markers: ES15_c12071_400 and ES01_c13432_100 (Figure 4.1 - 05021-14.1) and explained 16% of the phenotypic variation. The positive allele for this QTL, detected in 8 of 12 environments, was contributed by the later parent 'ND030299' and increased heading date by 1 day. The third major QTL, which accounted for 19% of the phenotypic variation, was identified on LG 05021-20 (oat chromosome 20D) in a region flanking by the EST marker ES01_c9095_194 and the DArT marker DS_oPt-2653_332 (Figure 4.1 – 05021-20). The positive allele for this QTL that increased heading date by 1.2 days was contributed by 'ND030299' (Table 4.3-VI).

Five QTL for heading date in five different linkage groups were identified in the population 05026. Together, the main effect of these QTL accounted for 55% of the phenotypic variation observed for heading date. 'ND030299', the later parent, contributed alleles that increased heading date at four QTL on linkage groups LG 05026-20.1, LG 05026-19, LG 05026-6, and LG 05026-7.1 which respectively accounted for 18, 8, 10, and 9% of the phenotypic variation. 'Souris', the earlier parent, contributed alleles for decreased heading date at one QTL on linkage group LG 05026-3 and accounted for 10% of the phenotypic variation. The region on LG 05026-20.1 flanked by the EST markers ES01_c22619_143 and ES01_c9095_194 (Figure 4.2 – 05026-20.1), showed the strongest association with heading date and was consistently detected in all the environments evaluated. The 'ND030299' allele at this locus was associated with an increase in 1.2 days to heading. The second most important locus associated with

heading date involved a group of 6 EST markers, 3 DArT markers, and 1 GBS marker on LG 05026-3 (Figure 5.2 – 05026-3). At this locus, the ‘Souris’ allele contributed to a decrease in 0.9 day to heading (Table 4.5-VI).

4.4.7. Biochemical and other agronomic traits in population 05021

A total of 12 QTLs in 9 LGs were associated with β-glucan content, oil content, groat percentage, dehulling efficiency, and lodging (Table 4.4). At 1 cM from the EST marker ES17_c24_198 on LG 05021-12 (Figure 4.1 – 05021-12), homologous to oat chromosome 12D, only one QTL was detected for β-glucan content. This locus, derived from the parent with higher β-glucan content ‘ND030299’, accounted for 22% of the total phenotypic variation with an additive effect of 0.4% (Table 4.4-I).

The variation in oil content was associated with 2 QTLs. The two loci explain 43% of the total phenotypic variation among the progenies. One of them with the largest effects was detected on LG 05021-16 (oat chromosome 16A) on a distorted region flanked by two EST markers (Figure 4.1 – 05021-16) and explain 35% of the phenotypic variation. The positive allele was derived from ‘ND030299’ with an additive effect of 1.4% (Table 4.4-II). Linked to EST marker ES02_c3604_151 (Figure 4.1 – 05021-1.1), the second QTL with minor effects was identified in a distorted region on LG 05021-1.1 homologous to oat chromosome 1C. This locus explained only 8% of the phenotypic variation and was derived from ‘ND991151’ with an additive effect of 0.6% (Table 4.4-II).

Two QTLs found on LG 05021-13 and 05021-14.1 explained 28% of the phenotypic variation observed in groat percentage. Both alleles that increased groat percentage were contributed by ‘ND991151’. The QTL on LG 05021-13 was linked to GBS marker GBS_16970 (Figure 4.1 - 05021-13) at position 95.4 cM and it is also associated with dehulling efficiency

(Table 4.4-IV) whereas the one on LG 05021-14.1 was located at 1 cM from EST marker ES_CC7903_312 (Table 4.4-III). Lodging was associated with two minor QTLs on LG 05021-1.1 and 05021-18, and together explained 23% of the total phenotypic variation. Both loci were derived from 'ND991151' (Table 4.4-V).

4.5. Discussion

4.5.1. Comparison to other QTLs in oat

A good comparison of QTL across populations requires maps with homolog or homeolog linkage groups (Dekoeyer et al., 2004). The Kanota/Ogle mapping population (O'Donoughue et al., 1995) was the most used for the last 20 years in the identification of genomic regions associated with agronomic, disease, and grain quality traits in cultivated oats. The other maps published in the last ten years including the Terra/Marion (TM) population (Dekoeyer et al., 2004), the Kanota/Marion (KM) population (Groh et al., 2001), the Ogle/TAM O-301 (OT) population (Portyanko et al., 2001), the MN841801-1/Noble-2 (MN) population (Portyanko et al., 2005), the Ogle/MAM17-5 (OM) population (Zhu and Kaeppler, 2003), the Aslak/Matilda (AM) population (Tanhuanpaa et al., 2012), the OT207/Kanota (OK) population (Milach et al., 1997), and the Dal/Exeter (DE) population (Hizbai et al., 2012) had been aligned, when possible, with the KO mapping population. The alignment between the recent anchored oat consensus map (Oliver et al., 2013) and the expanded KO map (Tinker et al., 2009) make possible accurate comparison of QTLs between population 05021, 05026, and the populations mentioned before using the oatgenes database (http://avena.agr.gc.ca/oatgenes).

Table 4.3. Genomic regions significantly associated with agronomic traits identified by composite interval mapping (CIM) in population 05021.

LG	OC	Environments observed	Peak position cM	Flanking position cM	LOD Score Average	R^2 Average	Additive effect	Positive allele
				I. Grain yield				
05021-7.1	7C-17A	10/13	34.1-34.7	24.1-45.3	3.0	0.10	328	ND030299
			79.8-81.5	75.4-84.0	3.3	0.09	245	ND991151
			93.7-97.7	90.2-100.5	3.6	0.13	306	ND991151
05021-8	8A	5/13	66.2-70.9	65.1-72.8	4.2	0.14	332	ND030299
			76.3-84.3	75.3-87.0	4.1	0.15	268	ND030299
05021-34	-	7/13	5.0-6.7	1.8-8.7	7.5	0.25	324	ND991151
		5/13	12.2-13.7	10.2-15.2	4.9	0.17	170	ND991151
				II. 1000 kernel weight				
05021-14.1	14D	3/3	62.8	60.6-65.1	4.3	0.08	1.0	ND991151
05021-16	16A	3/3	19.9	19.4-23.1	14.6	0.34	2.3	ND991151
05021-18	18D	3/3	94.0	88.4-95.4	3.8	0.07	1.0	ND991151
				III. Test weight				
05021-14.1	14D	9/12	41.0-54.0	35.7-59.6	4.8	0.13	12.0	ND991151
05021-16	16A	7/12	19.4-36.8	11.8-40.4	3.1	0.09	8.7	ND030299
05021-34	-	9/12	0.0-12.7	0.0-19.0	3.5	0.12	6.0	ND030299
				IV. Thins kernels				
05021-16	16A	12/12	19.4-22.9	14.0-26.9	6.4	0.14	1.9	ND030299
05021-31	-	5/12	15.2-16.1	13.0-17.1	4.9	0.09	1.9	ND030299
				V. Plant height				
05021-19	19A	12/12	50.6-58.0	43.4-62.1	5.4	0.17	3.6	ND991151
05021-6	6C	3/12	66.8-71.8	64.8-81.0	4.3	0.14	3.5	ND030299
				VI. Heading date				
05021-1.2	1C	11/12	31.4-46.7	27.7-61.6	6.5	0.19	1.1	ND991151
05021-14.1	14D	8/12	63.3-64.3	60.6-66.0	5.5	0.16	1.0	ND030299
05021-20	20D	7/12	47.5-57.5	44.4-58.7	5.8	0.19	1.2	ND030299

Table 4.4. Genomic regions significantly associated with biochemical and other agronomic traits identified by composite interval mapping (CIM) in population 05021.

LG	OC	Peak position	Flanking position	LOD Score	R^2	Additive effect	Positive allele
		cM	cM	Average	Average		
			I. β-glucan content				
05021-12	12D	130.7	125.4-135.3	6.7	0.22	0.38	ND030299
			II. Oil content				
05021-16	16A	44.3	44.0-45.4	10.9	0.35	1.35	ND030299
05021-1.1	1C	17.5	16.0-19.1	3.4	0.08	0.61	ND991151
			III. Groat percentage				
05021-13	13A	95.4	94.3-97.7	3.9	0.13	1.39	ND991151
05021-14.1	14D	77.0	70.0-82.1	5.0	0.15	1.53	ND991151
			IV. Dehulling efficiency				
05021-13	13A	95.4	93.9-98.6	3.4	0.12	1.37	ND991151
			V. Lodging				
05021-1.1	1C	0.0	0.0-1.2	3.5	0.13	0.45	ND991151
05021-18	18D	80.8	78.2-86.8	3.8	0.10	0.45	ND991151

Table 4.5. Genomic regions significantly associated with agronomic traits identified by composite interval mapping (CIM) in population 05026.

LG	OC	Environments observed	Peak position cM	Flanking position cM	LOD Score Average	R^2 Average	Additive effect	Positive allele
				I. Grain yield				
05026-5.1	5C	8/13	67.2-76.8	53.5-78.0	3.4	0.12	156	Souris
05026-16.1	16A	6/13	41.4-51.4	40.1-55.5	2.7	0.10	140	Souris
05026-19	19A	9/13	37.7-48.2	23.4-48.2	2.9	0.11	171	Souris
				II. 1000 kernel weight				
05026-1.1	1C	3/3	12.3-15.8	8.5-19.8	2.8	0.10	0.7	Souris
05026-5.1	5C	3/3	79.2-80.3	76.8-85.5	2.6	0.09	0.7	ND030299
05026-5.2	5C	3/3	34.6	29.3-42.1	2.2	0.08	0.7	Souris
				III. Test weight				
05026-1.1	1C	9/12	21.6-23.4	15.7-27.4	3.4	0.11	6.0	Souris
			0.6	0.0-5.3	2.9	0.08	4.1	Souris
05026-4	4C	10/12	7.2-16.1	4.6-19.9	3.5	0.12	5.7	Souris
05026-5.1	5C	7/12	13.0-18.5	4.9-25.2	4.1	0.16	7.1	Souris
05026-18	18D	5/12	3.4-5.1	1.6-7.5	4.0	0.13	8.4	ND030299
				IV. Thins kernels				
05026-1.1	1C	11/12	15.2-16.8	7.2-21.8	3.9	0.11	0.145	ND030299
05026-4	4C	8/12	4.6-9.6	0.0-13.3	3.2	0.09	0.130	ND030299
05026-8.1	8A	12/12	5.1-8.2	0.0-10.4	7.8	0.23	0.196	Souris
				V. Plant height				
05026-19	19A	12/12	42.7-47.0	33.9-47.6	6.0	0.21	2.6	Souris
05026-6	6C	11/12	31.9-40.0	21.1-51.8	3.2	0.11	2.0	ND030299
				VI. Heading date				
05026-3	3C	12/13	112.2-129.1	102.8-139.1	3.3	0.10	0.9	Souris
05026-20.1	20D	12/13	6.0-7.0	0.0-7.5	6.1	0.18	1.2	ND030299
05026-19	19A	13/13	40.7-47.6	24.5-48.2	3.0	0.08	0.3	ND030299
05026-6	6C	6/13	4.8-7.2	0.9-19.9	3.7	0.10	0.9	ND030299
05026-7.1	7C-17A	10/13	56.9-64.5	51.9-69.5	2.8	0.09	0.8	ND030299

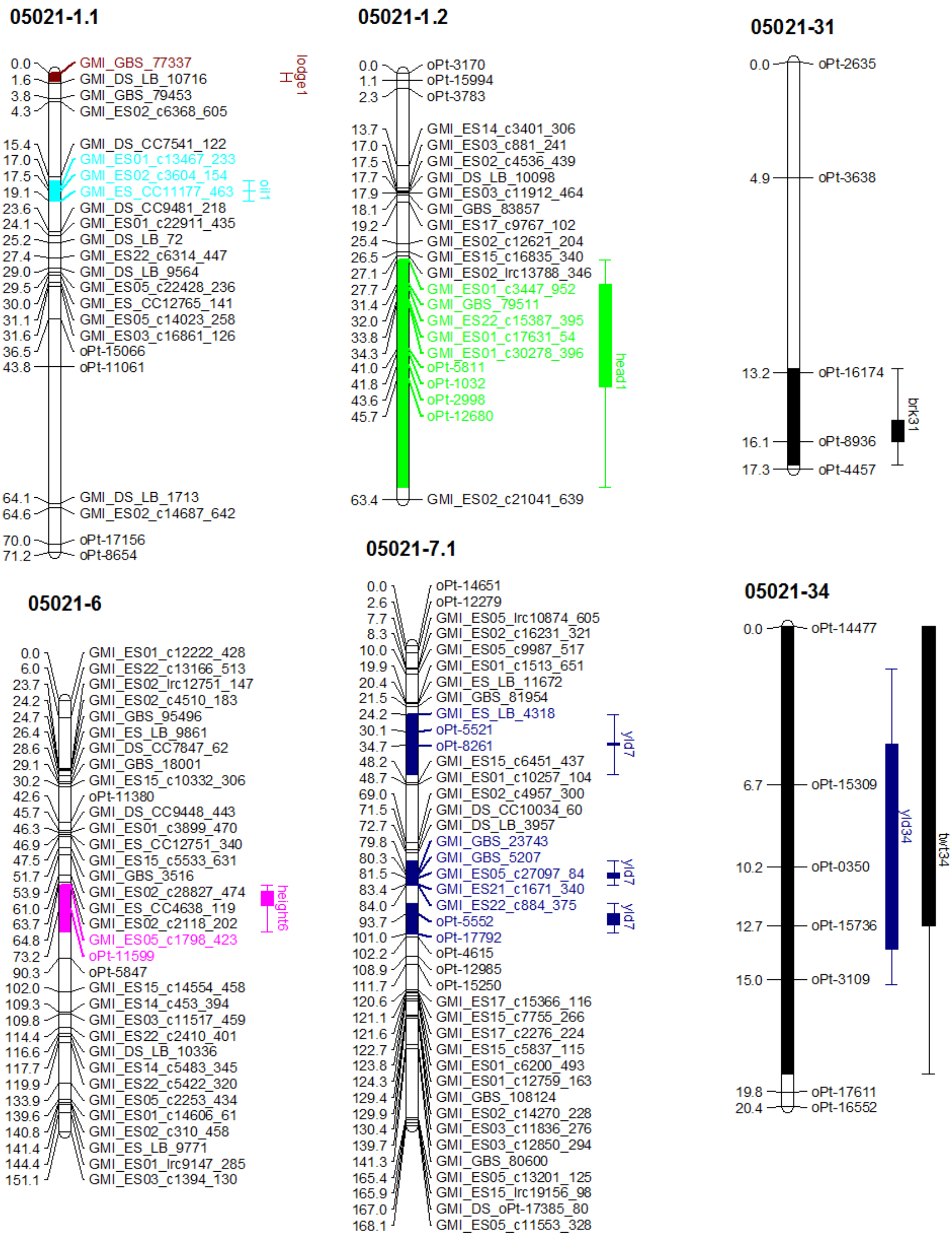


Figure 4.1. Linkage groups from the framework linkage map developed for the ‘ND991151’ x ‘ND030299’ F_6 recombinant inbred oat population (Population 05021) showing significant QTLs – yld=grain yield, kwt=1000 kernel weight, twt=test weight, brk=thin kernels, head=heading date, height=plant height, bg=β-glucan content, groat=groat percentage, dehull=dehulling efficiency, lodge=lodging.

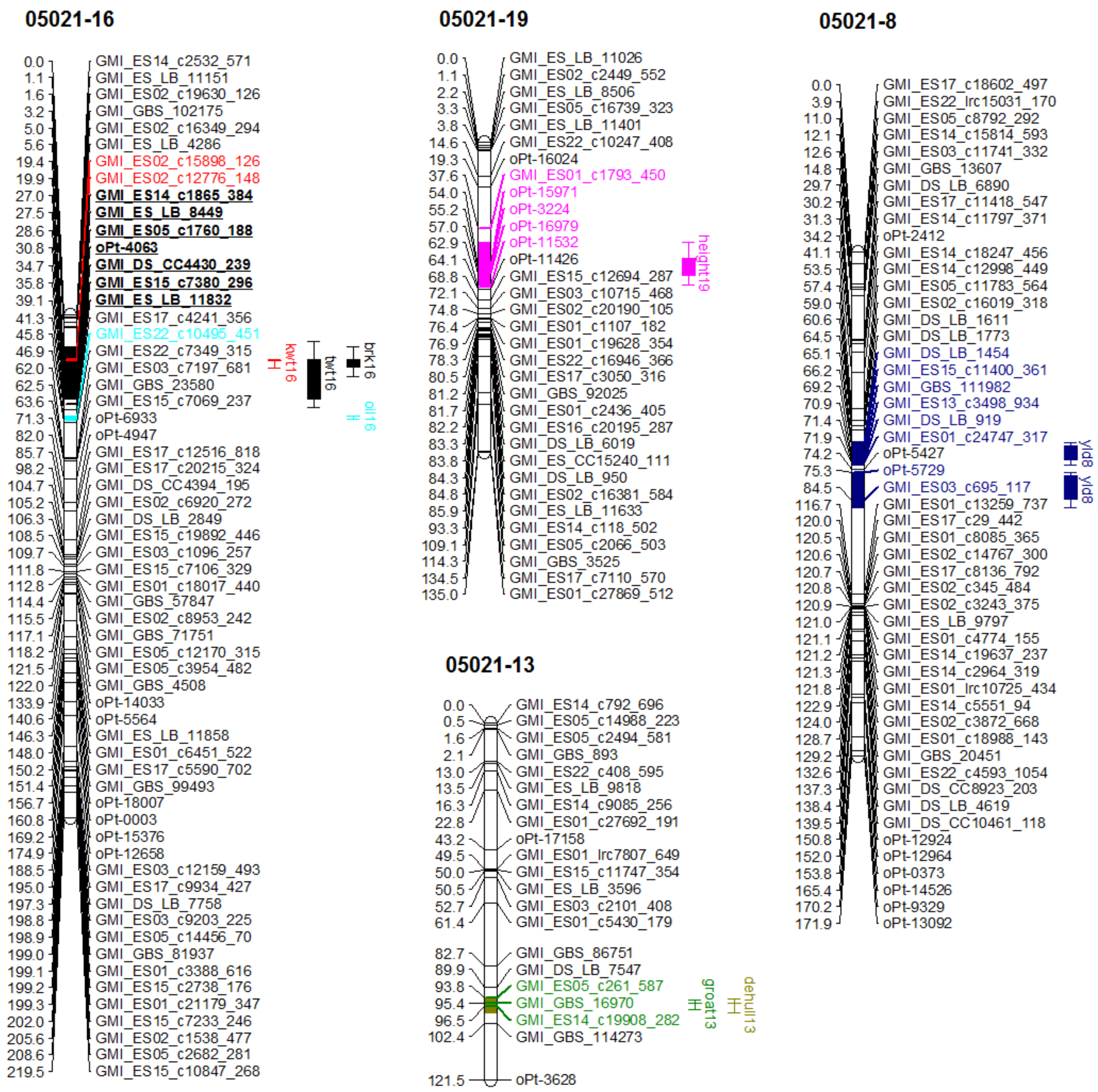


Figure 4.1. Linkage groups from the framework linkage map developed for the 'ND991151' x 'ND030299' F_6 recombinant inbred oat population (Population 05021) showing significant QTLs – yld=grain yield, kwt=1000 kernel weight, twt=test weight, brk=thin kernels, head=heading date, height=plant height, bg=β-glucan content, groat=groat percentage, dehull=dehulling efficiency, lodge=lodging (Continued).

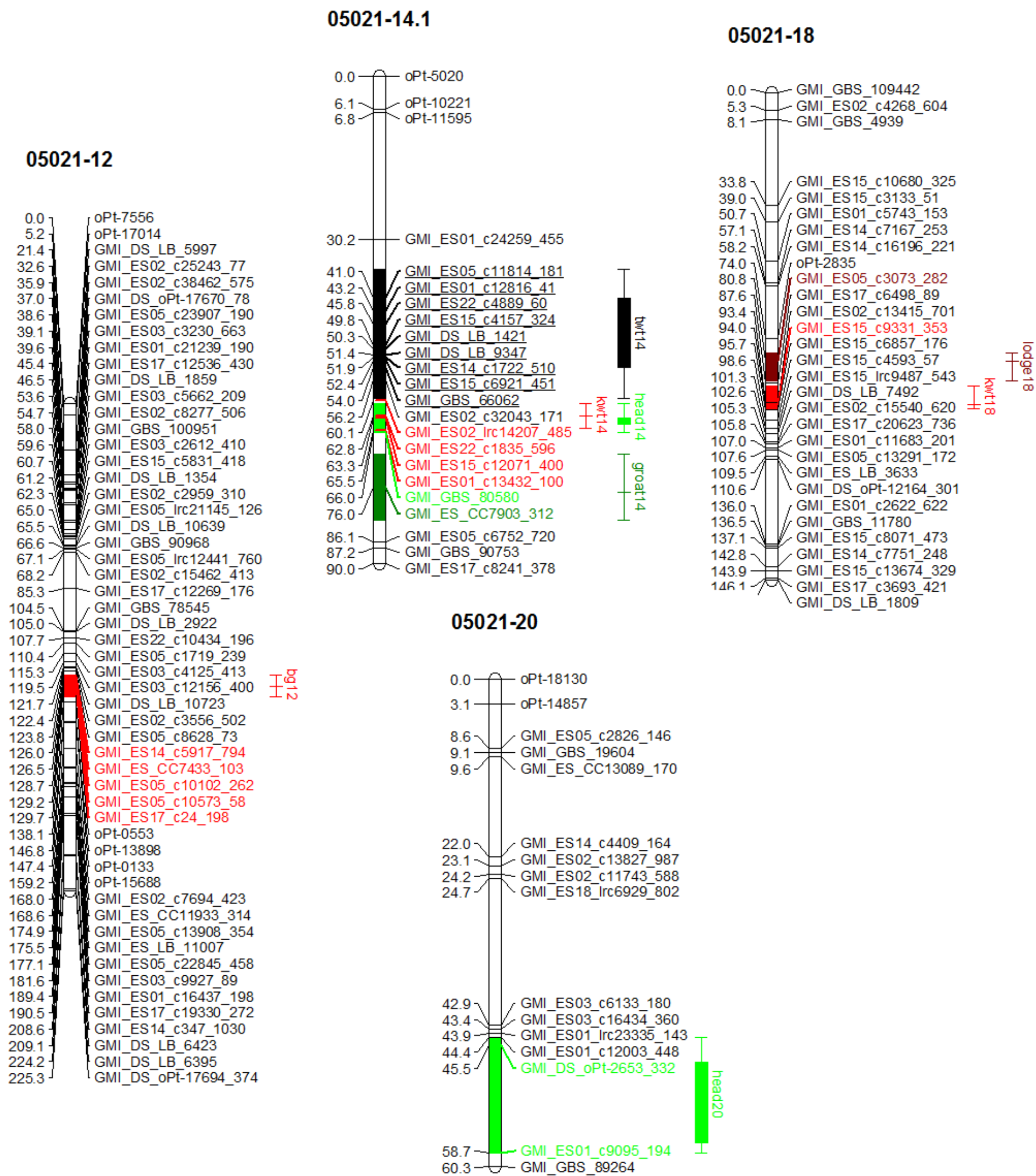


Figure 4.1. Linkage groups from the framework linkage map developed for the 'ND991151' x 'ND030299' F_6 recombinant inbred oat population (Population 05021) showing significant QTLs – yld=grain yield, kwt=1000 kernel weight, twt=test weight, brk=thin kernels, head=heading date, height=plant height, bg=β-glucan content, groat=groat percentage, dehull=dehulling efficiency, lodge=lodging (Continued).

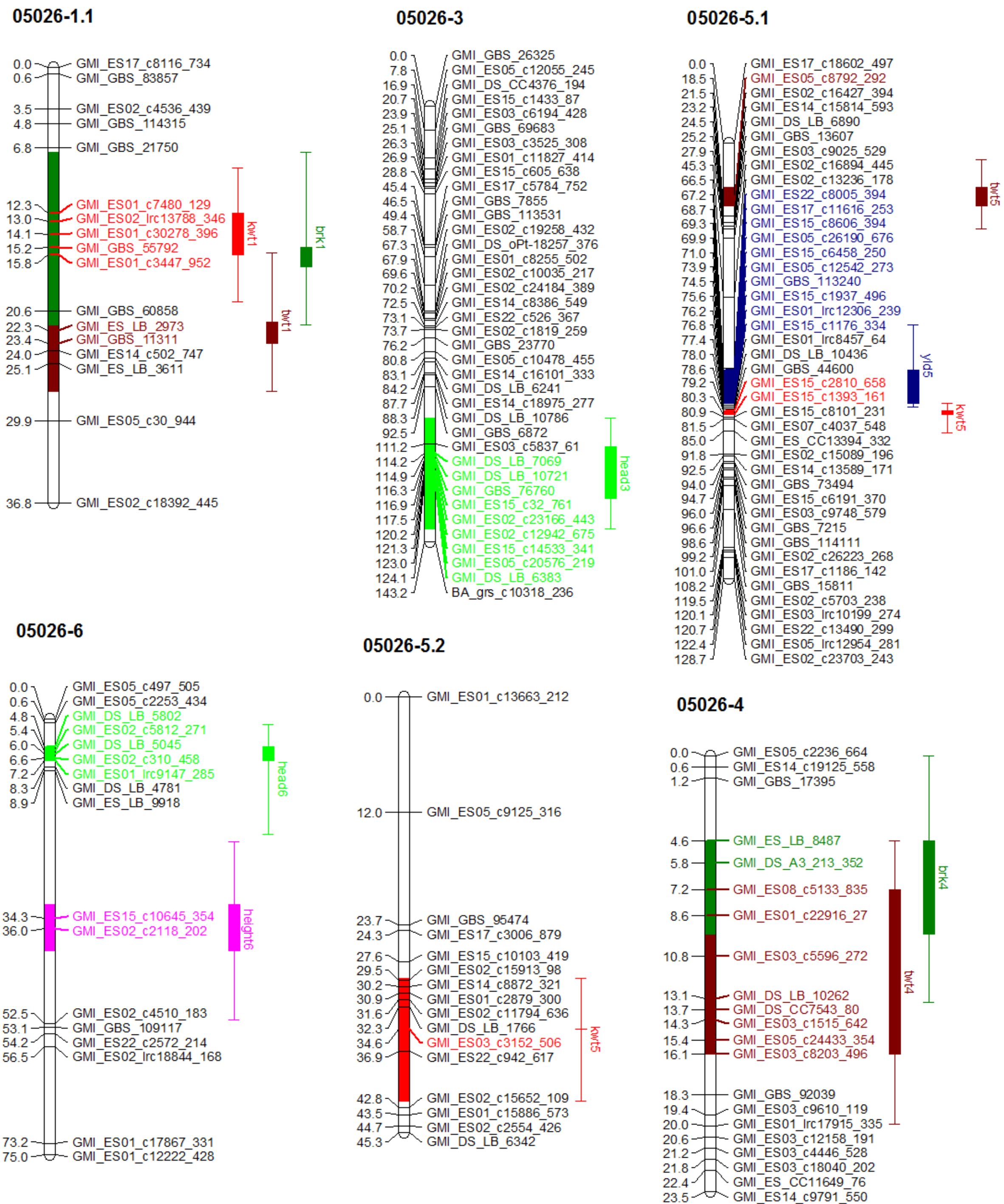
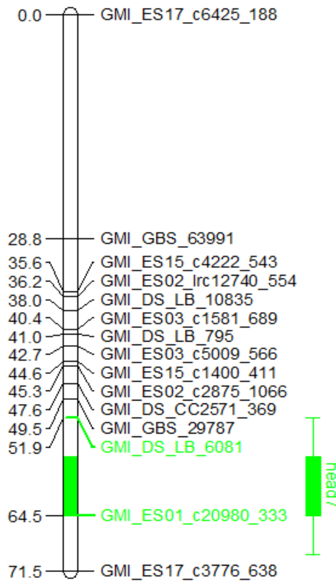
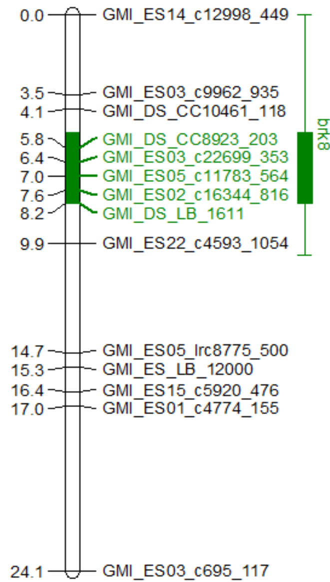
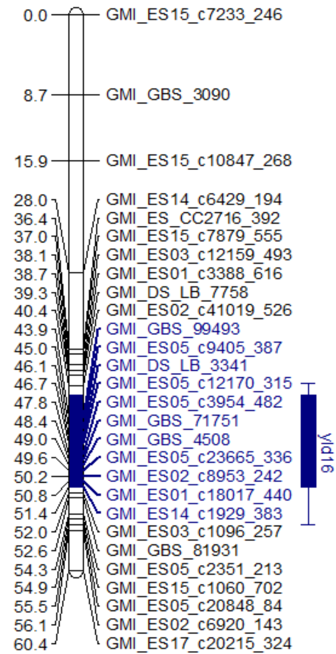
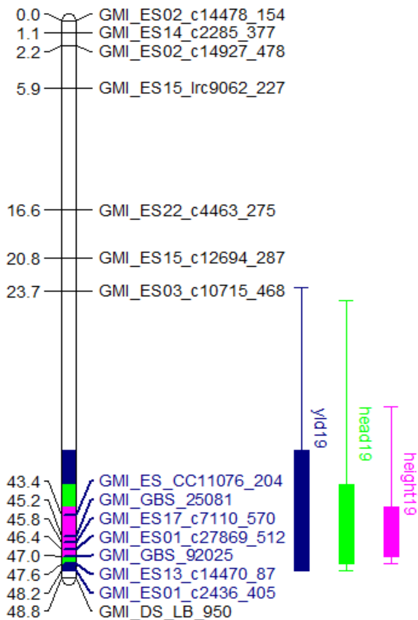


Figure 4.2. Linkage groups from the framework linkage map developed for the ‘Souris’ x ‘ND030299’ F_6 recombinant inbred oat population (Population 05026) showing significant QTLs – yld=grain yield, kwt=1000 kernel weight, twt=test weight, brk=thin kernels, head=heading date, height=plant height.









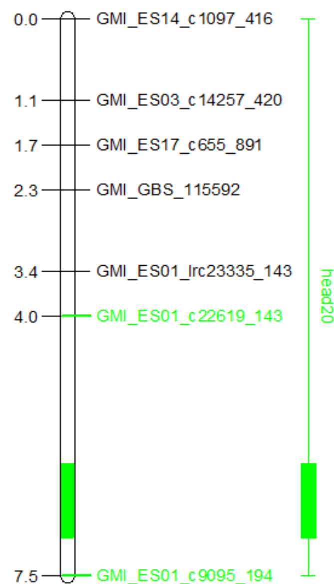


Figure 4.2. Linkage groups from the framework linkage map developed for the ‘Souris’ x ‘ND030299’ F_6 recombinant inbred oat population (Population 05026) showing significant QTLs – yld=grain yield, kwt=1000 kernel weight, twt=test weight, brk=thin kernels, head=heading date, height=plant height (Continued).

QTLs for grain yield had been found in this study on LG 05021-7.1, 05021-8, 05021-34 for population 05021, and on LG 05026-5.1, 05026-16.1, 05026-19 for population 05026. Similarly, Siripoonwiwat et al., 1996 reported also major QTL associated with grain yield in the Kanota/Ogle mapping population at two positions along LG KO 16_23, homologous to oat chromosome 8A. The QTL located at position 84 cM is approximately from 15 cM to the one reported in this study on LG 05021-8. A QTL associated with grain yield and linked to RFLP marker bcd1261a had been reported by Siripoonwiwat et al., 1996 on LG KO 1_3_38 homologous to oat chromosome 7C-17A and LG 05021-7.1. The position of the QTL identified on LG 05026-5.1 collocates with a QTL linked to RFLP marker cdo1312b in a homologous region in the Terra/Marion mapping population TM 22 (Dekoeyer et al., 2004). The QTL detected on LG 05026-16.1 is in agreement with the previous report of a QTL for grain yield in the putatively homologous genetic region KO_24_26_34 and KO 11_41_20_45 on the Kanota/Ogle population (Siripoonwiwat et al., 1996; Dekoeyer and Stuthman, 2001), TM 5 on the Terra/Marion population (Dekoeyer et al., 2004), LG 12 in the Aslak/Matilda mapping population (Tanhuanpaa et al., 2012). Siripoonwiwat et al., 1996 found two major QTL affecting grain yield in the Kanota/Ogle population KO 22 at position 112 linked to RFLP marker cdo708b, and at position 93 close to RFLP marker cdo484a. Both of them were identified in the present study on LG 05026-19 homologous to KO 22.

A QTL for 1000 kernel weight had been reported by Tanhuanpaa et al., 2012 in the Aslak/Matilda population LG 7b, and Beer et al., 1997 in the Kanota/Ogle mapping population at two positions on LG KO 16 homologous to oat chromosome 14D and LG 05021-14.1. Similarly, Dekoeyer et al., 2004 reported a QTL associated with 1000 kernel weight linked to phenotypic marker n1(responsible for the hulless character in oat) in the Terra/Marion mapping population

LG TM 5. This region showed homology with oat chromosome 16A, which corresponds to the location identified in this study on LG 05021-16. Tanhuanpaa et al., 2012 reported also this QTL on LG AM17 homologous to LG 05021-16.

Two QTLs associated with test weight had been reported by Siripoonwiwat et al., 1996 in the Kanota/Ogle mapping population LG KO 11_41+20. The first one linked to RFLP marker cdo836arv was mapped at 30 cM, and the second one linked to RFLP marker cdo1090c at 61 cM. This region on KO 11_41+20 showed homology with oat chromosome 16A. Another QTL identified on KO 14 homologous to oat chromosome 14D had been reported by Siripoonwiwat et al., 1996 in a region similar to the QTL found on LG 05021-14.1. Dekoeyer et al., 2004 reported a QTL associated with test weight in the Terra/Marion mapping population TM 5 flanking by the RFLP marker aco118b and the phenotypic marker n1. Linkage group TM 5 is syntenic to oat chromosome 16A and the QTL is located in the same region reported on this study on LG 05021-16. The position of the QTL reported for test weight on LG 05026-1.1 suggests that it had been mapped at approximately 10 cM to a QTL linked to RFLP marker isu2287a reported by Siripoonwiwat et al., 1996 on the Kanota/Ogle population LG KO 11_41+20. The map position of the QTL identified on LG 05026-4 suggests that it corresponds to a QTL reported by Siripoonwiwat et al., 1996 in the Kanota/Ogle population LG KO 32, homologous to oat chromosome 4C.

Dekoeyer et al., 2004 reported a major QTL affecting thin kernels and linked to phenotypic marker n1 in the Terra/Marion mapping population TM 5 homologous to oat chromosome 16A and LG 05021-16.

QTLs have been reported for plant height in the Kanota/Ogle mapping population KO 22, homologous to oat chromosome 19A, LG 05021-19, LG 05026-19, at position 108 cM (Holland

et al., 1997), 141, 98, and 116 cM (Siripoonwiwat et al., 1996; Milach et al., 1997). A QTL in a similar region has been reported by Tanhuanpaa et al., 2012 on LG 17 of the Aslak/Matilda population. Holland et al., 1997 reported also a QTL on KO 7_10_28 and KO 29_43 homologous to oat chromosome 6C, LG 05021-6, LG 05026-6.

Siripoonwiwat et al., 1996 reported 3 QTLs associated with heading date in the Kanota/Ogle mapping population, two of them on KO 11_41+20 and the last one on KO 37. Holland et al., 2002 reported the same QTL on the Ogle/TAM 0-301 mapping population OT 34 homologous to KO 11_41+20. This region is syntenic to oat chromosome 1C from 49.3 to 73.1 cM which in turn is less than 5 cM from the region identified on LG 05021-1.2. The QTL found in LG 05021-1.2 had been previously reported in a similar region by Tanhuanpaa et al., 2012 on LG AM10 and LG AM12. Another two QTLs associated with heading date had been reported on KO 14, homologous to oat chromosome 14D, at position 14 cM (Holland et al., 1997) and 37 cM (Siripoonwiwat et al., 1996) which correspond to the same region identified on LG 05021-14.1. This QTL had been also discovered by Tanhuanpaa et al., 2012 on LG TM7a. Three QTLs had been reported in two similar genomic regions on LG KO22 and LG OT1 at position 108 cM (Holland et al., 1997), 156 cM (Holland et al., 2002), 171 cM (Siripoonwiwat et al., 1996; Holland et al., 1997). Those two regions in the Kanota/Ogle population and the Ogle/TAM O-301 population are homologous to LG 05026-19. The QTL detected on LG 05026-3 is located in the exact same genomic region of the QTL reported on LG KO42 flanked by RFLP markers isu707a and hkt1c (Siripoonwiwat et al., 1996; Holland et al., 1997).

A QTL located at the same region, to the one found on LG 05021-12 for β-glucan content, had been reported by Dekoeyer et al., 2004 in the Terra/Marion mapping population TM 21 homologous to oat chromosome 12D. The position of the QTL identified on LG 05021-1.1

and affecting oil content collocates with a QTL linked to RFLP marker cdo665 in the Kanota/Ogle mapping population KO 11_41+20 (Kianian et al., 1999), RFLP marker rz69 in the Ogle/Marion mapping population OM 3 (Zhu et al., 2004), and DArT markers oPt-17088_A and oPt-6135 in the Dal/Exeter mapping population DE 13 (Hizbai et al., 2012). The same QTL was also reported by Tanhuanpaa et al., 2012 in the Aslak/Matilda mapping population LG 11 and 15 homologous to LG 05021-1.1. Similarly, a QTL for oil content was identified by Dekoeyer et al., 2004 and linked to phenotypic marker n1 in the Terra/Marion mapping population TM 5 homologous to oat chromosome 16A. This QTL was also found by Tanhuanpaa et al., 2012 on LG 12 and 17 homologous to 05021-16. The position of the QTL on LG 05021-14.1 associated with groat percentage corresponds to that of the QTL identified in the Kanota/Marion mapping population KM 14 and linked to RFLP marker cdo1358f (Groh et al., 2001).

4.5.2. Linkage and/or pleiotropic effects

It is interesting that the same distorted genomic region on LG 05021-16, from position 19.4 to 45.8 cM including the common EST markers ES02_c15898_126, ES02_c12776_148, and ES14_c1865_384, affects thin kernels, test weight, 1000 kernel weight, and oil content. Also, two QTLs affecting grain yield and test weight has been mapped at the exact same position on LG 05021-34 from 0 to 15 cM, and overlapped by the DArT markers oPt-14477, oPt-15309, oPt-0350, and oPt-15736. Similarly, on LG 05021-14.1 the region affecting test weight is 9 cM apart from the region associated with 1000 kernel weight, heading date, and groat percentage. These associations were consistent with the phenotypic and genotypic correlations observed between test weight and heading date, test weight and groat percentage, heading date and groat percentage, thin kernels and 1000 kernel weight, thin kernels and oil content, 1000 kernel weight and oil content, grain yield and test weight (Chapter 2-Tables 5, 7, 8). On LG 05021-14.1,

ND99151 alleles increased 1000 kernel weight, test weight, groat percentage, and decreased heading date whereas on LG 05021-16, the ND030299 alleles increased oil content and percentage of thin kernels, but decreased test weight and 1000 kernel weight. Tanhuanpaa et al., 2012 found also that groat percentage and heading date mapped to the same genetic location. These correlated responses could be due to linkage of the underlying QTLs or to pleiotropy. We point out both genetic actions because the resolution of the maps used in this study is not strong enough to discriminate QTLs with pleiotropic effects from tightly linked QTLs affecting the different traits.

The QTLs observed for oil content on LG 05021-1.1 and LG 05021-16 were identified in distorted regions. The QTLs for oil content reported by Kianian et al., 1999 on KO 11_41+20, Zhu et al., 2004 on OM 3, and Hizbai et al., 2012 on DE 13 are all located in the same homologous distorted region. The identification of QTLs affecting oil content in four mapping populations in the same distorted region furnishes strong evidence that the mechanisms underlying segregation distortion may directly or indirectly affect oil content.

LG 05026-19 loci, from position 23.7 to 47 cM had strong effects on heading date, plant height, and grain yield. Markers ES03_c1075_468, ES_CC11076_204, GBS_25081, ES17_c7110_570, ES01_c27869_512, and GBS_92025 are shared between the three traits. QTLs associated with grain yield, plant height, and heading date had been also reported by Dekoeyer and Stuthman, 2001. Loci in adjacent regions on LG 05026-6 from position 0 to 52.5 cM also had significant effects on heading date and plant height. Correspondingly, the QTL influencing grain yield on LG 05026-5.1 is approximately 3 cM apart from the region correlated with 1000 kernel weight. The same genomic region on LG 05026-4 overlapped by EST markers ES08_c5133_835, ES01_c22916_27, ES03_c5596_272 affect thin kernels and test weight. GBS

marker GBS_55792 and EST marker ES01_c3447_952 exhibited also strong effects on thin kernels, 1000 kernel weight, and test weight. 'Souris' allele increased test weight on LG 05026-1.1, LG 05026-4, LG 05026-5.1, 'ND030299' allele decreased thin kernels on LG 05026-1.1, LG 05026-4, and increased 1000 kernel weight on LG 05026-5.1. These associations are congruent to the phenotypic and genotypic correlations observed between grain yield and 1000 kernel weight, test weight and 1000 kernel weight, thin kernels and 1000 kernel weight, test weight and thin kernels (Chapter 3 – Table 5). On LG 05026-19, 'Souris' allele increased grain yield, and decreased plant height, and 'ND030299' allele increased heading date whereas on LG 05026-6, 'ND030299' allele increased plant height and heading date. These pleiotropic and/or linked genomic regions may have contributed to the significant positive phenotypic and genotypic correlations observed between heading date and plant height, and between grain yield and plant height in the population 05026 (Chapter 3 – Table 5).

4.5.3. Comparing the two populations for common QTLs

QTLs are investigated with the objective to use them in molecular breeding. In order to be exploited in a marker-assisted selection program, the QTL of interest identified should be integrated and validated on other population so that a clearer understanding of the QTL can be gained (Rines et al., 2006). This is a reason why comparative QTLs among population is important.

A QTL with major effect on heading date has been identified on LG 05021-20 of population 05021 from position 47.5 to 57.5 cM. This QTL has also been detected on LG 05026-20.1 of population 05026 from position 6 to 7 cM. Two EST markers (ES15_c13627_458, ES01_c9095_194) were shared between the two homologous genomic regions. The positive allele on both QTLs was contributed by 'ND030299', the common parent between the two

populations. The effects of both QTLs are quite similar, on the population 05021 the QTL explained 19% of the phenotypic variation whereas on the population 05026 it explained 18% of the variation observed.

A QTL identified for plant height on LG 05021-6 of population 05021 was also observed on LG 05026-6 of population 05026. The shared EST marker (ES05_c1798_423) is responsible for the effects observed. The positive allele was also contributed by the common parent 'ND030299'.

An important genomic region on LG 05026-1.1 of population 05026, from position 12.3 to 23.4 cM, associated with 1000 kernel weight, test weight, and thin kernels had been mapped for heading date in the population 05021 on LG 05021-1.2 from position 31.4 to 46.7 cM. The homologous segment between the two populations comprised 6 EST markers (ES01_c30278_396, ES01_c17631_54, ES05_c1532_208, ES01_c3435_183, ES15_c14779_89, and ES01_c3447_952), 1 DArT marker (DS_LB_1139), and 1 GBS marker (GBS_55792). The positive allele, except for thin kernels, was contributed by the non-common parent, 'ND991151' for population 05021, and 'Souris' for population 05026.

4.6. Conclusion

Identification of molecular markers linked to genes controlling important functional traits provides a fast and reliable selection of desirable genotypes. Consequently, the identified markers can play an important role in a plant breeding programme and can seed up considerably the selection of genotypes with better agronomic properties (Okon and Kowalczyk, 2012). In this present research, we identified SNP markers that cosegregate or are closely linked to QTL conditioning many important agronomic traits in oat. Future works, to successfully apply those QTLs in a marker-assisted selection (MAS) program, should focus in validating the QTLs

reported on another genetic background and more environments, and developing an efficient and economic marker assay for screening large breeding populations. According to Xu and Couch, 2008 there is still a high discrepancy between QTL studies and application of these studies in a MAS program. One of the principal reasons is because the mapping populations, used on those studies, were from parents highly distinctive in their phenotype, and as noted by Bernardo, 2008 it is possible that these QTL will be population specific and therefore less useful in the context of breeding programs. In our case, since we use parents from a breeding program, the QTL reported in this research, have a higher probability of being valid across elite populations. The results, reported in this study, can also serve as an additional resource in the understanding of genetic mechanisms underlying important agronomic traits in cultivated oats.

4.7. References

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CHAPTER 5

5.1. General conclusions

Despite more than 20 years of genomic research, cultivated oats has been lagged behind and has not kept pace with other small grains such as wheat and barley in term of genome sequence, QTL analysis and marker-assisted selection. This dissertation provides supplemental steps towards selection of new oat cultivars with desirable combination of genes. Two recombinant inbred oat populations were evaluated in this research for genotypic and phenotypic relationships among important agronomic traits including grain yield, test weight, 1000 kernel weight, thin kernels, plant height, heading date, β-glucan content, and oil content. Two genetic linkage maps were developed from the two populations and QTLs associated with the agronomic traits were assessed and identified. This dissertation comprises 3 main parts beside the general introduction and that conclusion.

The first main part addressed the issues of the efficiency of indirect selection and the magnitude of response to selection by assessing genotypic and phenotypic correlations among traits. Some important conclusions drawn from that part include:

- Genotype main effect was highly significant for all the agronomic traits under study and showed considerable amount of variation. Such wide variation is an indication of the capacity to improve the two populations evaluated with respect to grain quality.
- The absence of significant genotype-by-environment interaction for 1000 kernel weight suggested that it should be practical to select for 1000 kernel weight.
- The absence of strong correlations among traits indicated that improvement of agronomic traits should not be difficult.

- Grain yield was positively correlated with test weight, plant height, β-glucan content, and dehulling efficiency suggesting that when quality traits are improved higher yields can be achieved.
- The high heritabilities observed suggested that all the traits measured should respond well to selection on a family-mean basis.

The second main part explored the issues relative to cytogenetic abnormalities in the oat genome and the difficulty of mapping by the construction of two genetic maps and the comparison of those maps with the recently published oat consensus map. The most important conclusions include:

- Thirty linkage groups using 1168 polymorphic markers were formed for population 05021, whereas population 05026 comprised 33 linkage groups using 1024 polymorphic markers.
- The 30 linkage groups of population 05021 varied in size from 15.8 to 225.3 cM, and contained from 3 to 62 markers for a total map size of 2601.7 cM.
- The 33 linkage groups of population 05026 comprised from 2 to 42 markers, varied in size from 2.3 to 143.2 cM for a total map size of 1174.2 cM.
- Twenty six of the 30 linkage groups from population 05021 can be placed on 19 of the 21 oat chromosomes.
- Thirty one of the 33 linkage groups from population 05026 showed homology with 20 of the 21 oat chromosomes.
- The differences noted in the ordering of markers between the two populations and the oat consensus map, are an indication that genomic rearrangements and intervarietal chromosome interchanges exist in the genome of cultivated oat.

- The shorter map observed in population 05026 may be the result of inadequate polymorphism in some regions with similar ancestry.

The purpose of the third main part was to address the QTL mapping in two oat populations, to compare the identified QTLs with those previously published, and to assess linked and/or pleiotropic regions between several agronomically important traits. Key findings discovered in that chapter include:

- Nineteen genomic regions on 14 linkage groups were significantly associated with agronomic and grain chemical traits in population 05021.
- Fourteen genomic regions on 12 linkage groups were identified for agronomic traits in the population 05026.
- The same genomic region on LG 05021-16, from position 19.4 to 45.8 cM, affected thin kernels, test weight, 1000 kernel weight, and oil content.
- Two QTLs affecting grain yield and test weight has been mapped at the exact same position on LG 05021-34.
- The region affecting test weight on LG 05021-14.1 was located 9 cM apart from a region associated with 1000 kernel weight, heading date, and groat percentage.
- Markers on LG 05026-19, from position 23.7 to 47 cM, had strong effects on heading date, plant height, and grain yield.
- The QTL influencing grain yield on LG 05026-5.1 was mapped at approximately 3 cM from a region correlated with 1000 kernel weight.
- All these correlated responses could be due to linkage of the underlying QTLs or to pleiotropy.

- The identification of QTLs affecting oil content in 4 mapping populations in the same homologous distorted region furnished strong evidence that the mechanisms underlying segregation distortion may directly or indirectly affect oil content.

In conclusion, many linkage maps, over the last 20 years, had been developed for several oat populations. Quantitative trait loci have been identified to be associated with several agronomic traits in cultivated oats. Marker-assisted selection (MAS) has been applied for some economic traits and mostly for disease resistance. Three AFLP markers associated with BYDV had been cloned for MAS purposes (Jin et al., 1998; Jin et al., 1999). Six SCAR and CAPS markers, also linked to BYDV, had been used for MAS (Pal et al., 2002). SNP markers were developed for Pc68 (Chen et al., 2004) and Dw6 dwarfing genes (Kiviharju et al., 2004). Nevertheless, nothing big had been done yet for other agronomic traits. In this point of view, this research will help the oat community with better understanding of the genomic regions underlying some important agronomic traits. In a near future, while waiting for the complete sequencing of the oat genome, the next goal will include the following:

- The utility of the SNP markers, identified and linked to major QTL in oat, needs to be validated in other genetic backgrounds, different locations than the ones used in this study, and years.
- The validated QTLs could be incorporated into breeding cultivars through the use of marker-assisted selection and could also be used in finding candidate genes that explain the genetics of the traits evaluated.

5.2. References

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APPENDIX

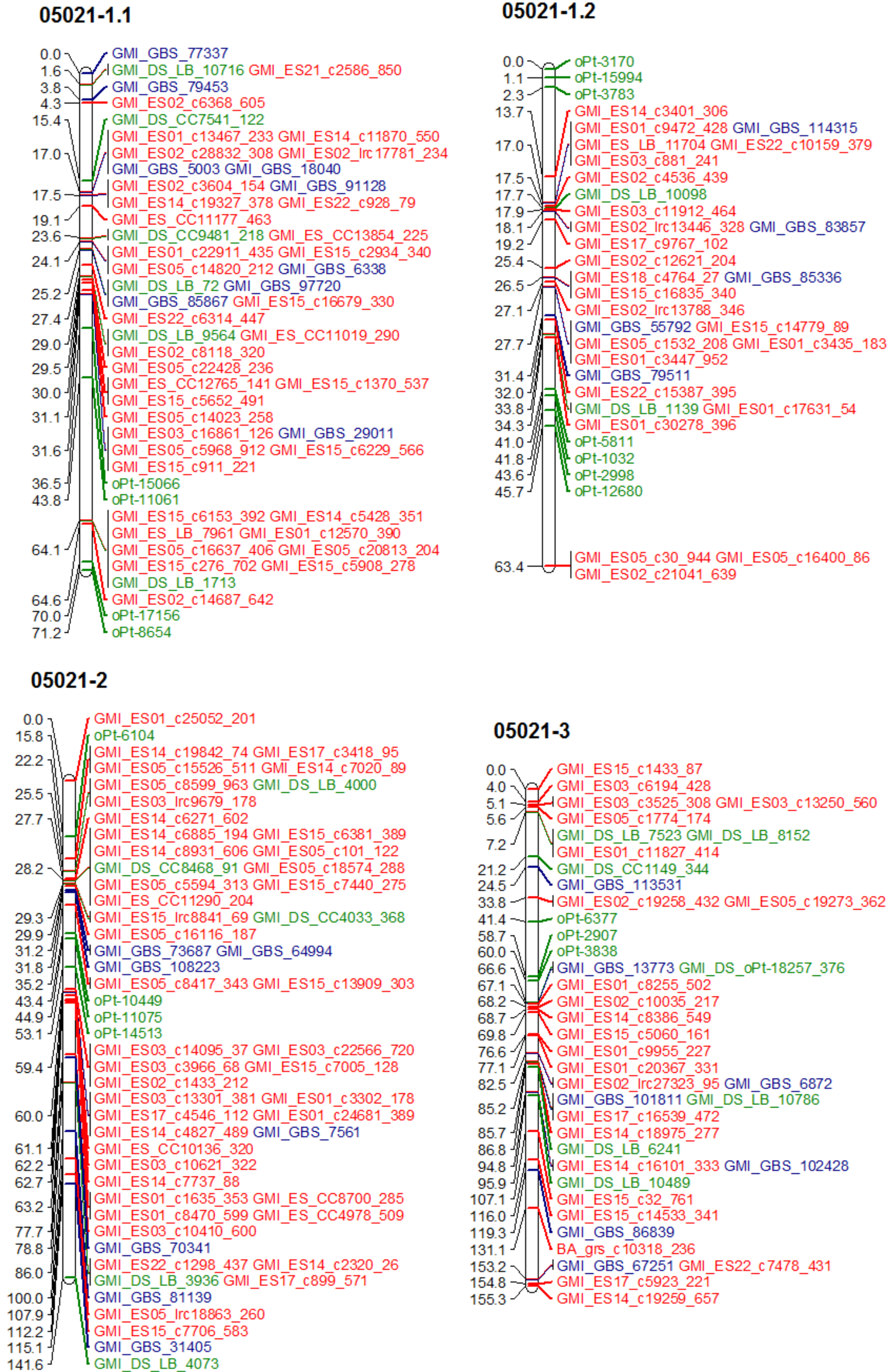
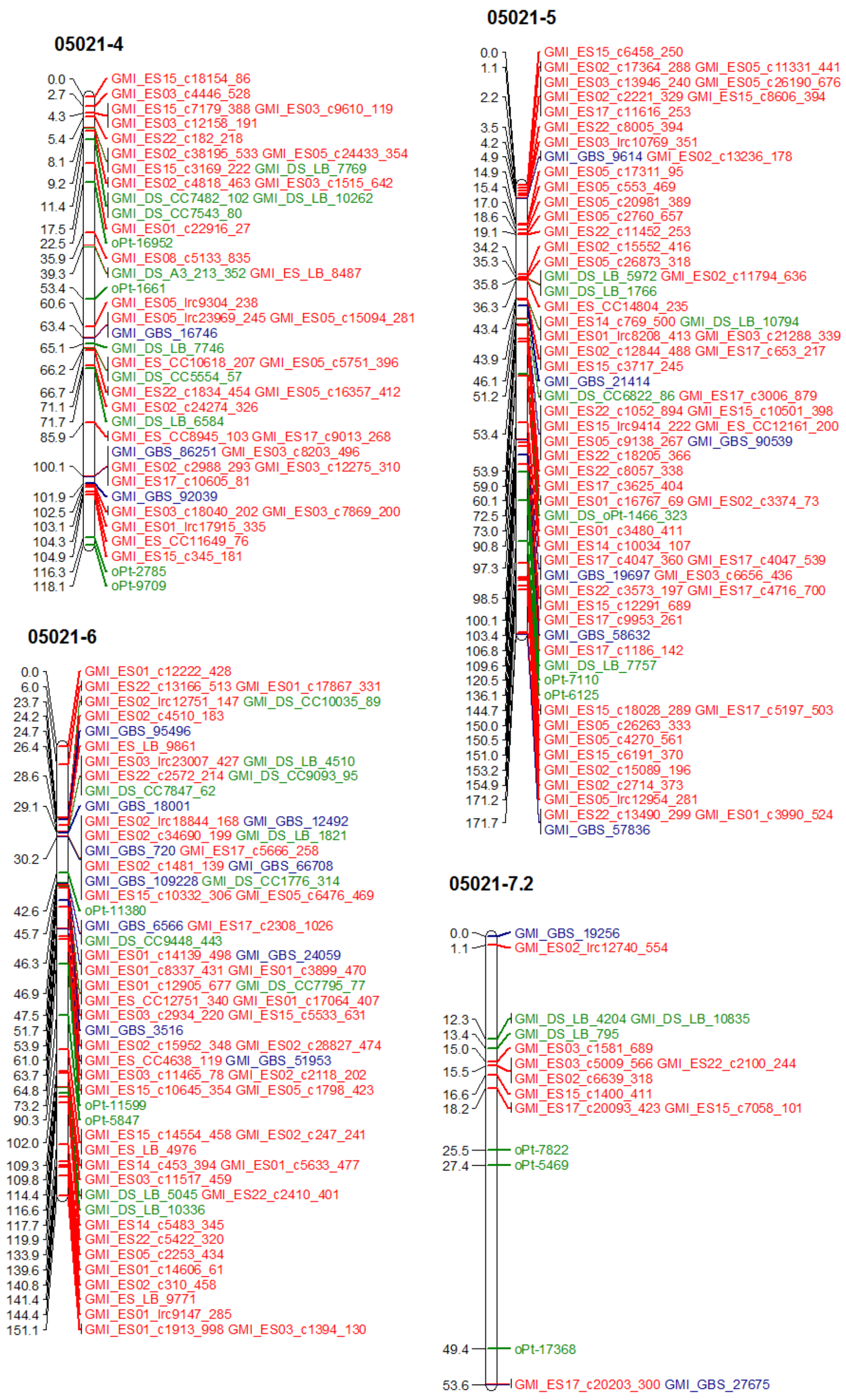


Figure A1. Molecular marker linkage map from 'ND991151' x 'ND030299' F_6 recombinant inbred oat population (Population 05021) (red=EST markers, blue=GBS markers, green=DArT markers).



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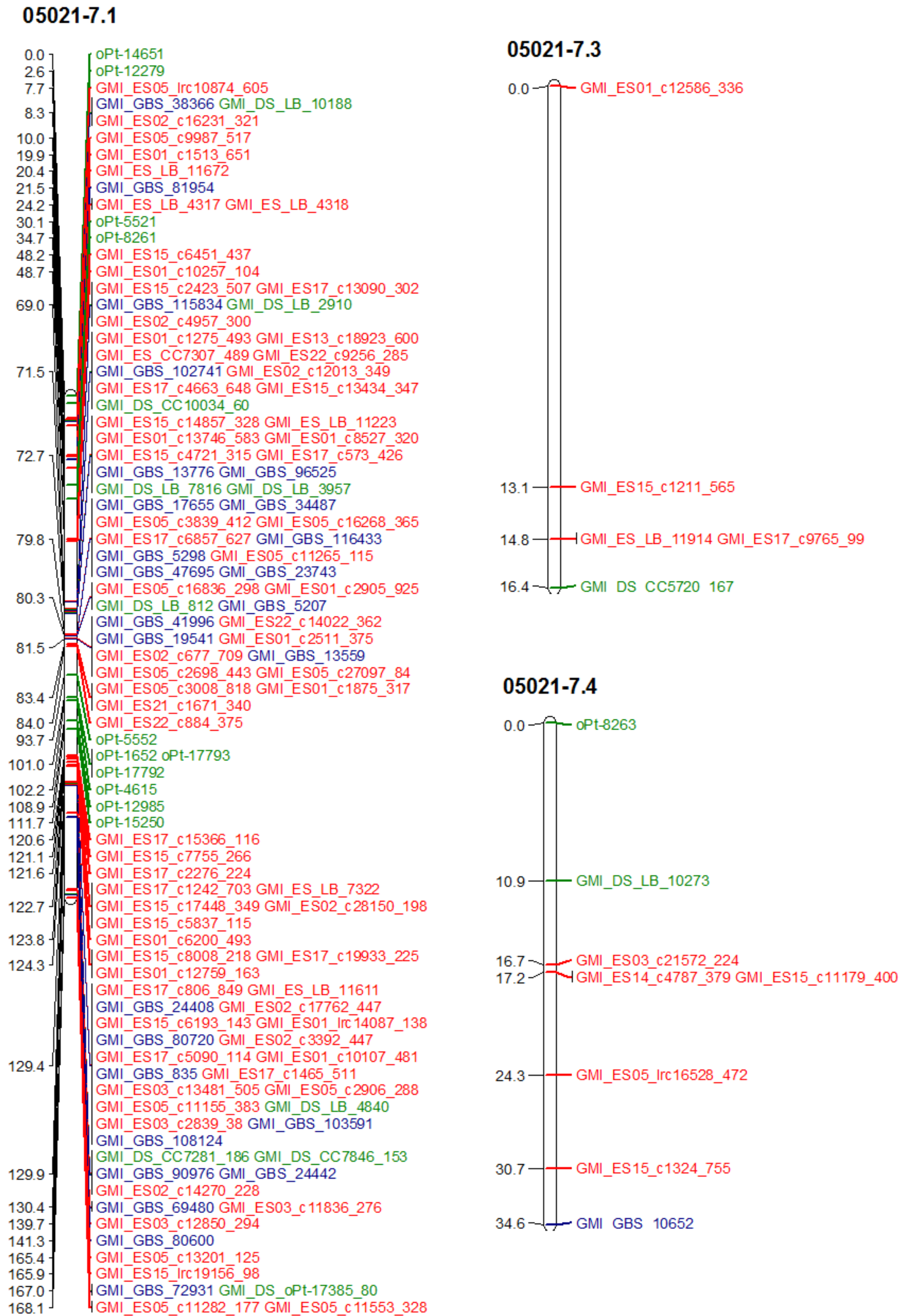

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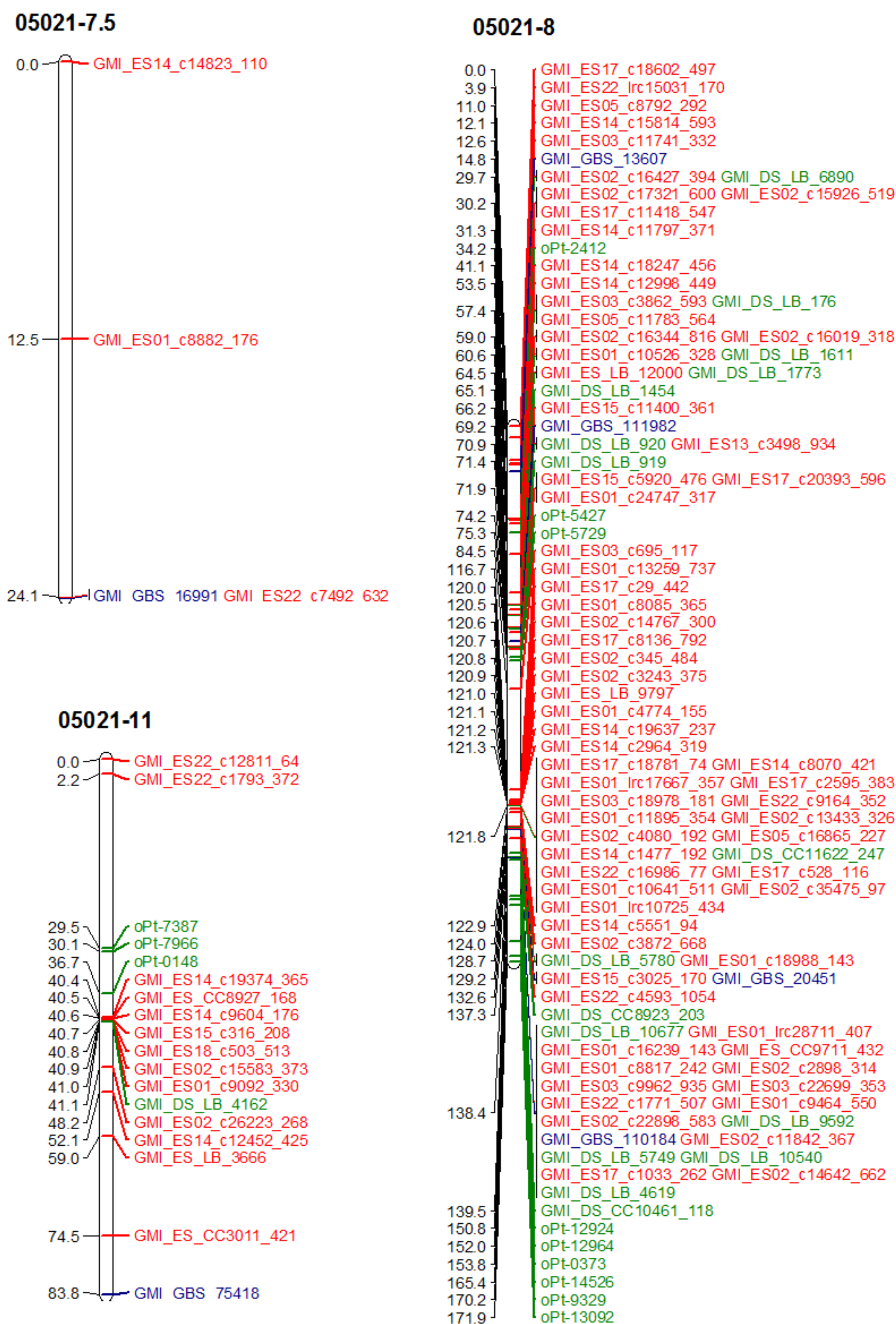

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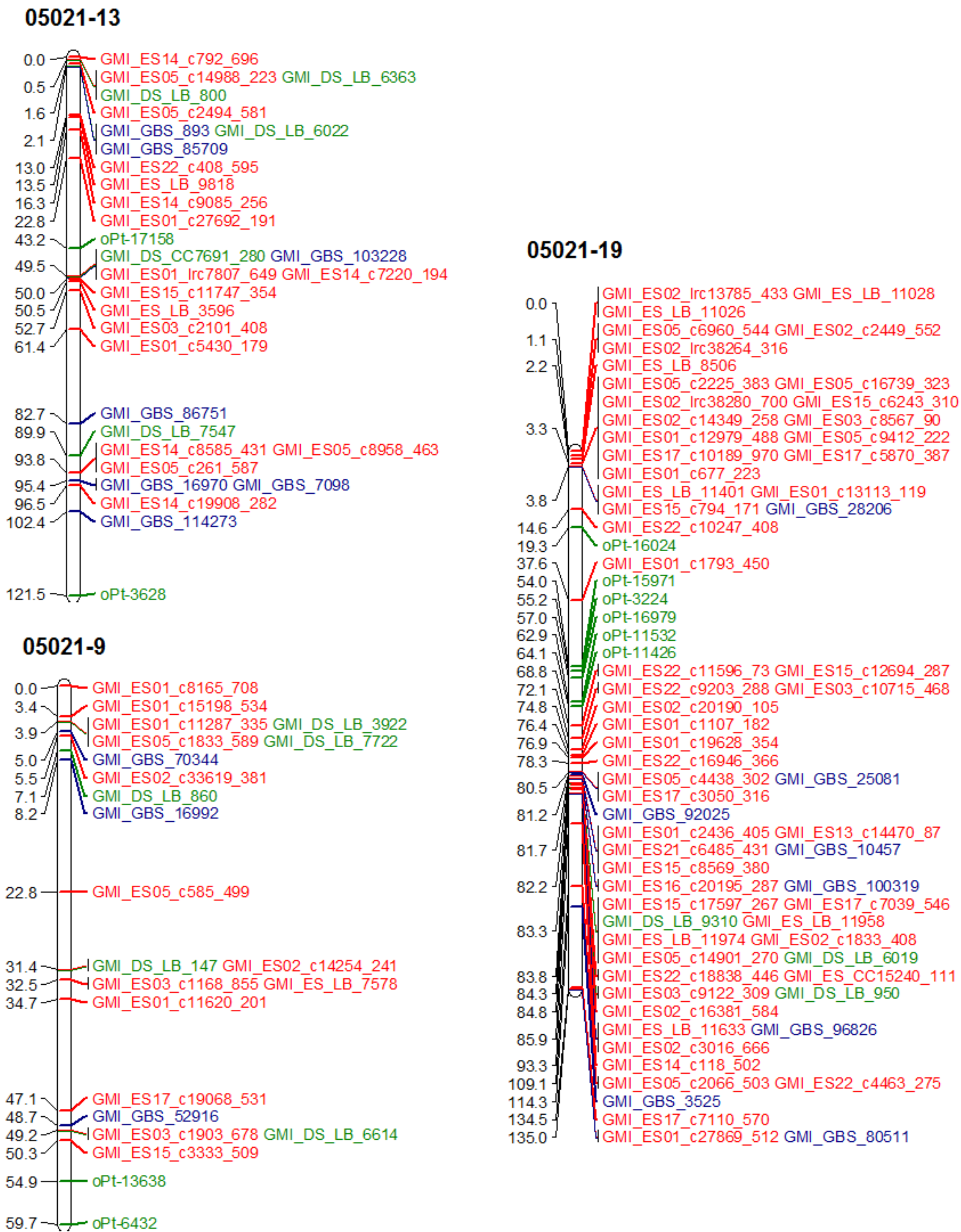

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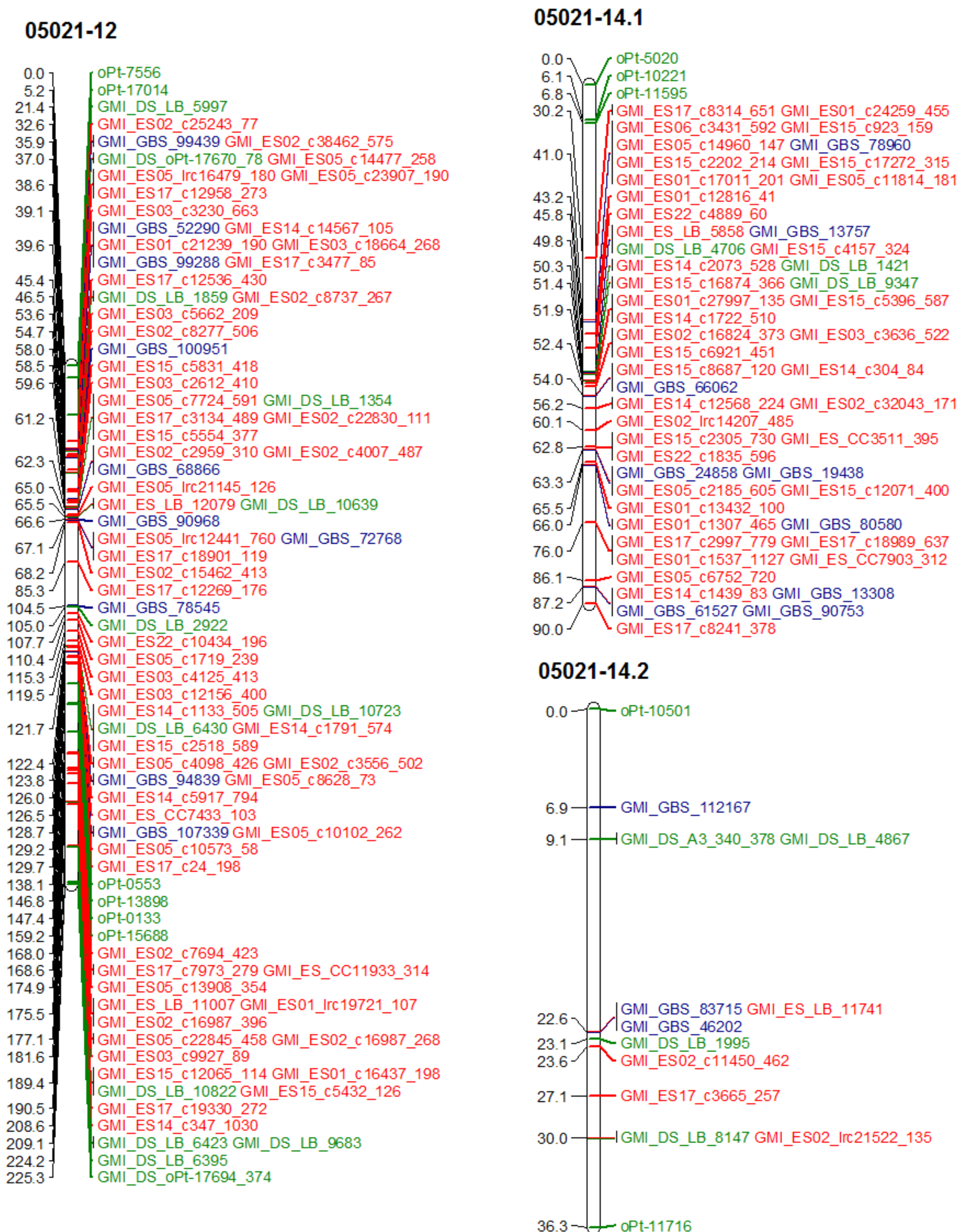

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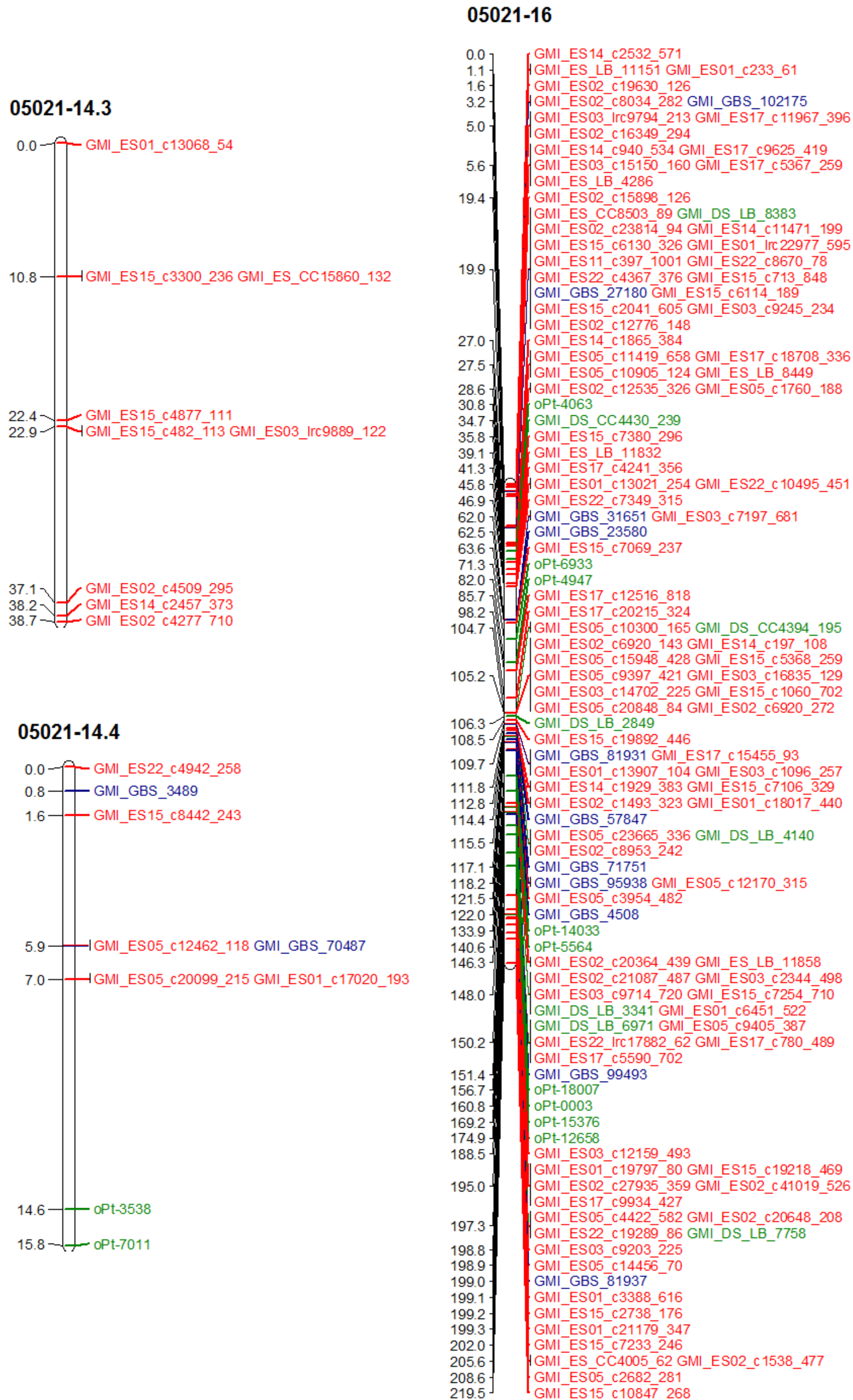

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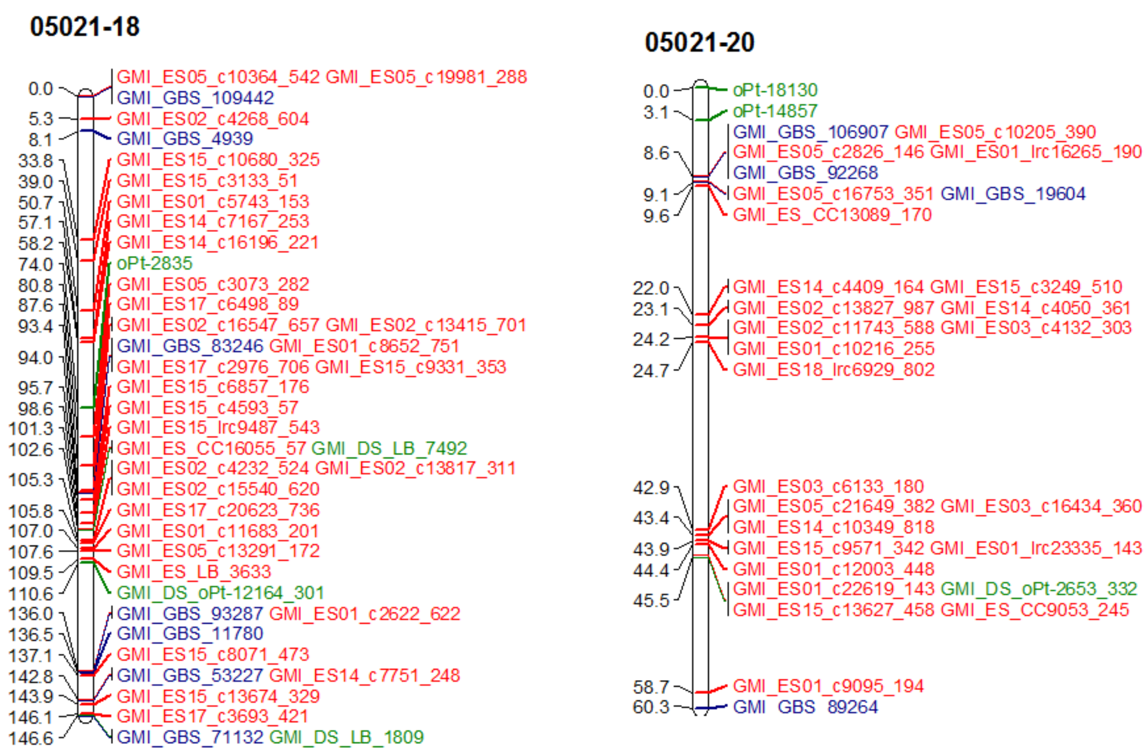

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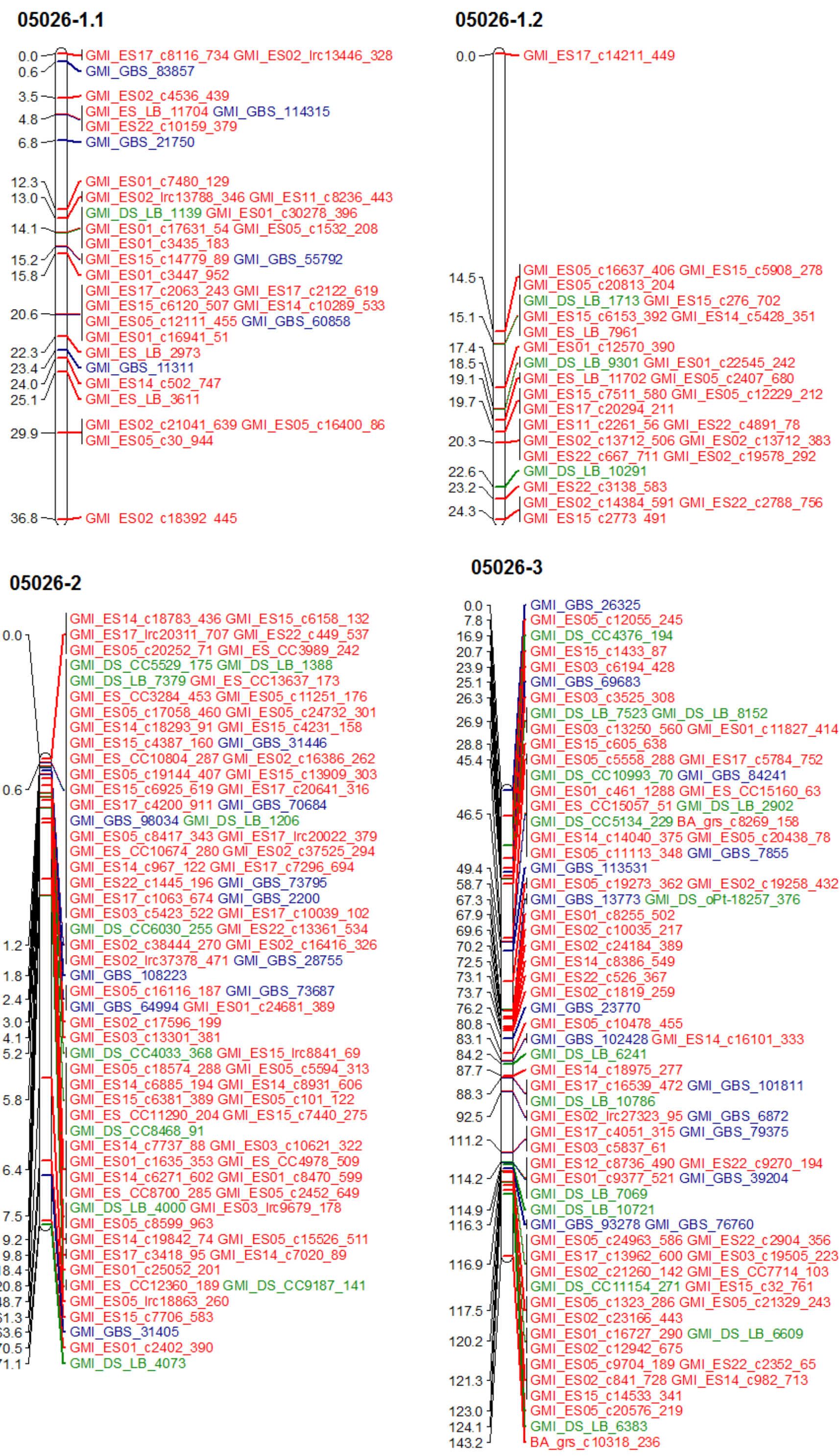
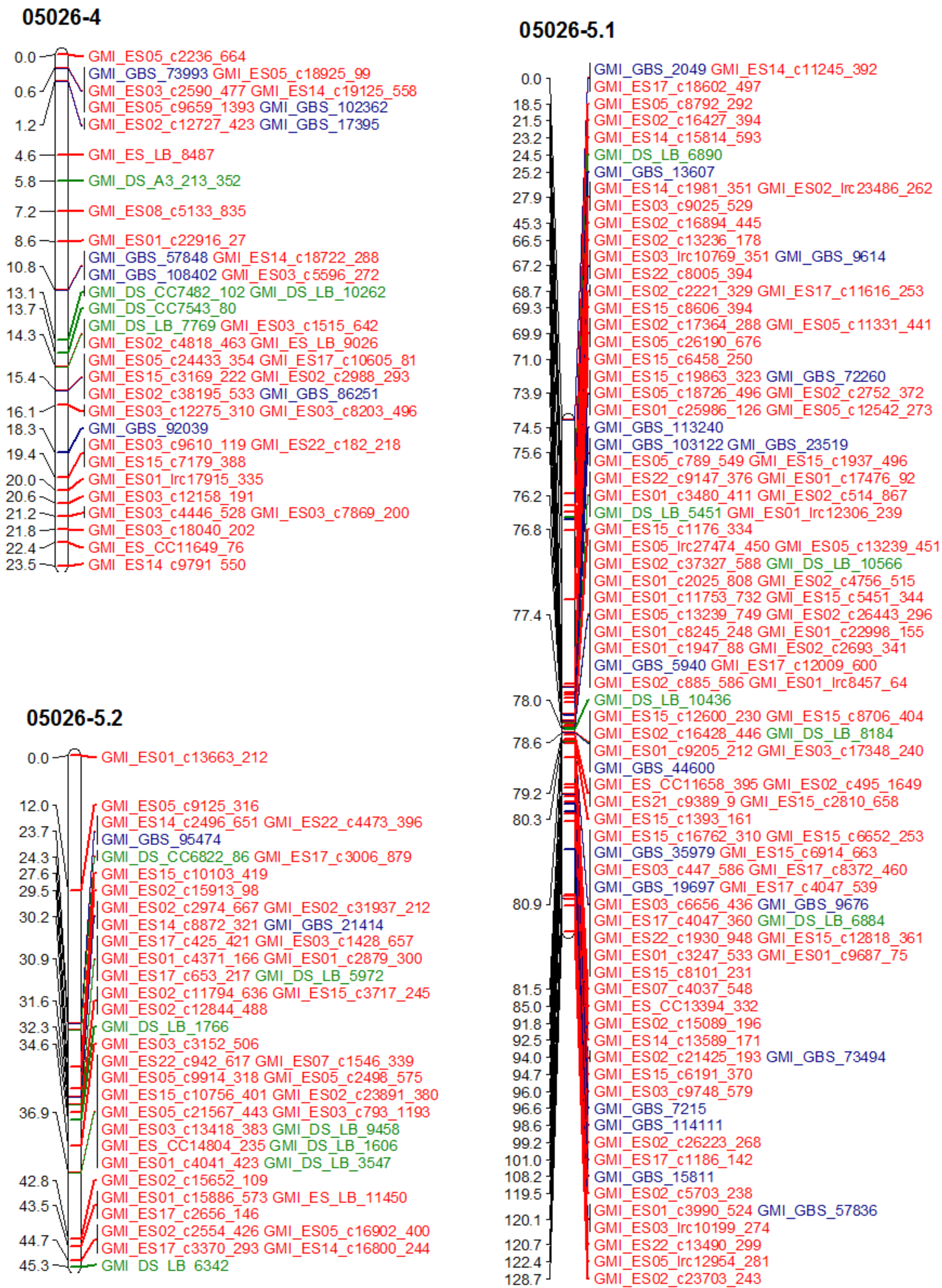


Figure A2. Molecular marker linkage map from 'Souris' x 'ND030299' F6 recombinant inbred oat population (Population 05026) (red=EST markers, blue=GBS markers, green=DArT markers).



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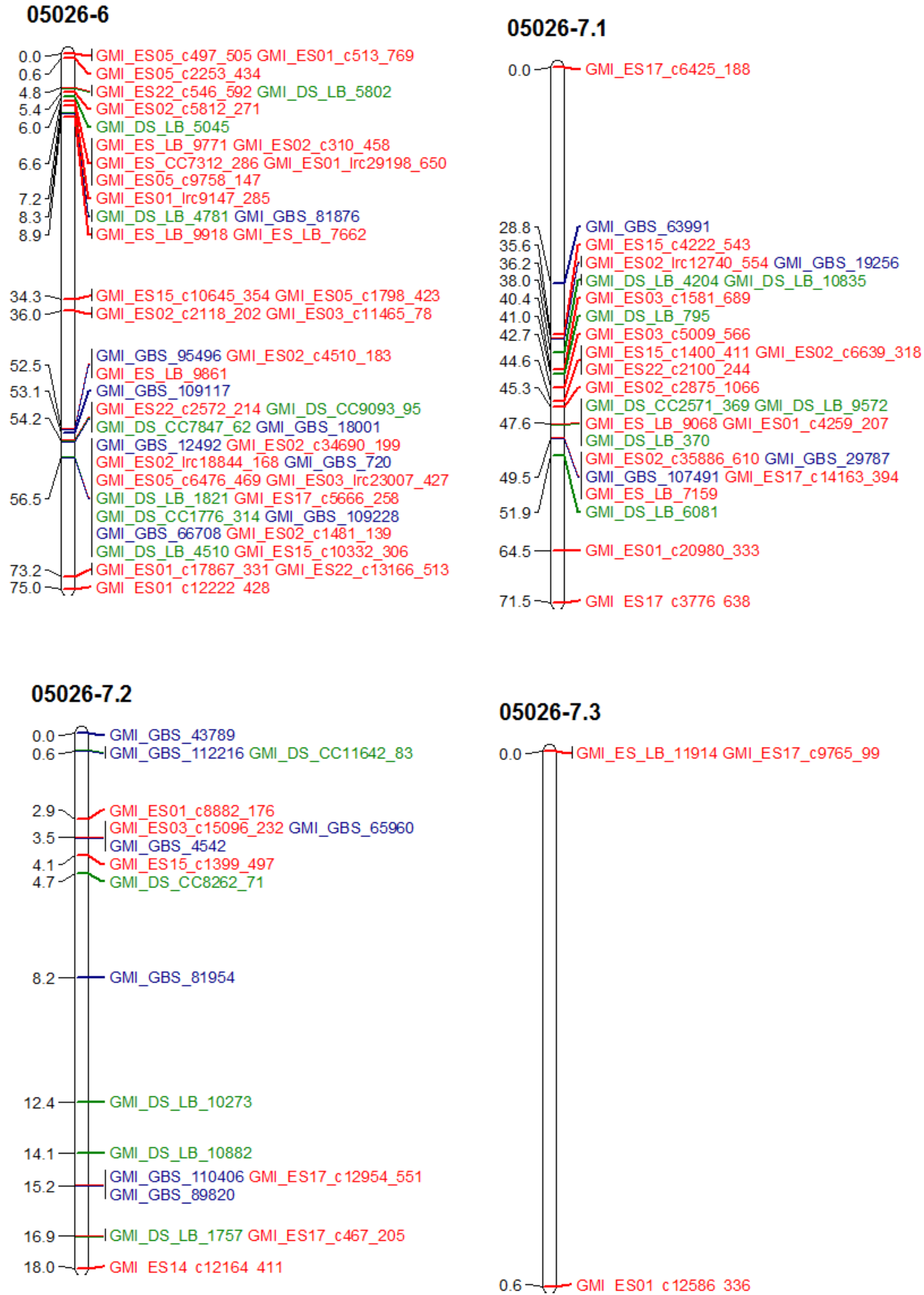

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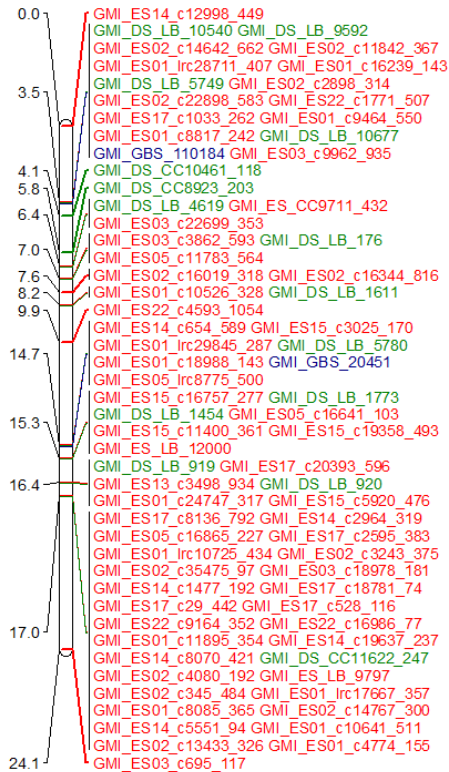

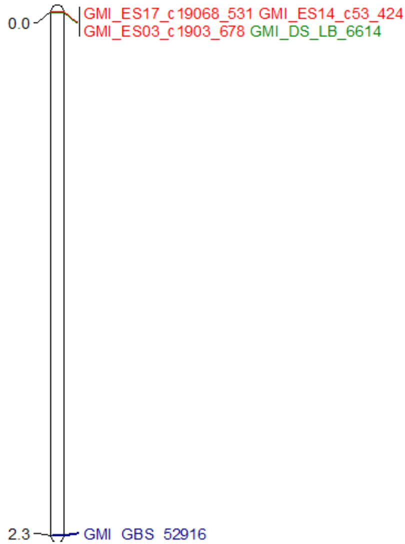

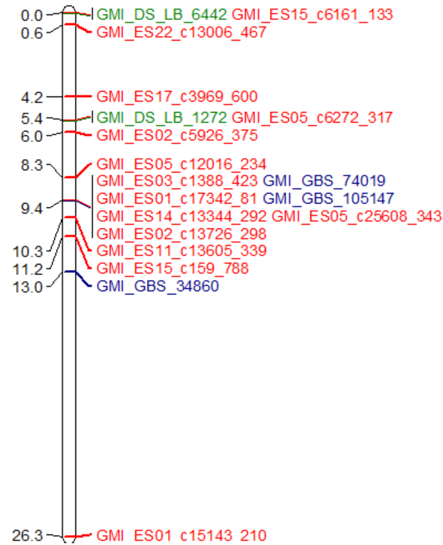

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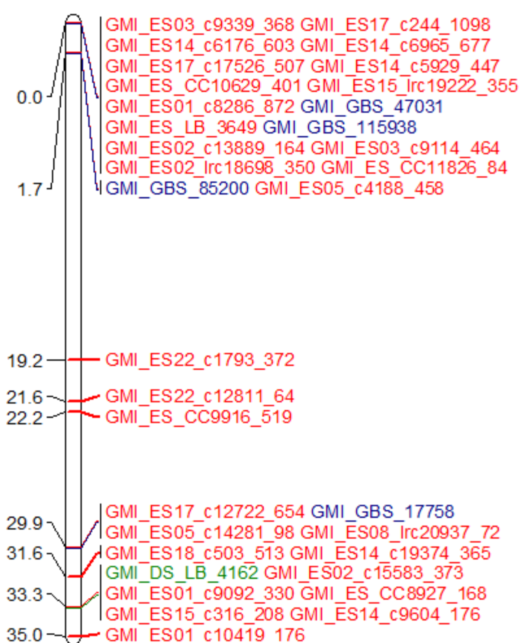

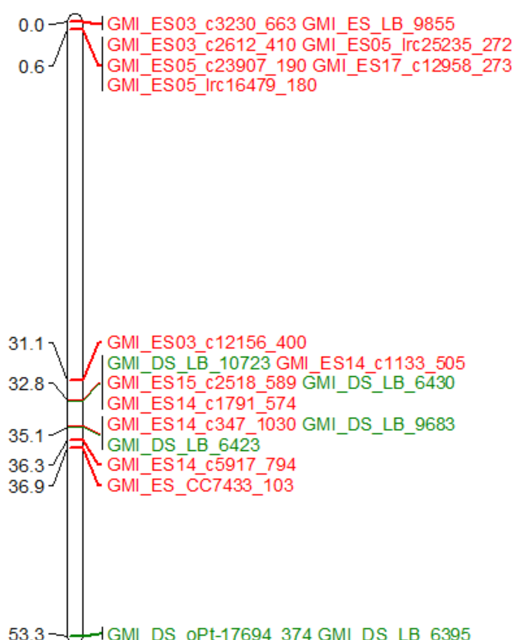

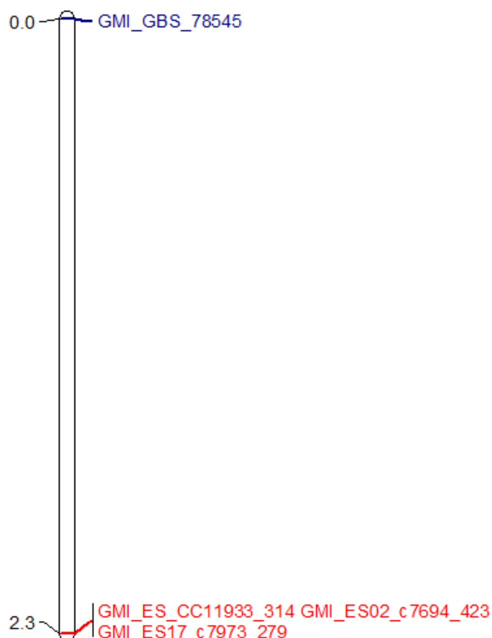

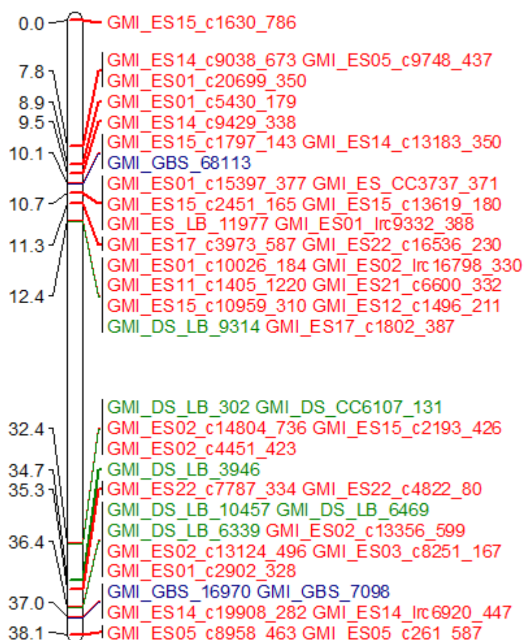

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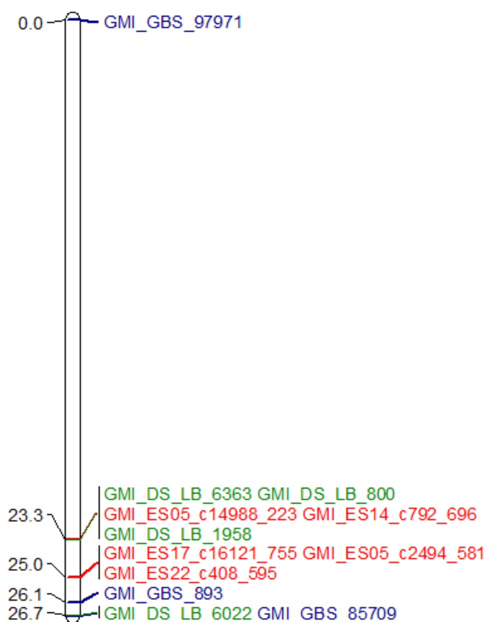

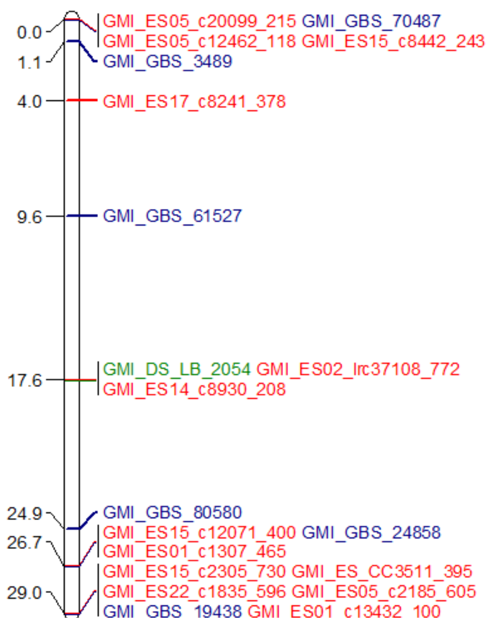

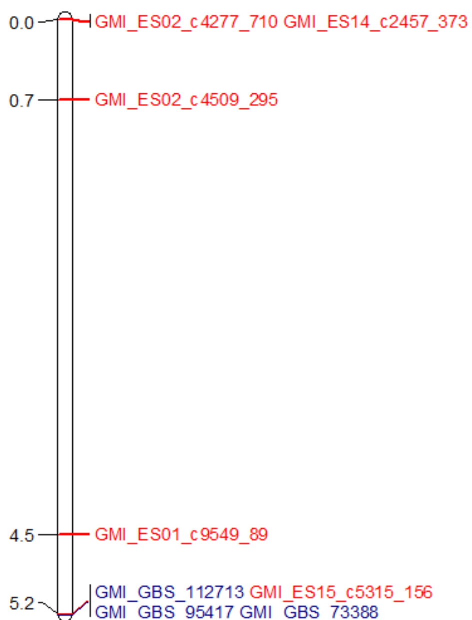

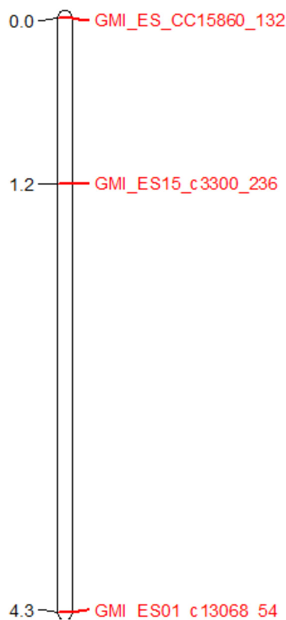

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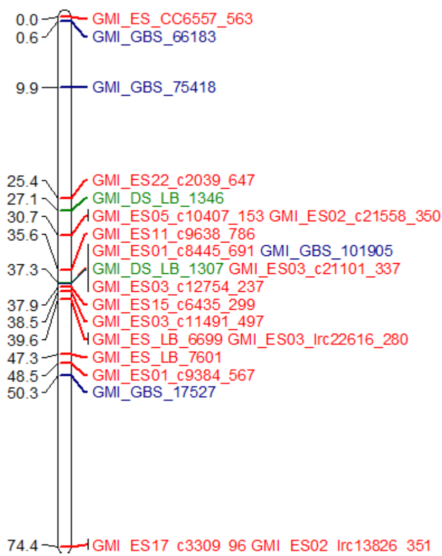

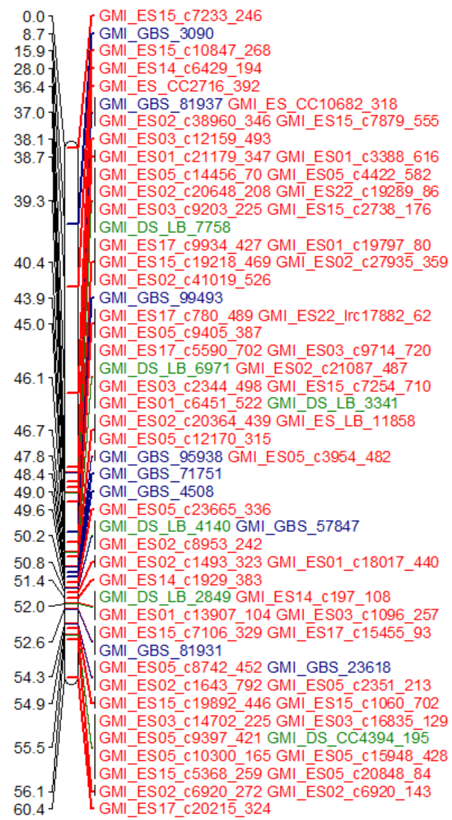

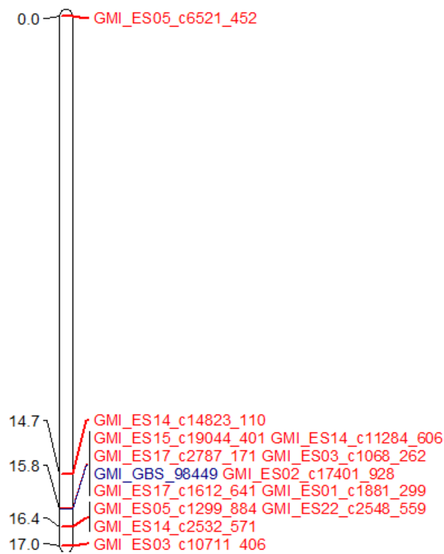

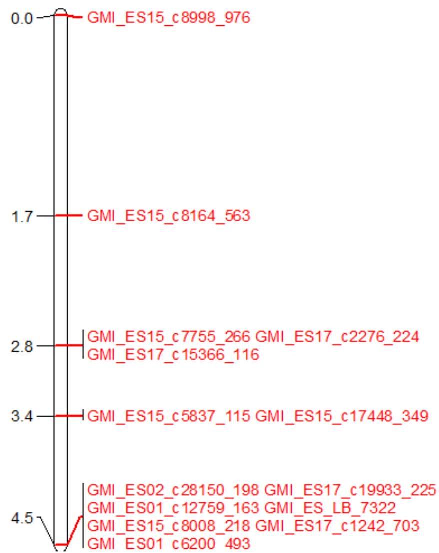

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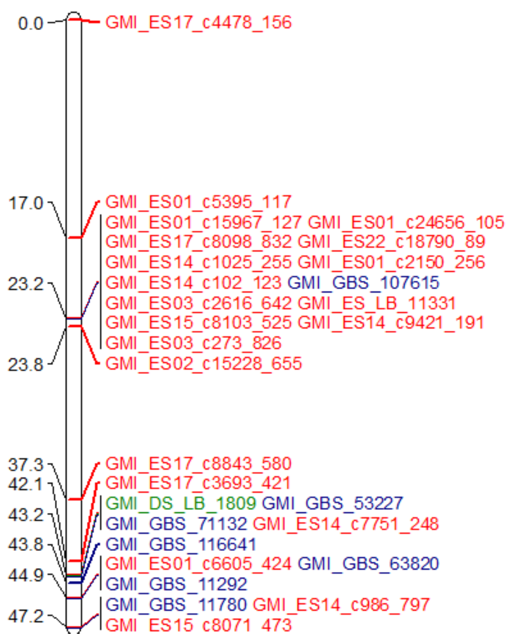

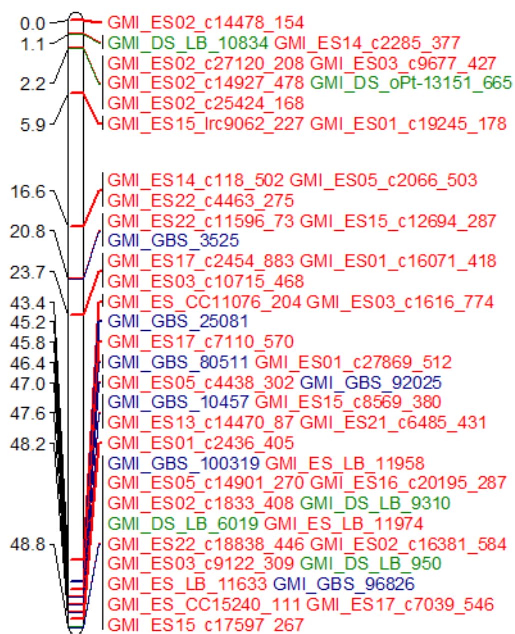

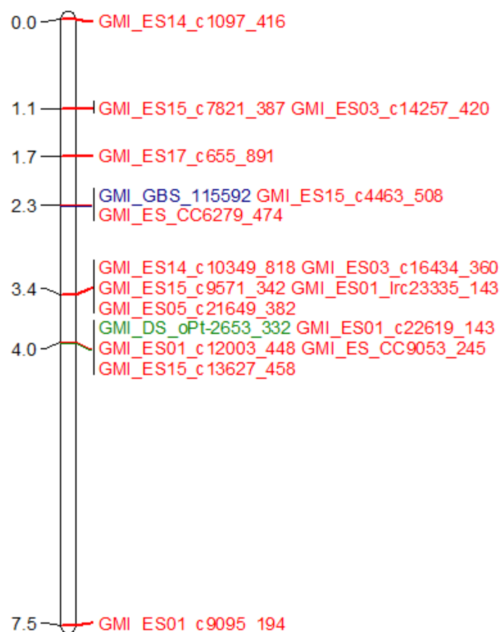

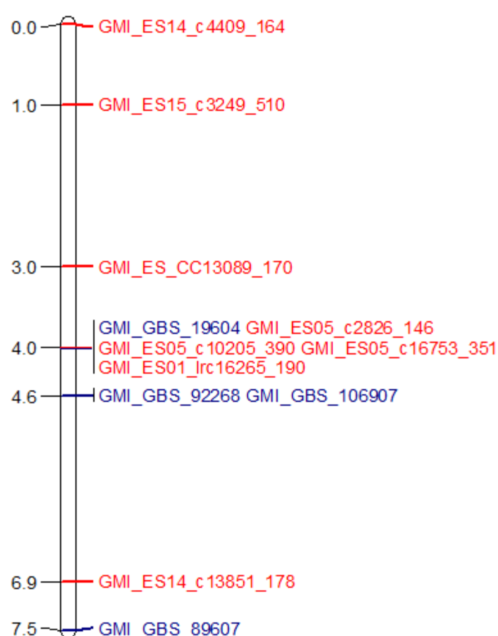

Figure A2. Molecular marker linkage map from ‘Souris’ x ‘ND030299’ F_6 recombinant inbred oat population (Population 05026) (red=EST markers, blue=GBS markers, green=DArT markers) (Continued).