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# Multiple Alleles with Multiple Applications: A New Set of SSR and Avenin DNA Markers for Oat.

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## Introduction

A large amount of molecular marker data of varying types has been collected for oats. Each marker system has its advantages and disadvantages with respect to cost, reproducibility, and ease of use (including data interpretation). While systems such as AFLP (Amplified Fragment Length Polymorphisms) can highlight many loci throughout the genome quite quickly, the markers are generally dominant in nature, and the banding patterns are very complex. SSR (Simple Sequence Repeat) markers, on the other hand, generally highlight very few loci, but are often multiallelic and much simpler to use.

We present a set of fifteen new SSR markers that are multiallelic (MAMA_1 to 15), as well as one that has been monomorphic across all lines tested to date but is useful for assessing DNA quantity and quality (MAMA_0). Two SCAR (Sequence Characterized Amplified Region) markers for avenin genes are also presented. It was expected that these markers would be multiallelic because of the large numbers of avenin bands seen on protein gels.

## Materials and Methods

**Marker development and use**

SSR markers:

DNA from the oat cultivar 'Ogle' was sent to Vizon SciTech, Inc., Vancouver, BC, Canada, for the production and isolation of clones containing AC, TC, and TCTA repeat sequences. The clones were then sequenced at ECORC. Contigs were assembled using the program 'Lasergene7' (**www.dnastar.com/**), and primers flanking the repeat regions were designed using the program 'Primer3' (**frodo.wi.mit.edu/**). The forward primer of each pair was end-labelled using $^{33}$P-γ-ATP and used for PCR. A standard touchdown program (67°C-58°C) was used to amplify the template. Amplification products were separated on 4 or 5% polyacrylamide gels run for 2-5 hours. Gels were dried down and the bands visualized using Kodak BioMax MR film.

Avenin markers:

Twenty-four avenin sequences (protein and/or DNA) from oat were downloaded from GenBank (NCBI: **www.ncbi.nlm.nih.gov/**). These sequences were aligned using Lasergene7 and clustered into groups. Primers for two of the groups were designed in conserved regions using Primer3. The forward primer of each pair was end-labelled as above and PCR performed using an annealing temperature of 62°C. Products were separated and visualized as above.

**DNA sources and preparation**

A set of lines intended to represent the diversity of germplasm available in North American cultivars (the "Standard Oat Allele Panel", or SOAP) was selected after consultation with researchers from across North America. Lines were increased from a single seed originating from each source. DNA was extracted from each of these lines as well as from the lines comprising the 'Kanota' x Ogle (KO) and 'Terra' x 'Marion' (TM) mapping populations using the large scale extraction method described in Wight, *et al.* (2003).

Five random seeds from each of the nineteen lines comprising the 2007 Ontario Performance Trial (PT) lines were selected from seed grown in four-row plots at ECORC and harvested for yield and quality assessments only. These were planted in cyg™ seed germination pouches (**www.mega-international.com/**) containing tap water. When the seedlings were approximately five cm tall, the leaves were harvested, frozen at -70°C, and the DNA extracted using the method of Edwards, *et al.* (1991).

**Data analysis**

Data from the KO and TM mapping exercises were incorporated into the existing maps (Wight, *et al*. 2003, Dekoeyer, *et al.* 2004) using the program 'M5' (Tinker 1999). Comparative mapping was done using the program 'C2 Maps', available as part of the M5 program. The *A. atlantica* x *A. hirtula* (AH) map used for comparison was that of O'Donoughue *et al.* (1992).

The presence or absence of each SSR or avenin marker band was recorded using the program 'GELATO' (Bergeron, *et al.* 2006). Dendograms illustrating how well the different lines could be separated using these markers were produced using the program 'R' (**www.r-project.org/**).

## Results and Discussion

Thirteen of the SSR markers and both avenin markers could be mapped in either or both of the KO and TM hexaploid oat populations (Table 1). The avenin markers map to the locations of the avenin protein loci, as expected, and the SSR markers map throughout the genome. By comparative mapping, all seven *A. atlantica* x *A. hirtula* diploid linkage groups are represented.

All of the markers were tested across the set of 35 SOAP lines. Twelve of the SSRs were seen to represent single loci, with the number of alleles ranging from one to thirteen. The four other SSR markers had more complex patterns. One avenin marker (MAvn_1) identified all three avenin gene blocks and 31 different alleles, while the other (MAvn_2) identified two gene blocks and eleven alleles (Table 1).

While marker efforts in oats have been focussed on their development for use in QTL (Quantitative Trait Locus) analysis and marker-assisted selection, the simplicity and multiallelic nature of the markers described here could also make them useful for diagnostic purposes. Phenotypic identification alone cannot always detect genetic variations that may affect the results of breeding programs or genetic studies nor detect such problems as seed lot contamination or misidentified lines.

Figure one illustrates not only the variation in alleles seen using the marker MAMA_1 with the SOAP lines, but also the genetic variation that can be found in the variety Ogle. The two lines represent the parents used in establishing the KO and Ogle x 'TAM O-301' mapping populations and are known to differ quite significantly (Hu, *et al.* 2007). It is also apparent that the variety 'Troy' is heterozygous at this locus.

To test how useful these markers would be in a real-world scenario, the markers were used to genotype five individuals from field plots of each of the 19 lines comprising the 2007 Ontario Performance Trial, which contained many closely related lines (Figure 2). All of the lines could be separated using the five best quality SSR markers (Figure 3) or the avenin marker MAvn_1 and the two best SSR markers (data not shown). In the latter case, fewer markers might have been needed if so many of the lines had not contained the rust resistance gene *Pc*68, which is linked to the avenin gene block on KO group 4_12 (Figure 2b). Variation in genotype could be detected in some of the lines, and contamination of four lines was also identified (as were some of the contaminants) (Figures 2a, 3). This was not surprising, as the seed from performance tests is combine-harvested and is not intended for pure-line maintenance, but it emphasizes the need for the careful consideration of seed sources when any crossing is to be done or genetic studies are to be undertaken.

**Table 1.** Results and map placements for the markers MAvn_1, MAvn_2, and MAMA_0 to 15.

| Marker name | # loci | # SOAP alleles | # PT alleles | KO group/marker* | TM group/marker* | AH group ** |
|---|---|---|---|---|---|---|
| MAvn_1 | 3 | 31 | 24 | 4 (bcd1482b), 5 (cdo393b), 6 (PGI) | 3(KO4) (POP6a), UL | A |
| MAvn_2 | 2 | 11 | 8 | 4 (bcd1482b), 5 (cdo393b) | no PM | A |
| MAMA_0 | 1 | 1 | 1 | no PM | no PM | ? |
| MAMA_1 | 1 | 9 | 4 | 7 (cdo1461) | no PM | B |
| MAMA_2 | 1 | 4 | 4 | no PM | no PM | ? |
| MAMA_3 | 1 | 10 | 4 | 3 (bcd1660) | no PM | C or A |
| MAMA_4 | 1 | 6 | 2 | 30 (umn5697) | UL | F |
| MAMA_5 | 1 | 11 | 7 | 17 (cdo53) | 9 (umn441x) | D |
| MAMA_6 | 1 | 13 | 12 | 16 (cdo1360) | no PM | E |
| MAMA_7 | 1 | 6 | 6 | 9 (bcd978) | no PM | B |
| MAMA_8 | 1 | 7 | 9 | no PM | 34 (agc_cta215) | ? |
| MAMA_9 | 1 | 7 | 5 | 13 (cdo585b) | 8(KO13) (cdo585b) | C |
| MAMA_10 | 1 | 5+ | 5+ | 3 (aco221b) | no PM | A |
| MAMA_11 | 1+ | 5 | 5 | 11 (cdo1090c) | 2(KO11) (act_caa166) | G |
| MAMA_12 | 1 | 2 | 2 | no PM | no PM | ? |
| MAMA_13 | 3+ | 9 | 8 | 1 (cdo393c), 3 (aco221b), 13 (isu2064b) | 4_16(KO1…) (isu1367x) | A or C |
| MAMA_14 | 3+ | 19+ | 16 | 21 (bcd1250), 21 (cdo1469bDI), 36 (bcd1761a) | 4_16(KO1…) (cdo358a), 25(KO36) (aco245a), 25(KO36) (cdo1321a) | F, G, or A |
| MAMA_15 | 1+ | 11+ | 4+ | 36 (cdo1321a) | 11 (bcd327x) | G |

*marker = closest framework marker from the map of Wight, et al. (2003) (KO) or Dekoeyer, et al. (2004) (TM)
**most likely group based on comparative mapping

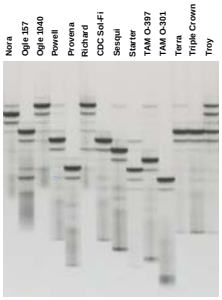


**Figure 1.** Subset of lines used in the Standard Oat Allele Panel amplified using MAMA_1.

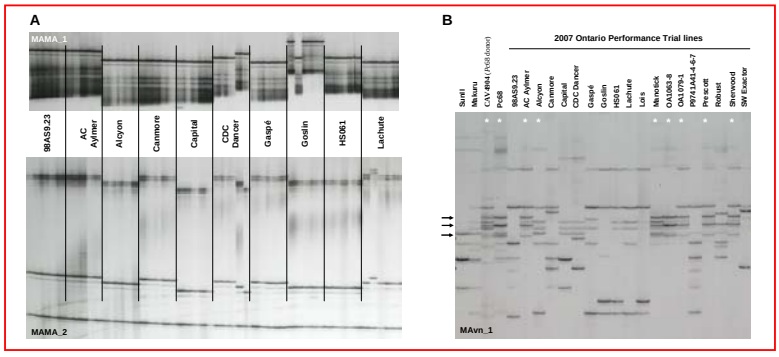


**Figure 2.** Examples of patterns seen using MAMA_1 and 2 (panel A) and MAvn_1 (panel B). Panel A illustrates some of the genotypic differences seen within seed lots and cultivars using two of the SSR markers. Panel B shows the number of different alleles highlighted by marker MAvn_1. It is also evident that three of the bands (arrows) are linked (but not tightly) to the *Pc68* gene (present in starred lines). The line Pc68, containing the *Pc68* gene derived from the *A. sterilis* line CAV4904, and a progenitor of many lines, has the pedigree Makuru*2//CAV4904/2*Sun II.

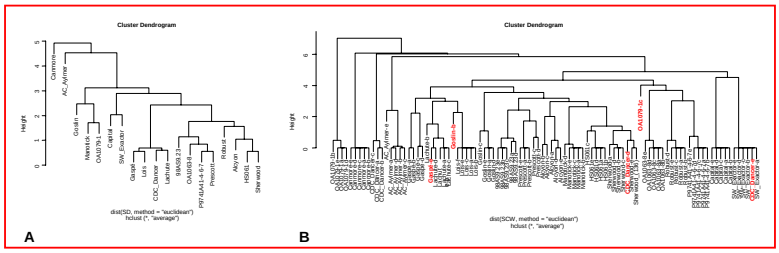


**Figure 3.** Dendograms showing how the markers MAMA_1 to 5 were able to distinguish the PT lines (Panel A) and how their use (along with markers 6 to 12) allowed the identification of genetic variation within the lines comprising the PT set, as well as the identification of contaminants (individuals in red type) (Panel B). The height scales are not meant to imply genetic distance; merely help illustrate which lines show identical marker patterns.

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