Molecular Characterization of Factors Affecting Flowering Time in Hexaploid Oat

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Introduction

Flowering time is a decisive factor in the adaptation of oat to sub-tropical environments. Varieties grown in southern Brazil show varying response to low temperature-dependant floral initiation, a process called vernalization. Vernalization is defined as "the acquisition or acceleration of the ability to flower by a chilling treatment" (Chouard 1960). Genetic analyses have identified at least three genes that affect vernalization requirement in wheat and barley: *VRN1*, *VRN2*, and *FT* (*VRN3*). These genes are implicated in both the vernalization response as well as the response to day length.

VRN1 is induced by vernalization and promotes the transition to reproductive development. *VRN1* encodes an *APETALA1*-like MADS box transcription factor that regulates meristem identity in a range of plants (Preston & Kellog, 2006). *VRN2* is a floral repressor that delays flowering until plants are vernalized and encodes a protein with a zinc-finger motif, which might mediate DNA binding and a CCT domain (Yan *et al.*, 2004). *VRN3* accelerates flowering in long days and encodes a polyethanolamine binding protein (PEBP), a class of protein involved in cellular signaling (Trevaskis *et al.*, 2007). The high level of molecular identity and similar map locations indicate that wheat and barley vernalization genes are orthologous (Dubcovsky *et al.*, 1998).

The objectives of this study were to clone and characterize genes associated with vernalization in hexaploid oat based on orthology with other grass species, and to identify genetic markers linked to those genes.

Materials and Methods

Grass sequences for *VRN1*, *VRN2*, and *VRN3* were obtained from NCBI. Heterologous alignments containing genomic as well cDNA sequences from wheat, barley, and *Lolium* were assembled for each candidate gene using CLUSTAL W (Thompson et al., 1994) in BioEdit Sequence Alignment Editor (Hall, 1999). A consensus template sequence was derived from each alignment, and primers anchored in conserved coding regions were designed using Primer3 (Rozen & Skaletsky, 2000).

Twenty-two prime pairs were used to amplify oat sequences from varieties 'Kanota', 'Ogle', and four parents of the crosses UFRGS 8 x UFRGS 930605 and UFRGS 881971 x Pc68/5*Starter. Of these, Kanota, UFRGS 930605, and UFRGS 881971 are known to show greater response to vernalization. PCR reactions were conducted using a Mastercycler Gradient thermocycler (Eppendorf, Hamburg, Germany). PCR products of the size expected based on the alignments of template sequences were extracted from agarose gels using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI). The purified DNA was cloned in the pCR®4-TOPO vector (Invitrogen, Carlsbad, CA). For each ligation, eight to twelve random clones were picked and sequenced.

The similarity of oat cloned sequences to known gene sequences from GenBank was determined using BLAST from NCBI (**http://www.ncbi.nlm.nih.gov/**). Sequences without BLAST similarity to known genes were not considered further in this study. The sequence variability in oat was estimated by the frequency of single nucleotide polymorphisms among parents using the program SeqMan (DNASTAR Inc., Madison, WI).

Results and Discussion

Oat coding sequences associated with vernalization genes were isolated in this study based on orthology with other grass species. The consensus template sequence derived from each heterologous alignment was similar in size (number of nucleotides) among the candidate genes. However, the number and size of individual introns and exons varied greatly for each gene (Figure 1).

Vrn1: Oat cloned sequences corresponding to the *VRN1* gene were amplified from the primers Ver1-1, Ver1-6, Ver1-18, and Ver1-19 (Figure 1b). Sequence derived from *VRN1*-based primers showed high similarity to cloned sequences of the same gene from wheat, barley, *Lolium*, and *Festuca*. Sequences derived from Ver1-18 and Ver1-19 also showed high similarity to the MADs box transcription factor isolated from *Avena sativa* and *Avena strigosa* (Preston & Kellog, 2006).

Vrn2: Oat sequence derived from *VRN2*–based primer did not show direct similarity to grass sequences of *VRN2*. Cloned sequences showed some similarity to a protein with a zinc-finger motif and a CCT domain (ZCCT) from *Triticum monococcum*. Proteins like ZCCT1 and ZCCT2 are components of *VRN2* in wheat and barley (Yan et al., 2004). Parts of these sequences were most similar to a retrotransposon (OARE-1) derived from *Avena sativa*. This suggests the possibility that a non-functional version *VRN2*, disrupted by a retrotransposon insertion in the first intron, was cloned.

Vrn3: Cloned oat sequences corresponding to the *VRN3* gene were isolated from the primers Ver3-23 and Ver3-24 (Figure 1b). These sequences showed high similarity to *VRN3* sequences from wheat, barley, and *Lolium* (Yan *et al.*, 2006). Oat sequences also showed high similarity to the *Hd3a* gene, which is involved with flowering time in rice, and is an orthologue of the barley and wheat *VRN3* gene and the *Arabidopsis thaliana FT* gene (Yan *et al.*, 2006).

Sequence diversity: The sequence variability in oat, estimated by the frequency of single nucleotide polymorphisms among parents, was highest for the *VRN3* genes. The highest number of single nucleotide polymorphisms were detected between the parents U71 and Pc68 (Figure 2a) for both *VRN1* and *VRN3*. These results are consistent with phenotypic results, where greater differences in vernalization requirement was observed between these genotypes (Figure 2b).

Mapping: Although most PCR product sizes for a given primer pair were consistent among genotypes, detectable size polymorphisms were observed for *VRN3* (Figure 3a). This polymorphism was used to map *VRN3* in populations from the crosses UFRGS 8 x UFRGS 930605, UFRGS 881971 x Pc68/5*Starter, and Kanota x Ogle (KxO). This was mapped relative to new DArT markers in all three populations (see Tinker *et al.*, oral presentation at this conference). The mapping of *VRN3* in all three populations relative to DArT markers and previously mapped KxO markers demonstrates that this gene lies within colinear regions corresponding to KxO linkage group 6 (Figure 3b).

Conclusion: These results demonstrate the first isolation and characterization of orthologous regions of key vernalization-related genes in oat, and the map location of one homoeolog of *VRN3* in three hexaploid oat populations.

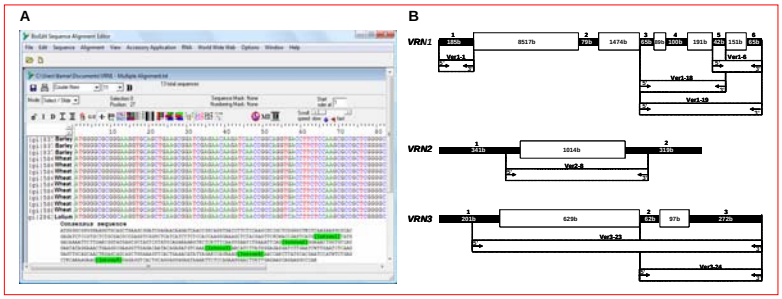

Figure 1. Example of heterologous alignment (panel A) and the identification of introns and exons in the consensus sequence for *VRN1*, *VRN2* and *VRN3* (panel B). Panel A illustrates a heterologous alignment of the *VRN1* gene containing genomic and cDNA sequences from barley, wheat, and *Lolium*. Panel B shows the location and size of introns (represented by boxes) and exons (represented by black bars) in the consensus sequence for each candidate gene. Panel B also shows the name and location of the primer pairs that produced coding sequences in oat associated with vernalization genes.

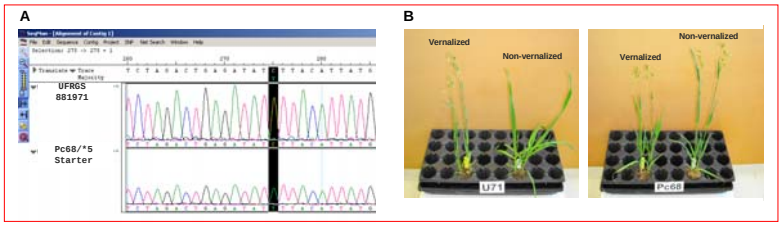

Figure 2. Sequence variability in oat (panel A) and the effect of vernalization on flowering time (Panel B). Panel A shows an example of sequence variability between the parental genotypes UFRGS 881971 (U71) and Pc68/*5Starter (Pc68). The sequence variability was estimated by the frequency of single nucleotide polymorphisms. Panel B illustrates the flowering time of the parents U71 and Pc68 when plants were submitted to different vernalization treatments (vernalized and non-vernalized).

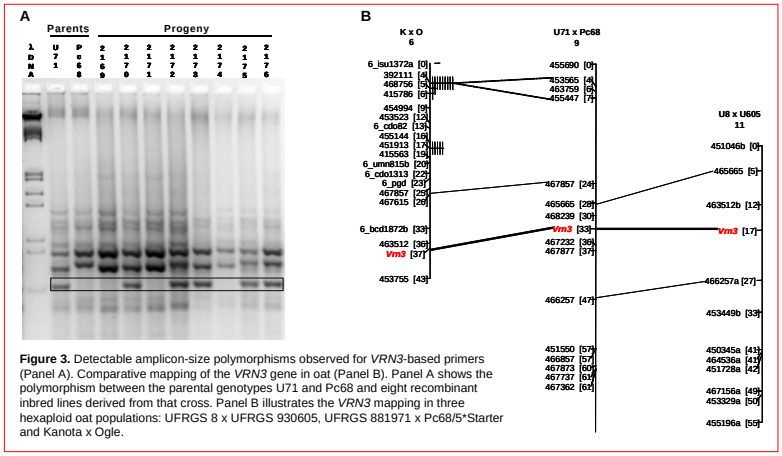

Figure 3. Detectable amplicon-size polymorphisms observed for *VRN3*-based primers (Panel A). Comparative mapping of the *VRN3* gene in oat (Panel B). Panel A shows the polymorphism between the parental genotypes U71 and Pc68 and eight recombinant inbred lines derived from that cross. Panel B illustrates the *VRN3* mapping in three hexaploid oat populations: UFRGS 8 x UFRGS 930605, UFRGS 881971 x Pc68/5*Starter and Kanota x Ogle.

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"Important note regarding results presented in scientific posters: The research reported here is in progress, and therefore the study results have not been subjected to scientific peer review."