Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

Identification and Characterization of Flowering Time Genes in Tall Wheatgrass

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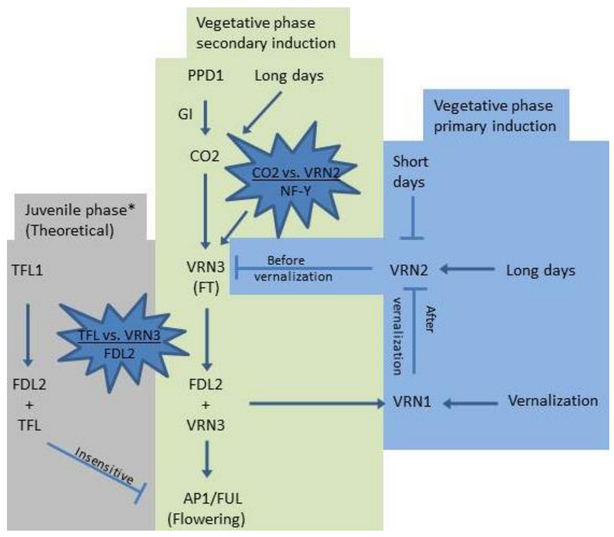
Introduction

Compared to annuals, perennial cereals confer significant environmental and economic benefits [1]. Tall wheatgrass, *Thinopyrum elongatum* and *Th. ponticum*, are long lived perennial grasses closely related to wheat and provide an important source of perennial habit for the development of perennial wheat. Despite the large amount of molecular information in annuals, limited plant development and molecular information is available for *Thinopyrum sp*. With the existing knowledge of flowering time pathways in annual and perennial plant species, we identified key flowering time candidate genes for sequencing and expression analysis.



Candidate genes	Expressed in
VRN1 (VERNALIZATION1)	Leaf and meristems
VRN2 (VERNALIZATION2)	Leaf
VRN3 (VERNALIZATION3)	Leaf and meristems
CO2 (CONSTANS2)	Leaf
PPD1 (PHOTOPERIOD1)	Leaf
FDL2 (FD LIKE2)	Leaf and meristems
TFL1 (TERMINAL FLOWER1)	meristems
GI (GIGANTEA)	Leaf

Vegetative to reproductive phase transition requires a co-ordinated action of vernalization and photoperiod. While *VRN1*, a MADS-box meristem identity gene, is up-regulated by vernalization [2], *VRN2*, the central repressor of flowering is down-regulated [3]. *VRN3* (TaFT) is induced by long days once vernalization requirement is met [4]. As such, CO2, which is up-regulated in long days, outcompetes *VRN2* to create a complex with NF-Y proteins and this CO2-NF-Y complex promotes *VRN3* expression [5]. Further, *GI*, which encodes a nuclear-localized membrane protein, functions upstream of *CO2* and *FT* [6]. *PPD1*, activated by phytochrome C, is required for high levels of *FT* (*VRN3*) under long days [7] which in turn induces *VRN1* expression, through interaction with *FDL2* [8], creating a positive feedback loop. In *Arabidopsis*, *TFL1* is antagonistic to *FT* and is proposed to compete for binding with *FDL2* to repress flowering. Reduced *TFL1* expression in *Arabis alpina* (perennial weed) is linked to shorter juvenile period [9] suggesting its potential role in perennial habit.

Material and methods

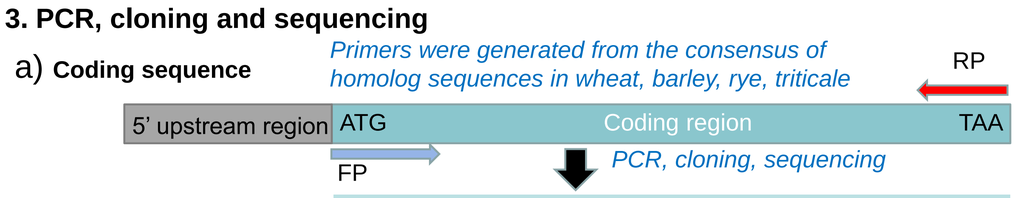
1. Plant materials:

Species	Accessions	Ploidy level	Growth habit
Triticum aestivum	Chinese spring	2n=6X=42	Spring, annual
Thinopyrum elongatum	PI 531717	2n=2X=14	Spring, perpetual flowering
	PI 531718	2n=2X=14	Spring, perpetual flowering
	PI 531719	2n=8X=56	Winter, perennial
Thinopyrum ponticum	PI 206624	2n=10X=70	Winter, perennial

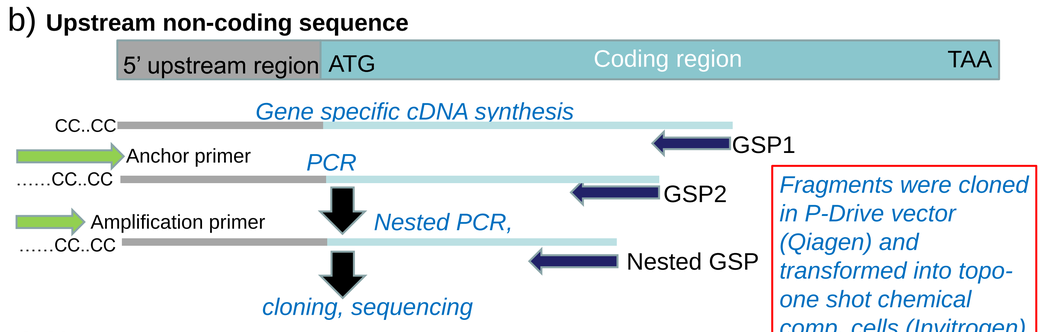
2. RNA extractions: Total RNA was extracted from young leaves and/or meristems (3 leaf stage)

3. PCR, cloning and sequencing

a) **Coding sequence**



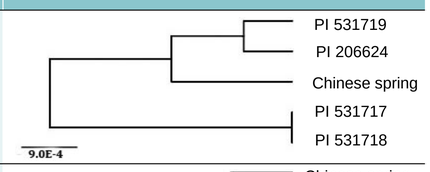
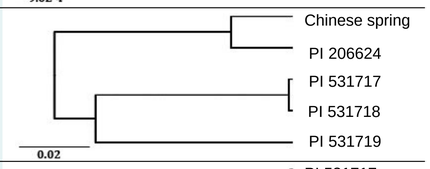
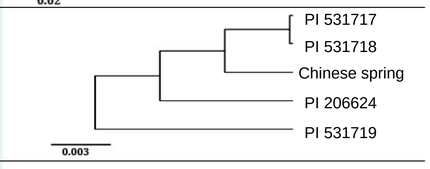
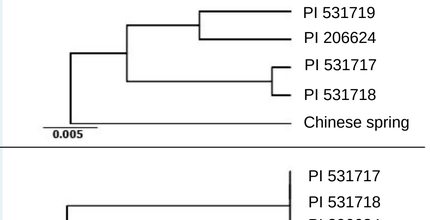
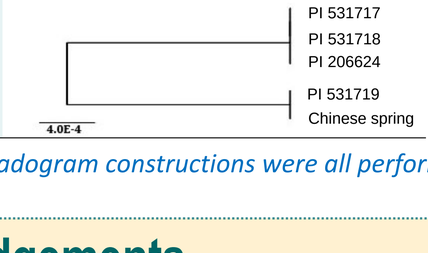
b) **Upstream non-coding sequence**



4. Next steps

Genome walking will be performed to elucidate the genetic makeup of the intron regions. The expression data and effects of the candidate genes on phenotype and flowering will be carried out through quantitative RT-PCR and virus induced gene silencing (VIGS) respectively.

Results and discussion

Genes	Expected length for Chinese Spring	*Thinopyrum* Accessions	Length of consensus sequence	% similarity to Chinese spring	Major insertion/deletions w.r.t. Chinese spring	Cladogram
CO2	647	PI 531717	647	99.5	Highly conserved.	
		PI 531718	647	99.5		
		PI 531719	647	99.3		
		PI 206624	647	99.5		
VRN2	592	PI 531717	613	84.5	Insertion of bases in multiples of 3 without changes to the conserved CCT domain.	
		PI 531718	613	83.8		
		PI 531719	604	82.5		
		PI 206624	616	92.9		
FDL2	375	PI 531717	375	98.4	Conserved with few SNPs.	
		PI 531718	375	98.8		
		PI 531719	375	96.5		
		PI 206624	375	97.5		
VRN1	701	PI 531717	701	97.8	Conserved with few SNPs.	
		PI 531718	701	97.8		
		PI 531719	701	97.2		
		PI 206624	701	97.6		
Ppd1	1918	PI 531717	2068	87.8	Insertion or deletion of bases in multiples of 3 without changes to the conserved Response-regulatory and CCT domains.	
		PI 531718	2062	87.9		
		PI 531719	1879	93.0		
		PI 206624	1882	92.4		
TFL1	429	PI 531717	429	97.2	Conserved with few SNPs.	
		PI 531718	429	97.8		
		PI 531719	429	97.7		
		PI 206624	429	96.9		

Progress

Gene	Coding region	5' upstream region
VRN1	Sequenced	In process
VRN2	Sequenced	In process
VRN3	In process	In process
Ppd1	Sequenced	In process
CO2	Sequenced	In process
FDL2	Sequenced	Sequenced
GI	In process	In process
TFL1	Sequenced	In process

A 144 bp insertion in PI 531717 and PI 531718 for *Ppd1* gene aligns to a region of *Ppd-B1* exon 7 in *Triticum turgidum, T. durum,* etc.

Sequencing was completed at McGill University and the Genome Quebec Innovation centre. Sequence alignments, computation of similarity and cladogram constructions were all performed in **gene**ious *6.1.6 software.*

References

[1] Bell et al (2010). Crop Pasture Sci. 61: 679-690. **[2] Yan L et al (2003).** PNAS 100(10): 6263-68. **[3] Yan L et al (2004).** Science 303(5664): 1640-44. **[4] Yan L et al (2006).** PNAS 103(51): 19581-86. **[5] Li C et al (2011).** Plant J. (67): 763-73. **[6] Tseng TS et al (2004).** Plant cell 16(6): 1550-63. **[7] Chen A et al (2014).** PNAS 111(28): 10037-44. **[8] Li C and Dubcovsky J (2008).** Plant J 55(4): 543:54. **[9] Wang R et al (2011).** Plant Cell 23(4):1307-21.

Acknowledgements

Project 13-1178 was funded by AAFC through peer reviewed projects. Germplasm used in this study was kindly provided by the USDA, ARS, National Genetic Resources Program. *Germplasm Resources Information Network - (GRIN)*.

2014

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