Copy number and natural haplotype variation at *HvFT1* locus associated with accelerated flowering time

Rebecca Nitcher[1], Assaf Distelfeld[1,2], Jorge Dubcovsky[1]

[1] Department of Plant Sciences, University of California, Davis, Davis, California 95616, USA

[2] Department of Mol. Biol. and Ecology of Plants, Tel-Aviv University, Tel-Aviv 69978, Israel

INTRODUCTION

The *Flowering Locus T* (*FT1=VRN3)* is a central integrator of photoperiod and vernalization signals in temperate cereals. As a mobile signal, FT1 facilitates the conversion of environmental cues received in the leaves to a plant reproductive response in the apical meristem. In barley (*Hordeum vulgare* L.), natural variation in the promoter (9 linked SNPs and indels) and first intron (2 linked SNPs) of *HvFT1* has been associated with flowering time differences. Here we describe copy number variation (CNV) at *HvFT1* in line BGS213, which accounts for contrasting flowering time of seemingly identical alleles, as well as for some of the effects previously attributed to SNPs in intron one. The understanding of the effect of *HvFT1* variation on flowering time will help breeders to engineer barley varieties better adapted to changing environments.

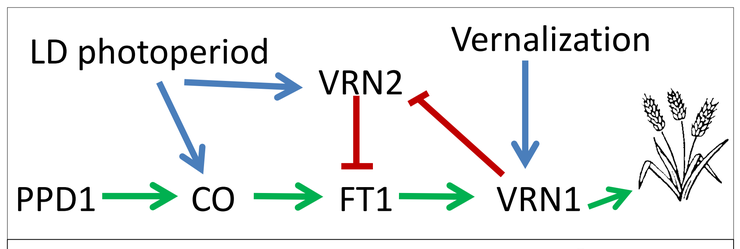

Figure 1. Flowering time pathway in wheat.[2]

METHODS

Two distinct haplotypes were identified in 10 varieties sequenced before.[1] Differences were found both in the promoter and the first intron (Table 1). The *HvFT1* alleles from BGS213 (genetic stock of dominant *Vrn-H3*) and Iwate Mensury C showed different effects on flowering time in spite of having identical *HvFT1* haplotypes. These two lines were crossed and F_2 lines were selected for the dominant *VrnH2* allele (winter) and tested for flowering time and for markers linked to *HvFT1*. Experiments were conducted in greenhouses under long day at non-vernalizing temperatures (20-25 C). Statistical analysis using a factorial analysis of variance (ANOVA) was performed using SAS version 9.1. CNV was tested by relative quantitative method by performing SYBR Green qPCR on genomic DNA, and comparing probes in the *HvFT1* region with a known single copy control gene SNF2[3].

Parent	Haploid CNV	*VrnH1*	*VrnH2*	*HvFT1*										
				Promoter									Intron 1	
				1137	1442	1505	1667	2533	3932	4419	4493	5548	2700	3884
BGS213	4	*W*	W	C	C	T	i	T	C	A	G	4GC	A	G
Iwate Mensury C	1	*W*	*S*	C	C	T	i	T	C	A	G	4GC	A	G
H. spontaneum	1	*W*	W	T	G	C	d	C	G	G	A	8GC	T	C

Table 1. Genetic composition of parental lines. *VRN-H1* and *VRN-H2* are shown as winter (W, *vrnH1/VrnH2*) or spring (S, *VrnH1/vrnH2*). Positions are reported as bp upstream (promoter) or downstream (intron) from the start codon in *H.spontaneum.*

RESULTS

In 164 IMC x BGS213 F_2 lines selected for dominant *Vrn-H2* allele a 3:1 ratio of spring:winter lines was observed. The plants carrying the BGS213 *HvFT1* allele flowered more than 2 months earlier than the plants with the IMC allele (P<0.0001). Therefore, we decided to test for CNV in *HvFT1*. Using at least ten biological replicates of each parent and several sites across the *HvFT1* gene, we found 1 copy of *HvFT1* in IMC lines and 4-5 copies in BGS213 lines. This variation correlates perfectly to flowering time in the F_2s (Fig 2). We also tested 9 other lines, and found most have single copies of HvFT1 (Fig 3).

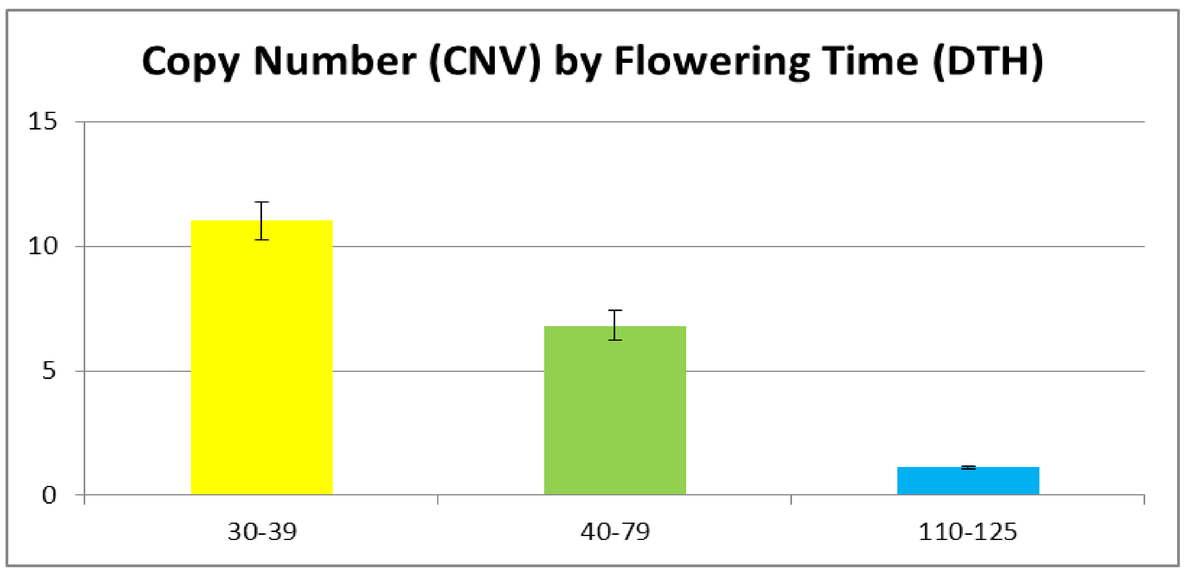

Figure 2. CNV at *HvFT1* correlates to flowering time in BGS213 x IMC recombinant F2 lines tested.

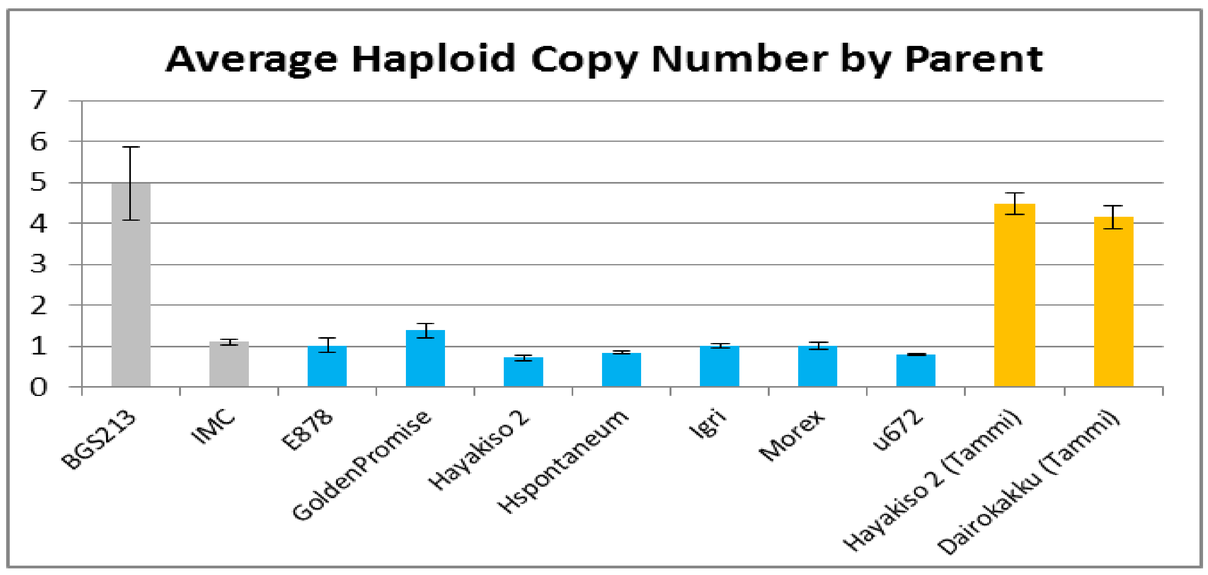

Figure 3. BGS213 and two Hayakiso 2 and Dairokakku lines carrying an introgression from Tammi (a parent of BGS213), also show matching increases in copy number.

CONCLUSIONS

1. Flowering time effects of BGS213 previously attributed to SNP differences are actually a result of CNV at the *HvFT1* locus. This may also be true in other populations where flowering time differences were previously unexplained.
2. Our results show that CNV contributes to barley genetic diversity at important reproductive genes and to its adaptation to different environments. Similar examples have been recently described for the photoperiod and vernalization genes in wheat[4].

REFERENCES

1. Yan L, Fu D, Li C, Blechl A, Tranquilli G, Bonafede M, Sanchez A, Valarik M, Dubcovsky J (2006) The wheat and barley vernalization gene VRN3 is an orthologue of FT. Proc. Natl. Acad. Sci. U.S.A. 103: 19581–19586.
2. Distelfeld A., Li C., Dubcovsky J. (2009) Regulation of flowering in temperate cereals. Curr. Opin. Pl. Biol. 12:178–184.
3. Yan, L., V. Echenique, et al. (2002). "Cereal genes similar to Snf2 define a new subfamily that includes human and mouse genes." Mol Genet Genomics 268(4): 488-499.
4. Diaz, A., M. Zikhali, et al. (2012). "Copy number variation affecting the Photoperiod-B1 and Vernalization-A1 genes is associated with altered flowering time in wheat (Triticum aestivum)." PLoS One **7**(3): e33234.

ACKNOWLEDGEMENTS

This project was funded by the National Research Initiative Competitive Grant no. 2007-35301-18188 from the USDA National Institute of Food & Agriculture, and by the Henry A. Jastro Graduate Research Scholarship Award at UC Davis.

United States Department of Agriculture — National Institute of Food and Agriculture