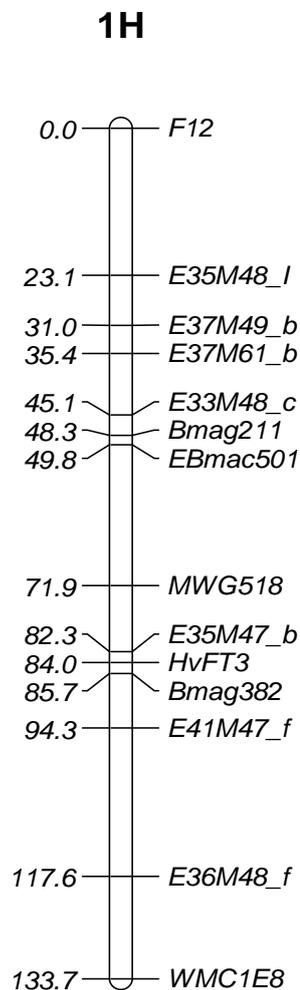


Expression analysis of vernalization and day-length response genes in barley (*Hordeum vulgare* L.) landraces with intermediate vernalization requirement.
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Supplementary File 1. Mapping of *HvFT3* in the Beka x Mogador population

HvFT3 was genotyped in 120 doubled haploid lines of the Beka x Mogador population (Cuesta-Marcos et al. 2008) as a presence/absence marker, with the primers reported. *HvFT3* was mapped using JoinMap 4.0 (Kyazma B.V.), on the long arm of chromosome 1H, at 84.0 cM, between markers E35M47_b and Bmag382. The position of *HvFT3* in this population is similar to that of the Igri x Triumph (Faure et al. 2007) and Steptoe x Morex (Kikuchi et al. 2009) populations.



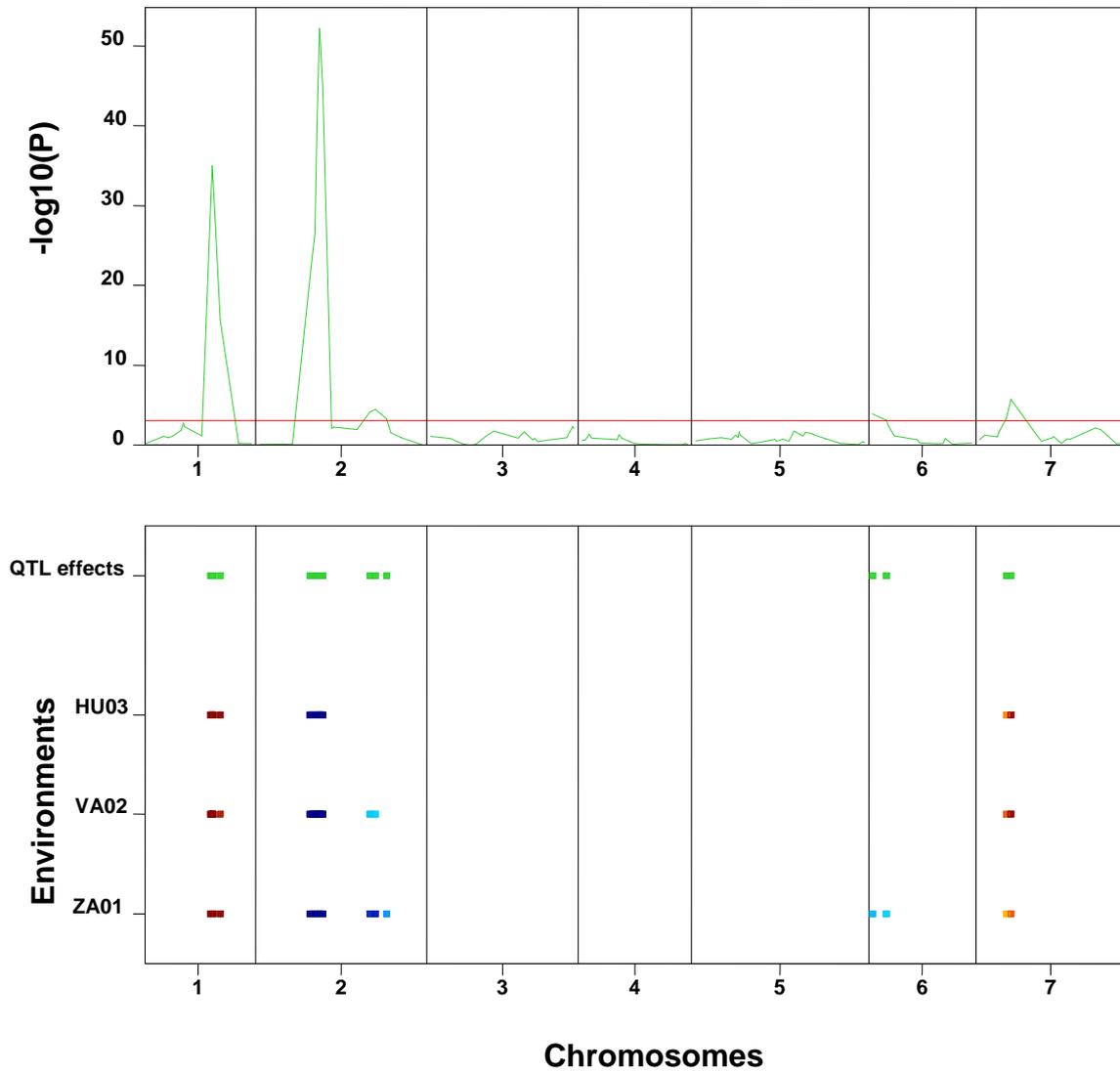
Supplementary File 2 – QTL analysis for flowering time related traits in the Beka x Mogador population.

Data used for the analyses were reported in Cuesta-Marcos et al. (2008), as the description of field and greenhouse trials, and plant materials. QTL analyses were carried out with the software GenStat 12.1 (VSN International Ltd) using the QTL module, multiple environments, Genotype by Environment analysis. Independent analyses were run for days to heading in the field (three autumn-sown trials) and final number of leaves on the main shoot in greenhouse treatments (no vernalization, short photoperiod and vernalization followed by short photoperiod). A single environment QTL analysis was run for the effect of photoperiod without vernalization (Pho_NV as the difference in the number of leaves between the NV_SP and the NV_LP treatments). QTL scans and the effect of the identified QTLs is reported.

Significant effects are indicated by either a '+' or '-' sign, depending on whether the allele from the winter parent (Mogador) increased or decreased the trait value (Malosetti et al. 2008). Brown and orange indicate strongly positive and moderately positive contributions from the allele coming from the Mogador parent, while dark and light blue indicate the superiority of the allele coming from the Beka parent (Cooper et al. 2009). The uppermost green line is a summary of the significance for the overall test on QTL presence as shown in the top panel.

HvFT3 was situated in the center of the QTL peaks previously identified as an effect of *PpdH2* in chromosome 1H (Cuesta-Marcos et al. 2008), thus confirming its status as *PpdH2* candidate.

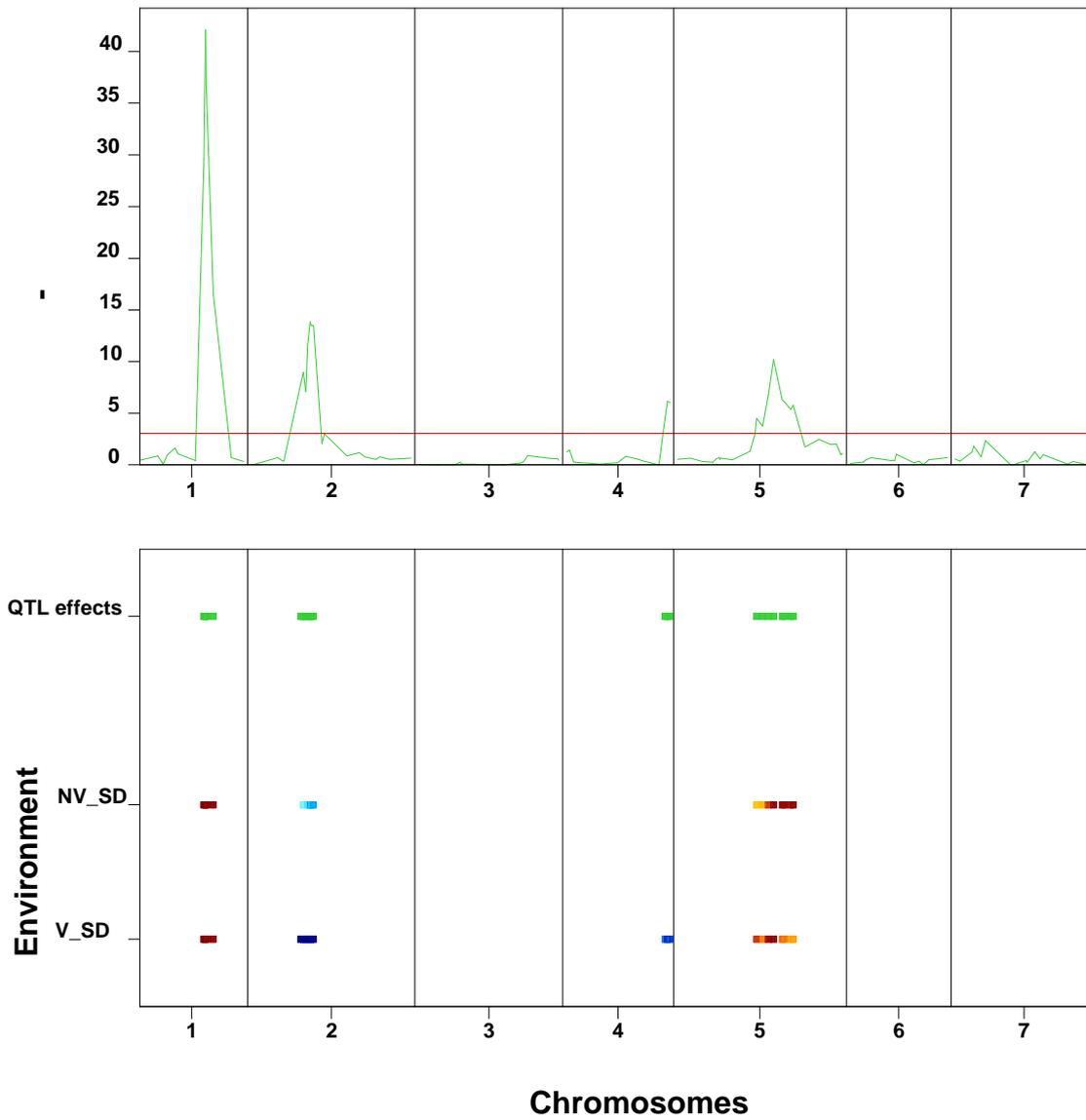
DAYS TO HEADING IN FIELD TRIALS



THRESHOLD=3.07

Locus	Chr.	cM	Wald	DfWald	PrWald	HU03	VA02	ZA01
HvFT3	1	84	76.57	3	0.0000	2.50	1.39	2.31
Bmac132	2	75	317.04	1	0.0000	-2.71	-2.71	-2.71
EBmag793	2	145	13.41	1	0.0000	-0.59	-0.59	-0.59
E35M47_c	6	0	6.74	3	0.0000	0.04	-0.08	-0.58
E41M47_e	7	39	31.93	1	0.0000	0.86	0.86	0.86

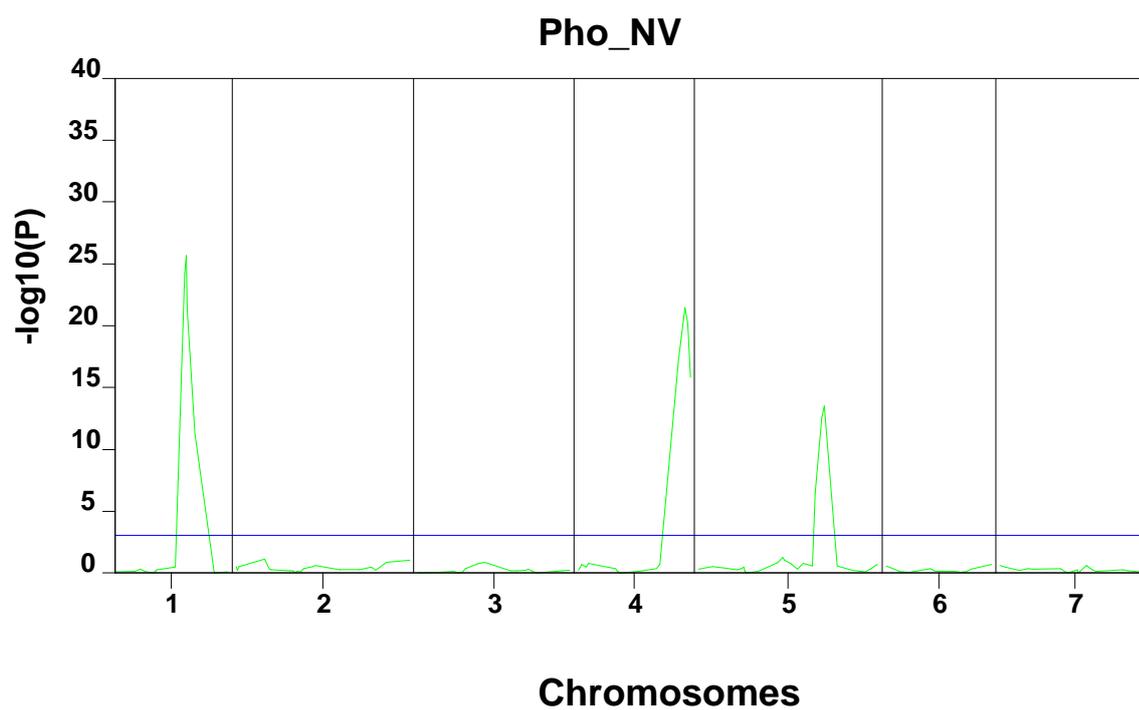
GREENHOUSE TREATMENTS UNDER SHORT PHOTOPERIOD



THRESHOLD=3.07

Locus	Chr.	cM	Wald	DfWald	PrWald	NV_SP	V_SP
HvFT3	1	84	163.82	2	0.0000	1.21	0.89
Bmac132	2	75	83.89	2	0.0000	-0.53	-0.91
HvZCCT	4	129	16.57	2	0.0000	0.03	-0.41
E35M48_c	5	123	54.09	1	0.0000	0.42	0.42

EFFECT OF PHOTOPERIOD WITHOUT VERNALIZATION (Pho_NV)



THRESHOLD=3.07

Locus	Chr.	cM	Wald	Effect
HvFT3	1	84	118.21	1.17
HvZCCT	4	126	98.78	-1.05
E36M48_g	5	148	57.65	-0.82

Supplementary File 3 - Sequencing *HvFT3*

To sequence *HvFT3*, primers were designed to amplify five overlapping fragments. Positions and amplicon size are labelled with respect to the coordinates of the 'Morex' *HvFT3* (AB476614) sequence (Kikuchi et al. 2009).

Primer	Sequence	Position	Size	Tm
1F	ACTAAGCATGCAGTTGAAACGA	29	517	60
2R	GGAACGGTTGATGTAAATGGAT	545		
3F	CAAGGCTAAGGCTGTTAATTGG	433	461	60
4R	GGCGAGAATAAGAAAATGTTGC	893		
5F	AAGATATTGGTGGATCCAGACG	796	511	60
6R	ACAATGGCCTTGTCTCAAAGTT	1306		
7F	GGTGCCAGCTTTGGTATGAT	1028	617	60
8R	CTGCAAAAGCACCCCAGTAT	1644		
9F	AGGTTTCGAAGAGTTTGCTTTG	1435	532	60
10R	CTGCACATTATTTGTGATGCAA	1966		

Sequencing was carried out in four genotypes: 'Alexis', SBCC058, the Spanish cultivar 'Pane' (SBCC167) and the French spring cultivar 'Beka' (SBCC169). Amplicons from two independent PCR reactions were sequenced from both ends with forward and reverse primers. Sequences were assembled and searched for polymorphisms using the software package ClustalW2 (Larkin et al. 2007).

Nucleotide sequences from the four genotypes (1922 bp) were identical and a few polymorphisms were identified with the sequence from 'Morex': an SNP in intron 2 at 983 bp, where Morex carries a "C" and these cultivars a "T"; an SNP in exon 3, at 1039 bp where Morex carries a "T" and these cultivars a "C". This is a conserved change, silent at the amino acid level. There are four polymorphisms in intron 3, SNP at position 1106, Morex has a "G" and these cultivars have an "A"; two indels at 1184 (TTC_/TTCC), and 1365 (GTT_/GTTT, Morex and these cultivars, respectively), plus another SNP at 1567 bp, where Morex has a "G" and these cultivars an "A". mainly in intronic regions.

A BLAST search (Altschul et al. 1997) against sequences present in GenBank revealed another 25 *HvFT3* sequences from different barley cultivars (GenBank accessions EU331874-EU331898). Multiple comparisons of these sequences showed the same polymorphisms detected in this study. The only observed polymorphism within the coding sequence is the same one that we have seen, in exon 3. The marker used to genotype *HvFT3* can be efficiently used to discriminate two alleles of the *HvFT3* gene.

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