Flowering time and genetic control in wide barley crosses

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Introduction

Barley, of all domesticated cereals, is the most diverse and drought tolerant crop. Range expansion alongside with natural and artificial selection over thousands of years caused adaptation patterns to arctic, subarctic and tropical environments, that are conserved in local landrace material and former cultivars.

Understanding the complex genetic and environmental control of the key developmental stages, such as flowering, has become a substantial scientific goal to minimize abiotic and biotic stresses under changing climate conditions.

Objective

This project aims to identify phenotypic segregation and diagnostic markers in the bi-parental subpopulations of crosses between the European elite cultivar RGT Planet and three landrace accessions across the geographical range from Syria (WB-340) and Iraq (WB-468) to Mexico (WB-168). These lines were chosen to represent the variation of flowering times when sown under field conditions in Dundee, as part of an EU legacy project (Wheat and barley legacy for breeding improvement -WHEALBI). (Figure 1)

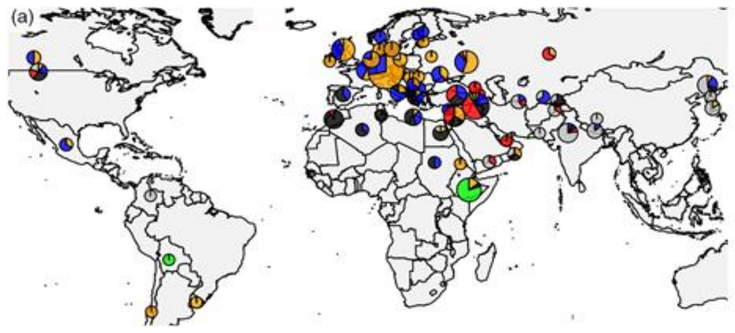

Fig.1 Geographical distribution and proportion of genotypes of six subpopulations for 371 WHEALBI accessions, The Plant Journal, Volume: 99, Issue: 6, Pages: 1172-1191, First published: 20 May 2019, DOI: (10.1111/tpj.14414)

Material and Methods

Segregating Populations

Recombinant inbred lines (RILs) and backcrosses (BC1 from a nested association mapping (NAM) population) (Fig. 2) were used in this study and are listed in Table 1:

cross	state	generation	numbers
RGT Planet x WB-168	BC1	F6	82
RGT Planet x WB-168	RILs	F5	34
RGT Planet x WB-468	BC1	F6	61
RGT Planet x WB-468	RILs	F5	79
RGT Planet x WB-340	BC1	F6	97

Controls	replications	numbers
WHEALBI lines 2-row, spring	1	163
Selection 12 random WB-lines	3	36
Parental landrace lines (WB-168, WB-340, WB-468)	9	27
RGT Planet	9 (+244)	9
TOTAL		588

588 individual plants were randomized in an augmented block design to compare development and flowering times, scored as days from sowing to awn emerging 2 cm over last leaf sheath at BBCH49 (DAE). 163 two-row spring lines from the WHEALBI collection were used as validation of phenotypic and genotypic results. Three individuals of the parental lines and one individual of a random selection of twelve of the WB-lines were used as partial replication on each bench. (Photos)

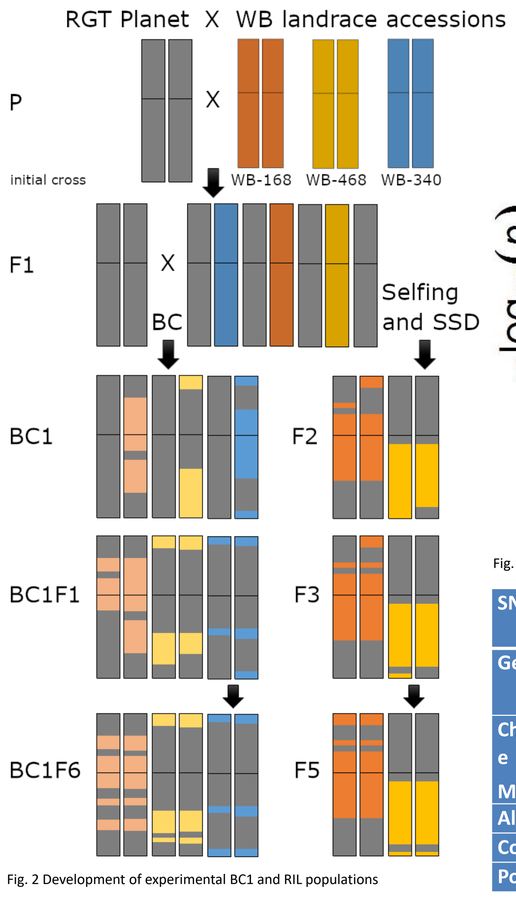

Fig. 2 Development of experimental BC1 and RIL populations

Development of KASP markers for key flowering related genes

Based on quantitative trait loci (QTL) from previous studies Kompetitive allele specific PCR (KASP) were designed and used to determine segregation of characteristic single nucleotide polymorphisms (SNP) on chromosomes 2H, 3H and 5H (Fig. 3, Table 2).

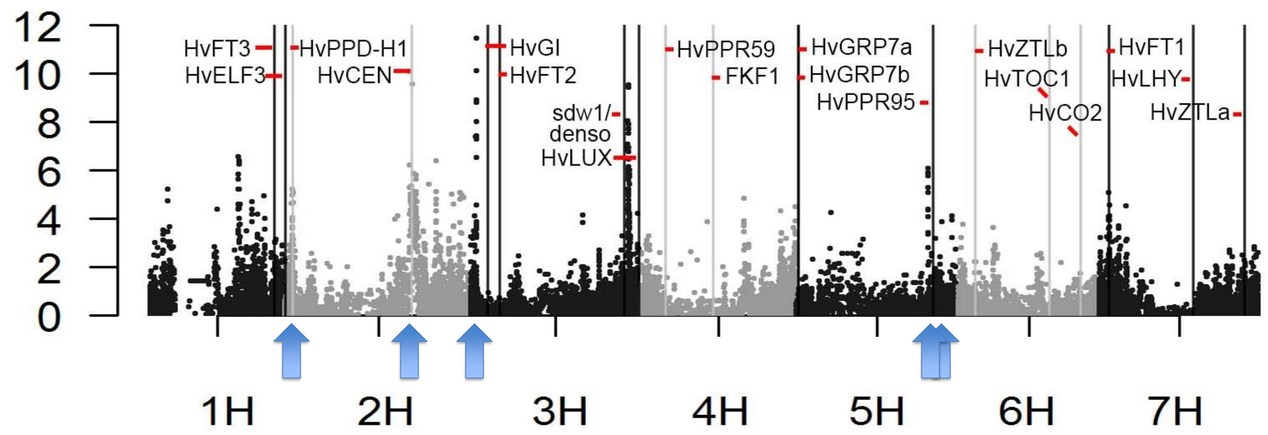

Fig. 3 SNP locations marked in manhatten plot for days to heading from multi-environment genome-wide association scans of individual single nucleotide polymorphism profiles for 371 domesticated barley lines.

SNP	12_30870	jhi-hv50k-2016-73422	vcZ4VQNB	vcZ0GALF	jhi-hv50k-2016-158769	12_30869
Gene	HORVU2Hr1G013400 (HvPPDH1)	HORVU2Hr1G013400 (HvPPDH1)	HORVU2Hr1G072750 (HvCEN)	HORVU5Hr1G081620 (HvPRR95)	HORVU3Hr1G010310	HORVU5Hr1G095530 (HvPhyC)
Chromosome Morex_V1	2H r 29126143	2H r 29126332	2H r 523378515	5H f 565158047	3H f 22622622	5H f 598563697
Alleles	C/A	G/A	G/C	T/A	G/C	T/C
Consequence	missense variant	synonymous variant	missense variant	missense variant	missense variant	3 prime UTR variant
Populations	WB-340	WB-468, WB-340	WB-168, WB-468	WB-340	WB-468, WB-340	WB-168

Fig. 4 Photos of the experimental design of the glasshouse trial

Results

Wide range of flowering times

Based on previous data (WB-168 48 DTH, WB-340 56.5 DTH, WB-468 50 DTH) we expected variation and greatest segregation within the WB-168 and WB-468 crosses.

We predicted the backcross generations would be more similar to RGT Planet than the WB-parent and expected more variation and earlier flowering for the RILs.

Flowering time was scored between 33 and 110 days. Transgressive segregation was observed in all the subpopulations, in agreement to field and glasshouse scores on the BC1 lines in 2019 and 2020 and ongoing glasshouse multiplication of the RILs.

In the WB-168 subpopulations flowering started about one week later than both the other crosses. The spread of flowering times in BC1 subpopulations WB-168 and WB-468 was greater than in the corresponding RILs, which indicates reduced diversity of the RILs, potentially due to rounds of speed breeding under constant light. The interquartile range of the WB-168 subpopulations was between the average DAE of the parents while half or more of the WB-340 and WB-468 subpopulations were flowering significantly earlier than both the parental lines, as shown in Figure 5.

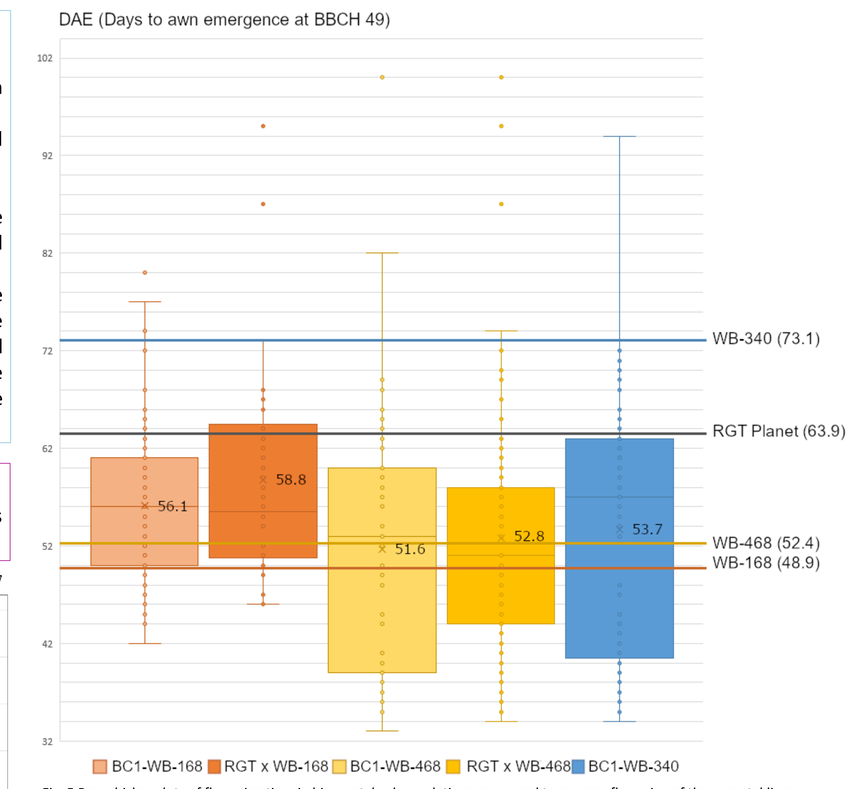

Fig. 5 Box whisker plots of flowering time in biparental subpopulations compared to average flowering of the parental lines

Segregation of flowering time related genes

The SNPs in *HvPPDH1* segregated in subpopulations WB-340 and WB-468 and were expected to have a strong effect on flowering time from QTL analyses. Other KASPs were used to track *HvCEN* and *HvPRR95* as well as candidate regions at QTLs on chromosomes 3H and 5H.

Fig. 6 Boxplots of KASP calls related to flowering times in the subpopulations

Correlations between phenotypes and KASP results

The most obvious correlation was found for *HvPRR95* in the BC1-340 subpopulation with early flowering phenotypes carrying the landrace allele. The box whisker plots (Fig. 6) show all the other significant correlations. Relations between landrace alleles at *HvPPDH1* and early flowering phenotypes were evident in BC-individuals WB-468 and WB-340 with lines carrying landrace allele A flowering on average about eight days earlier. At *HvCEN* the average lines with the Planet allele G flowered about 5 days later and 3 days later for C at *HvPhyC* with a clear discrimination in the extremes. The comparison with the flowering time of the parental lines with the crosses (Fig. 4) suggests complicated genetic interactions with transgressive segregation.

Conclusions

The transgressive differentiation of days to awn emergence and particularly extremely early flowering in the biparental subpopulations could be just partially explained with this limited set of KASP markers representing several major flowering related genes This is evidence of more complex genetic interactions and additional effects in control of flowering.

Future work

The single flowering genes have been well described but complex interactions of these genes and correlations with other development related factors are still not fully understood. A selection of early and late flowering extreme lines from the WB-168 and WB-468 crosses have been identified and will be genotyped using the 50k SNP chip (Bayer et al., 2017) to genetically dissect the segregation patterns observed in days to awn emergence.

Additionally, other traits such as plant height and thousand grain weight as well as grain colour (black in WB-468) may be interesting for future investigations given the diversity in parental material (Fig. 7)

As well as glasshouse trials for the RILs, lines from the BC1 populations have been multiplied in Spain as part of the European collaborative project BARISTA, and these will be sown in the coming year in field trials across Europe from Southern Spain to Finland.

Fig. 7 Ears showing diversity of WHEALBI lines within the glasshouse trial

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