Different daylength – different effect

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Introduction

The determination of the flowering time (FT) is one of the most important decision in plant life. Plant have to process plenty of external and internal signals to avoid unfavorable conditions (cold, hot and dry period) and to ensure seed propagation.

Major gene classes that control flowering process in wheat include photoperiod (*Ppd*), vernalisation (*Vrn*), and earliness *per se* (*Eps*).

In 1987 Košner identified **delaying** of FT under short days (SD) conditions in Sandra CP3B* (S3B) substitution line. On the contrary, the **acceleration** was reported under long days (LD) conditions (Košner and Pánková, 2002; our observations).
A subtle effect (2-7 days) we examined suggests an *Eps* nature of the novel gene located on chromosome 3B of Česká přesívka.

The **bipolar effect** of analyzed gene could be useful in future breeding programs to create the most suitable varieties for local environments.

The precise identification of the gene, its function analysis are the challenges we will be facing in near future.

***** *3B chromosome originated from Česká přesívka*

Mapping population

To localize the gene of interest a nearly isogenic lines (**NILs**) mapping population has been created (*Fig. 1*).

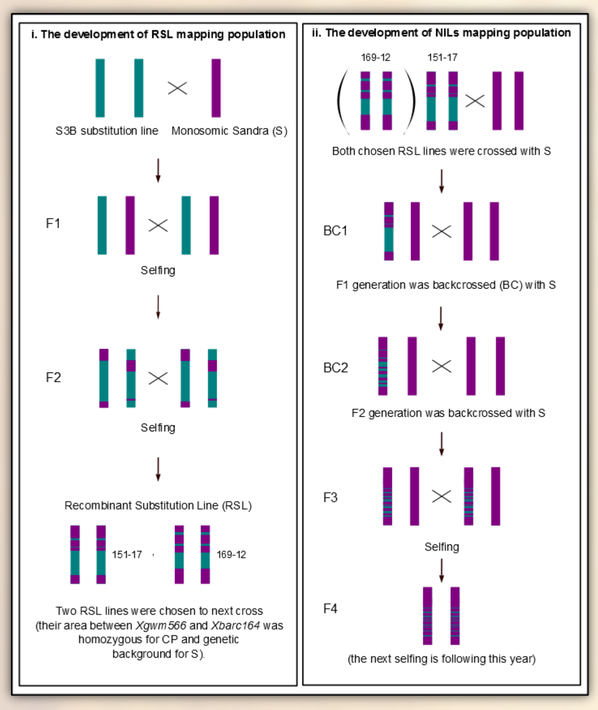

Fig. 1: Scheme of generation of NILs mapping population.

i. genotyping

We used two methods for identification of polymorphic markers: i. polyacrylamide gel electrophoresis **(PAGE)**(*Fig. 2*), ii. **fragmentation analysis** (*Fig. 3*). Besides mapping of known markers, Illumina sequencing data provided new sources of polymorphism which could be analyzed by **KASPar** genotyping system (*Fig. 4*).

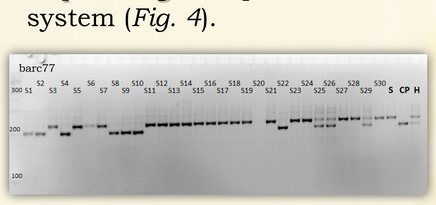

Fig. 2: The analysis of length polymorphism by PAGE.
S – Sandra, CP – Česká přesívka, H – heterozygot

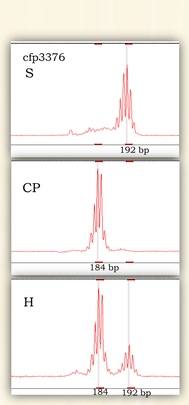

Fig. 3: The analysis of length polymorphism by fragmentation analysis. The amplicons were labeled with PET
S – Sandra, CP – Česká přesívka, H – heterozygot

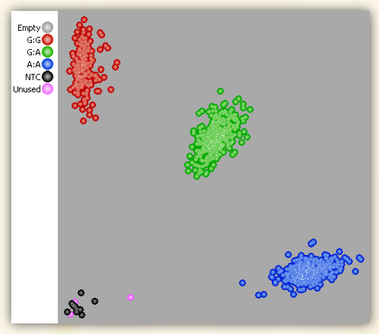

Fig. 4: Graphical view of KASPar genotyping data. (www.kbioscience.co.uk)

ii. phenotyping

Accurate phenotype identification is an important step in fine mapping of the gene. We have analyzed the different stage of apex development (*Fig. 5*) and the heading time (*Fig. 6*) in controlled conditions of fytotron chambers as well as in the field experiment.

Fig. 5: Apex development stage in S3B and S.
S3B – terminal spikelet stage, S – double ridge stage

Fig. 6: Heading time in S and S3B.

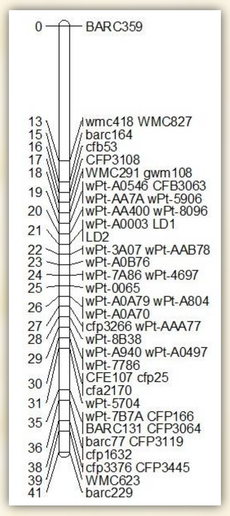

Fig. 7: The genetic map of 3B chromosome. The 30 cM region is spanning with 30 new markers.

iii. integration

The combination of data set gained from genotyping (*Fig. 8*) and phenotyping of mapping population was processed by JoinMap® 4 software. The resulted genetic map (*Fig. 7*) is showing the order and the relative distance between analyzed markers.

marker	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30
barc358	B	A	B	B	B	B	A	B	B	-	H	H	A	A	H	H	H	A	A	A	A	-	A	H	A	A	A	A	A	A
wmc418	B	A	B	B	B	B	A	B	B	B	H	H	A	A	H	H	B	A	A	A	A	B	A	H	A	A	A	A	A	A
wmc827	B	A	B	B	B	B	-	B	B	B	B	B	A	A	H	H	B	A	A	A	A	B	A	H	A	A	A	A	A	-
barc164	B	A	B	B	B	B	A	B	B	B	H	H	A	A	H	H	B	B	B	B	A	B	A	H	A	A	A	B	A	A
cfb53	B	A	B	B	A	A	A	-	B	B	A	B	A	A	H	A	B	A	A	A	A	B	A	H	A	A	A	A	-	A
LD1	B	A	B	B	A	A	A	B	B	B	A	B	A	A	B	A	B	A	A	A	A	B	A	A	A	A	A	A	H	A
LD2	B	-	B	B	A	A	A	B	B	B	A	B	A	A	-	A	B	A	A	A	-	B	A	A	A	A	A	A	H	A
cfp3266	B	A	B	B	B	B	A	B	B	B	B	B	A	A	B	B	B	A	A	A	A	B	A	B	A	A	A	A	A	A
cfe107	B	B	B	B	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	H	A
cfa2170	B	B	B	B	A	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A
barc131	A	H	B	B	B	H	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	H	A	B	A	A	A	A	A	A

Fig. 8: Genotyping of 30 (S1-S30) individuals of NILs mapping population.
A – Sandra, B – Česká přesívka, H – heterozygot

Conclusions

i. The bipolar effect of flowering time gene was examined:
   - delaying – LD (7 days difference)
   - accelerating – SD (4 days difference)

ii. The locus was localized into 30 cM region spanning *Xbarc164* and *Xcfa2170* markers and it has been saturated by 30 new polymorphic markers.

iii. The Illumina sequencing of 3B chromosomes from CP and S provided 147,852 SNPs, which will be use to delineate the region of interest *QFt.cri-3B.1*. However, efficiency of SNP transformation to PCR based markers is 30 % in allotetraploids (Byers et al. 2012), in allohexaploid wheat is possibly even lower.

iv. Presumably, the FT changes are caused by *Eps* gene. Our observations indicate interaction of analysed gene with photoperiod. Association of *Eps* gene with photoperiod gene (*Ppd-D1*) or temperature is proved in some other studies (Valárik et al., 2006; Kamran et al., 2013).

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Acknowledgments

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