Advanced approaches to the analysis of genes and alleles influencing growth, development, and yield components of wheat, with the aim of defining better adapted genotypes

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Introduction

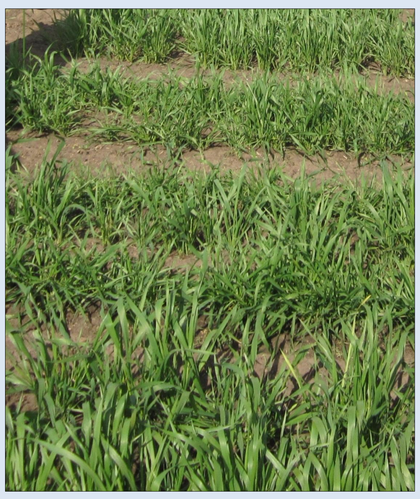
Breeding of wheat encounters a continuous call for improvement of yield and quality of production under changing climatic conditions. The genome of common wheat with its hecaploid constitution renders an intricate but extremely flexible system of genes, involved in the growth and development and thus influencing the yield. Diverse approaches to the analyses of these systems are presented here by the use of two types of recombinant inbred line mapping populations of wheat that have been produced with the aim of searching for better adapted combinations of genes and alleles influencing yield. These mainly include genes and alleles controlling growth habit and flowering time, and resistances to biotic and abiotic stresses.

Three mapping populations (SELGEN / CRI) have been produced based on crosses between three different genotypes of wheat, representing different genotype pools grown under the climatic conditions of the Czech republic,. These are being studied to evaluate the existing allelic variation, so as to discover the most favourable combinations of genes and alleles, for optimal growth and yield in the country.

Two CP 3B mapping populations based on substitutions of chromosome 3B of the Czech alternative landrace, Ceska Presivka, carrying a novel flowering time gene, *QFt.cri-3B,* have been subjected to detailed genetic and phenotypic analyses of the 3B chromosome region of interest. This region spans a large genetic distance between the markers *Xgwm285* and *Xcfa 2170*, and fine mapping using segmental recombinants is being carried out to genetically dissect the region.

SELGEN / CRI mapping populations

Three mapping populations have been produced based on the SSD (single seed descent) approach, until obtaining F6 generation. Parental wheat genotypes for the original crosses were chosen to represent genes and alleles most successful in the area of the Czech Republic. Sirael and Ilias are among the most important cultivars registered in the Czech republic, while the breeding line LINDA*SG-S3-93*SG-S1267-92 represents a high quality breeding, including the genes of the variety Linda. These genotypes are contrasting in the response to winter conditions.



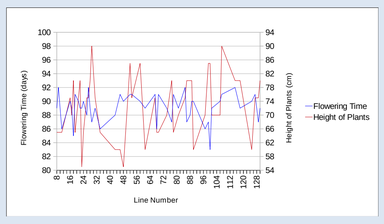
Sirael is a typical spring, medium early and medium high (73 – 85 cm) genotype with a high yield potantial. It was bred from a combination SG-S299-94*BANTI (Selgen, CR, 2005). Its resistance to diseases, especially to rusts, powdery mildew and fusaria is mostly high; good baking quality (C) is combined with high content of protein.

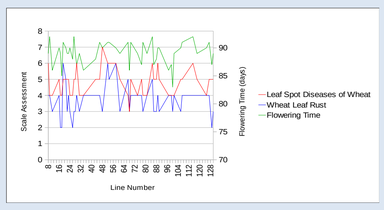
A medium early, medium high (75 – 84 cm) line SG-S5-01 (Selgen,a.s.) represents alternative type having a medium - high yielding quality. It comes from the cross LINDA*SG-S3-93*SG-S1267-92. Its resistance to diseases is medium high, achieves the quality group A.

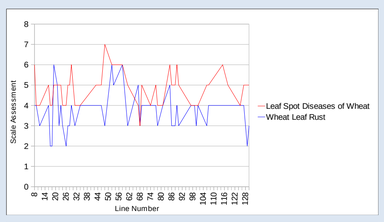
Ilias is a typical high yielding late winter cultivar, having a medium yielding potantial and high stem (90 – 98 cm); quality A. It was bred in Cebeco, Netherlands, in 2003.

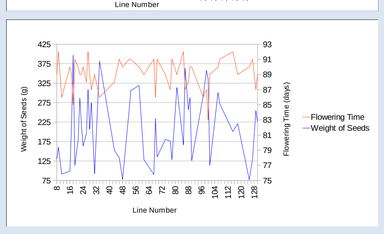
In 2012 the mapping populations were screened in a field trial for basic characteristics of the growth habit, earliness, disease resistance and yield. The diagrams show the obtained vatiabilitywhich will be a good starting point for the detailed genotyping and phenotyping of the lines evaluation:

I. SG-S5 – 01* ILIAS

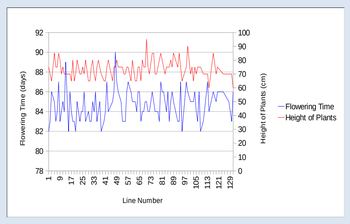


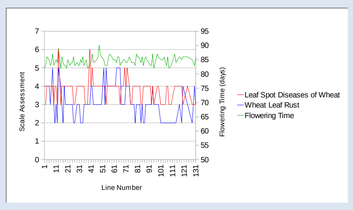


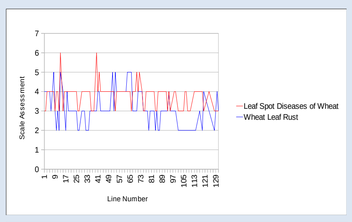


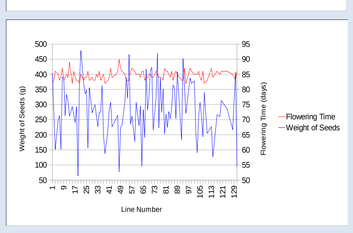


II. SIRAEL * SG-S5- 01.

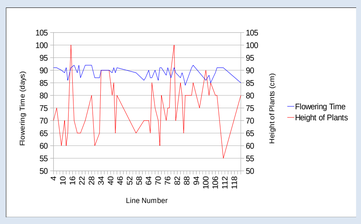


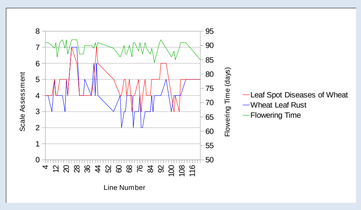


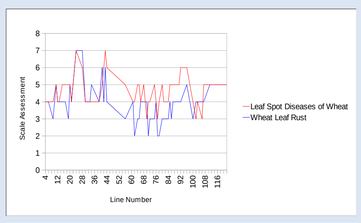


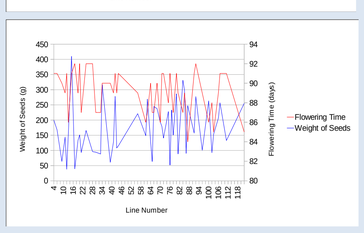


III. SIRAEL* ILIAS









ILLUMINA analyses (TraitGenetics) was carried out to estimate the suitability of the mapping populations for more detailed genotype and phenotypc analyses. The DNA samples of the first two of the mapping populations were fed into the illumina Infinium analysis pipeline with the 90K wheat array at TraitGenetics, then subject of the routinely performed mapping procedure at TraitGenetics starting with JoinMap for the grouping of the linkage groups and the pre-mapping of the markers on the respective groups. The parents of the two mapping populations most likely have been identical in large regions of the genome (identical by descent), and so a significant number of large gaps between marker groups exist. But the field trial has shown a nice segregation in the traits followed so the presence of the identical regions will most probably contribute to simplification of analyses of the target genes influencing growth and development.

CP 3B mapping populations

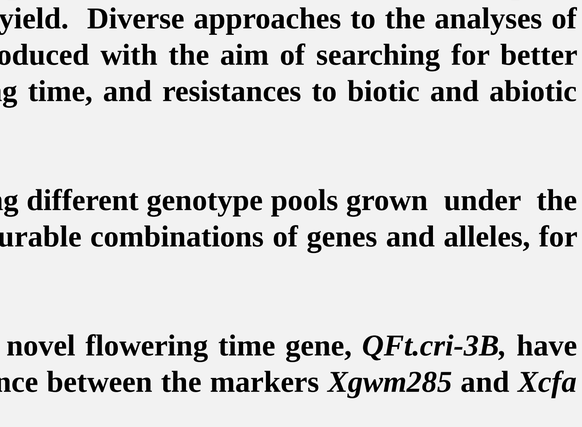
Effect on flowering time (FT) was revealed after the substitution of chromosome 3B of Czech alternative wheat landrace Ceska Presivka into backgrounds of two spring wheat cultivars, Sandra and Zlatka after growing the plants under the short day conditions (field shed), and this result was repeatedly confirmed (Košner,1986; Košner & Pánková, 2002).

To map the novel FT gene, two populations of recombinant substitution lines (RSL) were developed from back crosses of substitution lines Sandra (CP 3B) and Zlatka (CP 3B) carrying chromosome 3B of Ceska Presivka, by the technique described by Law and Worland (1973). The RSL were developed from the F1 hybrids; Sandra (CP 3B) x Sandra and Zlatka (CP 3B) x Zlatka. Each of the individual RSL plants was selfed to produce seed for mapping and phenotypic analyses. Disomic recombinants (expected at a 25% frequency) were identifed by SSR marker analysis as heterozygotes in the selfed generation. Phenotypic analyses included FT experiments under a long and short day controlled regimes.

Genetic analyses

Genetic maps of chromosome 3B were developed using the RSL mapping populations of Sandra//Sandra 3B/Sandra (CP 3B) and Zlatka//Zlatka/Zlatka (CP 3B). DNA was extracted from leaf samples using QiaGen and DNeasy kits. Mapping was carried out using publicly available single sequence repeat (SSR) primer sets. Primers giving clearly scorable polymorphisms on the recipient parents and 3B substitution lines were then genotyped on the entire mapping populations. DNA fragments were amplifed with PCR and run on 6% polyacrylamide gels for separation. The silver staining technique was used to visualize fragments (Bassam et al. 1991). Data for each population were accumulated into a genotype file, and genetic maps were developed using JoinMap software. The FT gene *QFt.cri-3B* was genetically mapped to the area between the markers *Xgwm285* and *Xcfa 2170* (Pánková et al. 2008).

Figure 3



FT experiment - JIC Norwich, 2012 / 2013
controlled regime (12 hours photoperiod, 20˚C)
It has brought a selection of the early flowering and non flowering RSL lines. They will be checked by a repeated genotyping and subjected to further analyses.

Most recent experiments and analyses

More analyses and more detailed study on growtth and development are being carried out to genetically dissect the region and describe the function of *QFt.cri-3B.*

Production, genotyping and phenotyping of segmental recombinant lines has been continued while new PCR based markers derived from the Darts have been obtained aiming to the fine mapping and candidate gene analyses.

In parallel, the apical development of parental cultivars, substitution lines and RSL populations has been studied in detail in more FT experiments under different controlled and field conditions (temperature, photoperiod) to define the distinction between present genotypes.

JIC Norwich 2012 /2013
Non flowering plants

Genotype	Line	gwm493	gwm566	barc164	barc229	wmc326
156	Zlatka 10-1-1 x Zlatka 2 189-9 12	a	a	b	a	a
1	Zlatka 3-3	a	b	b	b	b
9	Zlatka 3-5	a	b	b	b	b
95	Zlatka 10-1-1 x Zlatka 2 189-8 1	a	a	b	a	a
139	Zlatka 10-1-1 x Zlatka 2 189-9 6	a	a	b	a	a
145	Zlatka 10-1-1 x Zlatka 2 189-9 9	a	-	b	b	a
167	Zlatka 10-1-1 x Zlatka 3 284 5	a	a	b	b	a
31	Zlatka 8-5	a	b	b	b	b
32	Zlatka 8-5	a	b	b	b	b
35	Zlatka 8-5	a	a	b	b	b
97	Zlatka 10-1-1 x Zlatka 2 189-8 2	a	a	b	a	a
99	Zlatka 10-1-1 x Zlatka 2 189-8 2	a	a	b	a	a
132	Zlatka 10-1-1 x Zlatka 2 189-9 4	a	b	b	b	a
133	Zlatka 10-1-1 x Zlatka 2 189-9 4	a	b	b	b	a
218	Sandra 169-12 x Sandra 2 8-5 1	a	b	b	a	a

	all 5 non flowering plants
	4 non flowering plants/ 5 plants
	3 non flowering plants/ 5 plants

JIC Norwich 2012 /2013

Earliest flowering plants

Genotype	Line	Flowering time (days)	gwm493	gwm566	barc164	barc229	wmc326
7	Zlatka 3-5	127	a	b	b	b	b
14	Zlatka 3-15	128	a	b	b	b	b
25	Zlatka 4-2	129	a	b	b	b	b
27	Zlatka 8-4	127	a	b	b	b	b
61	Zlatka 12-2-3 x Zlatka 2 63-9 1	127	a	b	b	a	a
75	Zlatka 12-2-3 x Zlatka 2 63-9 4	125	a	a	b	b	b
79	Zlatka 12-2-3 x Zlatka 2 63-9 7	127	a	b	b	b	b
82	Zlatka 12-2-3 x Zlatka 2 63-9 9	117	a	b	a	a	a
102	Zlatka 10-1-1 x Zlatka 2 189-8 5	130	a	a	a	a	a
103	Zlatka 10-1-1 x Zlatka 2 189-8 5	130	a	a	a	a	a
107	Zlatka 10-1-1 x Zlatka 2 189-8 6	123	a	a	b	a	a
133	Zlatka 10-1-1 x Zlatka 2 189-9 4	127	a	b	b	b	a
135	Zlatka 10-1-1 x Zlatka 2 189-9 4	129	a	b	b	b	-
135	Zlatka 10-1-1 x Zlatka 2 189-9 4	132	a	b	b	b	-
181	Sandra 151-17 x Sandra 2 7-3 13	124	a	b	b	b	a
181	Sandra 151-17 x Sandra 2 7-3 13	128	a	b	b	b	a
182	Sandra 151-17 x Sandra 2 7-3 13	114	a	b	b	b	a
182	Sandra 151-17 x Sandra 2 7-3 13	124	a	b	b	b	a
201	Sandra 151-17 x Sandra 2 14-3 11	126	a	b	a	a	a
201	Sandra 151-17 x Sandra 2 14-3 11	116	a	b	a	a	a
202	Sandra 151-17 x Sandra 2 14-3 11	131	a	b	b	a	a
202	Sandra 151-17 x Sandra 2 14-3 11	129	a	b	b	a	a

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