The Maintenance and Exploitation of *ex situ* Genebank Collections – Association Mapping for Flowering Time in Wheat

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Summary

It is estimated that world-wide existing germplasm collections contain about 7.4 million accessions of plant genetic resources. Wheat (*Triticum* and *Aegilops*) represents the biggest group with about 900,000 accessions. One of the largest *ex situ* genebanks worldwide is located at the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben, Germany. This collection comprises wild and primitive forms, landraces as well as old and more recent cultivars of mainly cereals but also other crops. As on the global scale wheat is the largest group having almost 30,000 accessions. Beside the long term storage and frequent regeneration of the material phenotypic characterisation and evaluation data are collected as a prerequisite for gene identification and mapping. We report the outcome of an association-based mapping study to elucidate the genetic basis of flowering time in winter wheat. A core collection of 96 cultivars was subjected to a genome-wide scan using diversity array technology markers. The same set of accessions had been earlier evaluated for flowering time over six consecutive seasons. Some of the resulting marker-trait associations (MTAs) mapped to chromosomal locations in which known major genes affecting flowering time are known to reside. However, most of the MTAs identified genomic locations where no such genes are known to map, so providing new opportunities to exploit genetic variation for flowering time in wheat breeding programmes.

Key words

ex situ collections, genetic resources, association mapping, wheat, flowering time

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Introduction

Plant *ex situ* genebank collections comprise seed genebanks, field genebanks and *in vitro* collections. Species whose seed can be dried safely to a low moisture content can be readily conserved in seed genebanks, while field genebanks and *in vitro* storage are largely used for species that are either vegetatively propagated or whose seeds rapidly lose their viability when dried and stored for a long period. Some perennial species (in particular some of the major forage species) that produce only small quantities of seed, along with extremely long-lived plants (in particular trees) are also maintained this way. Worldwide, fewer than 10% of genebank holdings are maintained in field genebanks, and fewer than 1% are conserved *in vitro* (FAO, 2009; Börner, 2006). Currently about 7.4 million accessions are held in collections, and the largest single genus represented is *Triticum* (the wheats), for which 858,000 accessions are documented. In addition to these are 42,000 accessions of the closely related *Aegilops* spp. (FAO, 2009). Individual holdings of at least 25,000 *Triticum* and 1,500 *Aegilops* accessions are detailed in Tables 1 and 2. The German *ex situ* genebank, located at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben curates a substantial crop germplasm collection. Its 150,000 accessions include some 65,000 cereals, 28,000 legumes, 18,000 vegetables, 14,000 forage crops, 8,000 oil crops, 6,000 potatoes and 6,000 medicinal and spice plants. The cereal section includes 28,000 *Triticum* and 1,500 *Aegilops* accessions (Tables 1 and 2; Anonymous, 2008).

The successful exploitation of germplasm collections is frequently complicated by the size of the holding. As a result, efforts have been made to assemble representative core collections, which seek to capture, within a manageable number of accessions, most of the genetic variation present in the whole collection. These core collections are particularly well suited to association mapping, a technique which has been effectively used to understand the genetic basis of phenotypic variation in human populations, where controlled crossing is of course not an option. Here, we report the association analysis of flowering time within a 96 accession core wheat collection, genotyped using a set of diversity array technology (DArT) markers. Homologous and homoeologous relationships of the detected loci and comparable major genes or quantitative trait loci (QTLs) already described are discussed.

Material and methods

Plant materials and phenotypic evaluation

The 96 accession winter wheat core collection included cultivars bred in 21 countries over five continents. It represents a sub-set of the full core collection assembled by the Institute of Field and Vegetable Crops (Novi Sad, Serbia), the selection criteria for which were based on 20 important breeding traits (Kobiljski et al., 2002; Quarrie at al., 2003). The identity of the 96 cultivars is given in Table 3. The material was field grown in Novi Sad over six consecutive seasons (1994-1999), in six-row 1.2 m^2 plots, using an inter-row spacing of 20 cm. Each entry was represented by three plots in each year. Flowering time was recorded as the number of days (from January 1) taken for 50% of the spikes within a plot to have reached anthesis.

Genotyping and statistical analysis

DArT genotyping was performed by Triticarte Pty. Ltd. (http://www.triticarte.com.au/). A total of 874 markers were identified that were informative across the core. The allocation of 501 of these to linkage group (chromosome) was achieved by reference to Crossa et al. (2007) genetic map. The STRUCTURE program (Pritchard et al., 2000) was applied to a sub-set of 219 randomly distributed markers to enable an estimate of the population structure. Testing for marker/trait association (MTA) was performed using TASSEL v2.0.1 (Bradbury et al., 2007) software.

Table 1. Genebank collections holding at least 25,000 accessions of the genus *Triticum* (taken from FAO, 2009)

Institution	Country	Number of Accessions
CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo)	Mexico	110,281
NCGRP (National Center for Genetic Resources Preservation)	USA	57,348
ICGR-CAAS (Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Science)	China	43.039
NBPGR (National Bureau of Plant Genetic Resources)	India	35.889
ICARDA (International Center for Agricultural Research in the Dry Areas)	Syria	34.951
NIAS (National Institute of Agrobiological Science)	Japan	34,652
VIR (N. I. Vavilov Research Institute of Plant Industry)	Russia	35,950
IDG (Instituto del Germoplasma)	Italy	32,751
IPK (Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung)	Germany	26,877

Table 2. Genebank collections holding at least 1,500 accessions of the genus *Aegilops* (taken from FAO, 2009)

Institution	Country	Number of Accessions
ICCI-TELAVUN (Lieberman Germplasm Bank, Institute for Cereal Crops Improvement, Tel-Aviv University)	Israel	9,146
ICARDA (International Centre for Agricultural Research in the Dry Areas)	Syria	3,847
NPGBI-SPII (National Plant Gene Bank of Iran, Seed and Plant Improvement Institute)	Iran	2,653
NIAS (National Institute of Agrobiological Science)	Japan	2,433
VIR (N. I. Vavilov Research Institute of Plant Industry)	Russia	2,248
NCGRP (National Center for Genetic Resources Preservation)	USA	2,207
LPGPB (Laboratory of Plants Gene Pool and Breeding)	Armenia	1,827
IPK (Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung)	Germany	1,527

Table 3. Cultivar names/designations and countries of origin of the 96 accession core set analysed

Acciaio – ITA	L 1A/91 – SRB	Purdue 5392 – USA
Ai-bian – CHN	Lambriego Inia – CHL	Red Coat – USA
Al-Kan-Tzao – CHN	Lr 10 – USA	Renesansa – SRB
Ana – HRV	Lr 12 – USA	Rusalka – BGR
Avalon – GBR	Magnif 41 – ARG	Siete Cerros – MEX
Bankuty 1205 – HUN	Mex. 120 – MEX	Saitama 27 – JPN
BCD 1302/83 – MDA	Mex.17 bb – MEX	Sava – SRB
Benni multifloret – USA	Mex.3 – MEX	Semillia Eligulata – USA
Bezostaja 1 – RUS	Min. Dwarf – AUS	Slavija – SRB
Brigant – GBR	Mina – SRB	Sofija – SRB
Cajeme 71 – MEX	Mironovska 808 – UKR	Sonalika – IND
Capelle Desprez – FRA	Nizija – SRB	Suwon 92 – IND
Centurk – USA	Norin 101 – JPN	Szegedi 768 – HUN
Ching-Chang 6 – CHN	Norin 10/Brevor14 – USA	Tibet Dwarf – CHN
Cook – AUS	Novosadska crvena – SRB	Timson – AUS
Donska polupat. – RUS	Nova banatka – SRB	TJB 990-15 – GBR
Durin – FRA	NS 22/92 – SRB	Tom Thumb – CHN
F 4 4687 – ROM	NS 33/90 – SRB	Tr.Compactum – LVA
Florida – USA	NS 46/90 – SRB	Tr.Sphaerococcum – USA
Gala – ARG	NS 55-25 – SRB	Triple Dirk B – AUS
Hays 2 – USA	NS 559 – SRB	Triple Dirk B (bulk)–AUS
Helios – USA	NS 602 – SRB	Triple Dirk S – AUS
Highbury – GBR	NS 63-24 – SRB	UC 65680 – USA
Hira – IND	NS 66/92 – SRB	UPI 301 – IND
Holly E – USA	NS 74/95 – SRB	Vel – USA
Hope – USA	NS 79/90 – SRB	Vireo"S" – MEX
Inia 66 – MEX	Peking 11 – CHN	WWMCB 2 – USA
INTRO 615 – USA	Phoenix – USA	ZG 1011 – HRV
Ivanka – SER	PKB Krupna – SRB	ZG 987/3 – HRV
Kite – AUS	Pobeda – SRB	ZG K 3/82 – HRV
L – 1 – HUN	Purdue/Loras – USA	ZG K 238/82 – HRV
L 1/91 – SRB	Purdue 39120 – USA	ZG K T 159/82 - HRV

The general linear model applied incorporated the Q-Matrix from STRUCTURE to correct for population structure, since the structure analysed indicated the presence of two sub-populations among the 96 entries. MTAs were only considered as significant when the level of association was maintained at $p<0.05$ in each of the six growing seasons.

Results

The number of days to flowering ranged from 128.67 to 150,17, with a mean of 138.69 and a standard deviation of 5.26. Eighteen chromosomes were associated with flowering time, with only chromosomes 3D, 4D and 6D not being involved. Of the 501 mapped DArT markers, 85 were significantly associated with flowering time. Applying an MTA threshold of 10% of the phenotypic variation, it was possible to identify 14 MTAs (Figure 1), with four of these present on chromosome 6A. Three MTAs mapping to chromosomes 1B, 6A and 7A explained >15% of the phenotypic variation. The largest single effect was associated with a locus on chromosome 1B.

Discussion

Flowering time is known to be under the control of three main gene types, namely those responding to vernalization (*Vrn*), those responding to photoperiod (*Ppd*) and those that appear to be unresponsive to any environmental cue (*Eps*) (Snape et al., 2001). Because the cultivars investigated were all winter wheats, we did not expect to see any *Vrn* gene effects, and indeed none of the regions within the homoeologous group 5 chromosome long arms, where the major *Vrn* loci are sited, was identified as a location for an MTA. The MTA on chromosome 5BL maps about 10cM distal from the centromere, whereas *Vrn-B1* maps independent of the centromere on this chromosome arm (Leonova et al., 2003). The *Ppd* genes map to the short arms of the homoeologous group 2 chromosomes (McIntosh et al., 2008), and an MTA was detected on the short arms of both chromosome 2B and 2D. Although it was anticipated from breeding results that all the material in the core set was likely to be photoperiod insensitive, we cannot exclude the possibility that one or both of these MTAs reflect allelic variation at one or other of *Ppd-B1* or

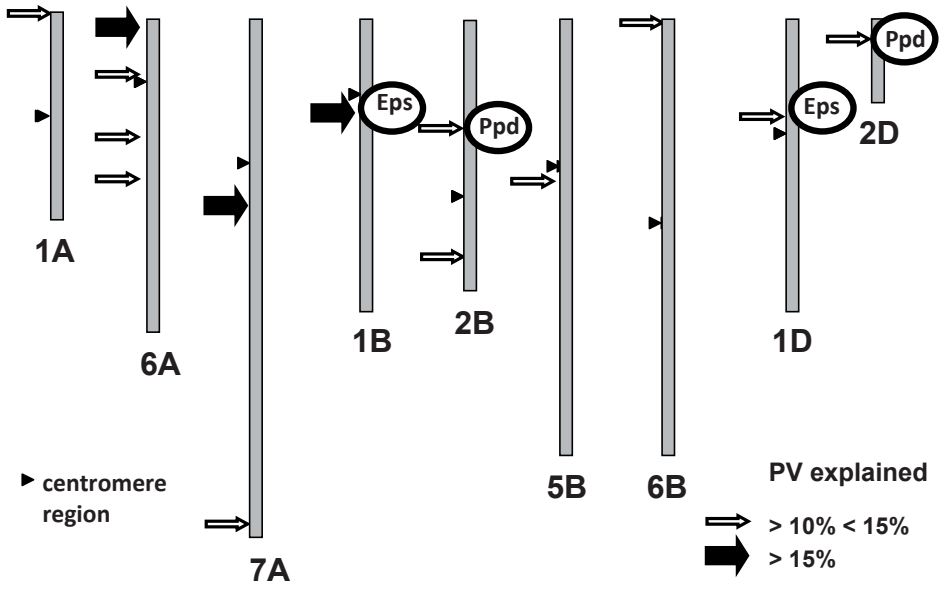

Figure 1. MTAs for flowering time detected. The positions of known loci affecting flowering time are indicated by circles on the right

Ppd-D1. A QTL associated with the photoperiod response has been described by Kuchel et al. (2006), but its location in the centromeric region of chromosome arm 7AS is inconsistent with that of the MTA on long arm of chromosome 7A.

The MTA mapping to the long arm of chromosome 1B was associated with the largest proportion of phenotypic variation; it could represent an *Eps* gene, since such a gene has been mapped to a very similar position in wheat by Toth et al. (2003) and to an equivalent region of barley by Laurie et al. (1995). Whether the 1D locus detected here is also syntenic can only be speculated since the centromere positions are only estimated. The MTAs on chromosomes 1A, 6A, 7A, 5B and 6B are not matched by any known flowering time gene

Conclusion

The association mapping approach is not as yet well developed in the cereals. In contrast to more conventional bi-parental segregation-based mapping, which can only capture the genetic variation between two individuals, association mapping detects MTAs from an analysis of many genetically unrelated individuals. This approach does require correction for population structure, since otherwise the false positive rate is too high. A large number of MTAs for flowering time were detected, and some - but not all – matched the location of known major genes. The identification of novel genes underlying the agronomically important trait flowering time may provide some interesting new opportunities to manipulate genetic variation for wheat improvement. The refinement of high-throughput genotyping has shifted the bottleneck for MTA detection to the acquisition of robust phenotypic data. Substantial volumes of such data are probably already in the hands of plant breeders and germplasm managers in the form of archived field trials.

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