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THE GENETIC APPROACH TO PHYSIOLOGICAL STUDIES IN BREAD WHEAT

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Summary: Genetics has long been applied as a tool to dissect biological processes in plants. Using genetic approaches, the phenotypic variation for many traits has received physiological and molecular genetic characterization. The natural variation for most physiological traits such as developmental processes, vegetative growth, nutrient uptake and utilization, stress tolerance and adaptation, etc., is continuous and determined by multiple genes. In addition, these traits are environmentally dependent and, therefore, their genetic analysis requires a quantitative trait locus (QTL)-based approach in which linkage maps are combined with phenotypic data. The large genome and the allopolyploid nature of bread wheat (*Triticum aestivum* L.) have long been substantial barriers to genetic dissection of quantitative traits. The boost in molecular marker technologies, the generation of dense linkage maps, coupled with improved statistical methods and development of precise genetic stocks have immensely increased the power of genetic analysis of complex physiological traits in wheat. In this paper, recent applications to two important phenology processes in bread wheat – seed germination and flowering time, are presented. These are examples of using QTL approach and novel segregating populations to map responsible loci followed by the use of the homology-based understanding of plant gene functionality to suggest the putative function of the candidate genes within the regions harboring the detected QTL. The gained knowledge could aid the fine tuning of seasonal phenology in wheat cultivars tailored for growing in specific environments and in response to climatic changes.

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The genetic approach to plant physiological processes

Genetics has long been applied as a tool to dissect complex biological processes in plants. The phenotypic variation for many traits has received physiological and molecular characterization using various genetic approaches (Cativelli et al. 2002; Galiba et al. 2009; Pearce et al. 2011). To apply these approaches, the genetic variation, both natural and experimentally induced, is essential. Most of the natural variation, particularly that related to physiological traits is continuous and determined by DNA polymorphisms at multiple loci referred to as quantitative trait loci (QTL). The expression of such traits is under polygenic control and is environmentally dependent which makes them hardly accessible for genetic analysis.

The last few decades brought up a number of new developments in genetics, such as the boost in DNA marker technologies (reviewed in Landjeva et al. 2007; Khlestkina 2014a), coupled with improved statistical methods and emerging platforms for efficient phenotyping. Due to the availability of competent molecular marker systems, the development of detailed genetic maps, and the generation of a wealth of mapping populations, the complex molecular basis of the quantitative traits are beginning to be elucidated. The identification of genomic regions responsible for this type of traits is establishing the position of QTL on the chromosome map based on the association of the trait phenotypic variation with genetic markers previously mapped to the chromosomes (Collard et al. 2005). This genetic mapping approach allows to position a QTL on the chromosome without knowing the biochemical function of the underlying genes. If there is co-mapping with genes encoding enzymes or other proteins, the 'candidate gene/s' can be supposed. Additionally, the existing extensive synteny among plant genomes allows the use of the comparative genetics approach, linking QTL with orthologous functional genes detected in even distantly related species. So, the knowledge gained in model plants enables to go further into the mechanisms determining the traits of interest. To understand the pivotal and pleiotropic effects of specific genes, more detailed analyses can then be performed through isolating and evaluating near-isogenic lines. In case of agronomically important traits, the obtained information on marker-trait associations could be used for marker-assisted selection (see Landjeva et al. 2007; Khlestkina et al. 2014).

The specific genetic tools in bread wheat

Bread wheat (*Triticum aestivum*) is one of the 'big three' globally important crops with a total output of nearly 660 million tonnes in 2013 (FAO 2014; http://www.fao.org/worldfoodsituation/). Given the projected demand for 70% growth in world food supply by 2050, major efforts are in progress worldwide to increase wheat production. Achieving this objective can be facilitated by analysing key agronomical traits, but also important physiological traits that can contribute to the yield increments, such as growth and development, photosynthesis, nutrient use efficiency, stress tolerance and adaptation.

The very large size and the polyploid nature of the bread wheat genome, however, have been substantial barriers to genome analysis and genetic dissection

of these complex traits. Bread wheat is hexaploid, with genome size estimated at ~17 Gbp and a large fraction of repetitive sequences (>80%). The wheat genome is composed of three component genomes (A, B and D) that had resulted from a series of naturally occurring hybridizations between three putative ancestor species. The 21 chromosome pairs of bread wheat are organized in 7 homoeologous groups consisting of one chromosome pair from each of the three sub-genomes (Sears 1969).

Although the size and the nature of the wheat genome has long been an obstacle to genetic analysis of quantitative traits, the triplication of genomic regions allows wheat to tolerate the loss of chromosomes, arms, and segments (Endo and Gill 1996). In addition, due to the homoeology existing between the three closely related sub-genomes wheat can tolerate a range of aneuploidy, as well as genetic substitutions and introgressions. This enabled a diverse set of genetic stocks to be developed through genetic manipulations including mono-, tri- and tetrasomics, ditelosomics, nulli-tetrasomics, translocation lines, etc. (reviewed in Khlestkina 2014b). This material allows mapping of genetic markers to particular chromosomes and chromosome arms and have been extensively used as pre-requisite in mapping studies (Börner et al. 2012). In addition, more than 400 deletion lines for the 21 wheat chromosomes have been isolated (Endo and Gill 1996). This unique material was used to physically map sets of RFLP, SSR and AFLP markers onto sub-arm chromosome regions delineated by neighbouring deletion breakpoints (the so called 'deletion bins') for all homoeologous groups (Sourdille et al. 2004). The deletion mapping strategy was used to construct a chromosome bin map of wheat for EST (Expressed Sequence Tag) loci (Peng et al. 2004). It is now possible to allocate QTLs to deletion bins where numerous ESTs that could be potential candidate genes have already been assigned. Orthologues or homologues for the genes in the identified genomic regions, as represented by ESTs, are known in model plants or in other species closely or distantly related to wheat (http://wheat.pw.usda.gov/wEST/). Thereby, the candidate homologues search can suggest the putative functions of the genes within the mapped QTL.

Conventional mapping populations in wheat include recombinant inbred lines and doubled haploid lines, near-isogenic lines for key genes, and mutant (TILLING) populations. Recently, novel precise genetic stocks have been constructed, for instance whole genome substitution lines, introgression lines, single chromosome substitution lines and recombinant single chromosome substitution lines. Such genetic resources could provide a powerful tool for high resolution QTL mapping, as they can detect the presence of minor QTL, which escape identification in conventional segregating populations (reviewed in Landjeva et al. 2007).

Two examples in bread wheat

Based on own results, the present paper is a brief demonstration of using these specific genetic tools in bread wheat to dissect two phenology related traits. The seasonal timing of critical developmental transitions, such as seed germination and initiation of reproduction, is under strong natural selection to coincide with favourable growing conditions. Seasonal

phenology has major influence on crop establishment and is among the most important plant responses to climatic and environmental fluctuations. Therefore, to optimize yield it is essential to adjust the key components of the plant life cycle to the specific agro-climatic conditions in which they are grown and in response to environmental changes. This objective can be achieved by gaining knowledge on the genetic control of the related physiological traits. As both germination and flowering time are physiologically complex quantitative traits (Lang 1952; Bewley and Black 1994) which are environmentally dependent, their genetic dissection required a QTL based approach.

Example 1. Seed germination

Germination is resumption of embryo growth. It initiates with release from dormancy and seed imbibition, followed by general activation of embryo metabolism, mobilization of seed nutrient reserves, synthesis of an array of metabolites used for growth and, finally, renewed cell division and cell enlargement in the embryonic axis. Germination *sensu stricto* is finished when the radicle emerges through the seed coat (Bewley and Black 1994). Studies using "-omics" technologies along with physiological approaches have revealed that seed germination is a very complex process involving multiple biochemical events, complicated metabolic pathway networks and hormonal cross-talks governed by plethora of genes (Yu et al. 2014).

To identify genomic regions affecting germination related traits, a unique set of D genome introgression lines was used. This genetic material represents a set of single chromosome recombinant inbred lines, each of which carries, in homozygous state, a single unique chromosome segment of the wheat D genome progenitor *Agilops tauschii* within a wheat background (Pestsova et al. 2006). The wild species is considered a possible donor of early vigour for improvement of wheat early growth and crop establishment (ter Steege et al. 2005). The material allows application of the bin-mapping approach to search for responsible QTL in D genome chromosomes. The following germination related traits were measured: capacity, timing and rate, characterized by a standard germination test, based on the 1 mm root protrusion (germination *sensu stricto*) (Landjeva et al. 2010).

In total five significant germination related QTL were identified on chromosomes 1D, 5D and 7D (Table 1). The gene content in the wheat deletion bins was inspected (http://wheat.pw.usda.gov/wEST/binmaps/) to consider possible underlying candidate genes. The assumed candidates might be involved in mobilization of deposited sucrose, starch, lipids and proteins, respiration and energy production, cell wall degradation and synthesis, establishment of signal and metabolic pathway networks, cell cycle regulation, or might be hormonal related genes (Table 1).

Example 2. Flowering time

Flowering time is another central event in the life cycle of plants. When timed correctly, it helps ensure the successful pollination and seed setting, and consequently affects reproduction and productivity. The complex regulation of this trait is governed by an intricate network of signaling pathways – vernalization (winter cold) and photoperiod (day

Table 1-1. Mapped QTL and examples of genes/gene products in the corresponding chromosome deletion bins, relevant to plant developmental traits in bread wheat.

QTL / nearest marker / chromosome bin	Genes or products	Function	Mechanism of action	Reference
Seed germination[a]				
QFingp.ipk-1D / Xgdm33 / 1DS5-0.70-1.00	*Gli* and *Glu-B3* genes	Encode the main storage proteins of mature wheat endosperm	Gliadins and glutenins nourish the embryonic plant after proteolytic breakdown; allelic variation at *Gli-1* loci correlates with differences in proteolysis rates during germination	Upelniek et al. (2003)
	auxin-response-factor (ARF)	Positive regulators of the ABA signaling pathway	ARFs control the expression of *ABI3* gene to stimulate ABA signaling thus coordinating seed dormancy and germination	Liu et al. (2013)
QMgt.ipk-1D QMgr.ipk-1D / Xgwm337, Xgwm1291 / C-1DS3-0.48	D-type cyclin (CYCD)	Regulatory subunit of cyclin-dependent kinase complexes thus playing an important role in the cell cycle responses to external signals	Over-expression of *CYCD* genes promotes cell cycle activation in radicle and the rate of germination in response to environmental triggers	Masubelele et al. (2005)
	cellulose synthase-1	Component of large multimeric cellulose synthase (CesA) complexes that synthesize cellulose	Involved in biogenesis of plant cell walls during germination	Endler and Persson (2011)
QFcgp.ipk-5D / Xgwm1454, Xgwm272 / 5DL5-0.76-1.00	SCARECROW like gene regulator (SCL)	Positive regulator of gibberellins signaling	*SCL3* gene modulates seed germination in Arabidopsis	Zhang et al. (2013)
	G-protein beta family (Gβ)	Negative regulators of ABA signaling	Loss-of-function *AGB1* mutants lacking Gβ have greater ABA hypersensitivity suggesting that *AGB1* is the predominant regulator of ABA signaling during germination	Pandey et al. (2006)

[a]Traits designation: Fcgp, first count germination percentage; Fingp, final count germination percentage; Mgt, mean germination time; Mgr, mean germination rate (for details, see Landjeva et al. 2010); Flt, Flowering time.

Table 1-2. Mapped QTL and examples of genes/gene products in the corresponding chromosome deletion bins, relevant to plant developmental traits in bread wheat.

QTL / nearest marker / chromosome bin	Genes or products	Function	Mechanism of action	Reference
Seed germination[a]				
QFcgp.ipk-5D / Xgwm1454, Xgwm272 / 5DL5-0.76-1.00	elongation factor (EF) 1-alpha	Component of the cell translation machinery	High levels of EFs-1 known to be actively engaged in translation are present in seedling tissues during germination	Gallie et al. (1998)
	prohibitin (Phb)	Regulator of cell cycle during seed germination	mRNA encoding Phb may have anti-proliferative activity, most probably related with hormonal control of translation	De Diego et al. (2007)
	beta-D-glucan exohydrolase	Required for the depolymerization of (1→3),(1→4)-β-D glucans, components of cell walls in Poaceae	The enzymes possibly participate in endosperm mobilization and in auxin-mediated cell elongation in germinating grains	Hrmova and Fincher (2001)
QFcgp.ipk-7D / Xgwm1187 / 7DS4-0.61-1.00	SPATULA protein	bHLH transcription factor, a repressor of seed germination and mediator of the germination response to environmental cues	The protein mediates the expression of the gibberellin biosynthetic gene GA3 oxidase (*GA3ox*) in response to light and temperature	Penfield et al. (2005)
	auxin-regulated GH3 protein	GH3 family enzyme controlling the level and activity of auxins and jasmonates through conjugating amino acids	Modulates signal transduction pathways responsible for plant growth and development	Westfall et al. (2010)
	pullulanase	Debranching enzyme catalyzing the hydrolysis of α(1→6) glucosidic branch linkages of pullulan, amylopectin and β-limit dextrins, active during seed germination	Possibly involved in the regulation of seed germination via fine mechanism that allows proper accumulation or degradation of starch	Repellin et al. (2008)

[a]Traits designation: Fcgp, first count germination percentage; Fingp, final count germination percentage; Mgt, mean germination time; Mgr, mean germination rate (for details, see Landjeva et al. 2010); Flt, Flowering time.

Table 1-3. Mapped QTL and examples of genes/gene products in the corresponding chromosome deletion bins, relevant to plant developmental traits in bread wheat.

QTL / nearest marker / chromosome bin	Genes or products	Function	Mechanism of action	Reference
Flowering time				
QFlt.icg-4D / *Xgwm4555* / C-4DL9-0.31 or C-4DS1-0.53	zinc finger protein	Product of a candidate for the cereal *Vrn-2* gene; zinc-finger domain is also found in B-box family proteins encoded by the CONSTANS (*CO*) gene in Arabidopsis	Participates in the vernalization pathway: *Vrn-2* gene is a repressor of flowering that is down-regulated by vernalization; *CO* gene and its orthologues in cereals are major candidates for photoperiod pathway loci[b]	Yan et al. (2004); Cockram et al. (2007)
	myb family transcription factor (TF)	Component of a super-family of TFs that play regulatory roles in developmental processes in plants	Genes encoding myb related TFs are involved in the photoperiod pathway and their putative function is being components of the central oscillator	Green and Tobin (2002)
QFlt.icg-7A / *Xgwm0060* / 7AS8-0.45-0.59	*TaFTA*	Belongs to the *Vrn-3* series that is orthologous to the Arabidopsis Flowering time (*FT*) gene	*FT* gene encodes a mobile protein, produced in leaves but translocated to the shoot apical meristem to activate flowering	Bonnin et al. (2008)
	Terminal Flower 1 (TFL1) protein	Negative regulator of flowering time	*TFL1* gene is a functional antagonist to *FT* gene	Kobayashi et al. (1999)

[a]Traits designation: Fcgp, first count germination percentage; Fingp, final count germination percentage; Mgt, mean germination time; Mgr, mean germination rate (for details, see Landjeva et al. 2010); Flt, Flowering time.
[b]In spring wheat, flowering time is regulated through the photoperiod pathway.

length) pathways which interact with the environment, and endogenous pathways that control the developmental rate independently of the temperature and day length. Therefore, variation in flowering behavior adapts plants to different geographical locations and climates. In wheat, there are at least three sets of genes that are responsible for the transition of vegetative to reproductive phase. Two of these, *Vrn* and *Ppd* genes respond to environmental cues, controlling plant vernalization and photoperiod sensitivity. The third set of genes controls the so called intrinsic earliness, or earliness *per se* (*eps*) independently of the environmental stimuli (Snape et al. 2001). The major developmental genes *Vrn-1* and *Ppd-1* have long been used for breeding wheat varieties adapted to different latitudes and climates. More precise regulation of flowering could be achieved by operating with minor genes whose allelic variation is still not well studied.

To detect minor loci associated with variation in flowering time, we applied the approach of homologous substitutions (Pshenichnikova et al. 2014). A set of intervarietal single chromosome substitution lines, in which each chromosome pair of the Russian early flowering variety Saratovskaya 29 (recipient) was substituted by the corresponding chromosome pair of the German late flowering variety Yanetzkis Probat (donor) was used. By this approach, the total effect of all responsible genes can be dissected and attributed to particular chromosome(s). The identified critical chromosome affecting flowering was a translocated one consisting of the entire donor chromosome 4D and an additional fragment of the donor chromosome 7A (Khlestkina et al. 2010). Further, a set of doubled haploid lines that were recombinant only for the critical T4D.7A chromosome was used. Thereby, the observed phenotypic variation for the trait of interest can be attributed to a single gene or locus (Worland and Law 1986). A multi-year multi-location experiment was performed in contrasting environments: one in Western Siberia (Novosibirsk, 55°01′N, 82°56′E), and two in Europe (Sofia, Bulgaria, 42°41′N, 23°19′E, and Gatersleben, Germany, 51°49′N, 11°16′E), differing for climate and day length during the plant vegetation cycle. Two significant QTL were detected (Table 1). One QTL, mapped to the pericentromeric region of chromosome 4D, was effective only in Europe following substantially earlier sowing and short days and was therefore regarded as minor locus for photoperiod response. Another QTL mapped to the chromosome 7A fragment was effective under both long (Western Siberia) and short days (Europe) thus probably representing an intrinsic earliness *per se* gene. The putative flowering time related genes in the corresponding chromosome deletion bins (http://wheat.pw.usda.gov/wEST/binmaps/) include sequences encoding for proteins that are involved in the vernalization or photoperiod pathways regulating the transition to reproductive growth (Table 1).

Conclusions

The combined use of precise genetic stocks and the availability of comprehensive genetic maps for all wheat linkage groups has immensely increased the power of genetic analysis in wheat. This allowed complex multi-faceted traits such as flowering time,

stress tolerance, nutrient use efficiency etc., that were previously recalcitrant to analysis, to become manageable. Such studies resulted in the identification of a number of individual major genes and QTL explaining the phenotypic variation for the traits of interest (reviewed in Landjeva et al. 2007, Cativelli et al. 2008, Fontaine et al. 2009). The benefit of these achievements for practice lies in the possibility to elaborate strategies for fine-tuning of the most critical components of the plant life cycle and/or introducing responsible genes or chromosome regions to aid crop improvement with respect to stress tolerance and efficient utilization of essential nutrients.

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