

Aus der Landessaatzuchtanstalt
der Universität Hohenheim
PD Dr. Tobias Würschum

Phenotypic and Genotypic Assessment of Traits with Relevance for Hybrid Breeding in European Winter Wheat

Dissertation
zur Erlangung des Grades eines Doktors
der Agrarwissenschaften
vorgelegt
der Fakultät Agrarwissenschaften

von
Diplom–Agrarbiologe
Simon Martin Langer
aus Illertissen

Stuttgart–Hohenheim
2015

Die vorliegende Arbeit wurde am 30.03.2015 von der Fakultät Agrarwissenschaften der Universität Hohenheim als „Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften (Dr. sc. agr.)“ angenommen.

Tag der mündlichen Prüfung: 21.04.2015

1. Prodekan:	Prof. Dr. M. Rodehutschord
Berichterstatter, 1. Prüfer:	PD Dr. T. Würschum
Mitberichterstatter, 2. Prüfer:	Prof. Dr. R. T. Vögele
3. Prüfer:	apl. Prof. Dr. B. Hausmann

Contents

Contents	ii
Abbreviations	iv
1 General Introduction	1
2 Publication I: Male Ideotype for Hybrid Wheat (Phenotypic Evaluation of Floral and Flowering Traits with Relevance for Hybrid Breeding in Wheat (<i>Triticum aestivum</i> L.) ¹)	9
3 Publication II: Flowering Time Control in Wheat (Flowering Time Control in European Winter Wheat ²)	11
4 Publication III: Plant Height Control in Wheat (Genetic Control of Plant Height in European Winter Wheat Cultivars ³)	13
5 Publication IV: Population Structure in European Wheat (Population Structure, Genetic Diversity and Linkage Disequilibrium in Elite Winter Wheat Assessed with SNP and SSR Markers ⁴)	15
6 General Discussion	17
7 Summary	33
8 Zusammenfassung	35
9 References	37
Acknowledgements	43
Curriculum Vitae	44
Erklärung	45

¹ Langer S. M., Longin C. F. H., Würschum T. (2014) *Plant Breed* 133, 433-441. doi: 10.1111/pbr.12192

² Langer S. M., Longin C. F. H., Würschum T. (2014) *Front Plant Sci* 5, 537. doi: 10.3389/fpls.2014.00537

³ Würschum T., Langer S. M., Longin C. F. H. (2015) *Theor Appl Genet.* 128, 865-874. doi: 10.1007/s00122-015-2476-2

⁴ Würschum T., Langer S. M., Longin C. F. H., Korzun V., Akhunov E., Ebmeyer E., Schachschneider R., Schacht J., Kazman E., Reif J. C. (2013) *Theor Appl Genet* 126, 1477-1486. doi: 10.1007/s00122-013-2065-1

Abbreviations

AEX	anther extrusion
BP	before present
CHA	chemical hybridizing agent
Eps	earliness <i>per se</i>
FLD	flowering duration
GCA	general combining ability
GS	genomic selection
GWAS	genome-wide association mapping study
MAS	marker assisted selection
PM	pollen mass
Ppd	photoperiod
PRR	pseudo-response regulator
QTL	quantitative trait locus/ loci
Rht	reduced height
SB	spike belt
SNP	single nucleotide polymorphism
SPS	number of spikelets per spike
SSR	simple sequence repeat
VAEX	visual scoring of anther extrusion
Vrn	vernalization

1 General Introduction

Wheat covers more of the world's surface than any other food crop. In 2013, harvested on 218.5 million hectare, the global wheat production was 713.2 million tons making it the second main food crop for human consumption after rice (FAO 2013; FAOSTAT 2014).

Bread wheat (*Triticum aestivum* L., spp. *aestivum*) can be produced under widely varying conditions and is grown all around the globe covering regions along the majority of latitudes. This remarkable adaptation ability is owed to the high genetic diversity caused by its genomic architecture. Bread wheat is a hexaploid ($2n=6x=42$) crop possessing 21 chromosomes in seven homeologous groups on three genomes, the A, B and D genome (Morris and Sears 1967).

The success story started 9,500 to 7,500 years before present (BP) when hexaploid wheat first appeared, originating in the Fertile Crescent, the region of today's eastern Turkey and northern Iran (Feldman 2001). Hexaploid wheats first migrated to central Europe ca. 6,000 years BP as an admixture of emmer and einkorn seeds (Zeven 1980). First pure bread wheat grains were found 3,700 years BP and already during the Bronze Age some pure bread wheat fields were present (Zeven 1980; Miedaner 2009). With the rise of the Roman Empire bread wheat cultivation increased strongly but declined again in significance with its fall. Not until the end of the 19th century bread wheat gained growing importance due to increased winter hardiness by explicit selection. With the further combination of English 'Squarehead' cultivars wheat yields could be significantly increased and by the first third of the 20th century bread wheat rose to the most important cereal crop in Europe (Porsche and Taylor 2001; Miedaner 2009).

Today, wheat has the largest production volume of all staple crops in Europe and a share of 31.6% of the global wheat production comes from Europe. In France, Germany and the United Kingdom wheat is grown on 5.3, 3.2 and 1.6 million hectare, respectively (FAOSTAT 2014). During the period from 1960 until the early 1990s yield for wheat has doubled. This massive yield increase was enabled by high-yielding varieties improved by innovative breeding technologies and large scale deployment of fertilizers and pesticides advances that were summarized and termed the 'Green Revolution' (Hedden 2003; Whitford et al. 2013). For the past 20 years, however, wheat yields have been stagnating, an intolerable development regarding the needs of the growing global

population. Maize yield improvement, by contrast, has continued undiminished which in part can be explained by advances associated with hybrid technology and hence the higher level of investment in research from the private sector (Whitford et al. 2013).

The stringent autogamous nature of wheat ensures self-fertilization. Wheat varieties are pure lines, homogenous and homozygous. In 2014 the ‘Bundessortenamt’ (German Federal Plant Variety Office) registered 136 winter bread wheat varieties in Germany of which all except for two are line varieties (BSA 2013). Regarding the time consuming breeding process of at least ten years prior to seed sales and hence the immense financial investments a possibly high return on investment is necessary to compensate costs of research and product development by the seed companies. In addition, a major disadvantage of line varieties is the very high rate of farm saved seed; 53% and 55% for winter wheat in Germany (BDP 2014) and soft wheat in Europe (EU 27) (ESA and BDP 2012), respectively. For comparison, rye had a farm saved seed rate in Germany in 2013/14 of 15% (BDP 2014), including hybrids, and for maize no farm saved seed was grown. This illustrates the advantages of hybrid breeding which promises to hold huge potential also for wheat varieties.

Hybrid Breeding in Wheat

Hybrid breeding in wheat has recently received increased interest, especially in Europe, and large private and public initiatives have been launched exploring hybrid wheat breeding (Longin et al. 2012, 2013; Mühleisen et al. 2013; Whitford et al. 2013; Zhao et al. 2013). Since the middle of the past century several attempts to realize hybrid wheat were started but could not be established successfully as some of the major issues could not be solved efficiently (for review see Pickett 1993).

A major advantage of hybrids is the exploitation of heterosis. Although lower for wheat as compared to allogamous species, an average superiority of 10.7% with maximum values up to 23.8% for grain yield of hybrids derived from elite breeding germplasm compared to the mean of their parents was reported in first large scale performance tests (Longin et al. 2013). In addition, advanced breeding technologies and a more intense screening for lines with high ‘general combining ability’ (GCA) will lead to an increased hybrid vigor (Longin et al. 2014). Furthermore, hybrids as compared to line varieties were confirmed to have a significantly higher yield stability and lower

susceptibility to abiotic and biotic stress (Longin et al. 2013; Mühleisen et al. 2013). The main benefit for seed companies is the advantage of an integrated variety protection by inbreeding depression and hence a higher return on investment by certified seed royalties (Edwards 2001).

The link between the profitability of a hybrid variety and the efficiency of a hybridization system is extremely close for wheat as compared to allogamous crops due to the lower heterosis. A major limitation for the breeding and production of hybrid wheat is the lack of a cost-efficient hybrid seed production system. Thus, a prerequisite for hybrid wheat breeding is the redesign of flowering and floral traits to ensure sufficient cross-fertilization in this selfing species. For mixed planting, blends with a minimum of male lines require appropriate amounts and spread of pollen.

Many traits contribute to pollination capability such as flowering time, flowering duration, plant height, extrusion of anthers and stigma, number of pollen grains per anther, adequate pollen dispersal outside the florets, opening of the glumes and longevity of pollen grains (de Vries 1971; Lelley 1966; Whitford et al. 2013). The male ideal form, or 'ideotype', should therefore extrude long anthers that shed large amounts of pollen outside the florets over an extended period of time (Figure 1). The pollen should be viable, long-lived and have good aerodynamic qualities (de Vries 1971, 1974a; Whitford et al. 2013). The female ideotype flowers ought to open glumes and extrude stigmatic hair during male flowering for increased receptivity. In addition, the flowering time for both parents has to be synchronized and males should be taller than females in order to facilitate cross-fertilization (de Vries 1972; Longin et al. 2013).



Figure 1 Good pollen shedding properties of the male line: wheat spikes extruding many and long anthers (left), and anthers releasing high amounts of viable pollen outside the florets (right).

For most of the traits phenotyping is difficult and time consuming, precluding a large scale use in applied plant breeding. In order to identify male lines that ensure constant pollination of females in seed production fields, appropriate phenotypic characteristics to efficiently select for pollination capability have to be defined. Suitable high-throughput phenotyping methods for floral and flowering traits are of utmost importance in order to facilitate the design of the male ideotype and hence increase the outcrossing rates of wheat for hybrid seed production.

Flowering Time Control in Wheat

Flowering time regulation is one of the most important and also most critical phenological stages in plant development. Wheat plants have to coordinate flowering during the year with respect to the greatest likelihood of reproductive success, particularly successful pollination and seed development (Laurie and Turner 2011). Adapted flowering allows early season resource accumulation, and facilitates avoiding high temperatures and drought stress during anthesis and grain filling (Bennett et al. 2012; Bentley et al. 2013). Already in the near future, extreme weather conditions such as high temperatures, extended drought periods and heavy precipitation can be expected to occur more frequently (Beniston et al. 2007; Olesen et al. 2012). Therefore, plant breeders need effective tools such as marker-assisted breeding to predict flowering time in order to transfer promising genotypes into different climatic regions. Furthermore, for hybrid wheat production adjusted flowering between male and female parental lines is crucial for appropriate seed setting. In addition, climatic stress during anthesis results in decreased pollen production thus hampering successful cross-fertilization (Pickett 1993).

Genes controlling flowering time in cereals are divided into three categories: The ‘vernalization’ (*Vrn*), ‘photoperiod’ (*Ppd*) and ‘earliness *per se*’ (*Eps*) signaling pathway (Worland and Snape 2001). The group of *Vrn* genes controls the temperature response. Wheat in its wild type form is a winter annual which needs exposure to a period of time at low temperature (vernalization) for the transition from vegetative to generative growth (Laurie and Turner 2011). Full vernalization of winter wheat is assumed to be obtained at temperatures between 3°C and 10°C for 33 days (Weir et al. 1984). Provided vernalization requirement has been met *Vrn* genes are of minor importance for flowering time control. *Ppd* genes genetically control the response to day length (photoperiod) (Laurie and Turner 2011; Kamran et al. 2014). Wheat is a long day plant and thus only

flowers after a critical day length has been reached. Major genes are the *Ppd-1* homeoloci located at colinear positions on the short arms of the group 2 chromosomes. These are members of the ‘pseudo-response regulator’ (*PRR*) family, which are part of the circadian clock in *Arabidopsis* and thereby involved in the plants’ internal day length measuring system (Beales and Turner et al. 2007; Laurie and Turner 2011). Mutations of the *Ppd-1* loci, however, enable wheat to flower irrespective of the day length. These photoperiod insensitive alleles are given an ‘*a*’ suffix whereas photoperiod sensitive wild types are given a ‘*b*’ suffix (Laurie and Turner 2011). In wheat, photoperiod insensitive alleles cause early flowering in long and short days. These mutations have been selected by plant breeders for several decades to enhance yield in certain climatic conditions by avoiding high summer temperatures through early flowering. *Eps* genes summarize effects of loci affecting flowering time independently of vernalization and photoperiod response (Kamran et al. 2014). *Eps* ‘quantitative trait loci’ (QTL) are located all over the genome and are assumed to contribute small effects to the fine-tuning of flowering time (Kamran et al. 2014; Worland 1996). *Eps* genes are still poorly characterized which is mainly due to joint appearance with larger *Ppd* or *Vrn* effects (Laurie and Turner 2011).

Flowering control in *Arabidopsis thaliana*, for example, is well understood (Srikanth and Schmid 2011) but further research is needed for major crops like wheat. There is a large amount of research that detected loci for heading and flowering time, predominantly based on biparental or rather diverse collections (Kamran et al. 2014). To date, diagnostic markers exist for important flowering alleles, however, only providing a rough classification of breeding germplasm. Accurate knowledge about effective alleles and their distribution in elite wheat breeding germplasm is still lacking.

Regulation of Plant Height in Wheat – A Key Trait of the Green Revolution

The regulation of plant height has probably been one of the most prominent traits in wheat breeding over the past century (Worland and Snape 2001). Plant height in wheat was reduced by half while in the past 50 years the human world population doubled (FAOSTAT 2014). Admittedly, these two trends are not dependent but the fact that increases in global cereal production matched the increase of the latter can certainly in parts be attributed to plant height adjustments (Hedden 2003). The reduction of wheat

plant height has been a main breeding goal and was responsible for increases in productivity between mid until end of the 20th century, the period of the ‘Green Revolution’. Plant height reduction led to increased grain yield and grain quality as it facilitated the use of technical achievements in the agricultural sector. Due to the higher lodging resistance an increased use of fertilizers was possible. In addition, wheat plants did no longer need to be tall to compete with weeds owing to the development of herbicide weed control (Worland and Snape 2001). The harvest index was improved as assimilates were put to greater use into the production of grain rather than straw. Nevertheless, despite these indirect positive effects on yield, plant height reduction is generally associated with reduced yield (Law et al. 1978).

The introduction of dwarfing and semi-dwarfing genes which are called ‘*reduced height*’ (*Rht*) genes enabled plant breeders to strongly modify plant height in wheat. The *Rht-1* homoeoloci, located on group 4 chromosomes are the major source of semi-dwarfism, predominantly alleles of the *Rht-B1* and *Rht-D1* genes. These important dominant (gain-of-function) mutations were carried by the Japanese variety ‘Norin 10’ and were brought to the United States after World War II and were distributed all around the world after being introduced to CIMMYT breeding programs (Hedden 2003; Borojevic and Borojevic 2005). The genetic control of plant height is very complex, determined by many major and minor genes (Worland and Snape 2001). There is a substantial body of literature on detecting QTL for plant height in wheat (e.g., Cadalen et al. 1998; Kato et al. 1999; Griffiths et al. 2012), however, the effects of key candidate loci and of loci responsible for the fine adjustment of plant height in European elite germplasm remain less clear.

Today, plant height as breeding goal has again gained increased importance in modern wheat breeding. As yield enhancement is most important the aim is to identify alleles that reduce height without negatively affecting the *per se* wheat yield potential. Furthermore, the fine-tuning of plant height will play an important role in order to implement an effective hybrid wheat production system by maximizing cross-pollination.

Objectives

The main goal of this thesis was to explore aspects with relevance for hybrid breeding in wheat such as the phenotypic and genotypic assessment of important traits, the development and evaluation of application-oriented phenotyping methods and the exploration of the population structure of European elite breeding germplasm. In particular, the objectives were to

1. identify and evaluate direct and indirect traits with relevance for hybrid breeding in wheat for increased cross-pollination,
2. develop methods for exact or high-throughput phenotyping of floral and flowering traits to facilitate the design of the male ideotype for hybrid wheat production and discuss their utility for breeding programs,
3. perform high-density genome-wide association mapping to identify main effect and epistatic QTL for flowering time and plant height in European elite winter bread wheat varieties,
4. assess the effect of specific candidate gene alleles on flowering time and plant height and investigate their frequency in dependence on the country of origin of the winter bread wheat varieties,
5. assess the population structure and genetic diversity of European elite winter bread wheat germplasm.

1 General Introduction

2 Publication I: Male Ideotype for Hybrid Wheat

Phenotypic Evaluation of Floral and Flowering Traits with Relevance for Hybrid Breeding in Wheat (*Triticum aestivum* L.)

Simon M. Langer, C. Friedrich H. Longin, Tobias Würschum

State Plant Breeding Institute, University of Hohenheim, 70593 Stuttgart, Germany

Plant Breed (2014) 133, 433-441, doi: 10.1111/pbr.12192.

The original publication is available at
<http://onlinelibrary.wiley.com/doi/10.1111/pbr.12192/full>

Abstract

Because of its promise to increase yield potential, hybrids have again received increased interest in wheat (*Triticum aestivum* L.) breeding. For autogamous crop species such as wheat, the profitability of hybrid varieties strongly depends on a cost-efficient system for hybrid seed production. Therefore, a sufficient level of cross-fertilization, especially based on improvements in the male pool, is highly important. Using 51 elite winter wheat lines, we developed and evaluated phenotyping methods for floral and flowering traits with relevance to improving cross-pollination. For most traits, high heritabilities and significant genotypic variance were observed, including for the putatively important traits of ‘anther extrusion’ and ‘pollen mass’. The results of this study suggest that the phenotyping approaches developed, as well as many traits measured, are highly suitable for use in applied plant breeding programs including the design of the male parent ideotype and increasing the level of out-crossing in hybrid wheat seed production fields.

3 Publication II: Flowering Time Control in Wheat

Flowering Time Control in European Winter Wheat

Simon M. Langer, C. Friedrich H. Longin, Tobias Würschum

State Plant Breeding Institute, University of Hohenheim, 70593 Stuttgart, Germany

Front Plant Sci (2014) 5, 537, doi: 10.3389/fpls.2014.00537.

The original publication is available at

<http://journal.frontiersin.org/Journal/10.3389/fpls.2014.00537/full>

Abstract

Flowering time is one of the most critically important stages in plant development. The timing of flowering during the year contributes to successful reproduction and is decisive regarding yield potential, yield stability and adaptation. The aim of this study was to assess the genetic architecture of flowering time in European winter bread wheat germplasm. A panel of 410 European winter wheat varieties was evaluated in multi-location field trials and genotyped using both a genotyping-by-sequencing approach and a selection of specific candidate gene markers. We found that the photoperiod regulator *Ppd-D1* had the largest effect on flowering time (58.2% of explained genotypic variance), whereas the plant height loci *Rht-B1* and *Rht-D1* had no significantly measurable effect on flowering time. In this cultivar collection, the fine-tuning of flowering time seems to be controlled by copy number variation at the *Ppd-B1* locus (3.2%), as well as six minor QTL which were identified in a genome-wide scan. Several epistatic QTL, all with small effects, were also identified.

4 Publication III: Plant Height Control in Wheat

Genetic Control of Plant Height in European Winter Wheat Cultivars

Tobias Wüschum, Simon M. Langer, C. Friedrich H. Longin

State Plant Breeding Institute, University of Hohenheim, 70593 Stuttgart, Germany

Theor Appl Genet (2015) 128, 865-874, doi: 10.1007/s00122-015-2476-2.

The original publication is available at

<http://link.springer.com/article/10.1007%2Fs00122-015-2476-2>

Abstract

Plant height has certainly been one of the most prominent traits in wheat (*Triticum aestivum* L.) breeding during the past century. Lowering plant height has had a major impact on global wheat production as it has direct and indirect effects on grain yield and quality. The aim of this study was to assess the genetic architecture of plant height in European winter bread wheat varieties. Four hundred and ten European winter wheat cultivars were tested for plant height in multi-location field trials. The panel was genotyped by a genotyping-by-sequencing approach yielding 23,371 markers with known positions, as well as for the specific candidate gene loci *Rht-B1*, *Rht-D1*, *Rht8*, *Ppd-B1* copy number variation and *Ppd-D1*. Analyses revealed that *Rht-D1* and *Rht-B1* significantly affect plant height in European bread wheat, explaining 40.9 and 15.5 % of the genotypic variance, respectively with *Ppd-D1* and *Rht8* accounted for 3.0 and 2.0 % of the variance respectively. In addition, a genome-wide analysis for marker-trait associations identified two medium effect QTL on the 6A and 5B chromosomes explaining 11.0 and 5.7 % of the genotypic variance, respectively. In addition, several small effect QTL and epistatic QTL were also detected. Our results show that the two *Rht-1* loci are the major sources of variation in plant height in European winter wheat, with a number of small and medium effect QTL and epistatic QTL also involved.

5 Publication IV: Population Structure in European Wheat

Population Structure, Genetic Diversity and Linkage Disequilibrium in Elite Winter Wheat Assessed with SNP and SSR Markers

Tobias Würschum¹, Simon M. Langer¹, C. Friedrich H. Longin¹, Viktor Korzun², Eduard Akhunov³, Erhard Ebmeyer², Ralf Schachschneider⁴, Johannes Schacht⁵, Ebrahim Kazman⁶, Jochen C. Reif⁷

¹State Plant Breeding Institute, University of Hohenheim, 70593 Stuttgart, Germany

²KWS Lochow GmbH, 29303 Bergen, Germany

³Department of Plant Pathology, 4024 Throckmorton Plant, Sciences Center, Kansas State University, Manhattan, KS 66506, USA

⁴Nordsaat Saatzuchtgesellschaft mbH, 38895 Langenstein, Germany

⁵Limagrain GmbH, 31226 Peine-Rosenthal, Germany

⁶Lantmännern SW Seed Hadmersleben GmbH, 39398 Hadmersleben, Germany

⁷Leibniz Institute of Plant Genetics and Crop Plant Research, 06466 Gatersleben, Germany

Theor Appl Genet (2013) 126, 1477-1486, doi: 10.1007/s00122-013-2065-1.

The original publication is available at
<http://link.springer.com/article/10.1007%2Fs00122-013-2065-1>

Abstract

For modern genomics approaches, high-throughput and high-density genotyping platforms are important. Currently, a SNP array is available for the polyploid species wheat (*Triticum aestivum* L.). In this study, a collection of 172 European elite winter wheat lines was used to assess the utility of the SNP array for genomic analyses in germplasm derived from wheat breeding programs. The results revealed differences in genetic relatedness and population structure using the SNP array as compared to SSR markers, which suggests that further research is necessary to determine the optimum approach for investigating kinship and structures in wheat populations. The analyses showed that linkage disequilibrium (LD) decays within approximately 5 to 10 cM and is variable along chromosomes. In order to obtain a higher coverage of the chromosomes, the number of SNPs needs to be increased further. Nevertheless, for genomics approaches and knowledge-based breeding of wheat SNPs can be an advantageous tool.

6 General Discussion

Hybrid breeding is a remarkable success in many allogamous crops, but despite over 60 years of research it is still not established in wheat. A major limitation for the breeding and production of hybrid wheat is the lack of a cost-efficient hybrid seed production system caused by the poor cross-fertilizing capability. Nevertheless, hybrid breeding in wheat has recently again received increased interest, especially in Europe, and large private and public projects investigating hybrid wheat breeding have been launched. Facing future challenges, it is of utmost importance to exploit advantages of hybrid breeding also for wheat (Whitford et al. 2013). Climate changes force the agricultural sector to respond. In wheat breeding, hybrid technology can be expected to further utilize the ability of wheat for broad adaptation.

Wheat Breeding with Regard to Climate Changes

Climate conditions directly influence plant development. Plant physiological stages and hence reproduction success (in hybrid breeding the seed production efficiency) strongly depend on weather conditions. Plant breeders have to consider this particular aspect when planning breeding programs and objectives. For future variety development, breeding for climate-stability gains importance as varieties need to perform high yielding under diverse weather conditions. Hybrid wheat varieties as compared to line varieties can be expected to perform more stable under variable environmental conditions (Mühleisen et al. 2013) and Longin et al. (2013) observed clear superiority of hybrids regarding abiotic and biotic stress.

The average temperature in Europe is projected to continue increasing over the 21st century (EEA 2012). The annual near-surface temperature is estimated to increase up to 2.5°C and 4.0°C for the periods between 2021-2050 and 2071-2100, respectively, compared to the period from 1961-1990 (van der Linden and Mitchell 2009). This trend can be seen since around 1980. Extreme high temperatures are projected to become more frequent and longer-lasting across Europe during this century (EEA 2012). Consequently, heat stress days are expected to occur frequently in future periods even in central Europe (Figure 2A).

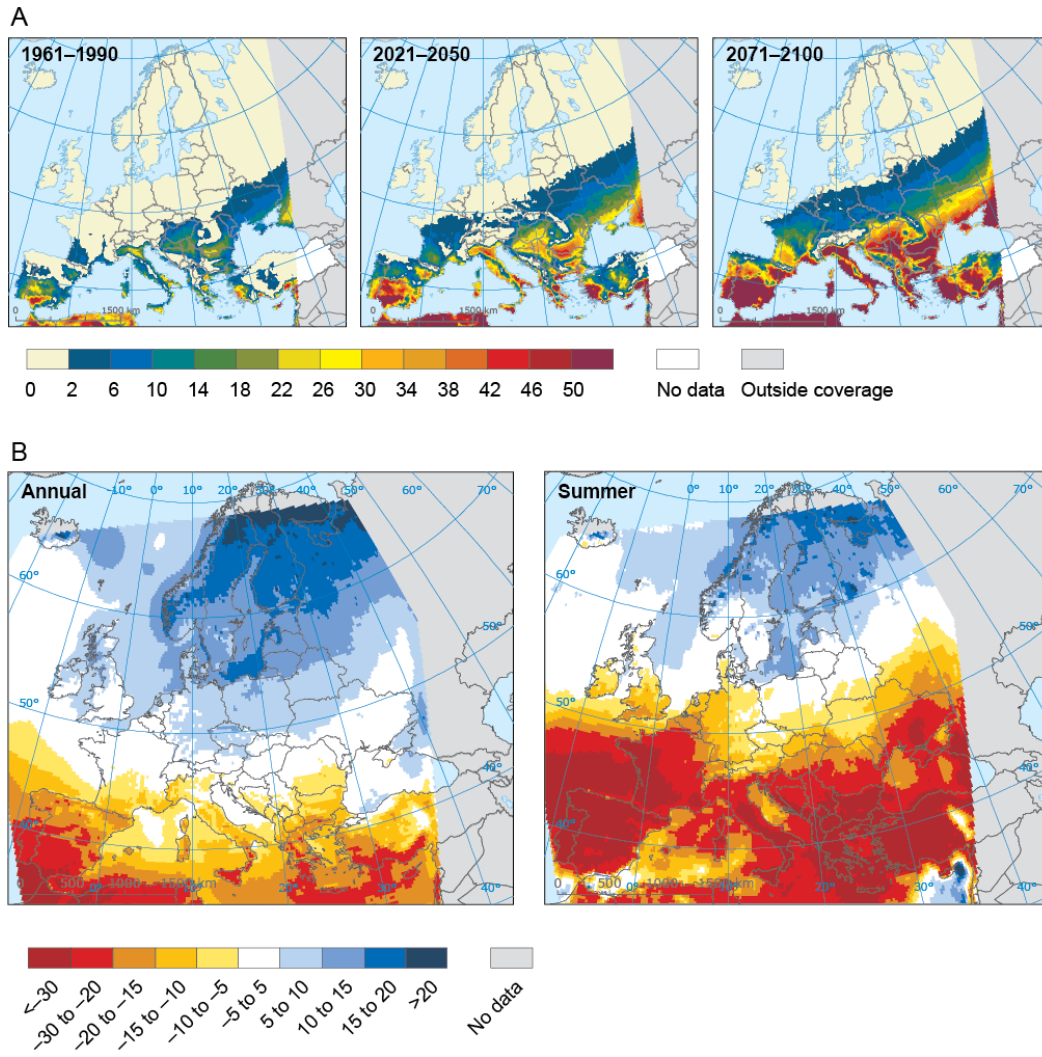


Figure 2 Projections of temperatures and precipitation in Europe. (A) Maps show changes in extreme temperature for two future periods, relative to 1961-1990. Extreme temperatures are represented by the combined number of hot summer (June-August) days ($T_{MAX} > 35^{\circ}\text{C}$) and tropical nights ($T_{MIN} > 20^{\circ}\text{C}$). (B) Projected change in annual (left) and summer (right) precipitation (%) in the period 2071-2100 compared to the baseline period 1971-2000. (adapted from EEA 2012, owners: UK Met Office, Ensembles FP6 Project)

Anthesis is a very vulnerable phase during plant development and adverse environmental conditions directly influence seed set and grain yield. To predict the exact time of flowering is therefore essential and will gain further importance as in future periods the end of frost and the start of heat stress during one year are expected to narrow down the window for optimum flowering time. For large parts of the main wheat

production areas over Europe precipitation during the summer is projected to decrease by up to 60%, especially in southern regions (Figure 2B) (van der Linden and Mitchell 2009). Severe drought stress will be a result hazarding grain yield in general but also influencing the success of hybrid wheat production. High temperatures cause decreased anther emergence and pollen viability in maize which is similar in wheat (Pickett 1993). In addition, genotypes respond differently to drought consequently passing through stages of plant development dissimilar resulting in a lower synchrony of male and female parents which reduces the chance for cross-fertilization.

Future European climatic events are projected to go into extremes: increased frost and drought stress, high temperatures, precipitation lower in the south and higher in the north with general increases in frequency and intensity of heavy precipitation; all events in quick and hard to predict alternation (JRC and EEA 2014). With regard to hybrid production in wheat, hybrid parents have to be compatible to the expected conditions even when environments for seed production are chosen carefully. In addition, breeding for specific traits for cross-pollination is very difficult as especially flowering and floral traits respond rather sensitive to environmental stress. A successful phenotyping of male lines for anther extrusion using the ‘visual scoring of anther extrusion’ (VAEX) method, for example, strongly depends on favorable weather conditions. Jacob et al (2014) projected heavy precipitation events during summer and early summer for the period 2071-2100 compared to 1971-2000 to increase by up to 25% for central Europe. Heavy rain and strong wind carries away extruded anthers which results in decreased repeatability in field trials. Rainfall during anthesis has a deleterious effect on cross-fertilization (Pickett 1993). Shed pollen is washed out of the air which severely reduces the reach and, in addition, lodging is more likely hence reducing the ability of female plants to receive pollen.

The Challenge of Cross-Pollination

During the domestication process, wheat went through the stages from allogamy to autogamy and even cleistogamy (d’Souza 1970). The cultivation of wheat outside the region of origin enhanced the isolation of self-fertilized types. The ability for cross-fertilization, however, is still available as can be seen by the extrusion of anthers.

Reach and altitude of pollen grains do not seem to be the major limiting factors for cross-pollination but rather pollen longevity and the amount of shed pollen (d'Souza 1970). Seed set on male sterile wheat could be recorded up to 20m from the pollen source (de Vries 1974b) and pollen mediated gene flow was detected even over a 100m distance (Loureiro et al. 2012). D'Souza (1970) reported pollen reach of 50m with an altitude of 0.9m above the floret of origin and a relatively low sink rate. The amount of pollen shed outside florets has to be increased though. Although up to 80% of pollen grains are shed outside, which is quite high as compared to 95% for allogamous rye, the number of pollen grains per anther is only 10-15% of that of rye (d'Souza 1970). Our results showed great variability for 'pollen mass' (PM) which is in line with reports of rates of airborne pollen between 3-80% (de Vries 1970). Anthers are extruded only after bursting and the rate of airborne pollen depends on the time needed between bursting and extrusion which is under direct influence of the prevailing meteorological conditions. For instance, the time required for the anthers of a floret to fully extrude is about two to five minutes and for full elongation of the filaments 15 to 40 minutes (Briggle 1967). But if and to what extent the anthers emerge also strongly depends on the prevailing weather conditions. Warm, dry weather and low humidity promote anther extrusion and pollen release. Increased temperature and drought, however, lower the longevity and fertilization capability of the pollen grains. There is thus a fine balance between success and failure of cross-fertilization regarding the requirements of pollen release and maintenance of pollen viability.

The exact phenotyping of many outcrossing related traits is hampered by their often strong environment dependency. For instance, the 'visual scoring of anther extrusion' method required presence on the field during the main occurrence of anther extrusion. Depending on the location the time between heading and the start of anther extrusion took up to seven days (data not shown). It was then very important to wait for the day-time of major anther extrusion as anthers may quickly shrivel in a strong noon sun shine which can influence the phenotyping results significantly. In addition, we empirically observed that rainy conditions tended to reduce anther extrusion which is consistent with reports in grasses where anthesis was retarded and anther extrusion decreased (Emecz 1962). However, as long as multi-location testing is given, the 'visual scoring of anther extrusion' method seemed to be well suited to quantitatively evaluate the pollen shedding potential in large scale breeding programs.

A more thorough analysis is the ‘anther extrusion’ (AEX) method based on counting, yet, more work-intensive and time-consuming. Extrusion of anthers is the basis for air-borne pollen and is significantly correlated with the pollen mass shed outside the florets. We evaluated the ‘anther extrusion’ and ‘visual scoring of anther extrusion’ method and certified both as appropriate for practical use. The large-scale use of ‘anther extrusion’ is mainly hampered by the counting of the anthers that remained in the florets, a work that takes place simultaneously to the work in the field. In order to level out peak work periods we tested to deep-freeze spikes and counted the anthers months later. The empirical results were as good as those of the direct counting which means the method gains flexibility for use in breeding programs. A major disadvantage of the ‘anther extrusion’ method was that only lateral florets are used for determining the anther extrusion capability of genotypes. The number of central florets varies, hence, a lot of potential may remain disregarded. Although ‘visual scoring of anther extrusion’ is the high-throughput version of ‘anther extrusion’ it does not share this disadvantage as the person who phenotypes is taking all extruded anthers into account. Especially experienced personnel are able to include also central florets in the phenotyping process.

A very important factor for pollination capability is quantity of shed pollen outside the florets. Several ways to detect this trait have been tested during the past decades, most of them suited for scientific purposes rather than for practical use. Usually adhesive coated surfaces placed near the pollen source were used. Although approaches were intended for applied breeding (e.g. Kempe et al. 2013), the use for large scale was not tested. As plant height is conspicuous for pollen concentrations in different distances to the pollen source (de Vries 1972) the position of the so called ‘pollen traps’ would have to be adjusted accordingly. This is not desirable for large scale use. This factor is no longer important when using the ‘pollen mass’ method described in Chapter 2.

Plant height is an important factor for hybrid seed production. In species such as maize the male floral parts are positioned above the female flowers which suggests a certain advantage of taller males relative to females in cross-fertilization (Pickett 1993). De Vries (1972) demonstrated a higher pollen concentration below spike level. Therefore, hybrid parents have to be chosen with consideration, especially when the hybridization system is based on ‘chemical hybridizing agents’ (CHA) which additionally have a shortening effect on plant height.

An optimized ratio of plant height between parents and a synchronized flowering time are important factors for a satisfying seed set in hybrid production. In order to give

the florets of the female parent time to open, the males should flower two days later on average (Pickett 1993). Additionally, an extended duration of flowering is favorable. Pollen should be shed over a couple of days depending on the spread of flowering in the female line. We tested the indirect trait for flowering duration, termed ‘spike belt’ (SB), however, without success. As some plant breeders use very similar methods for the same purpose, future research could test ‘spike belt’ with modified conditions. First, test plots should be larger and measurements then conducted in central parts of the plots to avoid side-effects which are unavoidable using double-row plots. Second, sowing rates should coincide with those used in hybrid production fields and third, late tillers need to be disregarded.

We observed very long ‘flowering durations’ (FLD) in Chapter 2 which may in part be explained by the relatively low seeding rate in double-row plots causing the production of secondary tillers and thus expanding differences in growth stages. In practice this effect could be used to increase the chance of successful cross-pollination. Female parents would then be planted at higher seeding rates hence minimizing the number of tillers and male parents would be sown at a lower rate (Koekemoer et al. 2011). As a result, female anthesis would take place at a very brief period of time while the male pollen load would be spread over a greater span. In addition, if sterilization is realized via CHA the probability of unsuccessful sterilization due to late tillers would be reduced.

Coincidence of the time of hybrid parents and an extended flowering duration are mandatory for successful seed production as incorrect phasing is a major cause for poor seed yield (Pickett 1993). It is therefore of utmost importance that consistency of these traits in different environmental conditions is given.

Early crosses between wheat and rye in order to increase the cross-pollination ability of wheat resulted in increased number of pollen grains but other important pollen traits were not improved (d’Souza 1970). Outcrossing rates of triticale, for example, are somewhere between wheat and rye. Besides the ambitious aim to improve the cross-fertilization capability important agronomical traits have to be held on a high level. For instance, the yield related ‘number of spikelets per spike’ (SPS) was negatively correlated with ‘anther extrusion’. Although more spikelets may compensate the fewer extruded anthers with regard to air-borne pollen, this correlation has to be broken in order to utilize the pollen shedding potential of long spikes.

Environmental conditions may play a similar or even more important role for cross-fertilizing success than the genotype. The interaction between genotypes and the environment for important traits such as ‘pollen mass’ and ‘anther extrusion’ was very high in our study which may indicate that some genotypes are less stable than others. The relatively high genotype-by-location interaction of ‘pollen mass’ and ‘anther extrusion’ could be explained by the increased number of cleistogamous flowers when low temperatures and rainy conditions prevailed. Nevertheless, high heritabilities in combination with high genotypic variances assures breeding success for outcrossing related traits. In addition, the outcrossing rate can be increased by selection due to the positive correlations among most floral traits (Abdel-Ghani et al. 2003). In rice, for example, selection for stigma length and the proportion of extruded stigmata was shown to improve hybrid seed production.

Molecular Breeding for Hybrid Parent Ideotypes

The application of genomics approaches in wheat is moving on rapidly making detailed genomic and genetic analyses possible (Snape and Griffiths 2011). ‘Genome-wide association mapping studies’ (GWAS) are suited to unravel the genetic architecture of complex traits (Reif et al. 2011). Both traits, flowering time and plant height are controlled by few major QTL and many minor and epistatic QTL. Epistasis refers to interactions between two or more genetic loci which in their specific combinations contribute to the phenotype of an individual (Würschum et al. 2011). The problem of GWAS is that also non-functional associations between the trait of interest and the underlying population structure of the test panel can be detected as QTL (Reif et al. 2011). Correction for population stratification and relatedness is necessary which unfortunately results in a lower power to detect especially minor QTL (Würschum et al. 2011). The purpose of association mapping approaches is to identify functional markers for ‘marker assisted selection’ (MAS) in breeding programs.

Many important traits with relevance for increased cross-fertilization in wheat have been identified, defined and evaluated in Chapter 2. In a further step molecular markers that are tightly linked to these traits could be identified in order to establish marker-based prediction for pollination capability of male lines. Assuming effective markers can be established the ‘visual scoring of anther extrusion’ method could be complemented using

MAS as the success of ‘visual scoring of anther extrusion’ strongly depends on weather conditions.

For complex traits, MAS has its limitations as only QTL with large contributions to phenotypic variation are of interest (Zhao et al. 2014). Regarding cross-fertilization, some relevant traits might be under simple and other under complex genetic control. The autogamous nature of barley for instance is controlled by only one gene, *cleistogamy 1*, which is responsible for flower opening (Nair et al. 2010). Also in barley, the number of spikelets per spike is strongly influenced by genes of the flowering pathway which indeed is complex but substantially controlled by few major genes like *Ppd-H1* or *VRN-H3* (Sreenivasulu and Schnurbusch 2012). GWAS for cross-pollination related traits would be a first step in order to get an idea of the complexity of the traits and to detect major regulators which could then be validated and subsequently implemented in breeding programs.

For plant breeders, knowing their breeding material is very important. Germplasm which is used for crosses needs to coincide with the breeder’s objectives and desired purposes of the future varieties. Therefore, MAS can be very useful for pre-breeding in order to create breeding programs more efficiently. The combination of flowering, floral and domestically important traits in hybrid wheat varieties has to ensure competitiveness and even clear superiority over classical line varieties. As cost-efficient hybrid wheat production highly depends on a deliberate combination of favorable traits, pleiotropic effects of specific alleles need to be considered. This is even more important as in this initial phase of establishing hybrid wheat the profit margin depends on a relatively low commercial heterosis. As shown in Chapter 3 *Rht* genes do not contribute to the timing of anthesis in wheat. *Rht8* may be pleiotropic to a certain extent as it explains a small amount of the genotypic variation but as there is a certain co-segregation with *Ppd-D1* due to their proximity of approximately 20cM this needs to be clarified in further research.

Clearly a MAS target for plant height breeding are the *Rht-B1* and *Rht-D1* semi-dwarfism alleles. A strong increase in introducing these alleles into Bulgarian breeding germplasm during the 1990s and 2000s was observed (Ganeva et al. 2005) which confirms our findings (Chapter 4: Figure 5). Plant height is, however, associated with grain yield. There seems to be a favorable height of around 90cm at which wheat grain yield peaks and severe yield reductions for shorter plants (Addisu et al. 2010). In order to gain flexibility for breeding programs and in particular for hybrid breeding where plant

height plays an important role plant breeders are interested in height regulating alleles independent from yield. In Chapter 4 we reported the plant height QTL ‘CloneID 1066954’ for which also a frequency increase during the past 20 years was observed especially in northern and western Europe (Chapter 4: Figure 4, Figure 5). Whether this locus is associated with yield would be of high interest and needs further investigation.

Regarding hybrid wheat production *Rht* genes may play an important role not only in optimizing the cross-fertilization between males and females based on optimized relative heights but also as *Rhts* could be correlated with pollen shed ability (Figure 3).

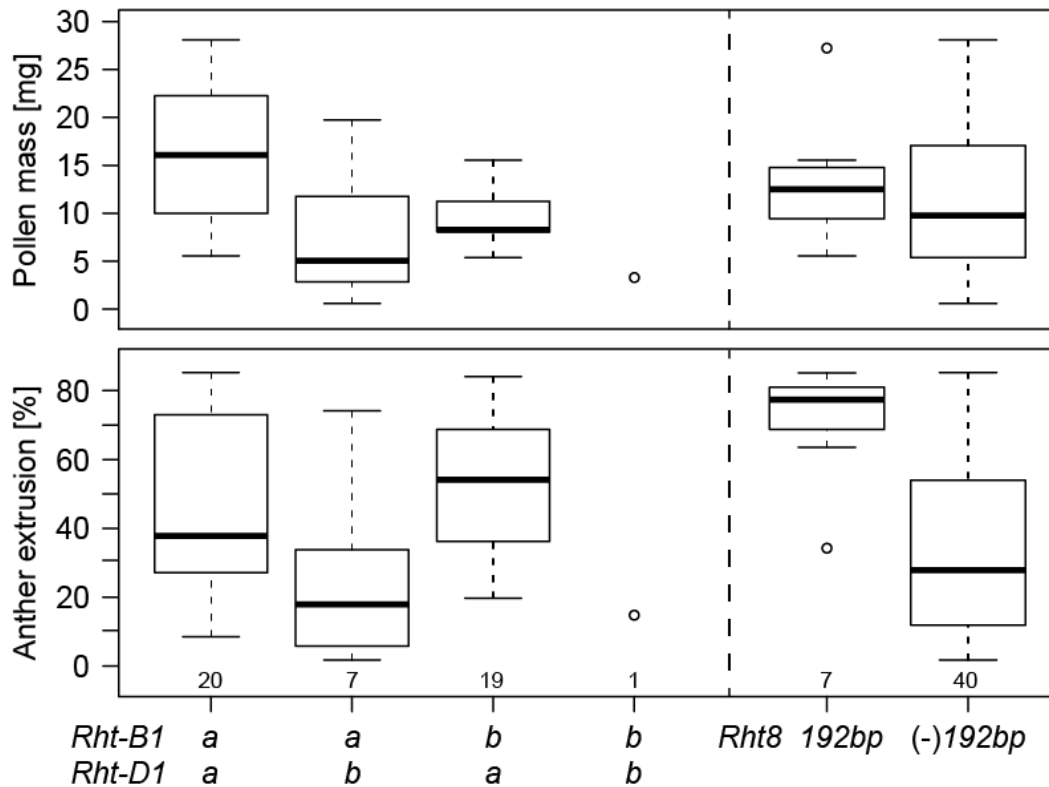


Figure 3 Boxplots showing ‘pollen mass’ (PM) (upper box) and ‘anther extrusion’ (AEX) (lower box) for genotypes carrying different allele combinations of *reduced height* (*Rht*) genes. 47 Genotypes from the Chapter 2 panel are grouped based on their *Rht-B1*/*Rht-D1* allele combination (left from dashed lines) and based on either carrying the *Rht8 192bp* allele or not (right from dashed line). The numbers underneath each boxplot indicate the number of genotypes in each group. *Rht-B1* and *Rht-D1*: *a* (tall allele), *b* (semi-dwarf). *Rht8*: *192bp* (semi-dwarf), *(-)192bp* (tall allele).

As stated in Chapter 2, dwarfing genes might have a negative effect on pollen traits. The findings shown in Figure 3 support this as ‘pollen mass’ as well as ‘anther extrusion’ tend to be decreased when the genotypes possess dwarfing genes. The extent of decrease for the different groups directly associates with the proportion of genotypic variation of plant height explained by the different *Rht* genes. *Rht-D1b* majorly affects plant height reduction and genotypes carrying this allele seem to perform worst for ‘pollen mass’ and ‘anther extrusion’. *Rht8 192bp* genotypes show a high anther extrusion and also a slightly higher pollen mass which would be in contrast to the initially mentioned hypothesis. It is noteworthy that all seven genotypes in this group also carry the *Rht-D1a* allele (data not shown) which may bias the results. Furthermore, sampling effects as well as co-selection could explain the findings to some extent. Whether either of these alleles truly influence the pollen shed capability, directly or indirectly, would be of highest interest for further research.

Ppd-D1a could be a MAS target for flowering time breeding. Although, it does give earliness too extreme for many parts of northern Europe thus, moderating the effect would be necessary (A. Bentley, pers. comm.). Therefore, it remains uncertain to what extent *Ppd-D1* will be used for flowering time control in breeding programs in the future as also its influence on yield for example can be negative or positive depending on prevailing environmental conditions (Beales and Turner et al. 2007). In general, photoperiod insensitive varieties flower earlier in the year which is advantageous in warm and dry environments as plants can complete development and grain filling prior to high summer temperatures and water deficit. That in mind, regarding climatic changes, photoperiod insensitivity alleles are likely to become more frequent also in central parts of Europe and tend to wander northward.

For future studies, either GWAS or GS, marker density in wheat has to be increased. Especially the D-genome shows wide regions with low marker coverage. As a substantial amount of genotypic variation could not be explained for flowering time as well as for plant height, studies with a larger number of markers would be beneficial. For instance, in Chapter 3 we were not able to detect the main flowering time QTL *Ppd-D1* without a targeted candidate gene analysis. Nowadays, this is still a problem but in the near future advanced ‘genotyping-by-sequencing’ with hundreds of thousands of markers will be possible and also ‘re-sequencing’ as already done in maize or rice.

Population Structure in European Elite Wheat

Knowledge about the population structure in (breeding) germplasm is very important. First, for genomic approaches and analyses and second, in order to identify and install genetically distant groups for effective hybrid breeding. Especially if no pedigree information are available, analyses based on marker data are necessary. Prerequisite for the successful implementation of hybrid wheat (besides a cost-efficient system for seed production and an adequate level of heterosis) is the establishment of heterotic groups and patterns to warrant future breeding progress (Edwards 2001; Koekemoer et al. 2011). These factors are necessary to provide a basis for planning reliability for breeding companies in order to keep up the interest for long-term investments.

Heterotic groups (or pools) are by definition “a group of related or unrelated genotypes from the same or different populations which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups” (Melchinger and Gumber 1998). The first and an important step to initiate a hybrid breeding program should be the establishment of heterotic pools (Fischer and Maurer et al. 2010) and thereby the optimization of the genetic diversity between female and male gene pools (Koekemoer et al. 2011). Experience gained from maize breeding suggests to evolve hybrid breeding from two different populations. In allogamous crops such as maize and rye the separated cultivation of populations based on their evolutionary history and geographic origin assisted in their classification into different heterotic groups (Koekemoer et al. 2011). In autogamous wheat, however, heterotic groups are not available or that easily to distinguish due to the past breeding history.

Concordantly, there was hardly any population structure and no subgroups within the panels used in this study. In Chapter 5, no population structure was observed whereas in Chapter 3 the slight population structure could be an effect of the increased number of varieties from eastern Europe and Russia. The development of breeding, especially after the second World war, has been slightly different from that of western and central Europe during the 20th century and in addition, exchange of breeding material between these regions has been at a lower rate (Angus 2001; Bonjean et al. 2001; Porsche and Taylor 2001; Merezhko 2001).

The absence of distinct sub-groups in wheat germplasm can be explained by the breeding history of wheat which is improved by line breeding with a constant exchange of germplasm between breeding programs (Chapter 5; Reif et al. 2011). For autogamous crops, breeding of pure line cultivars depends on deriving superior phenotypes from crosses between complementary parents obtained through a relatively widespread exchange of germplasm (Koekemoer et al. 2011). Additionally, the introgression of genes from wild relatives into modern wheat cultivars in order to incorporate new sources of tolerances or resistances regarding various abiotic and biotic stresses, contributed to a combination of germplasm from diverse genetic origins.

Today's modern European wheat cultivars show a relatively high degree of kinship. An idea gives the example of the derivation of a number of German wheat varieties shown in Figure 4 which have been released by many different breeders.

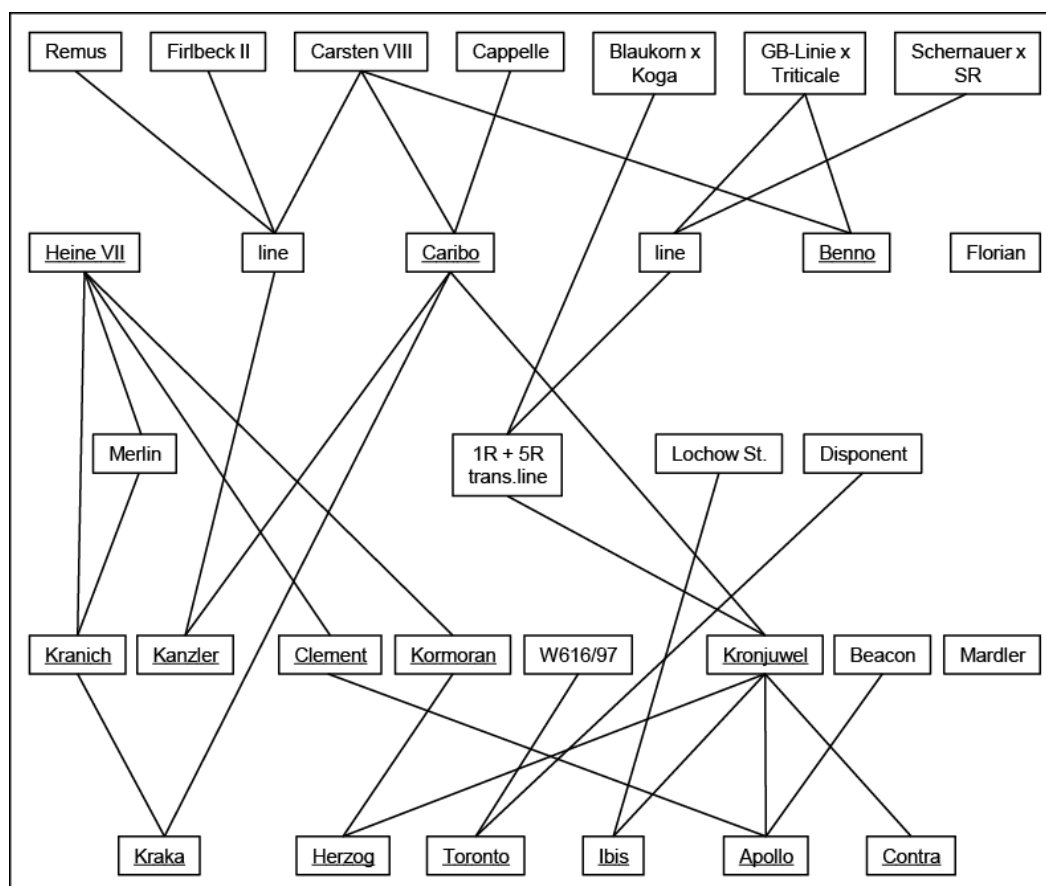


Figure 4 Pedigree of German wheat varieties. Names that are underlined were part of the experimental panels of the studies in Chapter 3 and 4. (adapted from Porsche and Taylor 2001)

There is a frequent use of promising breeding material depending on desirable traits and successful genotypes. For instance, ‘Caribo’, was a very popular variety of which more than 20 varieties were produced from direct crosses (Porsche and Taylor 2001). Despite the extensive use and exchange of favorable genotypes and alleles, which may paint a picture of a rather narrowed diversity, for most traits obvious genotypic variance and diversity is vast within European elite winter wheat germplasm (Figure 5).



Figure 5 Diversity in European elite winter wheat varieties. Observation plots with genotypes very different in appearance, viz. plant height, color, developmental stage, etc. (left). Spikes (dorso-ventral view) varying in shape, length, compactness, awns, waxiness, etc. (right).

The identification of heterotic pools could be done by evaluating testcross performance among available germplasm in the field, clustering based on molecular marker analyses or ‘genomic selection’ (GS) but latter has to be further investigated (Melchinger and Gumber 1998; Koekemoer et al. 2011; Longin and Reif 2014). Usually the development of new heterotic groups is difficult due to the gap in performance between improved breeding material and unimproved material is too large (Melchinger and Gumber 1998). Only a change in breeding goals provides good opportunities to install new heterotic groups. This situation is in effect regarding the switch in wheat breeding methodology. In order to trim genotypes towards a cost-efficient hybrid seed production, a certain grouping regarding male and female ideotypes will be inevitable. Male and female parental lines each need to possess a specific combination of favorable

traits. Hence, selection for these different characters may end up in phenotypically and genetically distinct groups. The combining ability between the groups could be in terms of an increased cross-fertilization rate. Pickett (1993) reported of indications that the pollen of certain male genotypes is more readily accepted by the female parent than others. Further research is needed to determine the extent of this factor but, assuming a genetically mediated favored pollen-stigma match exists this would be another way of determining heterotic groups in wheat.

For the quite radical step from line to hybrid breeding a lot of novel morphological characteristics are necessary; characteristics with a vast genotypic variation that are ad hoc available and maybe some characteristics that need to be pushed, for example, by including genetic resources material or even alien germplasm into modern breeding stocks. Therefore, the pre-breeding process will gain significantly in importance as breeding material, including none-elite or none-adapted germplasm, will be surveyed for desired classical as well as novel hybrid-relevant traits and for classification into potential heterotic groups (Longin and Reif 2014). In addition, for future breeding genetic resources will be used more frequently as these usually show substantially enhanced cross-pollination abilities, thereby enriching the diversity in modern wheat breeding material and hence providing opportunities for pooling. For the sake of completeness it should be mentioned that hybrid breeding for wheat, namely the necessity of cross-fertilization, bears the risk of another genetic bottleneck. The ‘ifs’ and ‘buts’ on this specific topic, however, can be discussed in further studies when we come to cross that bridge. Nevertheless, using fresh gene material is of utmost importance for future wheat breeding in order to assure breeding success and to enhance the potential to improve important traits such as grain yield, resistances and tolerances.

As the establishment of heterotic groups is one of the cornerstones of a long-term successful implementation of hybrid varieties, further investigations on the population structure of European wheat in order to discover different genetic pools will be a major topic for future research regarding hybrid wheat breeding.

Closing Remarks

By virtue of the rapidly growing global population, estimated 10.5 billion by 2050, the wheat production must increase strongly to even meet the projected demand of 760

million tons by the end of this decade (Singh et al. 2012; FAOSTAT 2014) and taking requirements of industrial processes into account an annual production enhancement of 2.5% per annum is required to satisfy the demand for wheat (Koekemoer et al. 2011). Current scenarios and projections of climate change and productivity stagnation require great effort and investment within and into the agricultural sector (Beniston et al. 2007; von Witzke 2010). Foremost and maybe the key discipline to tackle this challenge is plant breeding which still holds great potential for the improvement of bread wheat varieties.

The success of hybrid wheat strongly depends on a cost-efficient hybrid seed production. Low cross-pollination and hence a poor seed multiplication rate are the foremost limiting factors and pessimists consider this issue as unlikely to overcome. The results of this thesis, however, promise good chances to decrease the cleistogamy of wheat plants and confirm that basic prerequisites for profitable hybrid wheat breeding in Europe are given. European elite breeding material can directly be used to start selection for cross-pollination without the need to improve adaptedness. A high genotypic variance and heritability for important flowering and floral traits exists which is essential for further breeding success. In addition, specific targeted selection is just to begin and further opportunities such as the exploitation of genetic resources and novel (molecular) breeding tools are likely to contribute significantly to the success of hybrid wheat. Still, great research activity and effort is necessary from both, the private and public sector in order to finally realize the highly urgent next step in wheat breeding: from line to hybrid varieties.

7 Summary

Hybrid breeding in wheat has recently received increased interest, especially in Europe, and large public and private projects investigating hybrid wheat breeding have been launched. Hybrid breeding has been a great success story for allogamous crops and is seen as a promising approach to increase the yield potential in wheat. Wheat covers more of the world's surface than any other food crop and is the second main staple crop for human consumption. It can be produced under widely varying conditions and is grown all around the globe, yet, yield gain has declined and is lagging behind the needs of the constantly growing human population.

Future challenges in wheat breeding such as the establishment of hybrid varieties and the adaptation of breeding germplasm to increasing stresses caused by climatic changes also in Europe require knowledge-based improvements of relevant traits and phenotyping approaches suited for applied high-throughput plant breeding. A major limitation for the establishment and the production of hybrid wheat is the lack of a cost-efficient hybrid seed production system. This requires the generation of parental ideotypes which maximize the cross-fertilization capability. Male parents should have an extended time of flowering, extrude anthers and widely shed large amounts of viable pollen. Females need increased receptivity for male pollen by opening the glumes and extruding stigmatic hair. Furthermore, male plants should be taller than females and a synchronized timing of flowering between the two parents is also of utmost importance.

Employing a set of European elite winter wheat lines, we developed and evaluated phenotyping methods for important floral and flowering traits with relevance for improved cross-pollination (Publication I). We observed high heritabilities for important traits such as 'pollen mass' ($h^2=0.72$) and 'anther extrusion' ($h^2=0.91$). In addition, genotypic variances were significant which warrants further breeding success. Positive correlations were found among important flowering and floral characteristics which enables the improvement of outcrossing by indirect selection. 'Pollen mass' for example, was associated with 'anther extrusion', 'anther length' and 'plant height'. Our findings suggest the utility of the developed phenotyping approaches for applied plant breeding and the potential of the traits to assist in the design of the male ideotype for increased cross-fertilization.

We investigated the genetic architecture of flowering time and plant height (Publication II and III). A panel of 410 European winter wheat varieties was genotyped by a genotyping-by-sequencing approach and in addition, analyzed for the effects of specific candidate genes. The major factor affecting flowering time was the photoperiod regulator *Ppd-D1* (58.2% of explained genotypic variance) followed by *Ppd-B1* copy number variation (3.2%). For plant height, the two candidate loci *Rht-D1* (37.0%) and *Rht-B1* (14.0%) had the largest effects on the trait but contrary to reports in the literature did not contribute to flowering time control. In addition, we identified several small effect QTL and epistatic QTL responsible for fine-adjustments of these two traits.

Population structure and genetic relatedness in European elite wheat lines was assessed using different types of markers (Publication IV). Results for relatedness differed for the marker types but consistently showed the absence of a major population structure. Regarding the large wheat genome our results revealed that a high number of markers is necessary as there are regions with only low coverage. Concordantly, we were not able to identify the major flowering locus *Ppd-D1* without targeted candidate gene analysis. Observations on the findings on population structure could be confirmed in Publication II and III and in addition, the geographical distribution of important flowering time and plant height genes displayed the historical development of wheat breeding in Europe. This information on genetic relatedness among lines can also be employed to assist the establishment of hybrid wheat.

8 Zusammenfassung

Die Hybridzüchtung hat als neue Zuchtmethode bei Weizen großes Interesse geweckt. Vor allem in Europa wurden in den letzten Jahren große Projekte aus öffentlicher und privater Hand ins Leben gerufen, welche die Hybridweizenzüchtung voranbringen sollen. Das Züchten von Hybriden bei allogamen Kulturarten war von hohem Erfolg gekrönt und auch die Weizenzüchtung möchte damit das Ertragspotential von Weizen steigern. Weizen hat weltweit gesehen die größte Anbaufläche unter den Kulturarten und steht an zweiter Stelle der wichtigsten Grundnahrungsmittel. Weizen hat die Fähigkeit unter sehr variablen Bedingungen zu gedeihen und seine Anbauflächen spannen sich über den gesamten Globus. Allerdings ist der Ertragszuwachs eingebrochen und kann mit dem fortschreitenden Wachstum der Weltbevölkerung nicht mehr Schritt halten.

Die Weizenzüchtung muss auf zukünftige Herausforderungen reagieren. Der Wandel hin zu Hybriden muss vollzogen werden und das Zuchtmaterial muss für künftig erhöhten Umweltstress gerüstet sein, da klimatische Veränderungen auch in Europa deutliche Auswirkungen haben werden. Es sind daher wissensbasierte Verbesserungen von wichtigen Pflanzenmerkmalen notwendig und, denen entsprechende, effektive Zuchtstrategien, die in der angewandten Pflanzenzüchtung Verwendung finden können. Das größte Hindernis Hybridweizen zu züchten und herzustellen besteht darin, dass Hybridsaatgut noch nicht profitabel hergestellt werden kann. Dazu ist eine bestens abgestimmte Mischung agronomisch wichtiger Eigenschaften in Kombination mit einem hohen Maße an Auskreuzung zwischen den Elterlinien notwendig. Vaterlinien müssen in der Lage sein über einen ausgedehnten Zeitraum zu blühen und dabei ihre Antheren aus den Blüten heraus zu schieben und große Mengen an lebensfähigem Pollen zu verbreiten. Mutterlinien brauchen ein hohes Maß an Empfänglichkeit für den Pollen des Bestäubers, welche durch das Öffnen der Spelzen und dem Heraustreten der Stigmata erreicht wird. Zudem sollte der Pollenspenden höher gewachsen sein als die Mutterlinie und der Blühzeitpunkt beider Eltern muss exakt aufeinander abgestimmt sein.

Unter Verwendung von europäischen Elite-Winterweizenlinien haben wir Phänotypisierungsmethoden für wichtige Blüten- und Blüheigenschaften entwickelt und beurteilt, die zur Steigerung der Fremdbestäubung von Bedeutung sind (Publikation I). Wir konnten hohe Heritabilitäten für wichtige Merkmale, wie Pollenmasse (pollen mass) ($h^2=0.72$) und Antherenextrusion (anther extrusion) ($h^2=0.91$), feststellen. Zudem waren

genotypische Varianzen signifikant – Voraussetzung für eine erfolgreiche züchterische Bearbeitung. Wichtige Blüten- und Blühmerkmale waren miteinander positiv korreliert, welches eine Verbesserung der Auskreuzungsrate durch indirekte Selektion begünstigt. Pollenmasse korrelierte mit Antherenextrusion, Antherenlänge (anther length) und Pflanzenhöhe (plant height). Aufgrund der Erkenntnisse aus der Studie bescheinigen wir den darin entwickelten Methoden eine hohe Eignung für den Einsatz in der angewandten Pflanzenzüchtung. Bei der Entwicklung des idealen Phänotyps eines Hybridvaters mit erhöhter Auskreuzungseignung wiesen viele der beschriebenen Pflanzenmerkmale großes Potential auf, als Zuchtmerkmale Verwendung zu finden.

Wir untersuchten die genetische Regulation der Merkmale Blühzeitpunkt und Pflanzenhöhe (Publikation II und III). Zu diesem Zweck wurden 410 europäische Winterweizensorten mittels eines „genotyping-by-sequencing“ Ansatzes genotypisiert und mittels genomweiter Assoziationsstudie analysiert. Zusätzlich wurden die Effekte von ausgewählten Kandidatengen ermittelt. Hauptverantwortlich für die Blühregulation bei Weizen war der Photoperiode-Regulator *Ppd-D1* (58.2% der genotypischen Variation) gefolgt von *Ppd-B1* „copy number variation“ (3.2%). Die beiden Kandidatengen Loci *Rht-D1* (37.0%) und *Rht-B1* (14.0%) hatten den größten Einfluss auf die Wuchshöhe. Entgegen Berichten aus der Literatur konnte für die *Rht* Loci kein Effekt auf die Blühregulation festgestellt werden. Zusätzlich wurden für beide Merkmale kleine QTL und epistatische QTL detektiert.

Mittels unterschiedlicher genetischer Markertypen wurden Untersuchungen zur Populationsstruktur und Verwandtschaft in europäischen Elite-Weizenlinien durchgeführt (Publikation IV). Bezüglich Verwandtschaftsverhältnissen wurden unterschiedliche Resultate erzielt bei Verwendung verschiedener Markertypen, wohingegen bei keiner der Analysen eine Populationsstruktur festgestellt wurde. Unsere Untersuchungen ergaben, dass angesichts der Größe des Weizengenoms eine große Anzahl von Marker von Nöten ist. Weite Regionen wiesen nur eine geringe Markerdichte auf. Diese Beobachtungen bestätigten sich, da wir ohne eine gezielte Kandidatengenanalyse den wichtigsten Blühregulator *Ppd-D1* nicht detektiert hätten. Die Ergebnisse zur Populationsstruktur konnten in den Publikationen II und III bestätigt werden. Untersuchungen zur geografischen Verbreitung wichtiger Blühzeitpunkt- und Pflanzenhöheregulatoren spiegelten die historische Entwicklung der Weizenzüchtung in Europa wider. Die Informationen über die genetische Verwandtschaft innerhalb der Linien können genutzt werden, um Hybridweizen zu entwickeln.

9 References

- Abdel-Ghani A. H., Parzies H. K., Ceccarelli S., Grando S., Geiger H. H. (2003) Evaluation of floral characteristics of barley in the semi-arid climate of north Syria. *Plant Breed* 122, 273-275
- Addisu M., Snape J W., Simmonds J. R., Gooding M. J. (2010) Effects of reduced height (*Rht*) and photoperiod insensitivity (*Ppd*) alleles on yield of wheat in contrasting production systems. *Euphytica* 172, 169-181
- Angus W. J. (2001) United Kingdom wheat pool. In: Bonjean A. P., Angus W. J. (eds) The world wheat book – a history of wheat breeding. Vol 1, 103-125, *Lavoisier Publishing*, Paris
- BDP German Plant Breeders' Association, Federal Statistical Office (2014) Z-Saatgutwechsel bei Getreide 2013/2014. In: Getreideanbau und Saatgutwechsel 2013/2014. (in German). BDP Bundesverband Deutscher Pflanzzüchter e.V. (German Plant Breeders' Association), Bonn
- Beales J., Turner A., Griffiths S., Snape J. W., Laurie D. A. (2007) A *pseudo-response regulator* is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). *Theor Appl Genet* 115, 721-733
- Beniston M., Stephenson D. B., Christensen O. B., Ferro C. A. T., Frei C., Goyette S., et al. (2007) Future extreme events in European climate: An exploration of regional climate model projections. *Clim Change* 81, 71-95
- Bennett D., Izanloo A., Edwards J., Kuchel H., Chalmers K., Tester M., et al. (2012) Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions. *Theor Appl Genet* 124, 697-711
- Bentley A. R., Horsnell R., Werner C. P., Turner A. S., Rose G. A., Bedard C., et al. (2013) Short, natural, and extended photoperiod response in BC2F 4 lines of bread wheat with different *Photoperiod-1* (*Ppd-1*) alleles. *J Exp Bot* 64, 1783-1793
- Bonjean A. P., Doussinault G., Stragliati J. (2001) French wheat pool. In: Bonjean A. P., Angus W. J. (eds) The world wheat book – a history of wheat breeding. Vol 1, 127-164, *Lavoisier Publishing*, Paris
- Borojevic K., Borojevic K. (2005) The transfer and history of “reduced height genes” (*Rht*) in wheat from Japan to Europe. *J Hered* 96(4), 455-459

- Briggle L. W. (1967) Morphology of the wheat plant. In: Quisenberry K. S., Reitz L. P. (eds) Wheat and wheat improvement. Agronomy series No. 13, 19-88, *American Society of Agronomy*, Madison
- BSA Federal Plant Variety Office (2013) Descriptive variety List. Bundessortenamt (Federal Plant Variety Office), Hannover
- Cadalen T., Sourdille., Charmet G., Tixier M. H., Gay G., Boeuf C., et al. (1998) Molecular markers linked to genes affecting plant height in wheat using doubled-haploid populations. *Theor Appl Genet* 96, 391-397
- d'Souza L. (1970) Untersuchungen über die Eignung des Weizens als Pollenspender bei der Fremdbefruchtung, verglichen mit Roggen, Triticale und Secalotricum. (in German). *Z Pflanzenzucht* 63, 246-269
- de Vries A. P. (1971) Flowering biology of wheat, particularly in view of hybrid seed production – a review. *Euphytica* 20, 152-170
- de Vries A. P. (1972) Some aspects of cross-pollination in wheat (*Triticum aestivum* L.). 1. Pollen concentration in the field as influenced by variety, diurnal pattern, weather conditions and level as compared to the height of the pollen donor. *Euphytica* 21, 185-203
- de Vries A. P. (1974a) Some aspects of cross-pollination in wheat (*Triticum aestivum* L.). 3. Anther length and number of pollen grains per anther. *Euphytica* 23, 11-19
- de Vries A. P. (1974b) Some aspects of cross-pollination in wheat (*Triticum aestivum* L.). 4. Seed set on male sterile plants as influenced by distance from the pollen source, pollinator: male sterile ratio and width of the male sterile strip. *Euphytica* 23, 601-622
- Edwards I. B. (2001) Hybrid wheat. In: Bonjean A. P., Angus W. J. (eds) The world wheat book – a history of wheat breeding. Vol 1, 1019-1045, *Lavoisier Publishing*, Paris
- EEA European Environment Agency (2012) Climate change, impacts and vulnerability in Europe 2012 – An indicator-based report. EEA Report No 12, Copenhagen, ISSN 1725-9177, Office for Official Publications of the European Union, Luxembourg
- Emecz, T. I. (1962) The effect of meteorological conditions on anthesis in agricultural grasses. *Ann Bot* 26, 159-172
- ESA European Seed Association, BDP German Plant Breeders' Association (2012) Return on investment – Statistics ESA survey 2012, EU 27. ESA European Seed Association, Brussels

- FAO Food and Agriculture Organization of the United Nations (2013) Statistical yearbook 2013: world food and agriculture. Food and Agriculture Organization of the United Nations, Rome
- FAOSTAT Food and Agriculture Organization of the United Nations Statistics Division (2014) Compare data on production domain crops. <http://faostat3.fao.org/compare/E>
- Feldman M. (2001) Origin of cultivated wheat. In: Bonjean A. P., Angus W. J. (eds) The world wheat book – a history of wheat breeding. Vol 1, 3-56, *Lavoisier Publishing*, Paris
- Fischer S., Maurer H. P., Würschum T., Möhring J., Piepho H.-P., Schön C. C., et al. (2010) Development of heterotic groups in triticale. *Crop Sci* 50, 584-590
- Ganeva G., Korzun V., Landjeva S., Tsenov N., Atanasova M. (2005) Identification, distribution and effects on agronomic traits of the semi-dwarfing *Rht* alleles in Bulgarian common wheat cultivars. *Euphytica* 145, 305-315
- Griffiths S., Simmonds J., Leverington M., Wang Y., Fish L., Sayers L., et al. (2012) Meta-QTL analysis of the genetic control of crop height in elite European winter wheat germplasm. *Mol Breed* 29, 159-171
- Hedden P. (2003) The genes of the green revolution. *Trends Genet* 19, 5-9
- Jacob D., Petersen J., Eggert B., Alias A., Christensen O. B., Bouwer L. M., et al. (2014) EURO-CORDEX: new high-resolution climate change projections for European impact research. *Reg Environ Change* 14, 563-578
- JRC Joint Research Centre, EEA European Environment Agency (2014) Water limited crop production (CLIM032). Available online: <http://www.eea.europa.eu/data-and-maps/indicators/crop-yield-variability-1/assessment-1>
- Kamran A., Iqbal M., Spaner D. (2014) Flowering time in wheat (*Triticum aestivum* L.): a key factor for global adaptability. *Euphytica* 197, 1-26
- Kato K., Miura H., Sawada S. (1999) QTL mapping of genes controlling ear emergence time and plant height on chromosome 5A of wheat. *Theor Appl Genet* 98, 472-477
- Kempe K., Boudichevskaia A., Jerchel R., Pescianschi D., Schmidt R., Kirchhoff M., et al. (2013) Quantitative assessment of wheat pollen shed by digital image analysis of trapped airborne pollen grains. *Adv Crop Sci Tech* 1, 119
- Koekemoer F. P., van Eeden E., Bonjean A. P. (2011) An overview of hybrid wheat production in South Africa and review of current worldwide wheat hybrid developments. In: Bonjean A. P., Angus W. J. van Ginkel M. (eds) The world wheat book – a history of wheat breeding. Vol 2, 907-945, *Lavoisier Publishing*, Paris

- Laurie D. A., Turner A. S. (2011) Molecular characteristics of major genes controlling photoperiod response and vernalisation requirement in wheat. In: Bonjean A. P., Angus W. J. van Ginkel M. (eds) The world wheat book – a history of wheat breeding. Vol 2, 763-781, *Lavoisier Publishing*, Paris
- Law C. N., Snape J. W., Worland A. J. (1978) Genetic relationship between height and yield in wheat. *Heredity* 40, 133-151
- Lelley J. (1966) –observations on the biology of fertilization with regard to seed production in hybrid wheat. (in German with English abstract). *Der Züchter* 36, 314-317
- Longin C. F. H., Mühleisen J., Maurer H. P., Zhang H., Gowda M., Reif J. C. (2012) Hybrid breeding in autogamous cereals. *Theor Appl Genet* 125, 1087-1096
- Longin C. F. H., Gowda M., Mühleisen J., Ebmeyer E., Kazman E., Schachschneider R., et al. (2013) Hybrid wheat: quantitative genetic parameters and consequences for the design of breeding programs. *Theor Appl Genet* 126, 1-11
- Longin C. F. H., Reif J. C., Würschum T. (2014) Long-term perspective of hybrid versus line breeding in wheat based on quantitative genetic theory. *Theor Appl Genet* 127, 11635-1641
- Longin C. F. H., Reif J. C. (2014) Redesigning the exploitation of wheat genetic resources. *Trends Plant Sci* 19 (10), 631-636
- Loureiro I., Escorial M.-C., González Á., Chueca M.-C. (2012) Pollen-mediated gene flow in wheat (*Triticum aestivum* L.) in a semiarid field environment in Spain. *Transgenic Res* 21, 1329-1339
- Melchinger A. E., Gumber R. K. (1998) Overview of heterosis and heterotic groups in agronomic crops. Concepts and breeding of heterosis in crop plants, 25, 29-44, *CSSA*, Madison
- Merezhko A. F. (2001) Wheat pool of European Russia. In: Bonjean A. P., Angus W. J. (eds) The world wheat book – a history of wheat breeding. Vol 1, 257-286, *Lavoisier Publishing*, Paris
- Miedanre T. (2009) Geschichte des Weizenanbaus in Deutschland. In: Christen O. (ed) Winterweizen – das Handbuch für Profis. (in German). 9-10, *DLG-Verlags-GmbH*, Frankfurt am Main
- Morris R., Sears E. R. (1967) The cytogenetics of wheat and its relatives. In: Quisenberry K. S., Reitz L. P. (eds) Wheat and wheat improvement, Agronomy series No. 13, 19-88, *American Society of Agronomy*, Madison

- Mühleisen J., Piepho H.-P., Maurer H. P., Longin C. F. H., Reif J. C. (2013) Yield stability of hybrids versus lines in wheat, barley, and triticale. *Theor Appl Genet* 127, 309-316
- Nair S. K., Wang N., Turuspekov Y., Pourkheirandish M., Sinsuwongwat S., Chen G., et al. (2010) Cleistogamous flowering in barley arises from the suppression of microRNA-guided *HvAP2* mRNA cleavage. *PNAS* 107, 490-495
- Olesen J. E., Børgesen C. D., Elsgaard L., Palosuo T., Rötter R. P., Skjelvåg A. O., et al. (2012) Changes in time of sowing, flowering and maturity of cereals in Europe under climate change. *Food Addit Contam A* 29 (10), 1527-1542
- Pickett A. A. (1993) Hybrid wheat – results and problems. Supplement 15 to: Röbbelen G., Weber W. E. (eds) *Journal of Plant Breeding – advances in plant breeding*. Paul Parey Scientific Publishers, Berlin and Hamburg
- Porsche W., Taylor M. (2001) German wheat pool. In: Bonjean A. P., Angus W. J. (eds) *The world wheat book – a history of wheat breeding*. Vol 1, 167-191, *Lavoisier Publishing*, Paris
- Reif J. C., Gowda M., Maurer H. P., Longin C. F. H., Korzun V., Ebmeyer E., et al. (2011) Association mapping for quality traits in soft winter wheat. *Theor Appl Genet* 122, 961-970
- Singh S. S., Hanchinal R. R., Singh G., Sharma R. K., Tyagi B. S., Saharan M. S., Sharma I. (eds) (2012) *Wheat – productivity enhancement under changing climate*. Narosa publishing house Pvt Ltd., New Delhi.
- Snape J. W., Griffiths S. (2011) New technologies for wheat improvement and their integration into wheat breeding programmes. In: Bonjean A. P., Angus W. J. van Ginkel M. (eds) *The world wheat book – a history of wheat breeding*. Vol 2, 749-762, *Lavoisier Publishing*, Paris
- Sreenivasulu N., Schnurbusch T. (2012) A genetic playground for enhancing grain number in cereals. *Trends Plant Sci* 117, 91-101
- Srikanth A., Schmid M. (2011) Regulation of flowering time: all roads lead to Rome. *Cell Mol Life Sci* 68, 2013-2037
- van der Linden P., Mitchell J. F. B. (2009) ENSEMBLES: climate change and its impacts: summary of research and results from the ENSEMBLES project. Met Office Hadley Centre UK, 160
- von Witzke H. (2010) *Towards the third green revolution: agriculture – a key industry in the 21st century*. *Ölbaum Verlag*, Augsburg
- Weir A., Bragg P., Porter J., Rayner J. (1984) A winter wheat crop simulation model without water or nutrient limitations. *J Agric Sci* 102, 371-382

- Whitford R., Fleury D., Reif J. C., Garcia M., Okada T., Korzun V., et al. (2013) Hybrid breeding in wheat: technologies to improve hybrid wheat seed production. *J Exp Bot* 64, 5411-5428
- Worland A. J. (1996) The influence of flowering time genes on environmental adaptability in European wheats. *Euphytica* 89, 49-57
- Worland T., Snape J. W. (2001) Genetic basis of worldwide wheat varietal improvement. In: Bonjean A. P., Angus W. J. (eds) The world wheat book – a history of wheat breeding. Vol 1, 59-100, *Lavoisier Publishing*, Paris
- Würschum T., Maurer H. P., Schulz B., Möhring J., Reif J. C. (2011) Genome-wide association mapping reveals epistasis and genetic interaction networks in sugar beet. *Theor Appl Genet* 123, 109-118
- Zeven A. C. (1980) The spread of bread wheat over the old world since the Neolithicum. *Journ d'Agric Trad et de Bota Appl*, XXVII, 1, 19-53
- Zhao Y., Zeng J., Fernando R., Reif J. C. (2013) Genomic prediction of hybrid wheat performance. *Crop Sci* 53, 802-810
- Zhao Y., Mette M. F., Gowda M., Longin C. F. H., Reif J. R. (2014) Bridging the gap between marker-assisted selection and genomic selection of heading time and plant height in hybrid wheat. *Heredity* 112, 638-645

Acknowledgements

Foremost, I want to thank my academic supervisor PD Dr. Tobias Würschum for giving me the opportunity to work on this topic. I highly acknowledge his advice, suggestions and support during this thesis work.

I am very grateful to Prof Dr. Ralf T. Vögele and apl. Prof. Dr. Bettina Haussmann for serving on my graduate committee.

Many thanks to all my PhD colleagues at the State Plant Breeding Institute and at all the other institutes of the Competence Center of Plant Breeding for a great time.

My very special thanks to all the members of the Research Group Biotechnology for being supportive in so many ways and for their great help in the lab and in the field.

I am very grateful to the heads and all members of the Research Group Biotechnology, Research Group Legumes and Sunflower, Research Group Rye, Research Group Triticale, Research Group Wheat of the State Plant Breeding Institute and to the members of the experimental stations Eckartsweier, Heidfeldhof, Ihinger Hof, Oberer Lindenhof.

Many thanks to all the authors of the publications.

Funding came from the Deutsche Forschungsgemeinschaft (DFG) within the project 'Flowering time control: from natural variation to crop improvement' (grant number WU658/1-1).

To My Wife Ines,
I wish to express my heartfelt sense of gratitude and deep, deep love!

Thank You!

Curriculum Vitae

Personal Data

Name: Simon Martin Langer
Date, place of birth: December 25, 1985, Illertissen
Citizenship: German

Current Position

since 12/2014 Wheat Breeder, Bayer CropScience AG, European Wheat Breeding Center

Education

10/2011 – 12/2014 Doctoral student, State Plant Breeding Institute (LSA),
Research Groups Biotechnology, University of Hohenheim
10/2006 – 01/2012 Study of Agricultural Biology, University of Hohenheim
(Diplom-Agrarbiologe)
09/1996 – 06/2005 Gymnasium, Kolleg der Schulbrüder Illertissen

Work Experience and Internship

03/2011 – 06/2011 LSA, Research Groups Biotechnology, temporary employee
07/2010 – 12/2010 LSA, Research Groups Triticale, temporary employee
05/2010 LSA, Research Groups Rye, temporary employee
07/2008 – 08/2008,
08/2007 – 09/2007,
08/2005 – 10/2005 Cognis GmbH, Illertissen, temporary employee
01/2006 – 07/2006 Agricultural holding with crop and livestock farming,
Bergenstetten, intern

Simon M. Langer

Erklärung

Eidesstattliche Versicherung gemäß § 8 Absatz 2 der Promotionsordnung der Universität Hohenheim zum Dr.sc.agr.

Bei der eingereichten Dissertation zum Thema “Phenotypic and Genotypic Assessment of Traits with Relevance for Hybrid Breeding in European Winter Wheat” handelt es sich um meine eigenständig erbrachte Leistung.

Ich habe nur die angegebenen Quellen und Hilfsmittel benutzt und mich keiner unzulässigen Hilfe Dritter bedient. Insbesondere habe ich wörtlich oder sinngemäß aus anderen Werken übernommene Inhalte als solche kenntlich gemacht.

Ich habe nicht die Hilfe einer kommerziellen Promotionsvermittlung oder -beratung in Anspruch genommen.

Die Bedeutung der eidesstattlichen Versicherung und der strafrechtlichen Folgen einer unrichtigen oder unvollständigen eidesstattlichen Versicherung sind mir bekannt.

Die Richtigkeit der vorstehenden Erklärung bestätige ich. Ich versichere an Eides statt, dass ich nach bestem Wissen die reine Wahrheit erkläre und nichts verschwiegen habe.

Simon M. Langer