Genetic analysis of anther extrusion in wheat for hybrid seed production

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1 General Introduction

1.1 Wheat breeding

Breeding and improvement of crops stand primarily on the shoulders of selection, a process whereby plants with the most desirable traits are selected and those considered unfit are removed from a population (Acquaah 2007). The selected plants are preserved and their successful propagation in subsequent generations depends upon the nature of heredity of the traits for which they are selected i.e., the more heritable the traits, the more successful their genetic fixation will be. Since its domestication, wheat breeding has been practiced with a major focus to develop pure-line cultivars. The pure-line cultivars, as the name indicates, are highly homozygous and homogeneous in their genetical and morphological structure and are products of repeated self-pollination (Baenziger and Depauw 2009; Bernardo 2010). Wheat is a highly self-pollinated crop and as a result, its breeding has resulted in homozygous cultivars. The homozygosity maintains the performance of wheat cultivars when they are grown in subsequent years.

Historically wheat breeding relied on the methods of mass selection and pure-line selection on populations of genetically distinct landraces. In mass selection, extremely heterogeneous plants are removed from a population and the remaining homogeneous plants are harvested in bulk which eventually increases the uniformity of the crop. In pure-line selection, the best individual from a population is identified and propagated (Acquaah 2007). Due to homozygosity, pure-lines have a narrow genetic base and essentially become a dead end for further improvement. Understanding and utilization of the principles of hybridization and inheritance of traits led to more advanced methods of breeding e.g., pedigree selection, bulk selection, single seed descent, doubled-haploid breeding, backcrossing, synthetic development, hybrid breeding, etc. As selection does not create novel genetic variation in a population and works effectively only on heritable differences, the above mentioned methods create variation by means of hybridization or crosses of pure-lines (Acquaah 2007; Baenziger and Depauw 2009; Bernardo 2010). To further improve a variety, harnessing the genetic variation and understanding the heritability of desirable traits are vital.

1.2 Objectives of wheat breeding and significance of genetic resources

Wheat breeding programs start with certain objectives such as improvement of traits for physiological, morphological and economic significance e.g., resistance to diseases, biotic and abiotic stresses, improvement of end use quality, etc. All these traits collectively point to yield which is the amount of a crop plant harvested from a unit area or within a given period (Acquaah 2007). Therefore, the premium objective of virtually any wheat breeding program is to improve grain yield. Wheat improvement is mainly exploited by taking advantage of the genetic variability - a variability that is ascribed to genes that encode specific traits and transmit from one generation to another. The genetic variability arises mainly from genetic recombination and mutations (Bernardo 2010).

Genetic resources (genebank germplasm) are indispensable sources of variability since they hold the accessions (landraces, wild relatives, genetic stocks and breeding material of improved or elite cultivars) of diverse genetic and geographic backgrounds. These resources harbor a remarkable diversity and are storehouse of beneficial genes/traits which are not found in modern cultivars (Tanksley and McCouch 1997). Improvement of complex traits (traits controlled by several small effect loci), in particular, can benefit from genetic resources by mining the alleles holding the promise of added value and introgressing them in elite cultivars.

1.3 Breeding for hybrid wheat

Hybridization of inbred parents and harnessing their advantages in the first filial (F_1) generation has benefited agriculture for over 100 years since George H. Shull suggested a method for producing maize hybrids (Shull 1909). F_1 hybrids manifest performance that is superior to the parents from which they are derived - a phenomenon called hybrid vigor or heterosis (Shull 1948). The advantages and widespread cultivation of hybrid maize exemplify the profits of exploitation of heterosis where inbred parents are mated to produce hybrids. The benefits of heterosis have been exploited in other allogamous (cross-fertilizing) species viz., rye, sunflower, carrots, beets, onions, brassicas and cucurbits, and in autogamous (self-fertilizing) or partially out-crossing crops such as rice, tomato, sorghum and oilseed rape (Bernardo 2010; Kempe and Gils 2011). The success of F_1 hybrids is due to their intrinsic heterozygosity which results in increased yield (both biological and commercial), yield stability, vigorous root systems and improved responses to fertilizers. The heterozygosity adds to the commercial success of F_1 hybrids by ensuring the property rights of the breeders. Growers are forced to buy F_1 hybrid seed every year afresh because F_2 seeds derived from F_1 hybrids segregate and result in the loss of superior crop performance. Hybrid cultivars are beneficial if the heterotic advantages are substantial and hybrid seed production and distribution is efficient and cost-effective. Though pronounced in cross-pollinating species, the benefits are not prominent in most of the strictly self-pollinating species mainly due to the lack of high heterotic advantages and ease in production systems (Melchinger and Gumber 1998; Pickett and Galwey 1997). Heterosis can be measured as mid-parent heterosis (superiority of F_1 hybrids over the average of both parents), better-parent heterosis (superiority of F_1 hybrids over the better parent) and commercial heterosis (superiority of F_1 hybrid over the commercial check variety). Heterosis has its practical and economic value if it is better-parent or commercial heterosis. Previously it was reported that a massive amount of mid-parent heterosis (~40-60%) exists in wheat hybrids (Bailey et al. 1980; Barbosa-Neto et al. 1996; Walton 1971). Bruns and Peterson (1998), by conducting multiple year and multiple location preliminary and advanced regional trials in the USA, concluded that more than 10% of hybrid wheat advantage exists over pure-lines. However, most of the recent studies on heterosis in wheat report on an average of 10-15% of mid-parent heterosis (Dreisigacker et al. 2005; Gowda et al. 2010; Longin et al. 2012).

Although efforts to create wheat hybrids span over several decades, the advantages of F_1 wheat hybrids have not yet been fully materialized (Cisar and Cooper 2002; Pickett 1993; Pickett and Galwey 1997). Main features of a successful hybrid wheat platform include a cost-effective F_1 seed production system, adequate levels of heterosis and distinction and development of heterotic groups and patterns to ensure continuous progress in the program (Koekemoer et al. 2011; Melchinger and Gumber 1998; Zhao et al. 2015). A major hindrance in implementing the above-mentioned features at a commercial scale comes from the fact that wheat is a self-pollinating species. As crossing is necessary to make F_1 wheat hybrids, it involves changing the plant behavior and structure so that one plant could act as a father (male or pollen donor) and the other as a mother (female, pollen receiver or seed plant). The development, preservation and propagation of mother-lines (male-sterile) and subsequently recovering their fertility, and low rates of cross-pollination are among the major factors why wheat hybrids occupy only a minute fraction of the world wheat market (Longin et al. 2012).

1.4 Systems to induce male sterility in hybrid wheat production

Several systems to develop mother-lines by inducing male sterility on an industrial scale in wheat have been proposed i.e., use of cytoplasm-induced male sterility, nuclear or gene induced male sterility, conditional or photoperiod induced male sterility, genetic sterility by adding an additional chromosome, use of gametocides or chemical hybridization agents and genetic modification or use of transgene technologies (Jordaan 1996; Kempe and Gils 2011; Koekemoer et al. 2011; Pickett and Galwey 1997). Of these, the most widely adopted and viable systems for commercial hybrid wheat seed production are cytoplasm-induced male sterility (CMS) and the use of chemical hybridization agents (CHAs). CMS involves the replacement of wheat's cytoplasm mostly with *Triticum timopheevii*'s cytoplasm. The incompatibility between wheat's nuclear genetic content and *T. timopheevii*'s cytoplasm results in cytoplasm-induced male sterility. Although other cytoplasm donors causing male sterility exist, *T. timopheevii* has gained widespread use due to its neutral effects on agronomic traits and the deleterious effects of other cytoplasm sources (Koekemoer et al. 2011). The CMS method can generate effective male-sterile plants (mother-lines). F_1 hybrids are produced by crossing male-sterile mother-lines with fertile father-lines harboring fertility restorer (*Rf*) genes (Figure 1). However, the lack of effective *Rf* genes and their sensitivity to environments hinder the full exploitation of this system at present (Koekemoer et al. 2011; Pickett and Galwey 1997).

Sterility achieved by CHAs involves the application of chemicals which interact with the stamens of the plant and render them unfertile. This method is efficient and reduces the time of hybrid wheat breeding as it does not involve the development and propagation of mother-lines (Cisar and Cooper 2002; Rajaram 2001). However, difficulties to develop CHAs, their phytotoxic effects coupled with environmental sensitiveness and high costs make CHA application cost-ineffective (Cisar and Cooper 2002; Pickett 1993). Nevertheless, with the current pace of progress in technology and science, it can be speculated that operational male-sterility would be achieved at lesser costs. A general scheme for the production of F_1 wheat seed is described in Figure 1.

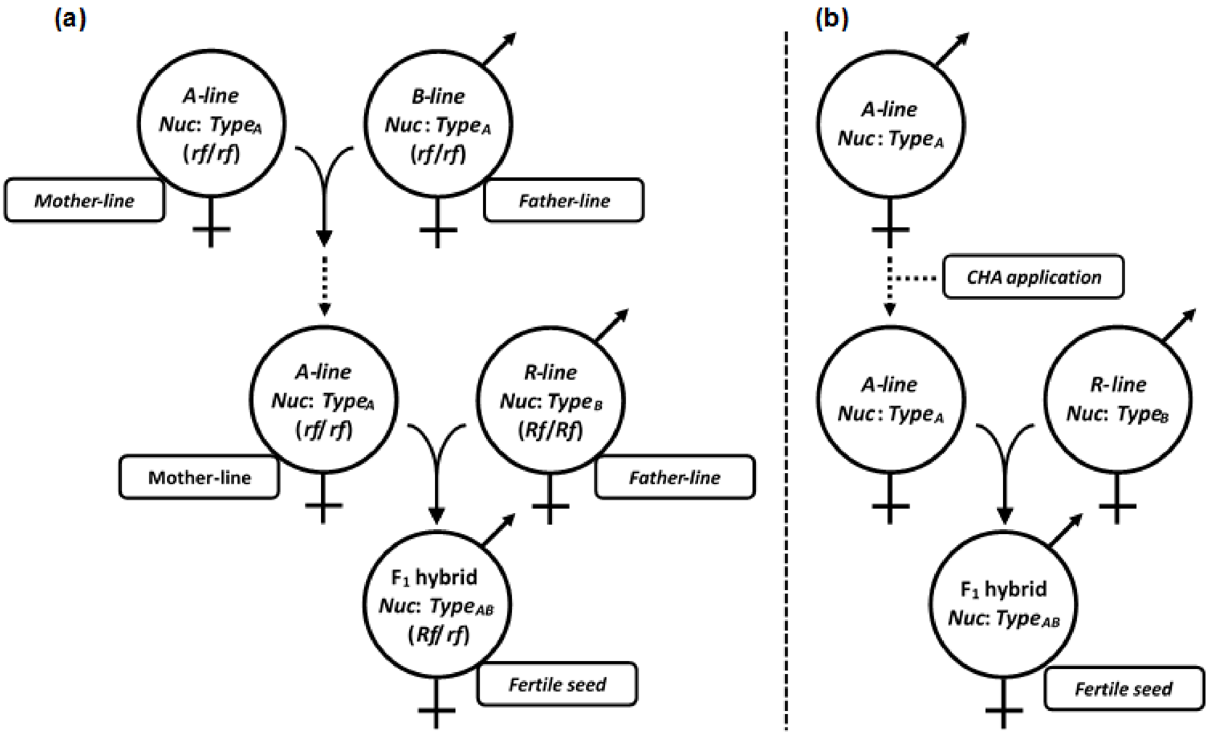

Figure 1. Scheme for F_1 hybrid seed production in wheat. **(a)** F_1 hybrid seed production by cytoplasm-induced male sterility system. Propagation of male-sterile mother-line (*A-line*) harboring sterility inducing cytoplasm is carried out by crossing to the fertile maintainer father-line (*B-line*) harboring normal cytoplasm. *A* and *B-lines* harbor the same nuclear genomes (*Nuc*: $Types_A$). After sufficiently propagating the *A-line*, a cross with a father-line (*R-line*) harboring fertility restorer (*Rf*) genes and different nuclear contents (*Nuc*: $Type_B$) is performed. The cross between "*A-line*; *Nuc*:$Type_A$" and "*R-lines*; *Nuc*: $Type_B$" produces fertile F_1 hybrid seed. **(b)** F_1 hybrid seed production by chemical hybridization agents (CHAs). CHAs are applied on fertile lines (*A-line*) to induce male sterility. Male-sterile mother-lines are crossed with fertile father-lines (*R-line*) to produce fertile F_1 hybrid seed.

1.5 Establishing male ideotypes for hybrid wheat seed production

High seed set on the mother-lines by efficient pollen reception reduces the F_1 hybrid wheat seed cost by increasing seed yield and quality (Koekemoer et al. 2011). However, floral and flowering biology of wheat impose barriers on father-lines to act as effective pollen donors. Main features of efficient cross-pollination are open flowering during early stages to help anther extrusion and production of abundant, viable and mobile pollen (De Vries 1971, 1972, 1973, 1974; Nettevich 1968; Wilson 1968; Wilson and Driscoll 1983). Anther extrusion is a phenomenon when anthers at yellow stage emerge and burst outside the florets during anthesis to shed pollen into the air. Open flowering is vital for father-lines (both *B* and *R-lines*; Figure 1) because closed-flowering leads to pollen shedding inside the florets with virtually no pollen availability for the mother-lines (*A-lines*) for fertilization and to set seed (D'Souza 1970; De Vries 1971). Literature suggests that floret opening is influenced by both genotype and ambient conditions with major factors being the smaller size of lodicule or its failure to be sufficiently turgid at flowering time, subsequent obstruction of glume openness and, size and structure of the lemma and palea (D'Souza 1970; De Vries 1971; Koekemoer et al. 2011; Pickett 1993; Rajaram 2001). As a result, unsuccessful floret opening causes inadvertent effects on pollen shedding capacity outside the florets and decreases cross-pollination potential of father-lines. Pollen shedding outside the floret depends on sufficient anther extrusion which hinge on adequate widening of florets and large length of the filament. Anther extrusion, anther length, pollen grain number, pollen mass and pollen viability were reported to be positively correlated with each other (De Vries 1974; Langer et al. 2014; Singh et al. 2007). Along with anther extrusion and anther size per se for increased pollen grain per anther, pollen surface features and aerodynamics are also critical factors to enhance cross-pollination (Athwal and Kimber 1970; Beri and Anand 1971; Dafni and Firmage 2000; Langer et al. 2014; Pickett and Galwey 1997). Relative pollen production in wheat is far less than in out-crossing species. As De Vries (1971) noted, one wheat inflorescence produces only 2.5% and 10% of pollen compared to one inflorescence of maize and rye, respectively. In addition, wheat pollen is heavy and its size is large due to its high ploidy level and large chromosomes (D'Souza 1970; Lelley 1966; Sax 1923) which makes it dense and reduces its flyability to female lines. Pollen grain longevity or viability is also crucial. Wheat pollens are viable for less than one hour (D'Souza 1970), which makes it crucial to find female lines which are flowering at the same time with large and receptive stigma to increase the success of cross-pollination. Finally, the duration of flowering is decisive in promoting success of cross-pollination because, the longer the period of flowering time, the higher the chances for anthers to extrude to maximize pollen shedding and the smaller the chances of failure with regard to the adaptation of flowering times (flowering time nick) of mother and father-lines (De Vries 1973; Koekemoer et al. 2011). Singh et al. (2007) reported a significant positive correlation of anther extrusion and duration of floral opening with anther and stigma length. Based on above mentioned corroborating evidences of significant positive correlations between anther extrusion and other floral and flowering traits in wheat, it seems possible to develop efficient father-lines to maximize cross-pollination for better seed set on mother-lines.

Besides improving the floral and flowering traits, success of cross-pollination also depends on height of the father-lines. Pollen shed by taller father-lines can travel longer distances and can be effectively received by shorter mother-lines. Recent studies show that plant height positively influences anther extrusion in wheat with taller plants showing improved anther extrusion (Buerstmayr and Buerstmayr 2015; He et al. 2016b; Langer et al. 2014; Lu et al. 2013; Skinnes et al. 2010). Therefore, improved anther extrusion via selection of taller father-lines appears to be a method to enhance cross-pollination.

1.6 Importance of improved cross-pollination for hybrid wheat breeding and genetic architecture of anther extrusion

The development of parental ideotypes and deployment of hybrids is costly and therefore, costs principally associated with male sterilization and cross-pollination must be compensated by increasing F_1 hybrid seed production and heterosis. As F_1 hybrid seed is only recovered from the mother-lines, its production (yield) can be estimated by following an expression suggested by Pickett and Galwey (1997) as:

$$F_1 \text{ Yield} = X \times Y \times Z,$$

where, X is the yield of the mother-line in tonnes per hectare (t ha^{-1}), Y is the proportion of area occupied by the mother-line and Z is the proportion of seed set on the mother-line. Let 7.0 t ha^{-1} be the yield of the mother-line, 67% of the area be occupied by the mother-line (female to male ratio = 2:1) and 40% be the seed set on the mother-line. F_1 hybrid seed yield would equal:

$$F_1 \text{ Yield} = 7.0 \times 0.67 \times 0.40 = 1.88 \text{ t ha}^{-1}$$

The percentage increase in cross-pollination potential of father-lines can impact the latter two components of the above expression by increasing the proportion of area and seed set on the mother-lines. Figure 2 shows a linear increase in F_1 seed production by increasing the seed set on mother-lines. Given the low seed multiplication rate in wheat, ~0.5 t ha^{-1} increase in F_1 hybrid seed by every 10% increase in seed set on mother-lines is remarkable. The commercial advantage is that 0.5 t of seed serves 10 hectares if the seeding rate is 50 kg ha^{-1}. Another method based on land area used for F_1 seed production also concludes that 2.3 times greater area than pure-line would be required, assuming 2:1 female to male ratio and an optimistic 65% seed set on females (Lucken 1986). These estimates show that increasing cross-pollination could result in the higher and cost-effective F_1 seed production which is a major bottleneck in hybrid wheat breeding programs. To generate efficient pools of pollen donors, it is important to find father-lines showing higher anther extrusion to increase the rate of cross-pollination (De Vries 1973; Longin et al. 2012; Whitford et al. 2013).

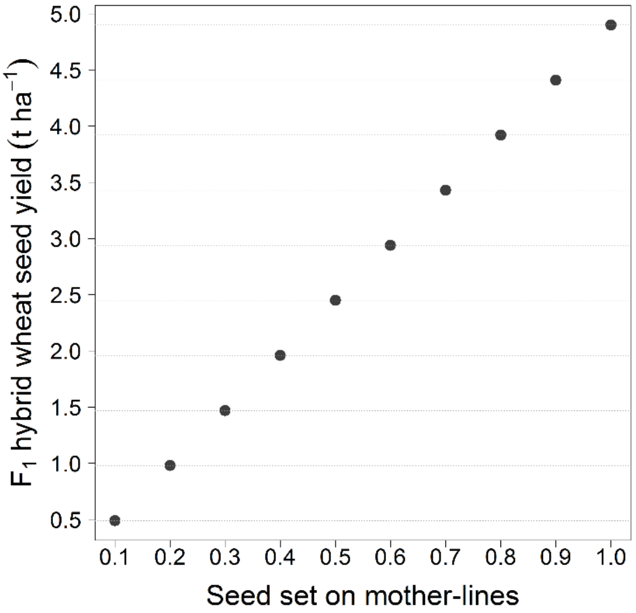

Figure 2. F_1 hybrid wheat seed production (yield) as a function of yield, proportion of area and proportion of seed set on the mother-lines calculated by setting mother-line yield as 7.0 t ha^{-1} and the area planted by mother-line as 70%.

A special emphasis has been given to the importance of anther extrusion in literature and a recent surge in hybrid wheat breeding to break the yield barriers also highlights the significance of this trait to generate pools of effective pollinators (Buerstmayr and Buerstmayr 2015; Langer et al. 2014; Longin et al. 2012; Longin et al. 2014; Mühleisen et al. 2014; Skinnes et al. 2010; Whitford et al. 2013). Many traits of agronomic importance are complex (Bernardo 2010; Mackay 2001). A wide range of variation exists for anther extrusion and analyses in various wheat populations suggest a complex genetic architecture for anther extrusion (Boeven et al. 2016; Buerstmayr and Buerstmayr 2015; Langer et al. 2014; Lu et al. 2013; Muqaddasi et al. 2017a; Muqaddasi et al. 2017b; Skinnes et al. 2010). This can be used in breeding to meet objectives viz., generating pools of lines with high anther extrusion and identification of contrasting lines for further crosses to understand its genetic background. As a good father-line does not ensure good combining ability with the mother-lines, identification of heterotic groups and patterns is important to exploit the advantages of general and specific combining abilities (Melchinger and Gumber 1998; Zhao et al. 2015).

1.7 Marker-assisted selection to accelerate wheat breeding

The availability of abundant molecular markers has helped breeders to speed up the process of breeding as they contribute to selection gain per unit time and cost by a procedure known as marker assisted selection (MAS) (Bernardo 2008; Bernardo and Yu 2007). MAS largely benefits from marker alleles significantly associated to the trait of interest and by performing selection based on the linked markers in breeding cycles.

Variation in a quantitative traits is wide and caused by multiple loci (quantitative trait loci or QTL) segregating in the population with small effects i.e., imparting minor phenotypic variation to the trait. Due to varying degree of phenotype, detection of QTL conferring small effects on the trait is not possible by studying segregation ratios in crosses or pedigrees (Mackay 2001). QTL mapping is a method used to map the markers on the genome and estimate their effects on the traits by statistical techniques. A general prerequisite to detect and locate QTL is to find marker loci in tight genetic linkage to the QTL. Those marker loci in close vicinity to the QTL show larger effects and by virtue of showing Mendelian segregation assist in selection (Mackay 2001). Generally, two types of QTL mapping viz., linkage and association mapping are used to map the genomic regions or find the markers associated with the traits. Linkage mapping is used to map the traits in a genetic population mainly derived from F_1 hybrids resulting from a cross between two inbred parents. The populations used in linkage mapping studies are generally F_2 or its derivatives e.g., F_3, F_4 etc., backcrossed lines, doubled-haploids, recombinant inbred lines, immortalized F_2 and near isogenic lines. On the other hand, association mapping is a widely used approach to establish marker-trait associations (MTAs) in a population exhibiting a diverse genetic and geographic background. Association mapping harnesses the benefits of a large number of marker polymorphisms, the extent to which these markers are in linkage disequilibrium (LD) to the genes controlling the trait, a long recombination history and large size of the investigated population (Bernardo 2010; Collins et al. 1997; Hamblin et al. 2011; Mackay 2001). Significantly associated (large effect) marker alleles could be used in MAS to incorporate the linked trait in high yielding advanced lines. Use of MAS to accelerate selection gains is described in Anderson et al. (2007) where a major QTL *Fhb1* for *Fusarium* head blight disease resistance with a large and consistent effect in wheat was identified. With the use of flanking markers, the effect of the *Fhb1* QTL was validated by introducing it into 19 different pairs of near-isogenic lines (for *Fhb1*) where a consistent effect of about 23% reduction in disease severity ratings and 27% reduction in infected grains was reported (Pumphrey et al. 2007).

MAS is profitable when much of a quantitative variation of a trait is controlled by a few genes with large effects. QTL exerting significant effects on the trait of interest are identified by linkage or association mapping and are used for QTL-introgression, F_2 enrichment, or marker-assisted recurrent selection by introducing or pyramiding them into elite germplasm to develop improved cultivars (Anderson et al. 2007; Bernardo 2008, 2010). However, MAS is unrealistic in cases where the trait is highly quantitative because of the challenge of stacking of many genes in a single cultivar. The significance test used to identify the trait-associated-markers implies that only a subset of markers will be used in the MAS i.e., markers crossing the significance threshold. As a result, MAS completely neglects the markers which do not cross the significance criteria however close their effects may appear to the markers crossing the significance threshold. To circumvent the limitation of significance tests in mapping studies, genome-wide selection or genomic selection (GS) can be exploited for selection of highly quantitative traits. In genomic selection, instead of using a subset of molecular markers with large effects, both large and small effects of all the markers on the trait are computed to predict the total genetic value (Meuwissen et al. 2001). The genetic value is estimated as the sum of individual's genetic values across all markers. Subsequent selection is performed based on these genome estimated genetic values (GEGVs). GEGVs therefore become particularly useful for traits which are highly complex and laborious to phenotype. Earlier studies indicated that genomic selection leads to high correlations between GEGVs and observed genetic values for quantitative traits (Meuwissen et al. 2001; Schaeffer 2006). In dairy cattle, the application of genome estimated breeding values (GEBVs) reduced the cost by 92% and increased genetic gain per year by two fold compared with a traditional progeny testing (Schaeffer 2006). Recent extensive studies in plant species like wheat, maize, barley, etc. also showed that the genetic values for quantitative traits can be predicted with high accuracy (Bernardo and Yu 2007; He et al. 2016a; Heffner et al. 2010; Heslot et al. 2012; Jiang et al. 2017; Lorenzana and Bernardo 2009). Popular approaches for genomic selection are based on genomic (GBLUP) or ridge regression best linear unbiased predictions (RRBLUP) and Bayesian models (Meuwissen et al. 2001). Overall, in plant breeding BLUP remains to be the method of choice because of its simplicity and swiftness (Lorenzana and Bernardo 2009).

1.8 Objectives

Strict self-pollination is needed to achieve homogeneity in wheat fields but F_1 hybrid seed production demands cross-pollination. Better anther extrusion can help to increase cross-pollination in wheat. The general aim of this thesis was the genetic analysis of anther extrusion in hexaploid wheat (*Triticum aestivum* L.) for hybrid wheat breeding by exploiting genetic resources and elite breeding lines and by using different molecular maker systems. The specific objectives of this thesis were:

1. phenotypic analysis of anther extrusion in various wheat panels
2. genome-wide association analyses of anther extrusion in various wheat panels by using different marker systems
3. identification of putative genomic target regions of anther extrusion
4. estimating the accuracy of genome-wide prediction of anther extrusion

2 Research Publications

2.1 Genome-Wide Association Mapping of Anther Extrusion in Hexaploid Spring Wheat

Published in:

PLoS ONE (2016) 11(5): e0155494.

DOI:10.1371/journal.pone.0155494

Authors: Quddoos H. Muqaddasi, Ulrike Lohwasser, Manuela Nagel, Andreas Börner, Klaus Pillen and Marion S. Röder

The original publication is available online at:

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0155494

2.2 Genetic Architecture of Anther Extrusion in Spring and Winter Wheat

Published in:

Frontiers in Plant Science (2017) 8:754.

DOI: 10.3389/fpls.2017.00754

Authors: Quddoos H. Muqaddasi, Jonathan Brassac, Andreas Börner, Klaus Pillen and Marion S. Röder

The original publication is available online at:

https://journal.frontiersin.org/article/10.3389/fpls.2017.00754/full

2.3 Genome-wide association mapping and genome-wide prediction of anther extrusion in CIMMYT spring wheat

Published in:

Euphytica (2017) 213:73.

DOI: 10.1007/s10681-017-1863-y

Authors: Quddoos H. Muqaddasi, Jochen C. Reif, Zou Li, Bhoja R. Basnet, Susanne Dreisigacker and Marion S. Röder

The original publication is available online at:

https://link.springer.com/article/10.1007/s10681-017-1863-y

3 General Discussion

3.1 Stability and wide genetic variation of anther extrusion can result in a strong selection response

Efficient cross-pollination determines the rate of seed-set on mother-lines during the course of F_1 hybrid seed production in wheat. Anther extrusion is a major contributing trait for effective cross-pollination. It is evaluated when anthers at yellow stage emerge and burst outside the florets during anthesis to shed pollen into the air. Anther extrusion can be assessed by means of i) empirical evaluation i.e., counting anthers retained inside the florets after anthesis and subtracting the count from the total number of anthers housed by those florets or ii) visual evaluation i.e., quantitative scoring of anther extrusion based on scale one (best) to nine (poor) or vice versa. Empirical estimation can either be performed on field or by harvesting the spikes from field, freezing them in the laboratory and counting the anthers days later after defrosting. Comparing both field and laboratory based empirical assessment of anther extrusion in spring and winter wheat accessions (genetic resources) yielded highly significant correlations (>0.80; $P < 0.001$) verifying that both methods produce similar results (Muqaddasi et al. 2017a; Muqaddasi et al. 2016). For research purposes, however, it is convenient that the laboratory based method of empirical anther extrusion scoring be adopted to avoid difficulties associated with handling of large numbers of lines, on field scoring and lodging of non-semi-dwarf lines. Although more subjective, visual scoring as a high-throughput version to evaluate anther extrusion also produces correspondingly suitable estimates for further genetic analyses (Muqaddasi et al. 2017b). These findings are in line with other reports where both methods to evaluate anther extrusion in wheat produced analogous results in genetic analyses (Boeven et al. 2016; Langer et al. 2014; Skinnes et al. 2010).

Wide variations and population frequencies approximating to statistical normal distributions among the observed spring and winter wheat genetic resources (Muqaddasi et al. 2017a; Muqaddasi et al. 2016) and elite lines (Muqaddasi et al. 2017b) are primary clues to the quantitative nature of anther extrusion. Heritability is the predictability of the transmission of the characters from one generation to another and determines the amount of genetic variance of a trait expressed as a proportion of total phenotypic variation (Falconer 1981). Therefore, introgression and selection of traits showing high heritability are expected to be easily and reliably implemented in breeding programs. Heritability of a trait is the property of the population and its environment (Falconer 1981). However, consistently high heritability estimates of a particular trait across several populations and environments are indicative of the reliability with which that trait can be selected. Broad-sense heritability (H^2) estimates of anther extrusion in wheat genetic resources (H^2 (spring) = 0.84; H^2 (winter) = 0.65)) and elite lines (H^2 = 0.64) were generally high with genotypic variance principally contributing to anther extrusion across the studied panels (Muqaddasi et al. 2017a; Muqaddasi et al. 2016; Muqaddasi et al. 2017b). These estimates correspond to recent reports where different populations (bi-parental and elite varieties or breeding lines) and methods to estimate anther extrusion (empirical and visual evaluations) in various environments yielded H^2 >0.80 (Boeven et al. 2016; Buerstmayr and Buerstmayr 2015; Langer et al. 2014; Lu et al. 2013; Singh et al. 2007; Skinnes et al. 2010). High genetic variation and heritability estimates suggest that on one hand a strong selection response can be expected for anther extrusion in breeding programs and on the other hand further genetic analysis to identify loci underlying anther extrusion will not be hampered. The latter can be the case when the quality of phenotypic data is low (Liu et al. 2013).

3.2 High marker density, sharp LD decay and correction of population stratification enable high-resolution mapping in wheat

Resolution of association mapping is largely contingent on i) the size and structure of mapping population, ii) a sharp decay in linkage disequilibrium (LD) and iii) density of molecular markers (genetic variants) and the extent to which these markers are in LD to the causative genes (Mackay 2001; Zondervan and Cardon 2004). This approach entails the principle that markers are in close vicinity (in LD) to the gene controlling the phenotype and the likelihood of co-inheritance of the marker and the causative gene is greater than it would be expected under independent assortment (Collins et al. 1997). Therefore, the probability of finding the causative gene becomes directly proportional to the population size, genome-wide marker density, marker polymorphism and rapid LD decay. The relevance of the above mentioned features for association mapping to dissect the genetic architecture of anther extrusion in spring and winter wheat populations are discussed in the following paragraphs.

Association mapping panels are usually of large size and represent as much diversity as possible to exploit more recombination events (smaller LD decay), resulting in increased resolution and detection of causal genetic variants. However, lines representing the association panels may not be completely independent and form subpopulations. As a result, allele frequency for a particular trait may be high in one subpopulation and low or completely absent in other subpopulations. In marker-trait association analysis, all markers that are fixed or in high allele frequency in one subpopulation are likely to be associated with that trait (Pritchard and Rosenberg 1999). Population stratification can therefore result in the detection of spurious marker-trait associations (MTAs). Among the popular methods to deal with population stratification and familial relatedness are genomic control (Devlin and Roeder 1999), structured associations (Pritchard et al. 2000), principal component analysis (Price et al. 2006) or by determining the coefficient of co-ancestry (kinship) among the lines (Lynch and Walsh 1998; VanRaden 2008; Yu et al. 2006). Correction for population stratification in linear mixed-effect models in association analysis can circumvent the problem of spurious MTAs. Wheat populations studied in this thesis were examined for the presence of pronounced population structure. Clear population stratification was seen between accessions (genetic resources) bred in Asia and Europe with DArT and SNP markers (Muqaddasi et al. 2017a; Muqaddasi et al. 2016). An obvious explanation for a rather pronounced clustering in genetic resources is that wheat panels were collected from Genebank (IPK-Gatersleben, Germany) and harbored accessions from all continents except Antarctica. On the other hand, absence of clustering in wheat lines representing elite breeding material from CIMMYT was observed (Muqaddasi et al. 2017b). A most likely reason that elite panel formed a more uniform group is the application of strict selection criteria for several traits to grow homogeneous and homozygous crop stands. Nevertheless, the correction for population stratification in association mapping via mixed models was performed to avoid spurious associations.

LD is the nonrandom association of alleles at two or more loci. It is measured as the squared allele frequency correlation (r^2) in a population. The genetic or physical distance over which LD decays gives a suitable measure for the number and density of markers required and determines the resolution in association mapping studies. As compared to self-pollinating species, patterns of LD decay are much sharper in cross-pollinating species, mainly due to the presence of a higher recombination frequency. For example, in maize which is a cross-pollinating species, Remington et al. (2001) reported patterns of local and genome-wide LD in 102 inbred lines representative of worldwide genetic diversity. They reported a rapid intragenic LD decay on a small distance (within 1500 bp) which promises high-resolution of association studies compared to linkage mapping where the QTL intervals span over several map units (cM) or kilo base pairs (kbp). On the other hand, self-pollinating species such as *Arabidopsis* (*Arabidopsis thaliana*) and barley (*Hordeum vulgare* L.) have longer LD patterns. In *Arabidopsis*, Nordborg et al. (2002) investigated the extent of LD on a genomic scale and showed that it decays within ~1 cM or 250 kbp. In barley, even larger genetic distances (>20 cM) over which LD decays have been reported (Hamblin et al. 2010; Malysheva-Otto et al. 2006). Fast decay of LD was observed in all wheat panels with low r^2 values between adjacent marker pairs. Chromosome and sub-genome-wise LD decay patterns varied within all observed wheat panels with LD declining sharply on group-B chromosomes followed by groups-A and D. Although the chromosome-wise LD decay differed, on average the genome-wide LD extended to ~5 cM at $r^2 \geq 0.20$ in all panels. This is in line with other investigations based on various wheat populations and marker types where the LD decay ranged from ~5-10 cM (Chao et al. 2010; Hao et al. 2011; Somers et al. 2007; Würschum et al. 2013). Compared to maize, the observed slower decay of LD in wheat has implications on association mapping. Given the size of the wheat genome, its self-pollinating nature and lower level of recombination, the relatively slower LD decay warrants that few markers are required to cover the genome. In addition, a selection process based on markers can be much faster and reliable if the causal variant/marker is successfully detected. At the same time, with such level of LD, the resolution is still not sufficient to warrant the detection of the exact gene and therefore the power of GWAS could be improved by increasing marker density and size of population.

Modern high-throughput genotyping platforms e.g., diversity array technology (DArT), single nucleotide polymorphisms (SNPs) and genotyping-by-sequencing (GBS) provide cost effective high density genome-wide polymorphisms (markers). High and uniform genome-wide marker density plays a vital role in association mapping studies as it helps in correct estimation of subpopulations (stratification) and counterbalances the problems associated with slower LD decay. For example, since LD extends to larger genetic distances in wheat and genome size is large, low and uneven marker density across the chromosomes or genomes might miss the LD blocks harboring the causal genes. Nevertheless, the use of marker platforms like DArT, SNPs and GBS in this thesis provided reasonable genome-wide coverage for the detection of marker-anther extrusion associations via mixed models.

3.3 Anther extrusion in wheat is controlled by a concerted action of several loci and strongly depends on the population’s genetic background

Because populations vary in allele frequencies and perform differently in different environments, the success of QTL mapping is a function of the population and the environment. Genetic background of anther extrusion was elucidated by association mapping in wheat panels comprising of genetic resources and elite lines. Association mapping in all populations revealed that anther extrusion is a complex trait, controlled by a concerted action of several minor to moderate effect loci.

Anther extrusion in spring and winter wheat was generally controlled by dissimilar genetic regions. The identified genetic regions differed from previously published linkage and association studies as well, pointing to a strong dependence of anther extrusion on population genetic background. Comparison with other studies, in general, is difficult because most of the mapping studies published on anther extrusion in wheat are linkage mapping studies (Buerstmayr and Buerstmayr 2015; He et al. 2016b; Lu et al. 2013; Skinnes et al. 2010). Although important, the significance of trait-linked markers rests upon the parents giving alleles responsible for variation, which reduces the genetic base and resolution of bi-parental populations. Moreover, most of the QTL published for anther extrusion in linkage mapping studies also do not coincide, making the comparison of genetic regions difficult. This may be a sign of the genetic complexity of anther extrusion. Nevertheless, comparison among association studies presented in this thesis and association and linkage studies by other authors yielded a few common genetic regions controlling anther extrusion. For example, chromosome 1A harbored a significant DArT marker at 126.67 cM in spring wheat accessions accounting for

4.58% of the phenotypic variance (R^2) (Muqaddasi et al. 2016). In winter wheat accessions, a QTL at 115.39 cM with R^2 = 14.51% was identified on the same chromosome with SNP markers (Muqaddasi et al. 2017a). Association study by Boeven et al. (2016) identified a significant SNP in elite winter wheat on chromosome 1A at 141.6 cM with a proportion of genotypic variance (p_G) equal to 4.25%. In a doubled haploid (DH) population, Skinnes et al. (2010) identified a 1A QTL spanning from 92 to 102 cM with a mean R^2 of 18.3%. Chromosome 1B featured a QTL in winter wheat accessions at 70.08 cM (R^2 = 10.72%). Boeven et al. (2016) detected QTL on chromosome 2B for both anther extrusion and visual anther extrusion in elite winter wheat at 74.4 cM imparting p_G of 6.90 and 8.53%, respectively. Likewise, Skinnes et al. (2010) reported a 2B QTL for anther extrusion (86-102 cM; R^2 = 7.4%) in a DH population. Another common QTL across populations was detected on chromosome 3B at 57.24 cM (R^2 = 14.14%) in spring wheat accessions (Muqaddasi et al. 2016). Recently, Buerstmayr and Buerstmayr (2015) identified a QTL for anther retention (equivalent of anther extrusion) in a population of recombinant inbred lines (RILs) on chromosome 3B spanning from 53.5 to 82.1 cM and accounting for a mean R^2 of 9.70%. QTL explaining more than 10% of R^2 were identified on the long arm of chromosome 6A in spring and winter wheat accessions at 138.31 and 140.87 cM, respectively (Muqaddasi et al. 2017a). Similarly, a QTL in winter wheat accessions on chromosome 6B (71.18-72.29 cM) imparted an average of 11.73% of phenotypic variation (Muqaddasi et al. 2017a). Buerstmayr and Buerstmayr (2015) detected QTL for anther retention on chromosome 6B over a genetic interval covering a distance of 25 cM (~70-90 cM) and explaining a mean R^2 of 11.3%.

QTL spanning over larger genetic intervals and with larger R^2 values are expected in linkage mapping studies because phenotypic diversity is generated from a controlled cross (taking opposing phenotypes) and fewer recombination events which populations have had during their development (Myles et al. 2009). Although the marker systems, populations and the loci controlling anther extrusion across populations are largely different, based on comparable locations of genetic regions in above examples, it can be assumed that they constitute the same QTL. To date, no other QTL for anther extrusion explaining more than 10% of phenotypic variance have been reported in the similar regions as stated in association studies of this thesis and therefore these QTL can be considered novel.

Interestingly, association studies performed on genetic resources identified markers which accounted for larger phenotypic effects on the trait (Muqaddasi et al. 2017a; Muqaddasi et al. 2016), contrary to elite panel where anther extrusion was controlled by several small effect loci (Muqaddasi et al. 2017b). An apparent reason is the demand of increased homogeneity in elite wheat which is necessary for variety release. Cleistogamous or strict inbreeding varieties are therefore preferred resulting in the reduced frequencies of alleles promoting open flowering in elite wheat populations. In addition, spring and winter wheat accessions (genetic resources) represented the worldwide diversity and were collected or released several decades ago (Muqaddasi et al. 2016) which could be the reasons for the increased frequency of alleles promoting open flowering and subsequently making genetic resources a valuable source for anther extrusion alleles.

Nevertheless, it is important to note that all recent reports suggest an extremely complex nature of anther extrusion which warrants that it is controlled by mostly small effect loci. This finding is in contrast to what was initially reported that anther extrusion has a low heritability and is controlled by only a few, possibly two genes (Sage and De Isturiz 1974). Moreover, as most QTL are non-overlapping in earlier studies, it becomes apparent that anther extrusion greatly depends on the genetic background of the mapping population. Moreover, association analyses could not capture the total phenotypic variance imparted by QTL. Several factors e.g., population size, uneven and low marker density along the chromosomes, epistasis and experimental error could result in failure to capture all QTL controlling a trait. These results are analogous to all previous reports where a large part of the phenotypic variance remained unexplained which shows that marker density could be improved to capture the small effect loci and unexplained phenotypic variance. However, it should be noted that the failure to detect QTL could also be due to the extreme quantitative nature of a trait. Even if the population size and marker density are reasonable, a number of small effect QTL contributing to trait variation would not cross the significance threshold. From the applied population sizes and marker densities in most of the linkage and association mapping studies, it can be concluded that anther extrusion has a strong quantitative genetic nature.

3.4 Demarcating QTL regions via intra-chromosomal LD and the study of genes/ transcript content is an appropriate method for spotting the putative candidate genes in wheat

Association mapping performed on a diverse and extensive set of lines takes the advantage of long recombination history and smaller LD decay (Mackay 2001). Bi-parental populations segregating for particular traits, on the other hand, have narrow genetic base and far shorter recombination history. Moreover, time and difficulty to develop these populations remain chief obstacles for breeders. Due to fewer recombination events, bi-parental populations used in linkage mapping studies have lower-resolution versus association mapping studies. This indicates that the association studies are particularly useful for dissection of complex traits to capture marker alleles located close to causal variants from a broader genetic base (Collins et al. 1997; Hamblin et al. 2011; Myles et al. 2009).

QTL regions can be demarcated by estimating the intra-chromosomal LD among the SNPs i.e., estimating the LD (r^2) between the marker showing the largest effect and the remaining markers on the same chromosome. Significant markers with large effects on the phenotype mostly act as proxies (by virtue of being in LD) for the causal genetic variant (Myles et al. 2009). Demarcating the QTL regions by the markers (most significant and those in LD with it) can help to study all the genes corresponding to the markers. Moreover, annotated functions, transcript profiles and expression level of the genes in demarcated QTL regions supply information about their possible roles. By following the above mentioned criteria of demarcating the QTL regions and studying the annotated function and expression profiles, genes with functions involved in GA production, stamen, anther and floral expansion were found (Muqaddasi et al. 2017a). Likewise, the expression profiles of some of these genes present in those QTL regions suggested a significantly higher ($P < 0.05$) expression in flowering tissues as compared to non-flowering above-ground tissues. Based on function and expression profiles, it seems that these genes could either be the genes-of-interest or linked to the true gene-of-interest. However, unless a complete, high quality and publically available sequence of the wheat genome is available and marker density is improved to the point that each gene is represented by a marker, this strategy does not provide a conclusive answer. For instance, additional genes in LD, which were not represented by markers (DArT, SNPs or GBS arrays) may be the causal genes for anther extrusion. Although further investigation and validation of the roles of these genes in wheat is needed, anther extrusion associated genes can be considered as possible candidate genes and provide a resource for future map based (positional) cloning - a strategy for isolation of genes controlling the phenotype (Krattinger et al. 2009).

3.5 Genome-wide prediction of anther extrusion is possible with high accuracy

The accuracy and efficiency of MAS in breeding programs depends upon the effect, reliability and stability of the significantly associated (diagnostic) markers. The results of association studies in various wheat panels indicate that anther extrusion is controlled by several small to modest effect QTL. Therefore, marker-assisted selection based on significant markers may not be a promising approach to improve anther extrusion in elite wheat varieties. On the other hand, genomic selection avoids the significance tests and predicts the total genetic values for a trait based on both significant and non-significant markers. Genomic selection is a tool of choice to improve complex traits and it holds the potential to boost selection gain per unit time and cost (Heffner et al. 2010; Meuwissen et al. 2001). Extensive studies in wheat and maize suggest that genomic selection can be used to assist commercial breeding programs by predicting the genetic values based on markers to improve traits of high economic value (He et al. 2016a; Heslot et al. 2012; Jiang et al. 2017; Lorenzana and Bernardo 2009). The potential of genome-wide prediction for anther extrusion was examined in CIMMYT spring wheat with high density GBS markers (Muqaddasi et al. 2017b). Cross-validated prediction accuracies amounting to 0.62 are in agreement with findings in winter wheat for anther extrusion (Boeven et al. 2016) which underline that anther extrusion, a time consuming trait to phenotype, can be predicted with fairly high accuracy using genome-wide prediction methods. Nevertheless, representative training populations have to be phenotyped and genotyped in order to guarantee high prediction accuracies of anther extrusion in applied hybrid wheat breeding programs.

4 Summary

Hybrid breeding is attractive to both plant breeders and geneticists to break the yield barriers and understand the genetics of heterosis. F_1 hybrid cultivars in a number of crop species are able to out-perform inbred varieties, both with respect to economic yield and to yield stability. Hybrid wheat breeding is a promising strategy to break the yield barriers. In an attempt to exploit hybridity for bread wheat improvement, practical difficulties associated with the production of hybrid grain which mainly arise from the strong cleistogamous nature of wheat have to be addressed. To obtain a reasonable F_1 hybrid seed yield, the female parent needs to be male-sterilized and its flowers must open sufficiently with receptive stigma in order to allow access for incoming pollen; meanwhile, the male parent must extrude its anthers prior to anthesis to shed pollens outside the floret. The extent of anther extrusion in father-lines, therefore, governs the rate of cross-pollination and seed-set on mother-lines.

The results in this thesis show that anther extrusion is a stable and highly heritable trait and can be reliably selected in breeding programs. The association mapping analyses of anther extrusion in spring and winter wheat panels comprising of genetic resources and elite breeding material have shown that it is under the control of a number of genetic loci, some of which act favorably and others unfavorably. As a result, there is potential for genetic advance for anther extrusion by selecting the former and excluding the latter. Market pressure to achieve homogenous crop stands has resulted in increased homozygosity of the wheat varieties, especially in terms of their closed flowering nature. Genetic resources harbor valuable alleles (accounting for higher trait-phenotypic variance) for improved anther extrusion, which can be introgressed into elite germplasm via marker-assisted selection (MAS). Intra-chromosomal linkage-disequilibrium (LD) estimation can help to demarcate the QTL regions on the respective chromosome and studying putative function and expression of the genes corresponding to the LD markers can help to spot candidate genes for future map-based cloning. As association analyses revealed a highly complex nature of anther extrusion in elite germplasm, a breeding strategy based on genomic selection can be applied as it provides more promising results in terms of gain per unit time and cost. The results of genomic prediction revealed high prediction accuracy for anther extrusion, paving the way for a

systematic utilization of heterosis in wheat. Understanding the genetic basis of anther extrusion will, therefore, serve to promote the exploitation of heterosis in this key crop, which relies almost entirely on homozygous inbreds.

Zusammenfassung

Hybridzüchtung ist für Pflanzenzüchter und Genetiker gleichermaßen attraktiv, um die Ertragsbarrieren zu überwinden und die Genetik von Heterosis besser zu verstehen. In vielen Kulturarten haben F_1-Hybride eine bessere Leistung als Inzuchtlinien in Bezug auf wirtschaftlichen Ertrag und Ertragsstabilität. Daher ist Hybridweizenzüchtung eine vielversprechende Strategie, um Ertragsbarrieren zu überwinden. Bei dem Versuch Hybride für die Verbesserung von hexaploidem Weizen zu nutzen, müssen praktische Schwierigkeiten bei der Produktion von Hybridsaatgut überwunden werden, welche durch die kleistogame Blühbiologie von Weizen bedingt sind. Um einen akzeptablen Ertrag an F_1-Saatgut zu produzieren, benötigt man männlich sterile Mutterpflanzen, deren Blüten mit rezeptivem Stigma genügend weit geöffnet sind, um Pollen von außen aufzunehmen. Währenddessen müssen bei den männlichen Elternpflanzen die Antheren vor der Anthese ausgestoßen werden, um Pollen außerhalb des eigenen Blütchens zu verstreuen. Daher ist der Grad der Antherenextrusion in den Vaterlinien ausschlaggebend für die Rate der Kreuzbestäubung und für einen verbesserten Samenansatz bei den Mutterlinien. Die Ergebnisse dieser Arbeit zeigen, dass Antherenextrusion ein stabiles und hoch heritables Merkmal ist, auf das in Züchtungsprogrammen erfolgreich selektiert werden kann. Assoziationskartierungsanalysen von Antherenextrusion in veschiedenen Sommer- und Winterweizenpanels, welche genetische Ressourcen und Elitezuchtmaterial umfassten, haben gezeigt, dass dieses Merkmal unter der Kontrolle verschiedener genetischer Loci ist, von denen einige einen positiven Effekt und einige einen negativen Effekt zeigen. Es kann geschlussfolgert werden, dass ein Potential für die genetische Verbesserung von Antherenextrusion durch gezielte Selektion auf Loci mit positivem Effekt und gegen Loci mit negativem Effekt besteht. Die Anforderungen des Marktes haben dazu geführt, dass für homogene Feldbestände gezüchtet wurde, welches in einer vermehrten Homozygotie und insbesondere geschlossen abblühenden Pflanzen resultierte. Genetische Ressourcen können als Quelle für wertvolle Allele (mit erhöhter merkmalsphänotypischer Varianz) für verbesserte Antherenextrusion dienen, welche via markergestützter Selektion (MAS) in Elitelinien eingelagert werden können. Schätzungen des intrachromosomalen Gametenphasenungleichgewichtes (LD) können dazu beitragen, die QTL-Regionen

auf den jeweiligen Chromosomen einzugrenzen. Die Analyse der putativen Funktion und Expression von Genen aus den mit Hilfe von LD Markern gefundenen Genomregionen kann zur Auffindung von Kandidatengenen für eine weitere markergestützte Genklonierung führen. Da die Assoziationsanalysen eine hoch komplexe Vererbung der Antherenextrusion im Elitematerial gezeigt haben, ist eine Züchtungsstrategie basierend auf MAS via genomischer Selektion vielversprechend, da hier verbesserte Ergebnisse in Bezug auf Zeiteinheit und Kosten zu erwarten sind. Die Ergebnisse genomischer Vorhersagen ergaben eine hohe Vorhersagegenauigkeit für Antherenextrusion und ebnen den Weg für eine systematische Nutzung von Heterosis in Weizen. Ein verbessertes Verständnis der genetischen Basis von Antherenextrusion kann daher die Nutzung von Heterosis in dieser wichtigen Kulturpflanze, welche bis jetzt hauptsächlich auf homozygoten Inzuchtsorten basierte, voranbringen.

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Eidesstattliche Erklärung

Declaration under Oath

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.

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