Systematic molecular and phenotypic investigation of

speltoid off-types in bread wheat germplasm

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1   General Introduction

Bread (*common*) wheat (*Triticum aestivum* L., 2n = 6x = 42, BBAADD) is one of the most important cereals grown and consumed worldwide and plays a fundamental role in food security. Nowadays, wheat becomes an object of plant genetic researches due to its ever increasing demand for the ever growing world population and changing environmental conditions caused by climate change. Since the green revolution in the 1960s, the development of modern agricultural high-performance and high-yielding varieties could be achieved leading to a worldwide transformation of wheat production recently reaching more than 90 % of the area in developing countries (Shiferaw et al. 2013). During the last decades, conventional breeding can be greatly facilitated by using modern cropping technologies to improve wheat plants in order to adapt them for biotic and abiotic stresses and enhanced quality traits, yield-related parameters and agronomic performances in a sustainable way. Breeding of new favorable cultivars, which can easily exceed more than ten years, demands for a long-term phenotypic selection process resulting in new wheat variety candidates followed by official admission tests. In Europe, the licensing process is implemented and harmonized by European rules (EU CPVO 2011) for distinctness, uniformity and stability (DUS test). Lack of homogeneity criterion can lead to the rejection of a cultivar candidate resulting in unreasonable high cost and loss of time for breeding companies.

The occurrence of speltoid off-types during the licensing process is one major cause for the rejection of the cultivar candidate, although new favorable features may have been integrated within the new developed lines. Speltoid off-types in bread wheat are usually phenotypically recognizable since they express elongated stems and spikes with tenacious glumes in contrast to the normally occurring wheat phenotype. Although plant breeders remove them from breeding stocks immediately at appearance, they emerge from time to time within multiplication plots. However, the genetic cause for the occurrence is widely undisclosed. Systematic investigations are required in order find solutions to avoid them during seed propagation.

Since the 1980s, DNA-based molecular marker techniques revolutionized genetic research programs having the potential to accelerate breeding of new beneficial cultivars of crop species. For example, molecular markers are applied for assessing the genetic variability and characterization of germplasm, detection of monogenic and

quantitative trait loci (QTL), fingerprinting of genotypes, genetic mapping, marker-assistant selection (MAS), and gene pyramiding. Nowadays, combining molecular techniques with conventional breeding methods is very attractive for breeding companies. The International Wheat Genome Sequencing Consortium (IGWSC) attempts to decode the sequence of the wheat genome in order to analyze genome organization, gene functions, and biological pathways, but the polyploid complexity along with the large genome size of approximately 16 Gb and the repetitive character impeded the decryption since a long time. In 2014, the IGWSC published a first draft sequence produced by sequencing of isolated chromosome arms with more than 120,000 annotated genes nearly equally distributed across all the three subgenomes (Mayer et al. 2014). Recently, a *de novo* sequence assembly of 9.1 Gb could be achieved using high-thoughput sequencing combined with parallel computing, and genetic mapping (Chapman et al. 2015). These insights into genome biology will contribute to understand molecular mechanisms and cellular processes in wheat in order to speed up gene isolation projects, rapid molecular marker development and precise breeding strategies to meet the demands for increased food supply for the growing world population.

The present study systematically investigates the inheritance of speltoid off-types in order to prevent them during seed multiplication increasing the chance to breed favorable homogeneous bread wheat cultivar candidates. Therefore on the one hand, molecular marker techniques combined with expression analysis are applied in different wheat germplasm to create a selectable marker system suitable for MAS against speltoids. One the other hand, biennial field trials, based on lines derived from speltoid plants of modern winter wheat cultivars, are conducted. Subsequently, scoring of speltoid off-types is carried out in order to analyze the effects of genetic background and cultivation conditions leading to the occurrence of speltoids during wheat breeding process.

1.1   Bread wheat - taxonomy, evolution, domestication and agronomic relevance

Wheat belongs to the genus *Triticum* (*T.*) subordinated to the tribe of *Triticeae,* which is a taxon of Poaceaes. The family of Poaceae includes approximately 11,000 species including many important crops and nearly 800 genera worldwide (Peterson

2013). Generally, taxonomy of wheat is controversially discussed since the middle of the 20th century causing confusions in the research community. Researchers use two adopted classifications as reviewed by Goncharov (2011). Western scientists follow an order based on the classification of Mac Key (1966; 1977), whereas eastern scientists prefer classification as described by Dorofeev et al. (1979). Hence, Goncharov (2011) proposed an improved version that better meets the present and future demands for taxonomic classification of *Triticum*. Wheat can be classified into diploid, tetraploid, and hexaploid species based on ploidy level. There exist two wild diploid wheats (2n = 2x = 14), *T. boeoticum* (genome A^{b}A^{b}) and *T. urartu* (genome A^{u}A^{u}). *T. boeoticum* is the ancestor of the probably first domesticated and cultivated wheat species *T. monococcum* (Einkorn) approximately 10,000 years ago in the Fertile Crescent (Peng et al. 2011). After hybridization between *T. urartu* and the diploid goat grass *Aegilops speltoides* (genome SS) 300,000-500,000 before present (BP) wild emmer (*T. dicoccoides*, 2n = 4x = 28, genome A^{u}A^{u}BB) arose. At the same time when *T. monococcum* was domesticated, spontaneous selection of wild emmer gradually created cultivated emmer (*T. dicoccum*). Due to hybridization of *T. dicoccum* with *Aegilops tauschii* (genome DD) approximately 9,000 BP, early spelt wheat occurred (*T. spelta*, 2n = 6x = 42, genome A^{u}A^{u}BBDD) (Kihara 1944; Mc Fadden and Sears 1946; Kerber 1964; Dvorak et al. 1998, Matsuoka and Nasuda 2004). A free-threshing spike type developed about 8,500 BP through natural mutation contemporaneously in emmer and spelt wheat, later evolved into durum or macaroni wheat (*T. durum*) and bread wheat (*T. aestivum*). Experimental investigations of Dvorak et al. (2006) indicated that *T. spelta* is not the ancestral form of free-threshing common wheat, instead the sources of cultivated wheat ancestry are more complex and influenced by various factors like gene flow from wild species. A schematic overview of the evolution of wheat is given in Fig. 1a.
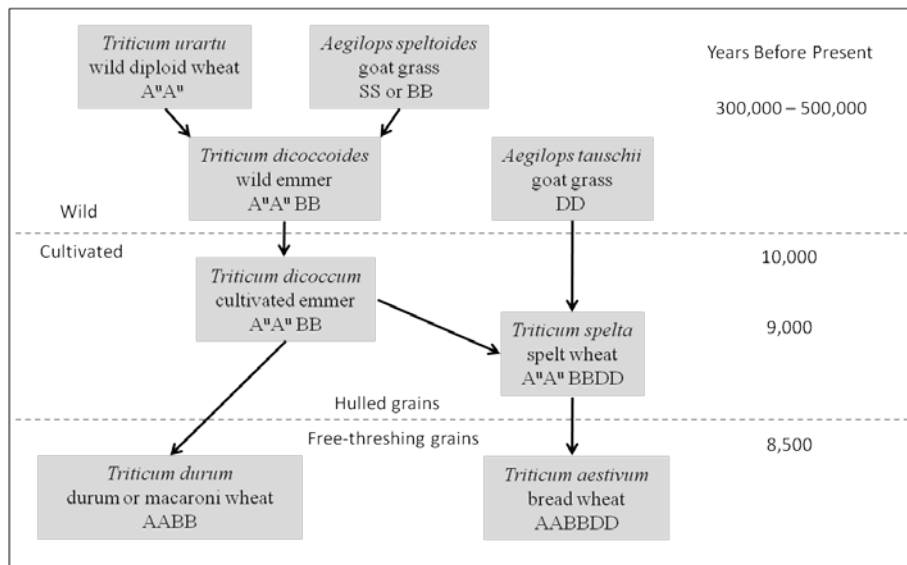
Fig. 1 Historical evolution of wheat from prehistoric diploid grasses to modern durum wheat and bread wheat (modified from Peng et al. 2011)

In general, the genome evolution of wheat in this regard could be accelerated by allopolyploidization events (Feldman and Levy 2012) induced by: (1) rapid genome alterations due to momentarily generation of germplasm with genetic and epigenetic changes, called "revolutionary" changes, and (2) sporadic genomic changes during species' evolution, which are not possible at diploid state. These alterations have led to a diploid cytological and genetic behavior of single subgenomes in a polyploid background. Marcussen et al. (2014) reported on hybridization of ancestral genomes of bread wheat. The authors were able to show that A and B genomes diverged from a common ancestor approximately 7 millon years ago. The genomes gave a rise to the D genome due to homoploid speciation 1-2 million years later. Thus, the hexaploid wheat genome nowadays is a product of multiple hybrid speciation events suggesting a complex, phylogenetic mosaic for the evolution of wheat.

The earliest indications of crop domestication appeared 10,000-12,000 years ago in the Fertile Crescent, in Central America, and in South China, involving various crop species. In the Fertile Crescent of the Near East domestication was founded on crop reliability, yield, and suitability for storage predominantly for wheat, barley, lentils and peas. In Central America and South China other crops species such as rice or potato were domesticated, respectively. Dubcovsky and Dvorák (2007) reviewed the genetic changes that differentiate domesticated plants from their wild ancestor designated as domestication syndrome. The requirements for wheat domestication were relatively straightforward and rapid. Increased seed sizes along with nonshattering spikes were the most important one. The latter characteristic includes multiple traits like brittleness of

rachis, threshability of spikes, and glume tenacity. Most wheat domestication studies focused on the three qualitative traits, which are controlled by the single major genes *Br* (Brittle rachis), *Q* (domestication gene *Q*), and *Tg* (Tenacious glumes) (Gill et al. 2007). The transformation of brittle rachis (*Br*) to non-*Br* was perhaps the first domestication trait leading to non-shattering spike at maturity (Peng et al. 2003). Theses findings can be interpreted as a key event for all major cereals (Konishi et al. 2006). At the end of ripening, the spikelets of wild ears fall apart due to fragmentation of the rachis, whereas genotypes with non-brittle rachis are characterized by suppressing the fraction zones at the rachis until harvest by man. Two major genes, *Br2* and *Br3*, located on the short arm of chromosome 3A and 3B, respectively, govern the brittleness effect between wild and domesticated emmer wheat (Chen et al. 1998; Nalam et al. 2006; Gill et al. 2007). Free-threshing spikes are characterized by thinner glumes and paleas that allow an early release of kernels after ripening in contrast to hulled wheat varieties that require drying after harvest to separate kernels from the chaff. Threshability is predominantly effected by two QTLs (Quantitative Trait Loci). Kerber and Rowland (1974) reported on a chromosome 2DS QTL (in correspondence to *Tg1* gene). Later, a second QTL on chromosome 5AL (in correspondence to *Q* locus) (MacKey 1966; Jantasuriyarat et al. 2004) could be identified. Nalam et al. (2007) fine-mapped the *Tg1* locus on 2DS and further studies by Sood et al. (2009) showed that the soft glume gene (*sog*) in Einkorn was located close to the 2AS centromere. This suggests that *Tg* in common wheat and *sog* have different evolutionary origins. The *Q* locus, as the second major gene effecting the free-threshing character, evolved due to a mutation event during wheat domestication (Simons et al. 2006). All non-free threshing wild relatives carry the recessive allele *q*, in contrast to tetraploid and hexaploid wheats carrying the *Q* allele. Kuckuck (1959) suggested that *Q* might arose due to a duplication event of *q*, but Simons et al. (2006) rejected this hypothesis and showed that the 5A*Q* allele might have arisen by a gain-of-function mutation. Free-threshing hexaploid wheat is characterized by the genotype *tgtg* and *QQ* since the suppression of the free-threshing phenotype is controlled by the partially dominant allele *Tg* originated from *Ae. tauschii* and thus resulting in tenacious glumes. Beside threshability and brittleness to select nonshattering spikes, other traits shared by all domesticated wheats were subjected to domestication by early farmers. The most important ones were reduced dormancy, increased seed size and yield as well as erected growth habit in order to achieve a high

germination rate and growth of seedlings in cultivated fields. Due to domestication, selection processes and ongoing breeding activities, elite bread wheat cultivars today are characterized by all of these advantageous traits.

Nowadays, bread wheat is cultivated with a production of approximately 716 million tons and 219 million hectares acreage throughout the world in the season 2013 (FAOSTAT). In Germany, wheat is the leading crop with 25 million tons and 3 million hectares followed by barley and maize (FAOSTAT). The average yield varies between 7.5 and 1.5 tons per hectare (t/ha) in Western Europe and Central Asia, respectively. Due to the ever increasing world population the demand for wheat production is predicted with more than 800 million tons in 2020. Nowadays, wheat is grown in a wide range of climatic environments of the two hemispheres form 69° north in Norway, Russia and Finland to 45° south in Argentina as well as the South Australia (Nevo et al. 2002). Depending on the growth habit, bread wheat is classified into two types. Winter wheat requires a period of cold temperatures and is sown in fall, whereas spring wheat is conventionally sown in spring or in the fall without vernalization. Thereby, cultivation under divers environmental conditions is practicable due to its great ecological adaptability.

Wheat as a staple food is mainly used to produce flour, bread, noodles, cereals, but also serve as substrate for beer and whiskey production. The sugar content of wheat can be processed in powdered form used as an ingredient of food as well as for textile and paper industry. Different wheat types with specific properties are more suitable for certain end-products. For example, tetraploid durum wheat is commonly used to produce pasta products, whereas hexaploid bread wheat is characterized by good baking qualities producing preliminary baked goods. Due to higher protein content of wheat compared to other crop species such as rice or maize, it is the leading cereal producing vegetable proteins for human nutrition and contributes about 20% of the worldwide dietary calories (Shiferaw et al. 2013). The productions of wallpaper paste and biofuel as well as livestock feeding are further applications using wheat by-products for end-users.

1.2   Speltoids and the domestication gene *Q*

The term 'speltoid' was firstly introduced by Nilsson-Ehle (1917; 1920) due to phenotypic similarity of bread wheat plants with *Triticum spelta*. Hereafter, numerous studies were carried out to clarify the genetic cause and inheritance of the spelt-like character in the *T. aestivum* ssp. *vulgare* background. Winge (1924) observed various speltoid off-types of wheat which are associated with chromosome number irregularities. The author found certain speltoids having 41 instead 42 chromosomes. The aberrant phenotype was controlled by genes located on a pair of chromosomes referred as C (Winge 1924) and later 5A by Sears (1958). The speltoid supression was considered to be controlled by a single locus (Unrau et al. 1950), which was designated as *Q* (Sears 1954). In addition to speltoid suppression, the 5A*Q* gene influences many other agronomic traits and morphological characters. Genes for maturity and plant height are located at chromosome 5A as identified by Tsunewaki and Jenkins (1961). However, later it was shown that *Q* influences these traits, too (Muramatsu 1963; Kato et al. 1999; 2003). Pleiotropic effects governed by *Q* in regard to spikelet size, spike length, seed fertility, glume tenacity, and threshability could be observed in radiation-induced speltoid mutants as reported by Singh (1969). The effect of the *Q* gene depends on its dosage (Huskins 1946; Sears 1954). Plants that are nullisomic, monosomic, disomic, trisomic and tetrasomic for chromosome 5A express a speltoid, semi-speltoid, squareheaded, subcompact and compact spike architecture, respectively. The wild relative *q* allele carried by all non free-threshing wild wheats is also active but to a much lesser degree. Muramatsu (1963) showed that a normal squareheaded phenotype derives from either two doses of *Q* or five doses of *q*. Spelt wheat harboring the *q* allele are actually more speltoid than speltoid mutants of *T. aestivum* due to the genotypic background with clearly visible spear-shaped spikes and tough glumes. Heterozygous plants (*Qq*) express intermediate spike types since *Q* is considered to be incompletely dominant to the wild allele.

The *Q* gene could be physical mapped on the long arm of wheat chromosome 5A using chromosome deletion lines (Miller and Reader 1982; Endo and Mukai 1988; Ogihara et al. 1994; Endo and Gill 1996). Kato et al. (1999) placed the *Q* gene on a recombination based map on the long arm of chromosome 5A close to a dwarfing locus. Subsequently, *Q* could be fine-mapped (Faris and Gill 2002) and identified as a gene

with similarity to *Arabidopsis APETALA2*-like (*AP2*) genes (Faris et al. 2003). Structural, transcriptional, and regulatory differences between *Q* and *q* alleles as well as dosage dependent and pleiotropic effects were investigated by Simons et al. (2006). The authors isolated the *Q* gene and verified its identity using knockout mutants and over expressing transgenic plants. Alterations in rachis fragility, glume shape, and glume tenacity as well as dosage effects on spike compactness and variation on plant height were observed. Further, they confirmed the high degree of similarity with members of the *AP2* transcription factor family and showed that the domesticated allele is more abundantly expressed than *q*. The highest expression level could be detected during early spike development followed by root, flag leaves, and young leaves. One conserved single nucleotide polymorphism between both alleles effects an amino acid change (isoleucin for *Q* and valin for *q*, respectively), leading to altered homodimer formation of *q* in yeast cells. Yeast two hybrid experiments revealed putative interactors of *Q*, actually protein kinases, transcription factors, and a stress responsive protein (Tai 2007). A function in signaling pathways for abiotic stress response may be assumed. Recently, organization, evolution, and function of homoeologous *Q* loci were investigated by Zhang et al. (2011). Due to combined phenotypic and expression analysis, the authors were able to show that homoeologous genes from 5B and 5D are transcriptionally active and mutually regulate the expression of each other. During evolution of wheat, the 5B*q* allele became a pseudogene contributing indirectly to the suppression of the speltoid phenotype, whereas the 5D*q* influences directly the effect, but to a much lesser degree than 5A*Q*. However, since sequences from all homoeoalleles, including the wild relative 5A*q*, became available (Simons et al. 2006; Ning et al. 2009) DNA-based molecular markers techniques enable the differentiation of alleles. Asakura et al. (2009) developed a PCR-RFLP method to distinguish 5A*Q/q* alleles for studying the origin of *Q* using consensus single nucleotide polymorphic (SNP) sites reported by Simons et al. (2006). In the present study, the available sequences (http://www.ncbi.nlm.nih.gov/) of *Q* alleles derived from chromosome 5A, 5B, and 5D are used to design differentiating pyrosequencing primers in order to clarify the genetic cause for the occurrence of speltoid off-types in cultivated *T.aestivum* background.

1.3   Wheat breeding, molecular markers, and applications of pyrosequencing

Wheat breeding has a long tradition and has been practiced since wheat was domesticated. As a result of wheat breeding, a cultivar with improved agronomic and quality traits is the major goal of wheat breeders today. Due to extensive breeding activities and enhanced agronomic and cultivation conditions during the last decades, yield of wheat could be continuously increased from 10.1 dt/ha in 1961 to 32.7 dt/ha in 2013 across the world (FAOSTAT). But since more than a decade only moderate wheat yield increase could be observed reaching a plateau in France, Germany and United Kingdom (Braun 2012). One major reason is the limited physiological potential of a plant to indefinitely produce more grain yield per hectare. If one remove the effect of nutrition by using fertilizers and if plants growth under well-watered conditions, only the effiency of photosynthesis and climate conditions contribute to crop yield as the limiting factors for the enhanced breeding process (Cassman et al. 2003).

To improve agronomic traits efficiently, knowledge of heritability, level of genetic variation and number of genes controlling a trait are essential. Wheat breeder commonly use bulk, single-seed descent, pedigree, production of double haploids (DH), and backcross to create novel lines or cultivar candidates (Carver 2009). One critical bottleneck to release a new cultivar is the factor time, with up to 12 years to develop a winter wheat cultivar. Nowadays, *in vitro* techniques as proposed by De La Fuente et al. (2013) for accelerating plant breeding might be suitable tools reducing cycling time of a plant generation using DH technologies. In combination with off-season cultivation it can be possible to speed-up the development of new favorable lines. During the last decades, the focus of many researchers was to improve yield related parameters as well as abiotic stress tolerance and biotic stress resistance. Yield potential could be continuously increased in many geographic regions of the world (Rajaram and Braun 2008). An overview of breeding strategies for raising of wheat yield potential was given by Reynolds et al. (2010) suggesting that it can be enhanced by up to 50% due to effective utilization of radiation use efficiency (RUE). This can be achieved by improving of structural and reproductive aspects of the crop in parallel. To increase RUE, the main focus will be the improvement of light interception, performance, and regulation of *Rubisco*, introduction of C4 like traits, and the improvement of photosynthesis of the spike. To guarantee a high and stable harvest index (HI),

increasing of photo-assimilates translated into higher yield must be accompanied with vegetative growth aspects. Plants have to be robust enough avoiding lodging, when grains are much heavier. As mentioned by Koebner and Summers (2003) the breakthrough to increase yield potential was due to the manipulation of few major genes, namely *Rht* (Reduced height, gene responsible for the semidwarf growth habit), *Ppd* (Photoperiod gene, adaption to day length), and *Vrn* (Vernalization gene, response to cold temperatures). Especially, the incorporation of *Rht* semidwarfing genes into rice (*Oryza sativa*) and wheat was the major improvement during the green revolution leading to non lodging varieties with higher yield (for review, see Hedden 2003). Pearce et al. (2011) molecularly characterized the genes and found that *Rht-B1b* and *Rht-D1b* genes in wheat encoding for DELLA Proteins inhibiting gibberellin signaling pathways.

To understand the inheritance of agronomic traits, molecular marker technologies became beneficial from a breeder's perspective to identify QTLs or genes in wheat (Campbell et al. 2003; Gupta et al. 1999). The molecular approaches can accelerate marker-assisted selection towards marker-assisted breeding. In general, applying molecular marker technologies in wheat is rather difficult to handle compared to other species due to the complexity of the genome with its large genome size, the three closely related genomes, and the low level of polymorphism. An important milestone in crop research was establishing of high-resolution genetic maps under exploitation of linkage between agronomic traits and molecular markers (Edwards et al. 1987). Röder et al. (1998) published the first robust wheat genetic map using SSR (simple sequence repeat) followed by a second ITMI (International Triticeae Mapping Initiative) map for the wheat D genome (Pestsova et al. 2000). Nowadays, high-density SNP arrays became available serving as powerful resources for diversity studies and marker-traits associations (MTAs) (Wang et al. 2014). To support conventional wheat breeding programs, MAS became suitable during the last decade, which is already practiced in several countries of the world. Numerous studies of MTAs have been published for agronomic and quality traits as well as disease resistance and biotic stress tolerance (for review see Gupta et al. 2009). Currently, MAS is practiced in wheat and other crops for simple traits, which are difficult to score, whereas MAS involving marker-assisted recurrent selection (MARS) and genome-wide selection (GS) is more appropriate for complex polygenic quantitative traits (Bernardo and Yu 2007; Heffner et al. 2009). Limitations and future prospects for marker-assisted wheat breeding were

summarized by Gupta et al. (2010) including examples for marker-assisted validation, marker-assisted back crossing (MABC), and marker-assisted gene pyramiding. The authors published a list of varieties released or improved due to MAS. Recently, commonly used molecular marker systems in wheat were reviewed and comparative prediction and relevant information about the usage were given (Khan et al. 2014). Also, selection against unfavorable traits via molecular markers in bread wheat became practicable, for example selection against yellow flour color (Landjeva et al. 2007).

Nowadays, SNPs markers are the marker of choice in plant and animal genome researches, since single basepair differences abundantly occur and high throughput genotyping platforms are available. Genetic recourses have been grown fast during the last years for the most important crop species based on SNPs (Close et al. 2009; Cavanagh et al. 2013; Wang et al. 2014). From a breeders' point of view, desirable traits have to be analyzed on a multitude of genotypes using one specific marker indicating that the allele of interest is present within the line (e.g. Tyrka et al. 2004). One common technique to detect SNPs is pyrosequencing, which is a real-time sequencing method (Ronaghi et al. 1998). The principle is based on the transformation of pyrophosphate, released during DNA synthesis, followed by enzymatic reactions and finally a conversation into visible light. The signal strength within the pyrosequencing run depends on the quantity of incorporated nucleotides enabling copy number quantification (Söderbäck et al. 2005, Ellison et al. 2013). For SNP genotyping, the method is summarized by Royo and Galán (2009) and can be easily automated with highly reliable and accurate results (Langaee and Ronaghi 2005). Pyrosequencing applications in plant genetic studies became suitable during the last years. Hanemann et al. (2009) developed pyrosequencing-based SNP markers for fine-mapping of the *Rrs2* scald resistance gene in barley. A further example for SNP genotyping via pyrosequencing in cereals is an association mapping approach using wild barley introgression lines identifying photoperiod and vernalization genes associated with QTLs for flowering time and agronomic traits (Wang et al. 2010). However, in wheat the pyrosequencing technique is more complicated due to the complexity of genome, but might be suitable comparing copy number variation or aneuploid detection between the three homoeologous genes derived from each subgenome (e.g. Zhang et al. 2013). Here, the methode is applied summarized in two publications of chapter 2 (Förster et al. 2012, Förster et al. 2013).

1.4 True homolog pairing and aneuploidy in allohexaploid wheat

The evolution of bread wheat genome is characterized by a two-fold allopolyploidization event, leading to formation of tetraploid wheat *T. turgidum* and subsequently to hexaploid *T. aestivum* (Feldman et al. 1995; Dubcovsky and Dvorák 2007). The three closely related wheat genomes are restricted to true homologous chromosome pairing during meiosis since pairing between homoeolgous genomes is suppressed. This is subjected to the two pairing homoeologous genes *Ph1* and *Ph2* (Riley and Chapman 1958; Riley et al. 1959; Sears 1976; Moore 2002). The *Ph1* locus located on chromosome 5B has the strongest effect and could be molecularly characterized by Griffiths et al. (2006). In analogy to mammalians, *Ph1* suppresses *Cdk2*-type activities effecting replication and histone H1 phosphorylation during premeiosis and meiosis suggesting that it has a major role in exclusively intragenomic chromosome pairing in wheat (Greer et al. 2012). Thus, the *Ph1* locus confers the diploid-like meiotic behavior in these polyploid species stabilizing the complex wheat genome.

Although chromosome pairing in wheat is controlled by one major gene, meiotic irregularities spontaneously occur leading to 1% aneuploids in natural varieties (Riley and Kimber 1961) and 3% in synthetic allohexaploid wheat (Mestiri et al. 2010). This phenomenon frequently occurs in polyploid organisms due to the propensity producing aneuploid cells during mitosis and meiosis (Comai 2005). The allohexaploid nature makes wheat more tolerant for loss or gain of whole chromosomes or chromosome arms (Sears 1954), but in generally they are less vigorous and less fertile than the euploid counterparts (Joppa and Williams 1977; Knott 1989; Birchler and Veitia 2012). Aneuploids originate from random segregation of univalent chromosomes of normal diploid (2n) plants, from progenies from meiotic mutants (asynaptic disomics or aneuploids), from unequal segregation of autopolyploids (2x X 4x), and numerical non-disjunction from multivalent formation generating trisomics and monosomics. As mentioned by Bayliss and Riley (1972) chiasma frequency, and thus cytological stability, depends on temperature. Low temperature regimes during meiosis increase the formation of univalent chromosomes. Subsequently, univalents can undergo transverse division resulting in unbalanced gametes (Sears 1952; Friebe et al. 2005). Additionally, meiotic irregularities or univalents occur in a progeny-dependent manner in synthetic

hexaploid wheat as shown by Mestiri et al. (2010). Variability could be found between lines (Worland and Law 1985; Storlie and Talbert 1993). Since the bread wheat genome tolerates aneuploidy due to compensation of homoeologous chromosomes, the opportunity for developing of aneuploid genetic tester stocks is given. Consequently, monosomic, nulli-tetrasomic, and ditelosomic lines were constructed to localize genes on certain chromosomes or study gene dosage effects as in case of the domestication gene Q (Sears 1954; Sears 1966; Sears and Sears 1978; Muramatsu 1963). Especially, monosomic lines have been extensively used to indentify candidate gens and map these in a relative distance to the centromere (e.g. Sears 1954; Knott 1989; Raupp et al. 2001; Singh et al. 2001). As mentioned by Shimelis and Spies (2011) in theory progenies of selfed monosomic plants segregate into 25% disomic or nullisomic plants and 50% monosomic plants. According to aneuploidy theory mentioned by Sears (1954) 24% disomic, 73% monosomic, and 3% nullisomic plants can be expected due to altered vitality of paternal and maternal gametes. Over a period of 18 years, Singh and Rajlakshmy (1994) obtained similar results with 36.6% disomics, 62.4% monosomics, 0.5% double monosomics, 0.5% nullisomics and trisomics in the progeny of monosomic plants. The phenomenon for the occurrence of aneuploid or monosomic plants in bread wheat remains largely unknown leading to considerable problems for wheat licensing (e.g. Giura et al. 2009). It might be assumed that aneuploidy in beedings lines is one probable cause for this phenomenon due to evidences of early studies (Winge 1924).

Recently, Zhang et al. (2013) deployed homoeolog-specific pyroseqencing combined with GISH (Genomic in situ hybridization) detecting unequivocal numbers of single chromosomes of the three subgenomes. Förster et al. (2012) focused on copy number quantification of the speltoid suppressor Q located chromosome 5A. Such approaches might contribute to understand the occurrence unbalanced gametes resulting in aneuploids in bread wheat. This in turn enables the development of breeding strategies to avoid aneuploids during the seed propagation process of new breeding lines.

1.5   Objectives of the study

The aims of the present study are systematic genetic and phenotypic investigations of speltoid off-types, increasing the chance to create homogeneous bread wheat cultivar candidates for the wheat licensing process. In detail, the following objectives can be summarized:

1. The analysis speltoid off-types using a combination of cytogenetic and molecular methods. In order to detect the 5AQ copy number and differentiate both alleles of the Q locus on wheat chromosome 5A, a quantitative pyrosequencing assay enabling the detection of speltoid bread wheat plants in seedling stage is developed. Wheat lines of cultivar 'Chinese Spring' (CS) with defined 5AQ copy number variations are used to evaluate the method.

2. The verification of the quantitative pyrosequencing technique analyzing single progeny plants from current bread wheat cultivars emerged from speltoid off-types in order to make the method attractive for application in wheat breeding programs. Additionally, selected offspring plants are morphological characterized regarding pleiotropic effects governed by major genes like Q located on chromosome 5A. Expression studies of 5AQ are performed to analyze the transcription level of Q in regard to copy number variation and the speltoid spike morphology.

3. The investigation of genotypic and environmental factors contributing to the occurrence of speltoid off-types in lines derived from speltoid plants. Based on propagated speltoid starting material, biennial field trials are conducted at three locations and two different sowing times to score speltoid off-types within field plots.

2   Original Papers (Abstracts)

The present thesis illustrates the systematic investigation of speltoid off-types in bread wheat genotypes summarized in three original papers. In chapter 2.1 (Förster et al. 2012) a quantitative pyrosequencing assay, including cytogenetic studies, is presented to discriminate 5AQ/q alleles and detect copy number variation of the 5AQ locus. Subsequently, the assay is verified in current bread wheat genotypes, which are characterized for 18 morphological traits, and the expression for 5AQ in various tissues is presented in chapter 2.2 (Förster et al. 2013). In order to identify genotypic and environmental effects, results of biennial field trails are summarized in chapter 2.3 (Förster et al. 2014).

2.1 Förster S, Schumann E, Weber WE, Pillen K (2012) Discrimination of alleles and copy numbers at the *Q* locus in hexaploid wheat using quantitative pyrosequencing. Euphytica 186:207-218

Abstract Speltoid spikes are characterized by pyramidal spike morphology featuring an elongated rachis and tenacious glumes. Speltoids are considered undesirable spike aberrants in wheat breeding leading to increased heterogeneity within a cultivar candidate. As a consequence, the presence of speltoids may result in rejection of a cultivar candidate during official field trials or denial of cultivar certification during seed multiplication. A reliable method is, thus, required to assess the occurrence of speltoids, early on in a wheat breeding program. The domestication gene *Q* located on the long arm of wheat chromosome 5A is known to suppress the speltoid phenotype in wheat. Here, a quantitative pyrosequencing assay was developed to distinguish between normal wheat plants, which possess two copies of the *Q* allele, and aberrants, which are either aneuploids lacking the correct number of chromosome 5A copies or plants which carry the primitive *q* allele. An accurate and reproducible determination of the *Q* gene copy number was achieved for different wheat genotypes based on homoeologous sequence quantification with two primer combinations at the *Q* locus. Single plants with one to four copies of the *Q* allele could be detected by quantitative pyrosequencing which corresponded to the occurrence of speltoid (1 *Q* allele), normal (2 *Q* alleles), and compact (more than 2 *Q* alleles) spikes. *Q* and *q* specific alleles could be differentiated at SNP position 2299 of the *Q* gene. This SNP is assumed to be related to the emergence of freethreshing wheat forms. To our knowledge this is the first report for detection of aneuploids and differentiation of *Q* alleles in bread wheat using pyrosequencing technology. In future, quantitative pyrosequencing assay can be applied in wheat breeding programs to carry out marker-assisted selection against the presence of speltoid spike aberrants.

2.2   Förster S, Schumann E, Baumann M, Weber WE, Pillen K (2013) Copy number variation of chromosome 5A and its association with the Q gene expression, morphological aberrations, and agronomic performance of winter wheat cultivars. Theor Appl Genet 126:3049-3063

Key message Our investigations combine chromosome 5A copy number variation associated with relative 5AQ gene expression and morphological and agronomic data to characterize the occurrence of speltoid plants in winter wheat cultivars.

Abstract The occurrence of speltoid aberrants in wheat breeding is a serious problem that may result in rejection of a candidate cultivar during licensing. The spear-shaped, hard threshing spike is caused by copy number reduction of the domestication gene Q, located on the long arm of wheat chromosome 5A. As a member of the *APETALA2-*like transcription factor family, the 5AQ gene is involved in flower development and pleiotropically controls other agronomic traits. In this report, a characterization of instability of chromosome 5A is given and effects due to the loss of the Q gene and other genes are discussed. Based on pyrosequencing, we correctly predicted the 5AQ copy number for 392 of 402 tested offspring plants (97.5 %) originating from single speltoid plants of eleven wheat cultivars. The findings indicate that the resulting speltoid plants were either reduced in chromosome 5A copy number or possessed a partial deletion of the distal end of chromosome arm 5AL. 5AQ specific real-time PCR analysis revealed varying transcription levels among cultivars. During early spike development, the relative transcription of the 5AQ gene was always lower in speltoids than in normal square headed wheat plants, most likely leading to the occurrence of the characteristic speltoid spike phenotype. The parallel analysis of 18 agronomic traits revealed pleiotropic effects governed by genes located on 5A. Our results demonstrate that through pyrosequencing one can identify aneuploidy or deletions within chromosome 5A to select against the occurrence of speltoid plants in wheat seedlings.

2.3 Förster S, Schumann E, Pillen K, Weber WE (2014) Genetic and environmental effects on the occurrence of speltoids in winter wheat cultivars. Plant Breed 133:342-347

Abstract The occurrence of speltoid off-types during seed multiplication is one of the major causes for rejection of a wheat cultivar candidate due to phenotypic inhomogeneity. These aberrant plants express an atypical spearshaped spike with tenacious glumes. To analyse the frequency of speltoids under field conditions, field trials were conducted at three locations and two sowing times over 2 years based on speltoid off-type spikes derived from 14 different wheat genotypes. One hundred single ear progeny were developed from the normal and speltoid progeny plants in 2007. A mean frequency of 21.1% and 36.6% speltoid off-types in plots from normal and speltoid lines was observed, respectively. Plots sown late in the season displayed on average 5.3% less speltoid off-types, in particular when both sowing times were far apart. Field trials from 2010 revealed 7.9% more aberrant plants compared with 2011. The percentage of speltoids within the field plots ranged from 0.2% to 83.7%, which indicates a large effect of the respective genotype. Finally, strategies to reduce the number of speltoid off-types are discussed.

3 General Discussion and Future Prospects

In the following chapters the results of molecular and phenotypic investigations of speltoids, published in three original papers, are discussed. In chapter 3.1 the selection strategy against speltoids employing quantitative pyrosequencing are highlighted and compared to other studies applying the pyrosequencing technology. Subsequently, the expression of 5AQ, morphological studies and agronomic properties of speltoid off-types in relation to chromosome 5A specific gene copy number variation are discussed (chapter 3.2). Meiotic irregularities as the genetic cause for the occurrence aneuploids including speltoids as well as genotypic and environmental factors contributing to this phenomenon are presented in chapter 3.3 and chapter 3.4, respectively. The last chapter (3.5) discusses possible future prospects regarding the emergence of speltoid off-types in order to understand the genetic cause of heritability.

3.1 Selection of speltoid off-types via quantitative pyrosequencing

The occurrence of undesirable speltoid off-types among single plants of a cultivar candidate during the wheat licensing process may result in rejection since the potential candiate does not comply with the homogeneity criterion according to DUS test (EU CPVO 2011). To detect speltoid off-types at a very early developing stage and to offer plants breeders a marker-assisted selections method, a quantitative pyrosequencing assay could be established. Based on available sequences of wheat homoeologous genomes (Simons et al. 2006; Ning et al. 2009), primers were developed for two SNPs of the Q gene (Förster et al. 2012). Within the assay, one functional SNP (Simons et al. 2006) was studied differentiating Q and q alleles on wheat chromosome 5A. The nucleotide change gave rise to the emergence of free-threshing wheat. Asakura et al. (2009) developed a CAPS (Cleaved amplified polymorphic sequence) marker approach differentiating both 5AQ/q alleles in divers bread wheat germplasm. A recently developed PCR protocol enables the differentiation of homoeoalleles from the three subgebomes (Qi 2015). But only the pyrosequencing assay allows gene copy number quantification of the 5AQ gene (Förster et al. 2012). The reduced 5AQ copy

number in cytogenetic tester stocks of spring wheat cultivar CS is expected due to the loss of one or two chromosomes 5A in monosomic 5A and nullisomic 5A-tetrasomic 5D (N5AT5D) plants. Similar results were observed for monosomic speltoid tester stocks of four bread wheat cultivars (Förster et al. 2012). However, the loss of a certain wheat chromosome associated with the speltoid phenotype was already observed in earlier studies (Winge 1924; Sears 1954). Screening of progeny plants from speltoid off-types derived from eleven wheat cultivars confirms the copy number dependent spike type expression with highly accurate prediction of 97.5% (Förster et al. 2013). Single plants with zero and one, two, and more than two copies of the 5A*Q* gene with speltoid, normal squareheaded, and subcompact spike type, respectively, could be identified using quantitative pyrosequencing. Söderbäck et al. (2005) used pyrosequencing to differentiate *CYP2D6* gene copy numbers. *CYP2D6* is involved in drug metabolism in humans. The authors were able to show a highly accurate prediction of zero to four copies. A further example was the differentiation of beta-defensin gene copy numbers via pyrosequencing-based paralogue ratio test (PPRT) in order to associate them with disease resistance (Fode et al. 2011). Comparing two methods for copy number quantification, in fact PPRT and qPCR, revealed less bias for PPRT. In both studies copy number variation refers to allelic variations of a certain gene. Further, the method enables the comparison of copy number variation between similar chromosomes in polyploidy species. In bread wheat, Zhang et al. (2013) developed homoeolog-specific pyrosequencing assays for each of the 21 chromosomes analog to the approach described by Förster et al. (2012). However, quantification of gene copy number using the sequencing-by-synthesis technology delivered highly reliable results in all previous studies, making the method attractive for large scale screening procedures in a with high prediction accuracy.

Wheat breeders use wild species for crossing in order to introduce new, favorable alleles into their breeding lines (for review see Hajjar and Hodgkin 2007). Thus, *q* allele might be introgressed leading to speltoid off-types. But negative selection or backcrossing can directly eliminate this undesirable trait governed by the spelta allele. As mentioned before, beside copy number quantification, the 5A*Q* locus can be genotyped at the functional SNP2299 (Asakura et al. 2009; Förster et al. 2012), the SNP effecting the free-threshing spike type of domesticated wheat (Simons et al. 2006). The *q* allele could not be detected via pyrosequencing within screening experiments based

on speltoid starting material consisting of eleven cultivars (Förster et al. 2013). The spontaneous occurrence of the wild relative *q* within domesticated bread wheat cultivars, selected and multiplied across several years, could only be explained by a rare mutation event of the domesticated allele *Q*. The inheritance of speltoid phenotype would follow the segregation pattern according Mendelians law, where *Qq* and *qq* genotypes express a more or less recognizable intermediate and speltoid spike type, respectively, depending on the genomic background (Muramatsu 1963). In contrast, segregation of speltoid off-types corresponded well to the aneuploidy theory or segregation of monosomic plants as mentioned by Sears (1954). These findings were supported by cytogenetic studies and measurement of the *Q*-ratio (Ratio of pyrosequencing peak heights of 5A*Q* versus 5B*q* and 5D*q*) using quantitative pyroseqencing (Förster et al. 2012; Förster et al. 2013). From time to time, fully awned speltoid off-types occur within multiplication plots, in which no pyrosequencing signal for 5A*Q* had been detected. A possible genetic cause will be discussed in chapter 3.3.

In future, wheat breeders might integrate the pyrosequencing technique in their breeding programs to increase homogeneity within newly developed breeding lines or cultivar candidates. Although the genetic cause for aneuploidy cannot be solved satisfactorily, the assay enables the detection of speltoid off-types at seedling stage long before the phenotype is recognizable. Quality controls regarding purity of seeds as well as detection of the wild relative *q* allele might improve the selection process of favorable lines having a low tendency to produce speltoid off-types, whether they occur spontaneously or due to crossing with wild species. A recently developed cost-effective pyrosequencing protocol (Silvar et al. 2011) makes the method more attractive for SNP detection in cereals. This in turn might contribute to support wheat breeders for the development of new varieties using molecular marker technologies as reviewed by Khan et al. (2014).

3.2 Expression of the 5A*Q* gene and agronomic performance of speltoid off-types

In order to clarify whether the copy number variation of the 5A*Q* is associated with altered expression, the relative transcription level of *Q* was measured in various

tissues of CS and during early spike development of ten different wheat cultivars. The 5AQ genome specificity of the assay was guaranteed by creating 3' overhanging ends for the forward primer (Förster et al. 2013) since 5Bq and 5Dq are also expressed (Zhang et al. 2011). The highest expression level of Q in CS during early spike development (Förster et al. 2013) was consistent with results shown by Simons et al. (2006) and Gil-Humans et al. (2009). This finding supports the theory that early expression of Q (actually an *APETALA2* transcription factor) contributes to spike type expression of the full emerged spike. Genes regulated by Q involved in the signalling pathway are still largely unknown. Yeast two-hybrid experiments delivered first evidences for putative interaction partners of Q in wheat predicted to have an effect on abiotic and biotic stress response (Tai 2007). In *Arabidopsis*, *AP2* transcription factors are known to regulate several developmental processes like determination of flower organ identity (Riechmann and Meyerowitz 1998). Within the 'ABC' model (Coen and Meyerowitz 1991), they belong to the A class to control floral organ transition processes (Jofuku et al. 1994). In addition, they contribute to seed mass (Ohto et al. 2005), seed coat development, and seed size (Ohto et al. 2009). Although *AP2* transcriptions factors are predominantly involved in determination of flower development, transcripts are also detectable in leaves, stems and seedlings (Jofuku et al. 1994; Ohto el al. 2001). The latter results are consistent with studies in wheat (Simons et al. 2006; Gil-Humanes et al. 2009; Förster et al. 2013). It is assumed that *AP2* transcription factors influence a wide range of physiological processes in plants. Pleiotropic effects, e.g. increased plant height in Q deletion mutants or speltoid off-types, might be explained by the reduced transcription level of Q in stems. The RNA level in young spikes differed clearly between the winter wheat cultivars, but was always slightly lower in speltoids compared to the normal squareheaded plants of the same variety (Förster et al. 2013). Simons et al. (2006) reported on conserved alterations in the promoter region between the wild and the domesticated allele on wheat chromosome 5A, presumably effecting differences in relative expression between both alleles. Zhang et al. (2011) showed that Q homoeoalleles mutually influence each other, suggesting that 5Dq plays a more reinforcing role in speltoid spike type expression, when 5AQ is not present. In general, *AP2* transcription factors are post-transcriptionally regulated by microRNAs (Aukerman and Sakai 2003; Chen 2004) suggesting complex regulatory mechanisms of Q. It remains unclear whether regulating

elements, the genomic background, mutual effects of homoeoalleles from 5B and 5D, or post-transcriptional processes influence the expression level of the 5A*Q* gene in winter wheat.

In order to analyze the phenotypic or morphological constitution in contrast to the normal occurring phenotype 18 agronomic traits were measured in speltoids to investigate dosage depend effects governed by *Q* (Förster et al. 2013). Since it must be assumed that speltoid off-types mainly occur due to the loss of the entire chromosome 5A (Winge 1924; Förster et al. 2012; Förster et al. 2013), each major gene located on this chromosome might contribute to agronomic or phenotypic changes between normal and speltoid plants. Snape et al. (1985) genetically analyzed wheat chromosome 5A and its impact on agronomic traits using single chromosome recombinant lines. The authors focused on three major genes on 5A, in fact the domestication gene *Q*, the vernalization gene *Vrn-A1*, and the awn inhibitor *B1*, with predominant pleiotropic effects on yield components. Zhang et al. (2010) applied meta-QTL analysis and confirmed the previously mentioned effects on yield parameters for *Q* and *Vrn-A1*, also located on chromosome 5A. However, Förster et al. (2013) discussed altered traits in speltoid off-types in respect to already described effects caused by loci on wheat chromosome 5A. Speltoids are characterized by morphological alterations governed by the 5A*Q* gene. They are increased in plant height and spike length, spike density and reduced in threshability and yield-related parameters. But in contrast to previous studies, speltoid off-types were delayed in heading and flowering time, whereas the CS 5A*Q* gene deletion mutant flowered earlier (Zhang et al. 2011). Birchler and Veita (2012) reported on aneuploidy affecting altered stoichiometric differences of macromolecular complexes and the interactome resulting in unbalanced biological and molecular pathways. Since speltoid off-types are predicted to be monosomic for chromosome 5A, this observation could be one potential explanation for the contrastive results with previous studies. Whether these findings are due to other genes located on chromosome 5A, caused by the gene balance hypothesis (Birchler and Veita 2012), or are specific for spring or winter wheat remains speculative. Díaz et al. (2012) reported about copy number variation of *Vrn-A1* in spring wheat resulting in altered flowing time as one probable explanation for the opposed results. Although it must be taken into account that pleiotropic effects observed for speltoid off-types are due to various genes located on wheat chromosome 5A, Förster et al. (2013) correlated expression results for *Q* with

agronomic data. Only a weak negative correlation with respect to tiller number was observed for normal as well as speltoid plants. Kato (2000) reported a QTL linked to the 5AQ gene controlling this trait. Further, Zhang (2008) tested a 5AQ mutant in the spring wheat cultivar 'Bobwhite' characterized by about half of Q gene expression level and reduced tiller number. These results might indicate possible pleiotropic effects on tiller number and related agronomic traits. Expression studies in winter wheat germplasm should be examined to a larger extent and under diverse environmental conditions to understand whether the transcription level of Q effects agronomic performances in winter wheat.

3.3   Meiotic instability and aneuploidy as the genetic cause for speltoid off-types

The occurrence of polyploidy of genomes is a widespread and important evolutionary process of eukaryotic organisms especially in plants (Adams and Wendel 2005; Soltis et al. 2009). The far-reaching geographic distribution in contrast to diploids suggests selective advantages during crop evolution (Stebbins 1950). Allopolyploids are characterized by at least two or more divergent homoeologous genomes arisen due to interspecific or intergeneric hybridization events. Allohexaploid wheat, as a representative of allopolyploid species with three divergent homoeologous genomes (B, A, and D), showed a similar meiotic behavior as diploids although each chromosome has more than one potential pairing partner. Comai (2005) reviewed the advantages and disadvantages of polyploidy and mentioned that one major drawback of polyploidization is the increased complexity of chromosome pairing, resulting in deletion or addition of whole chromosomes. The higher number of similar chromosomes compared to diploids complicates the true homologous pairing (Youzafzai et al. 2010). However, bivalent pairing of homologous chromosomes in wheat during meiosis is a prerequisite for stabilization of the hexaploid genome and is controlled by the *Ph1* gene located on wheat chromosome 5B (Griffith et al. 2006). *Ph1* suppresses the pairing between homoeologous chromosomes of the three closely related wheat genomes. Although, correct chromosome pairing is under genetic control, meiotic irregularities, like univalent or multivalent formation spontaneously occur. This can lead to unbalanced gametes resulting in aneuploid plants. One reason might be low

temperature conditions during meiosis. Bayliss and Riley (1972) observed a temperature-dependent influence on chiasma frequency, whereby cytological instability or aneuploidy can be one probable consequence. Resulting plants can undergo meiotic disturbance, and genomic background and environmental conditions may intensify this phenomenon (Meijer et al. 1981). In wheat, progeny-dependent meiotic irregularities in synthetics, associated with the occurrence of univalent chromosomes, could be observed by Mestiri et al. (2010). This finding supports the hypothesis of influences caused by modifying genes depending in the genetic background.

One critical bottleneck during meiosis I is the centromere behavior, that sister chromatids of homologous chromosomes can segregate toward one side of the spindle pole. One essential role of centromere formation plays the *cenH3* (histone H3 centromeric variant) locus, originally identified in humans (Earnshaw and Rothfield 1985). In *Arabidopsis*, CENH3 could be described by Talbert et al. (2002) which replaces histone H3 at the centromere region of chromosomes, in fact at the kinetochore. The kinetochore is the anchoring point where spindle fibers attached to chromatides during meiosis and mitosis to ensure an equal distribution of chromosomes to the cell poles. CENH3 is required to assemble the kinetochore protein complex and to forward the mitotic process regarding chromosome segregation (Howman et al. 2000; Oegema et al. 2001; Blower et al. 2006). The N-termial domain of CENH3 is necessary for plant fertility. Mutations in the N-termial domain produced viable plants (Ravi and Chan 2010; Ravi et al. 2011). But these plants showed meiotic disturbance and were sterile due to chromosome misdistribution at metaphase I. Recently, two CENH3 genes could be identified and their role on wheat evolution were discussed (Yuan et al. 2015). The histone folding domain of only βCENH3 underlied a positive selection during wheat evolution. However, it might be assumed that CENH3 in allopolyploid wheat contributes the meiotic behavior like a diploid organism. A possible function in meiotic irregularities, chromosome misdistribution, and appearance of aneuploids in winter wheat germplasm cannot be ruled out, but is largely unclear in wheat today.

The speltoid supressor *Q* has been localized on wheat chromosome 5A (Huskins 1946; Unrau 1950; Sears 1954). Using aneuploid lines the loss of this particular chromosome in breeding material can be assumed as the reason for the occurence of speltoids (Förster et al. 2012; Förster et al. 2013). Moreover, segregation patterns of speltoid off-types corresponded well with aneuploidy theory as mentioned by Sears

(1954) and observations by Singh and Rajlakshmy (1994). This applies to the segregation of awnless speltoid off-types of ten cultivars with 27.96% and 70.96% normal squareheaded and awnless speltoid progeny plants, respectively, predicted to be disomic and monosomic for wheat chromosome 5A (Förster et al. 2013). Results from field trial data (Förster et al. 2014) revealed 68.4% speltoid off-types derived from speltoid tenacious spikes during the first propagation step, which also corresponded well with the theory mentioned by Sears (1954). Fully awned speltoid off-types with a weak stature and low fertility with a Q ratio of almost zero can be found in progenies of awnless speltoids with a frequency of 0.81% (Förster et al. 2013). Most probably these plants are nullisomic for chromosome 5A, which can be expected with a frequency of 3% or 0.5% according to Sears (1954) and Singh and Rajlakshmy (1994), respectively, in progenies derived from monosomics. It seems to be likely that these off-types might be repressed or perished within multiplication plots since they are reduced in their fitness and fertility. The genetic cause for fully awned and fertile progeny plants of cultivar 'Biscay' with a Q ratio of nearly zero (Förster et al. 2013) might be different from those mentioned before. Most probably these plants appear due to a homozygous deletion event on wheat chromosome 5A including Q and the awn inhibitor $B1$. The CS 5AL-10 line, characterized by a deletion on the long arm of chromosome 5A, showed a similar phenotype (Sourdille et al. 2002). The missing PCR product from SSR $Xwmc110$ is a further indication (Förster et al. 2013). Breeding lines expressing such undesirable awned speltoid phenotypes are well recognizable and will be eradicated within the selection process by plant breeders.

In summary, the genetic basis for speltoid off-types is predominantly caused by the loss of the entire chromosomes 5A as shown by Förster et al. (2012). The pyrosequencing results of progenies from speltoid offspring plants (Förster et al. 2013) verified the chromosomal mutation as the most probable reason for the occurrence of speltoids in winter wheat germplasm. In future, the high prediction accuracy in offspring plants emerged from speltoids allows more profound investigations with defined starting material to disclose the genetic cause for off-type or aneuploid plants.

3.4   Genotypic and environmental factors affecting the occurrence of speltoids

Biennial field trials in 2010 and 2011 were conducted to assess whether sowing conditions contribute to the quantity of speltoid off-types and if predispositions in various germplasm for suceptibility is genetically determinated. Speltoid spikes as starting material were selected in the year 2006 and visually scored for the spear-shaped, speltoid-like character and glume tenacity. All progenies of only normal spikes with a squarehead phenotype and soft glumes expressed a similar phenotype in the next generation. In contrast, spikes classified as speltoid revealed segregating progenies for glume tenacity. While 68.4% speltoids from speltoid spikes with tenacious glumes corresponded well with the theory of Sears (1954), only 20.2 % speltoids were found in progenies of spikes classified as speltoid with soft glumes. Misclassification of some speltoid spikes cannot be ruled out (Förster et al. 2014). As shown by Singh (1969), tenacity of glumes is one trait effected by the 5AQ gene, but additionally by $Tg1$ located on chromosome 2DS (Sood et al. 2009). Due to the reduced 5AQ gene copy number, caused by the loss of the entire chromosome 5A (Förster et al. 2012; Förster et al. 2013), the reduced threshablity of speltoids seems to be solely controlled by Q or, alternatively, due to its epistatic interaction with $Tg1$. Selection of defined speltoid starting material is the most critical aspect to conduct informative field trials. Speltoid spikes with tenacious glumes might be the most appropriate genetic resource.

After biennal selection and one year propagation, 100 lines were selected. They were derived from 34 normal and 66 speltoid progeny plants of the initially speltoids from 2006. Based on these lines, random sampling and visual scoring of speltoids within multiplication plots revealed highly correlated field trial data between replications, early and late sowing times and different environments (Förster et al. 2014). Thus, phenotypic evaluation as a mass screening for off-types is only appropriate method to account for aberrant plants in multiplication plots. Due to one propagation step of seeds, approximately 50% aneuploid off-types were expected, but the frequency was below the expectation value with 36.6% for speltoids segregating out of speltoid starting material. However, this might be due to missclassification of starting material or natural selection caused by environmental effects, since it must be assumed that monosomic plants are reduced in certain fitness parameters. One recent example

confirming this hypothesis was published by Zhang et al. (2013) demonstrating altered seed-setting and reduced pollen vitality for aneuploid wheat. Although allopolyploid plants like bread wheat can tolerate the loss of whole chromosomes through compensating genes located on homoeologous loci, unbalanced protein interactions occure due to stoichiometric alterations of macromolecular complexes (Birchler and Veitia 2012). Unfavorable external factors such as sowing depth, temperature or soil moisture may have a reinforcing effect reducing the emergence of aneuploid plants in the next generation. As shown by Förster et al. (2014) sowing time has a large effect on speltoid formation with a mean difference of 5.3% less speltoid off-types for late sown plots. Since it can be assumed that emerging winter wheat seedlings were exposed to lower tempertures, survival rate of aneuploids or monosomic plants might be reduced. Consequently, the amount of speltoids might be controlled by a wheat breeder shifting the sowing date as early as possible to encourage speltoid formation in the next generation characterizing breeding lines for their tendency to produce speltoids or aneuploids. On the other hand, the amount of off-types can be decreased by late sowing as a pratical opportunity avoiding them before the wheat licensing process restricted by EU CPVO (2011).

The occurrence of fully awned speltoid plants is rather difficult to control. They are either progeny plants of speltoid awnless plants characterized by weak stature and reduced fertility or of fully awned speltoid plants expressing a stable and fertile phenotype in the next generation. Both, fully awned speltoid plants as well as subcompact plants with a 5AQ copy number higher than two can emerge out of awnless speltoid off-types (Förster et al. 2013). They have likewise to be eradicated within multiplication plots, since they also differ from the normal occurring squareheaded phenotype, and thus, they are not complying with the DUS test during wheat licensing process (EU CPVO 2011).

Beside environmental influences and fitness effects, the genotype seems to be more or less susceptible for the occurrence of speltoids. The heritabilty of speltoid off-types emerging from speltoids was already discussed previously since they segregate according to aneuploid plants (Sears 1954). However, a large variation could be observed among bread wheat germplasm, such as contrasting results for wheat cultivars 'Batis' (86.2% speltoid aberrant versus 13.8% normal plants) and 'Anthus' (45.7% speltoid aberrant versus 52.5% normal plants) as shown by Förster et al. (2013). Similar

results were obtained analyzing field trial data in 2010 and 2011 at three different locations (Förster et al. 2014). Predispositions of certain genotypes for increased suceptibilty was found, e.g. cultivar 'Mulan'. The analysis of variance showed significant genotypic effects for the frequency of speltoids. As mentioned by Mestiri et al. (2010), the progenitor-dependent meiotic instability in synthetic hexaploid wheat support these findings. In future, tracking pedigree data may help to discover susceptible breeding lines avoiding them during the propagation process as well as using them for prospective studies with defined predisposed seeds.

3.5   Future prospects

In addition to the prospective studies (Förster et al. 2012; Förster et al. 2013), the occurrence of speltoid off-types needs to be examined in more detail. For this propose defined starting material genotyped through quantitative pyrosequencing in order to clarify the genetic cause of heritabilty could be used. Progenies of these plants potentially segregating into many versus few speltoid off-types might be obtained and analyzed. In combination with high density genotyping platform, e.g. 90K iSelect chip (Wang et al. 2014), these plants could be used to carry out an association mapping approach. Additionally, the pyrosequencing assays as described by Zhang et al. (2013) could be integrated to understand the genetic basis for aneuploidy in general, since monosomic plants for most of the 21 wheat chromosomes are not phenotypically recognizable. But, this approche demands for a new selection of suitable speltoid material by researchers or wheat breeders.

In future, field trials could be conducted using various lines and in a wide range of environments to evaluate the influence of environmental factors contributing to the appearance of off-type plants. Especially, germination conditions of speltoids might be analyzed in more detail and under diverse temperature regimes in order to advice wheat breeders about optimal sowing times. This can be helpful to avoid speltoid off-types during plot propagation and to understand whether they are actually reduced in certain fitness parameters based on the theory of Birchler and Veita (2012).

It must be assumed that aneuploidy for chromosome 5A is the most likely reason for the spelt-like character in general. The identification of new genes controlling a

diploid-like behavior in hexaploid wheat during meiosis plays an essential role in order to understand the genetic mechanism of aneuploid formation. Molecular investigation, like expression studies or resequencing analysis, of already known genes involved in meiosis-regulating processes might contribute to understand the formation of univalent chromosomes resulting in aneuploid off-types. The *Ph1* or *CenH3* loci are proper candiates for such studies.

Althougth the 5A*Q* gene is molecularly well descibed in the spring wheat background, less is known about the regulation of *Q* in winter wheat varieties and its pleiotropic effects on agronomic and phenotypic traits. In chapter 2.2, 5A*Q* copy number-dependent transcription levels during early spike development were observed showing a weak correlation with the trait tiller number. In future, expression studies might be conducted to analyze the extent of gene dosage dependent effects governed by *Q* in various plant tissues, breeding lines, and cultivation conditions explaining its influence on agronomic parameters like yield-related traits. Additionally, signaling pathways governed by the *APETALA2* transcription factor could be examined in more detail. Molecular tools like yeast two-hybrid, microarray analysis, RNA-seq, and two dimensional protein electrophoresis would help to identify genes that directly interact with *Q* as well as those which are up- or down-regulated by *Q*.

4   Summary

The objectives of this study were systematic molecular and phenotypic investigations of speltoid off-types in bread wheat. These off-type plants are the main reason for the rejection of a wheat cultivar candidate during the licensing process leading to considerable costs and loss of time for wheat breeders. Phenotypically, speltoid off-types are characterized by spear-shaped spikes featuring an elongated rachis with tenacious glumes. Additionally, they express an elevated shoot length, whereby speltoids are usually well recognizable and removable within multiplication plots. Nevertheless, speltoid off-types occur from time to time during seed propagation, but the genetic cause is widely undisclosed. Since the early 20th century the domestication gene Q located on wheat chromosome 5A was known as the 'spelt factor' causing the free-threshing phenotype. Recently, the Q gene was cloned and molecularly characterized as a dosage dependent gene with pleiotropic effects on several morphological traits.

Based on sequence data, a quantitative pyrosequencing assay was developed to distinguish between normal wheat plants, which possess two copies of the 5AQ gene, and aberrant plants which are either aneuploids lacking the correct number of chromosome 5A or plants which carry the wild relative q allele. Therefore, the sequences of each of the three homoeologous Q/q alleles were multiple aligned and two primer combinations were developed for homoeologous sequence quantification based on pyrosequencing peak heights, designated as Q-ratio. Plants with one, two, and more than two 5AQ copies could be associated with speltoid, normal and compact spike morphology, respectively. Cytogenetic studies corresponded well with the respective phenotype and pyrosequencing results. Speltoid plants of four approved wheat cultivars with the loss of a single chromosome and reduced 5AQ gene copy number could be identified for both SNPs confirming the loss of 5A. Q and q specific alleles on 5A could be differentiated at SNP2299, a conserved nucleotide polymorphism between both alleles effecting the free-threshing spike during bread wheat domestication.

In order to analyze the genetic cause of speltoid off-types in a large range of bread wheat cultivars, the approach was additionally applied on progeny plants of speltoid off-types for eleven further wheat cultivars. A correctly predicted 5AQ copy

number for 392 of 402 (97.5%) single plants could be achieved in correspondence to the respective phenotype. The investigated plants were either reduced in chromosome 5A copy number or possessed a partial deletion of the distal end on the long arm of wheat chromosome 5A including the 5AQ gene. Additionally, quantitative real-time PCR analysis were carried out during early spike development, and a varying transcription level of 5AQ among winter wheat cultivars could be detected. The relative transcription of the 5AQ gene in speltoid off-types was always below the transcription level compared to normal squareheaded plants due to the reduced 5AQ gene copy number. Tissue specific expression studies in spike, stem, and leaf confirmed the highest transcription level in young spikes, as already shown by other researchers. The parallel analysis of 18 agronomic traits revealed pleiotropic effects governed by Q and other major genes like *Vrn-A1* located on chromosome 5A.

Besides cytogenetic and molecular investigations, biennial field trials based on speltoid starting material from 14 different wheat genotypes were conducted to investigate genotypic and environmental effects. To estimate the frequency of speltoid off-types field trials were carried out in five environments (three locations) at two different sowing times and in two replications per genotype. In total, 100 single ear progeny (lines) were developed from speltoid and normal plants. Therefore, spikes were harvest in 2006 and single progeny plants were selected in 2007. The 100 selected lines representing the 14 genotypes emerged from 66 speltoid and 34 normal descendent plants of the harvest in 2007. During the field trials, approximately 100 spikes were randomly harvested across each plot and subsequently scored for spike type. A mean frequency of 21.1% and 36.6% speltoid off-types in plots from normal and speltoid parental lines were observed, respectively. On average 5.3% less speltoid off-types could be found in plots sown later in the season, conspicuously when the sowing interval comprised more than 20 days leading to less speltoid off-types with 10.3%. Further, speltoid off-types always occurred more frequently in the first year of scoring with 7.9% more speltoids on average, although seeds for field trials in both years were derived from the same starting material. The experimental field data revealed highly reliable results since all correlation coefficients between replications, sowing times, and locations were significant at $P = 0.001$. Large genotypic variation between the lines could be observed ranging from 0.2% to 83.7%. This finding and results of analysis of

variance (ANOVA) indicate a strong dependency of the respective genotype or line leading to more or less speltoid off-types in multiplication plots.

Within the presented study the genetic basis for the occurrence of speltoid off-types could be widely clarified within current bread wheat cultivars. The loss of chromosome 5A including the domestication gene *Q* was the main cause leading to the respective speltoid phenotype. Pleiotropic effects governed by *Q* and altered expression patterns additionally confirmed this finding. The developed quantitative pyrosequencing procedure enables an accurate prediction of morphological aberrant plants caused by copy number variation of the *Q* gene or chromosome 5A at seedling stage. Thus, the method can be very useful for quality controls of seeds or MAS in wheat breeding programs. In addition, the introgression of the wild relative *q* allele can be analyzed when wheat breeders use exotic species for crossing and developing of novel wheat lines. For future investigations, defined starting material can be used for additional field trials or specific association studies with aneuploids for chromosome 5A. This might contribute to analyzing the genetic cause for aneuploids and chromosome misdistribution at all. The conducted field trials provided some evidences of environmental effects and genotype dependenies influencing the frequency of speltoid off-types. Thereupon, strategies to avoid these aberrant plants during seed multiplication and advices for growing conditions are discussed. In conclusion, the study can contribute to develop homogeneous bread wheat cultivar candidates reducing the amount of speltoid off-types to a considerable extent. Moreover, the results provide new approaches for future studies associated with meiotic misdistribution of wheat chromosomes and the occurrence of aneuploids.

5   Zusammenfassung

Die Ziele dieser Arbeit bestanden in den sytematischen molekularen und phänotypischen Untersuchungen von speltoiden Abweichern im Brotweizen. Diese abweichenden Pflanzen sind die Hauptursache für die Ablehnung eines Sortenkandidates während des Zulassungsprozesses und verursachen erhebliche Kosten und Zeiteinbußen für Weizenzüchter. Phänotypisch sind Speltoide durch spitzzulaufende Ähren mit verlängerter Spindel und festen Spelzen gekennzeichnet. Zusätzlich zeigen sie eine erhöhte Pflanzenlänge, wodurch sie für gewöhnlich in Vermehrungsparzellen gut erkennbar sind, und entfernt werden können. Dennoch treten Speltoid von Zeit zu Zeit während der Saatgutvermehrung auf, wobei die genetische Ursache weitestgehend unaufgeklärt ist. Seit dem frühen 20. Jahrhundert ist das Domestikationsgen Q auf Chromosom 5A vom Weizen als 'Speltoid Faktor' bekannt, wodurch der freidreschende Phänotyp verursacht wird. Das Q Gen konnte kürzlich kloniert und molekular charakterisiert werden, und zeigt als ein dosis-abhängiges Gen pleiotrope Effekte auf eine Vielzahl morphologischer Merkmale.

Basierend auf Sequenzdaten konnte in dieser Arbeit ein quantitativer Pyrosequenzierungsansatz entwickelt werden. Dieser ermöglicht die Unterscheidung von Pflanzen die einen normalen Phänotyp ausprägen mit zwei Q Kopien tragen und abweichenden Pflanzen, die entweder aneuploid für 5A sind bzw. das Wildallel q tragen. Dazu wurde die Sequenzen aller drei homöologen Q/q Allele aller Subgenome miteinander verglichen und zwei Primerkombinationen abgeleitet. Durch den Vergleich der detektierbaren Signalstärke zwischen den Subgenomen konnte die Q Kopienzahl ermittelt werden und im Anchluss definiert als 'Q-ratio' definiert werden. Pflanze mit einer, zwei und mehr als zwei Q Kopien und entsprechend speltoider, normaler und kompakter Ährenmorphologie konnten dabei identifiziert werden. Bei zytologischen Untersuchungen stimmte dabei der Phänotyp mit dem Ergebnissen der Pyrosequenzierung überein. Speltoide Pflanzen von vier zugelassenen Weizensorten zeigten den Verlust eines Chromosoms und eine reduzierte Q Kopienzahl für die beiden untersuchten SNPs, wodurch der Verlust von 5A bestätigt werden konnte. Die beiden 5A spezifischen Allele Q und q konnten am SNP2299 unterschieden werden. Dabei

handelt es sich um einen konservierten Nukleotid Polymorphismus zwischen beiden Allelen, welcher für die Domestikation von frei dreschendem Weizen verantwortlich ist.

Umfangreiche Analysen der genetischen Ursachen von speltoiden Abweichern erfolgte im Anschluss an Nachkommen speltoider Pflanzen von elf weiteren Weizengenotypen. Eine korrekte Vorhersage der 5AQ Kopienzahl konnte für 392 von 402 (97,5%) Einzelpflanzen im Vergleich zum entsprechenden Phänotyp erreicht werden. Die untersuchten Pflanzen waren entweder in der Chromosom 5A Kopienzahl reduziert oder trugen eine partielle Deletion am distalen Ende des langen Arms von Chromosom 5A inklusive des Q Gens. Zusätzlich wurden quantitative Real-time PCR Analysen während der jungen Ährenentwicklung durchgeführt, die ein verändertes Transkriplevel zwischen verschiedenen Weizensorten ergaben. Die relative Transkription von 5AQ in speltoiden Abweichern war immer unterhalb des Expressionsniveaus im Vergeleich zu den entsprechenden normalen Pflanzen, hervorgerufen durch die reduzierte 5AQ Kopienzahl. Gewebsspezifische Expressionsstudien in Ähre, Halm und Blatt bestätigten die höchste Transkription während der jungen Ährenentwicklung, wie bereits durch andere Wissenschaftler gezeigt werden konnte. Parallele Untersuchungen von 18 agronomischen Merkmalen verdeutlichten die pleiotropen Effekte, welche durch das Q Gen und anderer Hauptgene wie *Vrn-A1*, die ebenfalls auf Chromosom 5A lokalisiert sind, hervorgerufen wurde.

Neben zytogenetischen und molekularen Untersuchungen wurden, basierend auf Nachkommen von speltoiden Ausgangspflanzen von 14 Weizengenotypen, zweijährige Feldversuche in fünf Umwelten (drei Orte) mit zwei verschieden Aussaatzeiten und zwei Wiederholungen pro Linie durchgeführt, um genotypische Effekte und Umwelteinflüsse zu untersuchen. Insgesamt wurden 100 Einzelährennach-kommenschaften (Linien) von normalen und speltoiden Pflanzen entwickelt. Dafür wurden im Jahr 2006 Ähren gerntet, angebaut und in 2007 sowohl speltoide als auch normale Nachkommenschaftspflanzen für Feldversuche selektiert. Die 100 selektierten Linien beinhalten 14 Genotypent und sind aus 66 speltoiden und 34 normalen Nachkommenschaftspflanzen aus dem Jahr 2007 hervorgegangen. Während der Feldversuche wurden etwa 100 Ähren pro Parzelle zufällig geerntet und anschließend hinsichtlich des Ährentyps beurteilt. Eine mittlere Häufigkeit von 21,1% und 36,6% an speltoiden Abweichern konnte in Parzellen von entsprechend normalen und speltoiden Linien erfasst werden. Im Mittel wurden 5,3% weniger Speltoide in spät gesäten

Parzellen gefunden. Dies war besonders deutlich, wenn mehr als 20 Tage zwischen den beiden Aussaaten lagen, und dann zu deutlich weniger speltoide Abweicher mit 10,3% führte. Beim ersten Feldversuch im Mittel 7,9% mehr Speltoide erfasst, obwohl das Saatgut für beide Versuchsjahre vom gleichen Vermehrungsmaterial stammte. Im Allgemeinen lieferten die experimentellen Felddaten sehr zuverlässige Ergebnisse da alle Korrelationskoeffizienten bei P = 0,001 signifikant waren. Zwischen einzelnen Linien konnte eine deutliche genotypische Variation zwischen 0,2% und 83,7% beobachtet werden. Diese Befunde und Ergebnisse der ANOVA Analyse dokumentieren einen starken Effekt einzelner Genotypen bzw. Linien, die zu mehr oder weniger speltoiden Abweichern in Vermehrungsparzellen führen.

In der vorliegenden Studie konnte die genetische Grundlage für das Auftreten von Speltoiden in aktuellen Weizensorten ermittelt werden. Der Verlust von Chromosom 5A inklusive des Domestikationsgen *Q* war die Hauptursache, die zu einem entsprechenden speltoiden Phänotyp geführt hat. Pleiotrope Effekte, hervorgerufen durch *Q,* und ein verändertes Expressionsmuster bestätigen ebenfalls diesen Befund. Der entwickelte Pyrosequenzierungsassay ermöglicht eine exakte Vorhersage von morphologisch abweichenden Pflanzen, hervorgerufen durch die veränderte Kopienzahl vom *Q* Gen bzw. Chromosom 5A im Jungpflanzenstadium. Die Methode kann daher sehr nützlich für Qualitätskontrollen oder die marker-gestützte Selektion in Zuchtprogrammen sein. Zusätzlich kann die Einkreuzung des Wildallels *q* verfolgt werden, wenn Weizenzüchter exotische Spezies zur Entwicklung neuer Weizenstämme verwenden. Für zukünftige Untersuchungen kann definiertes Ausgangsmaterial für weitere Feldversuche erzeugt bzw. für spezifische Assoziationsstudien mit Aneuploiden für Chromosom 5A genutzt werden. Die durchgeführten Feldversuche lieferten Hinweise auf Umwelteffekte und Genotypabhängigkeit, welche die Frequenz an speltoiden Abweichern beeinflusst. Strategien zur Vermeidung dieser Abweicher während der Saatgutvermehrung unter Berücksichtigung der Kultivierungsbedingungen werden diskutiert. Darüber hinaus liefern die Ergebnisse neue Ansatzpunkte für weitere Untersuchungen die im Zusammenhang mit meiotischer Fehlverteilung von Weizenchromosomen und dem Auftreten von Aneuploiden stehen.

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Chapter 1.1

Figure 1: Historical evolution of wheat from prehistoric diploid grasses to modern durum wheat and bread wheat (modified from Peng et al. 2011).

Chapter 2.1

Figure 1: Multiple sequence alignment of the *Q/q* intron 7–exon 8–intron 8 region from *T. aestivum* cv. 'CS' and *T. durum* ssp. *dicoccoides* and the homoeoalleles on chromosomes 5B and 5D (*T. turgidum* ssp. *durum* and *Ae. tauschii*). *Dotted* and *dashed lines* indicate PCR and sequencing primer sites for SNP2236and SNP2299, respectively. *Empty* and *filled arrows* indicate the SNP positions. *Black*, *grey* and *white bases* show 100, 60–80 and less than 60% similarity, respectively. *Dashes* represent gaps.

Figure 2: Differentiation of two progeny types originating from a 5A monosomic CS plant. *Left* speltoid plant with 2n = 41, *right* normal plant with 2n = 42.

Figure 3: Fig. 3 Pyrograms using SNP2299 (a, c, e, g) and SNP2236 (b, d, f, h) differentiating *5AQ* from *5Bq* and *5Dq* alleles. a, b CS N5AT5D, c, d *T. spelta* var. *duhamelianum*, e, f CS monosomic 5A, g, h CS euploid 5A, *Filled arrows* indicate the *5Bq* and *5Dq* alleles, *grey arrow* indicates the *5Aq* allele, *empty arrows* indicate *5AQ* allele, *x* axis dispensation order of nucleotides, *y* axis light emission in relative light units, *grey boxes* calculated frequencies of *Q* and *q* alleles.

Figure 4: Box whisker plots of *Q* ratios for SNP2299 (a) and SNP2236 (b) of four German wheat cultivars in comparison to CS. Each plot includes 4 or 8 measurements of a single offspring plant, either monosomic or disomic, of the 4 cultivars and CS, respectively. Prior to pyrosequencing, the chromosome numbers were identified by chromosome counting. The *y* axis indicates the calculated *Q* ratio for alleles *Q* 5A versus *q* 5B and 5D. *Black arrows* to the *right* indicate the predicted *Q* ratio of euploid and monosomic plants for 5A. Abbreviations *s* and *n* represent the speltoid and normal (*square headed*) phenotypes, respectively.

Figure 5: Phenotypes of selfed offspring within wheat cross DPZ (a) and box whisker plots of *Q* ratios from SNP2299 (b) and SNP2236 (c). Each plot (in b and c) includes four measurements of a single plant (*x* axis). The *y* axis shows the observed *Q* ratio for *5AQ* versus *5Bq* and *5Dq* alleles. *Black arrows* to the *right* indicate the predicted peak height ratio of monosomic, euploid, trisomic and tetrasomic plants for 5A. The *table* indicates phenotype, chromosome number, plant height, and mean *Q* ratio for both SNPs. Abbreviations *s*, *n*, *sc* and *c* indicate speltoid, normal (*square headed*),

subcompact (*semi -clavate* to *clavate*) and compact (*fusiform*) phenotypes, respectively. Morphological spike phenotypes (a), box whisker plots (b and c) and *table* below have the same order of plants. *Dashes* indicate that chromosome counting was not successful due to poor sample quality.

Chapter 2.2

Figure 5: Phenotypes of selfed offspring within wheat cross DPZ (a) and box whisker plots of *Q* ratios from SNP2299 (b) and SNP2236 (c). Each plot (in b and c) includes four measurements of a single plant (*x* axis). The *y* axis shows the observed *Q* ratio for *5AQ* versus *5Bq* and *5Dq* alleles. *Black arrows* to the *right* indicate the predicted peak height ratio of monosomic, euploid, trisomic and tetrasomic plants for 5A. The *table* indicates phenotype, chromosome number, plant height, and mean *Q* ratio for both SNPs. Abbreviations *s*, *n*, *sc* and *c* indicate speltoid, normal (*square headed*), subcompact (*semi -clavate* to *clavate*) and compact (*fusiform*) phenotypes, respectively. Morphological spike phenotypes (a), box whisker plots (b and c) and *table* below have the same order of plants. *Dashes* indicate that chromosome counting was not successful due to poor sample quality.

Figure 2: Homoeoallele-specific PCR amplification of exon 8 in disomic and nullisomic-tetrasomic CS lines. Three primer combinations were tested on disomic 'Chinese Spring' (*CS*), nullisomic5A-tetrasomic5D (*N5AT5D*), nullisomic5Btetrasomic5A (*N5BT5A*), and nullisomic5D-tetrasomic5A (*N5DT5A*) lines.

Figure 3: Sequence alignment of the genomic region of exon 8 from the 5A*Q*, 5B*q*, and 5D*q* homoeoalleles of CS and ten investigated wheat cultivars. Each sequence was compared to complementary reference genome sequences of CS (AY702956), *T. turgidum* ssp. *durum* (DQ123819) and *Ae*. *tauschii* (EU350482). *Black*, *gray* and *white* bases indicate 100, 60–80 and <60 % similarity among all sequences, respectively. Primer binding sites of the 5A*Q* qPCR forward primer and the sequencing primer of the pyrosequencing assay at SNP2299 are indicated as *dashed* and *dotted lines*, respectively.

Figure 4: Relative transcript levels of the 5A*Q* gene from different tissues of monosomic 5A (*Q−*) and disomic 5A (*QQ*) CS lines (a) and from young spikes of ten normal (*hatched*) and speltoid (*gray*) wheat cultivars (b). Calculation of relative transcription was carried out according to Pfaffl (2001) using the internal reference gene actin. Expression levels are represented as fold-difference over CS spike (*QQ*), which is set to 1. Each measurement includes 3–5 biological replicates. *Vertical bars* and *asterisks* represent standard errors and significant differences at P = 0.05 (Student's *t* test), respectively.

Figure 5: Correlation plot of the relative 5A*Q* expression between normal and speltoid plants across ten wheat cultivars and CS. Regression line, linear equation (*y*),

correlation coefficient (*r*), and significance value (*P*) are indicated in the diagram. Each measurement includes 3–5 biological replicates.

Figur 6: Histograms with 18 morphological traits for normal (*hatched bars*) and speltoid plants (*gray bars*) within ten investigated wheat cultivars. a spike length in cm, b grain weight in g, c spike density (number of spikelets/cm spike length), d fertility (grain number/number of spikelets), e grain width in mm, f grain length in mm, g grain area in mm2, h thousand kernel weight in g, i threshability in %, j plant height in cm, k peduncle length in cm, l flag leaf width in cm, m flag leaf length in cm, n heading time in days after 1st May, o flowering time in days after 1st May, p number of spikelets, q number of tillers and r grain number/spike. All spike and grain parameters are based on the main spike of each single plant. *Vertical bars*, *, **, and *** represent standard errors and significant differences at P = 0.05, P = 0.01, and P = 0.001 (Student's *t* test), respectively.

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9 Abbreviations

Abbreviation	Explanation
ANOVA	Analysis of variance
AP2	*APETALA2*
BP	Before present
Br	Brittle rachis
CAPS	Cleaved amplified polymorphic sequence
CenH3	Histone H3 centromeric variant
DH	Double haploid
DNA	Deoxyribonucleic acid
dt/ha	Decitonnes per hectare
DUS	Test for distinctness, uniformity, and stability
Gb	Giga bases
GISH	Genomic in situ hybridization
GLM	General linear model
GS	Genome-wide selection
HI	Harvest index
ITMI	International Triticeae Mapping Initiative
IWGSC	International Wheat Genome Sequencing Consortium
L	Long arm of chromosome
MABC	Marker-assisted back crossing
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
mRNA	Messenger ribonucleic acid
MTA	Marker -trait association
N5AT5D	Nullisomic 5A-tetrasomic 5D plants
PCR	Polymerase chain reaction
Ph	Pairing homoeologous
RFLP	Restriction fragment length polymorphism
Rht	Reduced height gene
Ppd	Photoperiod gene
PPRT	Pyrosequencing-based paralogue ratio test
qPCR	Quantitative real-time PCR
Q	Domestication gene *Q*
Q -ratio	Ratio of pyrosequencing peak heights of 5A*Q* versus 5B*q* and 5D*q*
RNA-seq	Rebonucleic acid-sequencing
Rubisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
RUE	Radiation induced efficiency
S	Short arm of chromosome
SNP	Single Nucleotide Polymorphism
SSR	Simple sequence repeat
T.	*Triticum*
Tg	*Tenacious glumes*
t/ha	Tons per hectare
Vrn	Vernalization gene

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Declaration under oath

Eidesstattliche Erklärung/ *Declaration under Oath*

Ich erkläre an Eides statt, dass ich die Arbeit selbständig 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.

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