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Breeding winter durum wheat for Central Europe: Assessment of frost tolerance and quality on a phenotypic and genotypic level

Dissertation

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Life is a combination of
magic and pasta.

- Federico Fellini

ABBREVIATION

CBF gene	*C-Repeat Binding Factor* gene
CNV	Copy number variation
COR gene	*Cold Regulated* gene
DArT	Diversity Arrays Technology
DH	Double haploid
Fr-1, *Fr-2*	*Frost Resistance* gene 1 and 2
GBS	Genotyping-by-sequencing
KASP	Competitive allele specific PCR
LD	Linkage disequilibrium
NIR spectroscopy	Near-infrared spectroscopy
PCoA	Principal coordinate analysis
Ppd	*Response to Photoperiod* gene
SCT	‘Weihenstephaner Auswinterungsanlage’, semi-controlled test
SEWANA region	Mediterranean region, including South Europe, East Asia, and North Africa
SNP	Single nucleotide polymorphism
Vrn-1	*Vernalization* gene 1

1 GENERAL INTRODUCTION

Durum wheat (*Triticum durum* Desf., 2n=4x=28) is used for the worldwide pasta, bulgur, and couscous production. The major growing area of the tetraploid wheat is surrounding the Mediterranean region, including South Europe, East Asia, and North Africa (SEWANA region; Royo *et al.* 2009). Other production areas are North America and Canada as well as Central Europe, the Middle East, and South Australia. It only has minor importance on the global wheat market (about 5%) compared to bread wheat (*Triticum aestivum*) (FAOSTAT 2015; IGC 2014). In contrast to bread wheat, however, durum wheat is almost entirely used for food production, making quality traits an important aspect to consider in breeding programs.

DURUM WHEAT QUALITY – THE FOUNDATION FOR PASTA

Breeding has considerably improved the quality of durum wheat over the last 30 years. The demands of farmers, millers, pasta producers, and consumers, however vary in their importance (Table 1). The price for durum wheat is, in comparison to bread wheat, defined by the deviation of established standards and not by quality classes. Therefore, farmers are focusing on traits influencing the resale, like high test weight, protein content, and vitreosity as well as a low proportion of diseased kernels. A high milling yield, strong yellow pigmentation (Central Europe in particular) and low ash content are of interest for the semolina mills. Beside traits responsible for good processing and appearance of the final pasta, the pasta producers look for good cooking quality and stability as well as reduced deleterious ingredients and fine taste.

Table 1 Quality parameters important for the durum wheat industry in Germany from the perspective of farmers, millers, pasta producers, consumers, and the influence of environment (E) and genotype (G).

Trait	Min. value	Farmer	Miller	Pasta producer	Consumer	Influence
Test weight (%)	80	++	++	-	-	**E**, G
1000 kernel weight		-	+	-	-	E, G
Protein content (%)	14	++	+	++	-/0	E, G
Protein quality		-	0	+	-	G
Vitreosity (%)	75	++	++	-	-	**E**, G
Falling number (s)	220-300	0	0	+	-	**E**, G
Color	22	0/+	0	+	++	**G**
Black points (%)	5	+	+	+	0	**E**, G
Ash content		-	+	+	-	E, G
Cd content		-	0	+	+	E, G
Mycotoxins (DON, µg/kg)	1750	++	0	+	+	E, G

Bold letters indicate stronger influence of E or G; Kling and Münzing (2008) and Lafferty (2010), modified

Pasta is made of semolina, an endosperm granulate that is produced by milling durum wheat. In contrast to flour (<180 µm), the semolina particles are larger (300-1000 µm) (Heiss 2013). The expected semolina output during milling can simply be estimated by evaluating vitreous kernels. Vitreosity is the glassy and translucent appearance of a durum wheat kernels (Dexter *et al*. 1988) – the typical amber characteristic distinguishing durum wheat from all other wheats. Starch particles are coated with protein, giving the endosperm its tightly packed structure (Dexter *et al*. 1989; Sissons 2008). The kernels thereby become extremely hard and break in semolina instead of flour during milling (Sissons 2004). The grade of glassiness varies between fully-vitreous, piebald (mealy inclusions), and non-vitreous (or fully-mealy) kernels. The presence of mealy spots in the endosperms is influenced by the genotypic ability to form vitreous kernels as well as environmental factors harming the texture. Rainfall before harvest has particularly severe effects. Moisture changes the protein-starch matrix irreversibly and the exposed starch generates flour during the grinding process (Sissons 2004). Consequently, breeders routinely select for vitreosity after harvest.

Currently, the interest in durum wheat production stretches from the SEWANA region to countries with a more humid climate. Such area expansion in agriculture is combined with changes in production systems for an adaptation to varying environmental factors. In Central Europe for example, durum wheat is typically harvested in late summer, a season that is confronted with recurring rains in this region. Dexter *et al*. (1989) reported about the loss of vitreosity under humidity, varying between varieties, regions, and years. To avoid vitreosity losses caused by rainfall before harvest, it is necessary to investigate in the kernel performance in order to maintain the glassiness under humid conditions. Further, the stability of a pasta during cooking depends on a high grain protein content and protein quality (Sissons 2004). The relationship between protein content and vitreosity is controversially discussed. It has been reported that the proportion of starchy kernels reduces in genotypes with increased protein content (Dexter *et al*. 1989; Bilgin *et al*. 2010), while other studies did not identify an association between these two quality parameters (Pinheiro *et al*. 2013). Additional investigation is needed for more detailed information about the relationship between vitreosity and protein content, especially under the influence of humidity, to ensure a breeding success for pasta quality.

DURUM WHEAT ON A GLOBAL PERSPECTIVE - THE NECESSITY OF WINTER DURUM WHEAT PRODUCTION

The worldwide durum wheat production can be roughly divided into three growing systems: using the predominant spring type with an autumn sowing, the spring type combined with a spring sowing, and the winter type used exclusively with an autumn sowing. In a nutshell, the term winter type refers to plants or crops that are adapted to cold and frost. They need several environmental signals to begin the flowering process, like vernalization, i.e. a cold treatment, and some photoperiodic sensitivity (Levitt 1980). Spring types on the other hand are frost susceptible, less adapted to winter conditions and need neither vernalization, nor certain photoperiodic conditions to start flowering. A third type is the facultative type, which is an intermediate form, as it can be sown in autumn and in spring. Facultative types survive cold temperatures, but struggle with frosts, and signals like vernalization are not required for the initiation of flowering.

As mentioned before, the majority of durum wheat is grown in the SEWANA region (Royo *et al*. 2009). The Mediterranean climate, also found in East-Australia, is characterized by mild winters, warm springs combined with few rains and summers that are dry and hot. Here, spring durum wheat is sown in autumn (Figure 1; Kling *et al*. 2006; Lafferty 2010), allowing a vigorous vegetative phase, an optimal grain filling, and an early ripening, resulting in yield comparable to bread wheat (Marti and Slafer 2014). The cold semi-arid climate, found in the Northern Great Plains of the U.S. (North Dakota and Montana) and South-West Canada (Saskatchewan), with warm days and moderate rainfall in spring and summer as well as dry days before harvest, is still suitable for spring durum. However, in contrast to the SEWANA region, the spring type is sown between mid-April and the end of May, allowing a harvest between August and September. The area of East Europe and Central Asia on the other hand uses winter durum wheat sown in early autumn (September-October) for a harvest in late summer. In Europe all three production systems are present (Figure 1). South Europe as part of the SEWANA region, uses an autumn-sown spring type (see above), while Central Europe (Austria and Germany) need to sow durum wheat in early spring due to missing winter hardiness (Figure 2) of the spring type. Countries towards West Asia prefer the winter type to provide an appropriate vegetative phase.

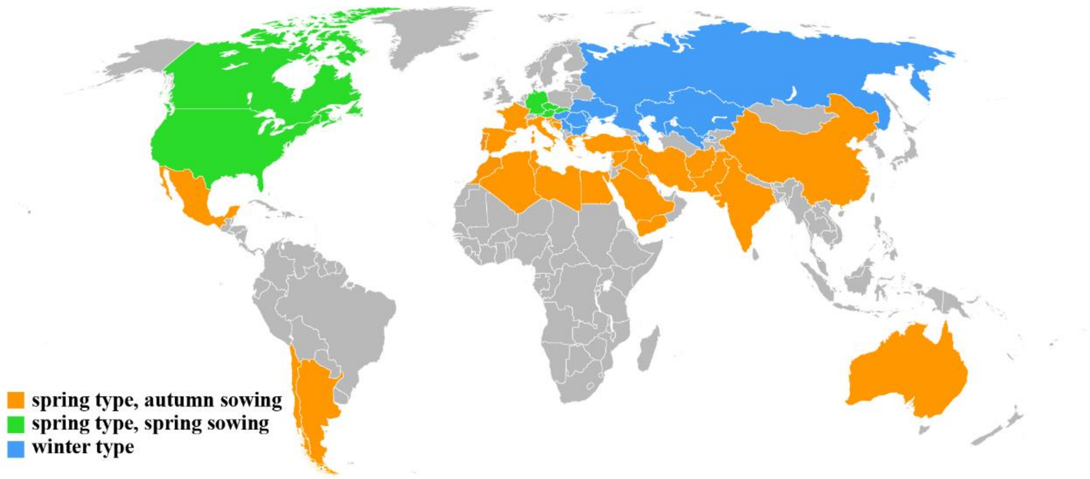

Figure 1 Durum wheat production systems for countries with economic importance of durum wheat (European Commission 2010).

An alternative for Austria and Germany, typical Central European durum wheat producers, could be the winter type. Major benefits would be an increased yield and yield stability (Lafferty 2010). Differences in yield performance of more than 20% were found for German spring and winter bread wheat (DESTATIS 2014). Due to a prolonged vegetative phase, plants are more vigorous and competitive to weeds. The prices for durum wheat (on a level between elite and quality bread wheat) in combination with increased yield of winter durum wheat, will be advantageous for farmers and favoring the choice towards durum wheat. Mills and pasta producer, however, did not accept winter durum thus far, due to poor and insufficient quality compared to the spring type (Kling and Münzing 2005). Quality improvement of winter durum in combination with higher yield and frost tolerance would result in an acceptance by the pasta industry. The increased durum wheat production in Central Europe will change the distribution of market shares. Mills and the pasta industry will be more independent of imports from e.g. Canada, receiving a raw product with slightly better quality, produced under more ecological conditions (Wagner *et al*. 2014). Winter durum improvement is also a part of risk management for climate changes as temperature is expected to increase in the next 50 years (DWD 2012). Spring and summer will become warmer and dryer and especially the change of the daily temperatures during spring will have dramatic effects. Spontaneous extreme temperatures during flowering can damage the spikelets, which causes yield and quality losses (Stone and Nicolas 1994; Spiertz *et al*. 2006). Using an early autumn sowing could mitigate the effects of such upcoming weather problems. Frosts with less than -20°C are still expected during winter, therefore an autumn-sown winter durum is required. The adaptation of durum wheat to cold stress in combination with high quality and yield will be a valuable contribution to food security.

HOW TO TEST FOR FROST TOLERANCE TO IMPROVE WINTER SURVIVAL

Winterkill is the damage caused by a mix of extreme cold temperatures, recurring frosts, winter diseases, desiccation and smothering due to ice and snow coverage (Figure 2). Such harm can result in (total) plant loss with the consequence of dramatic harvest failure. The complexity of winter survival is a network of different characteristics. A well-developed plant with a deep and advanced root system can tolerate the first cold temperatures during winter (Levitt 1980). Further features like a stable cell membrane and wall as well as an accumulation of non-freezing substances in the cells increase the cold tolerance in plant (Levitt 1980; Lamb 1987). On a genotypic level frost tolerance is not only important to survive freezing, but also plays a critical role in the interaction of photoperiodic and vernalization sensitivity to prevent a flowering before the last frost occurs (Galiba *et al*. 2009).

Figure 2 Phenotypic variation of winter damage in durum wheat test plots. Durum wheat lines with less or without frost tolerance are indicated by brown and dehydrated leaves.

A huge challenge for breeders is the selection for frost tolerance under field conditions. Frosts occur irregularly during a winter season or across years. The occurance of snowfall limits the impact of frost on the plants, since snow coverage has a protective and isolating effect. The need of alternative testing methods is high to get reliable and regular data for a good winter hardiness selection in cereals. Artificial frost tests are classified as direct or indirect frost tests (Sãulescu and Braun 2001). Direct methods expose plants to frost stress and measure the visible damage (Sãulescu and Braun 2001). There are several opportunities to simulate frost stress, examples include frost cabinets, climate chambers, pot tests, greenhouses or field-based tests, which differ in their amount of control. Beside the used planting system (micro-arrays, pots, field), time-, hardening-, and temperature-profiles can be influenced. Choosing a method mainly depends on the characteristic that is tested for, for example damaged plants, leaf color, root system development, or recovery. Controlled tests can be considerably time consuming, labor-intensive, expensive and require technical and biological knowledge. In bread wheat the ‘Weihenstephaner Auswinterungsanlage’ (Hoeser 1954) is successfully used for frost tolerance testing representing a semi-controlled test (SCT). This test uses wooden boxes placed one meter above the ground in the field. Seeds are planted by hand parallel to sowing in the field. The plants are exposed to all naturally occurring weather conditions, except for snow coverage. Movable glass lids can be put on top of the boxes, preventing any isolation by snow. The SCT enables to determine the proportion of plant survival after a frost period as well as the recovery after winter.

Indirect frost tests include the measurement of biochemical components that change during a cold period, which does not necessarily need a frost treatment (Sãulescu and Braun 2001). Molecular markers linked to winter survival traits also count as indirect frost tests. Further, using molecular markers can help to understand the genetic architecture of frost tolerance, moving towards a knowledge-based selection in breeding programs. Genes responsible for frost tolerance or features associated with winter survival have already been identified in wheat. Sutka and Snape (1989) detected *Frost Resistance-A1* (*Fr-A1*), a locus responsible for frost tolerance on chromosome 5A. A second locus (*Fr-A2*) was mapped within a distance of approximately 30 cM to *Fr-A1* (Vágújfalvi *et al*. 2003). Homoeologs of *Fr-1* and *Fr-2* were detected on group 5 chromosomes. A cluster of eleven *C-Repeat Binding Factor* (*CBF*) genes is located at *Fr-2* (Miller *et al*. 2006; Knox *et al*. 2008). *CBF* genes need cold treatment for induction, their function in turn is to activate other genes like the *Cold Regulated* (*COR*) genes that are required for an increased winter survival (Vágújfalvi *et al*. 2005). Deletions in the *CBF* cluster were reported to be associated with reduced frost tolerance (Pearce *et al*. 2013). Typical for tetraploid and hexaploid wheat is a deletion of six, nine, or eleven *CBF* genes at the *Fr-2* on the B genome (*Fr-B2)* (Pearce *et al*. 2013). For *Fr-A2* polymorphisms in the *CBF-A12* and *CBF-A15* genes were identified to form two haplotypes, frost susceptible (*Fr-A2-S*) and tolerant (*Fr-A2-T*) (Zhu *et al*. 2014). These haplotypes can be found in winter and spring types, but the *Fr-A2-S* haplotype is more frequent in spring varieties, whereas the *Fr-A2-T* haplotype occurs more often in winter varieties. Further associations were found for varying copy number of *CBF-A14* (*CBF-A14* CNV) and the *FR-A2-S/T* haplotype. Knox *et al.* (2010) also detected an increased *CBF-A14* CNV level in winter wheat, suggesting an association between *CBF-A14* CNV and winter survival.

As *Fr-1* maps close to the *Vernalization-1* (*Vrn-1*) locus, it is now accepted to be a pleiotropic effect of *Vrn-1* (Sutka and Snape 1989; Dhillon *et al*. 2010). The transition from the vegetative to the generative growth phase is controlled predominantly by *Vrn-1* which encodes a MADS-box like protein (Trevaskis *et al*. 2003). Those proteins are transcription factors responsible for vernalization requirement and flowering development processes. In addition to deletions in the gene, copy number variation of *Vrn-A1* (*Vrn-1* found on the A genome) is correlated with varying vernalization requirement, and additionally associated with the previously mentioned *Fr-A2-T* haplotype (Díaz *et al*. 2012; Zhu *et al*. 2014). To prevent frost damage at the spikelets, a complex interaction between *Vrn* genes with mutually upregulating and suppressing functions control flowering of the winter type (Galiba *et al*. 2009). Hereby, *Response to Photoperiod* (*Ppd*) genes have additional controlling tasks in responding to changes in day length. Little is known about the genetic control of frost tolerance in durum wheat and the effects of the above mentioned candidate genes.

EFFORTS NECESSARY FOR WINTER DURUM BREEDING

Before discussing the improvement of winter hardiness, it is necessary to evaluate the relationship between yield, quality, and winter hardiness to increase the acceptance of winter durum in the European pasta industry. The association between frost tolerance and quality is controversially discussed by breeders. Lafferty (2010) considers the combination as challenging, but not impossible and suggests a selection of quality on a level of classical quality spring durum varieties, moderate frost tolerance on bread wheat level and excellent yield performance. So far this might be difficult with regard to the short breeding history of winter durum (Palamarchuk 2005) and the small number of winter durum breeders focusing on different breeding goals. Breeders from the Ukraine and Russia concentrate predominantly on an extraordinary frost tolerance performance, while traits for the pasta production are rather neglected. To achieve frost tolerant durum wheat lines, interspecific crosses with bread wheat were made (Palamarchuk 2005). The achieved results were unsatisfactory and the productivity was still too low. Therefore, breeders conducted crosses between bread wheat with excellent winter survival and spring durum as well as winter durum. Several founder lines of the current winter material resulted from these efforts (Palamarchuk 2005). In the 1960s, first efforts began towards a winter type breeding in Central Europe (Walther 1978). Breeders and researchers scanned available genotypes with sufficient winter hardiness from different breeding programs as well as genebanks for potential quality. This also included some of the founder lines from Ukraine and Russia (Walther 1978). Breeding efforts over the last decades and continuous selection for the desired traits resulted in the first winter durum varieties accepted by farmers as well as the European pasta industry. The active material exchange between Austria, Hungary, and Germany might be a strong reason for this first success. A better understanding of the population structure, genetic relationship and diversity of the existing winter durum lines is necessary to make similar achievements as in spring durum breeding. No molecular marker analysis was conducted with winter durum so far. To further improve the breeding success of winter durum it is important to get a better understanding in the trait relationship of quality and winter hardiness, characterize the genetic kinship of winter durum, and evaluate frost tolerance in detail on a phenotypic and genotypic level.

OBJECTIVES

The aim of this thesis was to investigate frost tolerance in durum wheat on a phenotypic as well as on a genotypic level, to assess quality potential of winter durum wheat, and to examine the trait relationship between winter survival and quality.

In particular, the objectives were to

(i) Examine the potential of varieties to form vitreous kernels and the influence of humidity on the stability of vitreosity.

(ii) Evaluate a promising selection platform for frost tolerance, to further explore the phenotypical variability of frost tolerance in durum wheat.

(iii) Investigate the combination of frost tolerance with important agronomic and quality traits in durum wheat varieties.

(iv) Employ a genotyping-by-sequencing approach for a subsequent linkage disequilibrium analysis to determine the usability of a worldwide durum wheat panel for association mapping.

(v) Analyze the genetic diversity and population structure in winter durum wheat.

(vi) Identify major QTL underlying the genetic architecture of frost tolerance by a genome-wide association study.

2 PUBLICATION I: VITREOSITY AND PROTEIN CONTENT

VITREOSITY, ITS STABILITY AND RELATIONSHIP TO PROTEIN CONTENT IN DURUM WHEAT

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ABSTRACT

High quality requirements are set on durum wheat (*Triticum durum*) from semolina mills and pasta producers. For the production of semolina and pasta with good cooking quality, high grain proteincontent and vitreosity is required. The dependency of vitreosity on protein content as well as its stability under the influence of humidity was not well investigated up to now. We (1) compared two methods to determine vitreosity, (2) investigated the relationship between vitreosity and protein content, (3) developed a method to analyze vitreosity under humidity, and (4) examined the relationship between protein content and agronomical as well as quality traits in durum wheat. The results showed that the formation of vitreous kernels greatly depends on the protein content. To evaluate the stability of vitreosity under the influence of humidity a new method was elaborated and employed to assess the durum germplasm under study. This revealed that vitreosity of a durum wheat variety depends on the potential to form vitreous kernels but also to maintain this vitreosity under the influence of humidity. Our results further show that protein content is a central trait in durum wheat that strongly influences important traits like grain yield, vitreosity, and b-value.

3 PUBLICATION II: SELECTION TOOL FOR FROST TOLERANCE

EVALUATION OF A SEMI-CONTROLLED TEST AS A SELECTION TOOL FOR FROST TOLERANCE IN DURUM WHEAT (TRITICUM DURUM)

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ABSTRACT

Most durum wheat (*Triticum durum*) varieties possess only low winter hardiness due to their frost susceptibility. In North America and Central Europe, durum wheat is therefore typically sown in spring to circumvent the local winter conditions. However, the yield potential of durum in these regions could be much better exploited if durum varieties with increased frost tolerance were available, which could be sown in autumn. A factor limiting breeding for increased frost tolerance is the variation in the occurrence of frost stress across years. The 'Weihenstephaner Auswinterungsanlage' is a semi-controlled test that exposes the plants to all weather conditions. Snow coverage of the plants, serving as frost protection, is prevented by the movable glass lid of the semi-controlled test. In this study, different scorings for frost tolerance based on this semi-controlled test were evaluated and compared with frost tolerance data in the field. Our results illustrate the potential of the 'Weihenstephaner Auswinterungsanlage' as an indirect selection tool for frost tolerance in durum breeding programmes, especially when regular frost tolerance data from the field are not available.

4 PUBLICATION III: FROST TOLERANCE, YIELD AND QUALITY

COMBINING FROST TOLERANCE, HIGH GRAIN YIELD AND GOOD PASTA QUALITY IN DURUM WHEAT

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ABSTRACT

Growing in Central Europe winter instead of spring durum wheat would substantially increase yield potential but is currently hampered by the lack of knowledge of frost tolerance present in elite material. The objectives of our survey were to (i) study the genetic variability and heritability of frost tolerance and its association with other important agronomic and quality traits in durum wheat, (ii) examine the potential to combine frost tolerance with high quality and high grain yield and (iii) investigate the consequences of the heritabilities and associations among traits on the optimum design of a multistage selection programme for winter durum wheat. We investigated 101 elite winter durum wheat lines and four commercial checks in field trials at four locations. Four agronomic as well as nine quality traits were recorded. In addition, frost tolerance was evaluated using a semi-controlled test resulting in high-quality phenotypic data. Genotypic variances (r^2G) were significantly larger than zero for all traits, and heritabilities were moderate to high. Several elite durum wheat lines exhibited a frost tolerance comparable to that of two frost-tolerant *Triticum aestivum* varieties. Frost tolerance was not negatively associated with other important agronomic and quality traits. The high quality of the phenotypic data for frost tolerance evaluated in a semi-controlled test suggests that this is a cost-efficient approach to consider frost tolerance at early stages of a multistage durum wheat breeding programme.

5 PUBLICATION IV: GENETIC DIVERSITY

MOLECULAR CHARACTERIZATION OF WINTER DURUM WHEAT (*TRITICUM DURUM*) BASED ON A GENOTYPING-BY-SEQUENCING APPROACH

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ABSTRACT

Durum wheat (*Triticum durum*) is predominantly grown as spring type and depending on the production area autumn or spring sowing is used. For the durum production in Austria and Germany, autumn sowing has several advantages, such as yield increase and stability, but this requires the selection for winter hardiness including a good frost tolerance. The aim of this study was to support breeding of winter durum and to facilitate genomic approaches by molecularly characterizing a panel of 170 diverse winter and 14 spring durum lines employing a genotyping-by-sequencing approach. We obtained an unprecedentedly high number of 30,611 polymorphic markers covering the entire genome. The principal coordinate analysis and the cluster analysis revealed the absence of a major population structure but a tendency of lines to group according to their country of origin. Linkage disequilibrium was found to decay within a short distance of approximately 2–5 cM and also showed variable patterns along chromosomes. In summary, our results can assist breeding of durum wheat and pave the way for genomic approaches towards knowledge-based winter durum breeding.

6 PUBLICATION V: GENETIC CONTROL OF FROST TOLERANCE IN DURUM WHEAT

COPY NUMBER VARIATION OF *CBF-A14* AT THE *FR-A2* LOCUS DETERMINES FROST TOLERANCE IN WINTER DURUM WHEAT

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ABSTRACT

Frost tolerance is a key trait for successful breeding of winter durum wheat (*Triticum durum*) which can increase the yield performance in regions favoring autumn-sown winter cereals. The aim of this study was to investigate the genetic architecture of frost tolerance in order to provide molecular support for the breeding of winter durum wheat. To this end, a diverse panel of 170 winter and 14 spring durum wheat genotypes of worldwide origin was evaluated for frost tolerance in the field, as well as in a semi-controlled test. A total of 30,611 polymorphic genome-wide markers obtained by a genotyping-bysequencing approach and markers for candidate loci were used to assess marker-trait associations. One major QTL was detected on chromosome 5A, likely corresponding to *Frost Resistance-A2* (*Fr-A2*). Further analyses strongly support the conclusion that copy number variation of *CBFA14* at the *Fr-A2* locus is the causal polymorphism underlying this major QTL. It explains 91.6 % of the genotypic variance and a haploblock of two strongly associated markers in the QTL region also allowed to capture the variance of this QTL. In addition to this major QTL, a much smaller contribution of 4.2 % was observed for *Fr-B2*. We further investigated this major QTL and found that the copy number of *CBF-A14* and the frequency of the frost tolerant haplotype mirrored the climatic conditions in the genotypes' country of origin, suggesting selection through breeding. Two functional KASP markers were developed which facilitate a high-throughput screening of the haploblock and thus a marker-based breeding of frost tolerance in winter durum wheat.

7 GENERAL DISCUSSION

Available seed material, knowledge about breeding strategies, genetic diversity, informative selection tools, as well as a deep understanding of the quality and winter survival complexity are crucial for the success of winter durum wheat breeding. Different experiments were conducted as part of this thesis, gaining insights which can support plant breeders in these aspects. The following questions served as a guideline to build a framework for improving winter durum breeding:

- What is the extent of quality potential in the Central European winter durum material?
- Is it possible to test for frost tolerance in durum wheat?
- Can breeding combine winter hardiness with improved quality characteristics?
- Does a population structure exist in winter durum and what is the genetic diversity in winter durum material?
- Which genes influence frost tolerance in durum wheat?

The aim was to determine the variation of quality and frost tolerance in winter durum, improve selection methods for these characteristics and enhance the selection process on a phenotypic and genotypic level. Furthermore, molecular genetic approaches were used to determine the genetic architecture of frost tolerance in durum wheat in order to allow a knowledge-based selection in breeding programs.

WINTER DURUM WHEAT – AN ECONOMIC POINT OF VIEW

The Central European climatic conditions favor autumn-sown winter cereals. Using winter durum instead of spring durum wheat in this region has several advantages. Increased yield performance of winter over spring types was observed in bread (2.5-30.9%) as well as in durum wheat (2.2-10.9%) (Figure 3). The main reason is a prolonged vegetative phase, allowing a quick and fast transition to the generative phase after winter (cf. Diepenbrock *et al*. 1999). Furthermore, the growth advance causes plant vigor and competitiveness against weeds. Damage caused by weeds can be decreased and with this the herbicide application in early spring. The constant soil coverage during the winter season will improve the soil structure and fertility. Furthermore, soil moisture in spring can be exploited in a better way, and nutrition losses, especially nitrate leaching can be avoided. The growth advantage of the crop allows an earlier harvest, avoiding summer rainfalls that result in possible vitreosity losses.

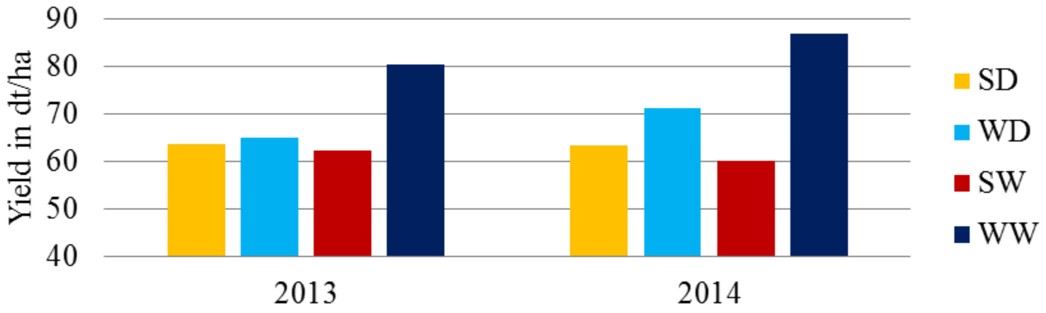

Figure 3 Yield comparison (in dt/ha) of spring durum (SD) and winter durum (WD) (results from variety testing) as well as spring bread wheat (SW) and winter bread wheat (WW) (DESTATIS 2014).

QUALITY POTENTIAL FOR WINTER DURUM WHEAT BREEDING

The pasta industry did not accept winter durum so far due to poor quality. Winter durum varieties have to improve at least to a moderate quality level of spring durum. German semolina mills and pasta producers demand a protein content of >14%, vitreosity of >75%, a b-value of >22, and a falling number of >220 s (Table 1). A major requirement to improve crops by breeding is a sufficient amount of genetic variability of a trait as well as a good transferability into the next generation (heritability). The genetic variation of quality traits in winter durum has been investigated insufficiently. The quality experiments of this thesis (Chapter 2, Chapter 4) showed significant (P <0.01) genotypic variances for different traits, like protein content and vitreosity. The genotype-by-environment interaction did have a smaller effect than the genotype. Heritabilities were moderate (protein content: $h^2 = 0.55$-0.59) to high (vitreosity: $h^2 = 0.67$-0.85). Diverse panels commonly have high genetic variation. The plant material used for both experiments were related breeding lines and consequently a reduced genetic variability and a lowered heritability might have been expected. However, the opposite was the case, giving breeders the chance to further enhance quality in winter durum. Results from variety testing could demonstrate that winter durum can perform on a similar level as spring durum or is even able to outperform spring durum (Figure 4). Particularly noticeable are yield differences with a better performance of winter durum of nearly 8 dt/ha (Figure 3).

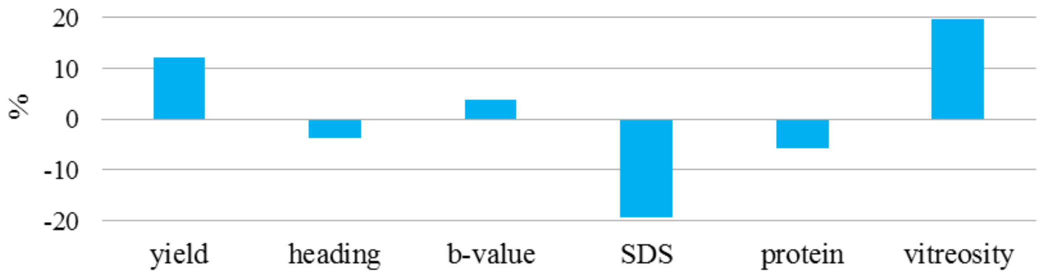

Figure 4 Winter durum performance of different important agronomic and quality parameters relative to spring durum (data from variety testing in 2014).

Concerning quality attributes available in durum wheat, a special focus was on vitreosity. The endosperm character of glassy kernels has a strong impact on semolina yield (Sissons 2004), and therefore is of great importance for semolina mills and pasta producers. Existing vitreosity evaluation methods, like the ICC standard method 129 (ICC 1980), are generally time consuming. Batches get separated roughly and not all kernels are taken into account, which may even falsify the results. An alternative could be a second determination method (VI_method2, Chapter 2), which was modified after a technique cited in Branković *et al*. (2014). In contrast to the ICC standard method, the second method only excludes shrunken or broken kernels and evaluates the surface of a cross-section. The technique is still time-intensive and destructive, but seems more precise. Surprisingly both methods yielded similar results (Chapter 2) and can be applied to evaluate glassiness in durum wheat. Nevertheless, they are time-consuming, labor-intensive, and rather subjective. The demand for alternatives providing fast and easy information about vitreosity is still high. Near-infrared (NIR) spectroscopy is a non-destructive and fast-working method (Dowell 2000). Results from a diverse winter durum panel used in Chapter 5, showed significant correlations (r = 0.79, P <0.001) between VI_method2 and vitreosity information determined using NIR spectroscopy (unpublished data). Additionally, in most of the breeding programs NIR spectroscopy is a standard method to analyze different parameters at the same time, like protein content.

The influence of humidity on the destruction of the protein-starch endosperm matrix in vitreous kernels is insufficiently understood. It was observed that rainfalls before harvest have a large negative effect on vitreosity (Dexter *et al*. 1989). Late summer rains are common in Central Europe and the use of spring durum forces a harvest between the end of June and the beginning of August. A consequence is the reduction of glassiness in durum wheat kernels. A time series using VI_method2 experiment was developed. Fully-vitreous kernels were evaluated and regularly wetted. A reduced vitreosity could be observed in all genotypes, but the extent of mealy inclusions in the kernel varied strongly. Since rains around harvest occur variable, the evaluation of vitreosity in breeding programs becomes more complicated (Chapter 2). It is necessary to evaluate the potential of a genotype to form vitreosity as well as to maintain it under humid conditions. Therefore a new trait, combing the two features, was introduced in this thesis: stability of vitreosity. It showed a wide range of genetic variation in the tested material and a moderate heritability (h^2 = 0.56) (Chapter 2). While it was used in combination with the improved evaluation method (VI_method2) it has disadvantages in practicability. A solution could be a combination with the NIR spectroscopy.

The glassy kernel character results from a typical protein-starch matrix in the endosperm (Dexter *et al*. 1989; Sissons 2008), and it was therefore expected that the protein content does have an impact on vitreosity. In Chapter 4, vitreosity was evaluated with a simple visual method in winter durum, and no correlation was found to protein content confirming results of other studies (Autran *et al*. 1986; Pinheiro *et al*. 2013). Bilgin *et al*. (2010) and Sissons (2004) on the other hand reported strong positive associations between the two traits. To clarify this issue, all genotypes were grouped into two classes, fully-vitreous and non-vitreous kernels (Chapter 2). The class of non-vitreous kernels showed a highly significant correlation between protein content and vitreosity, while the group of fully-vitreous kernels did not display any association between the two traits. Vitreosity thus appears to be correlated with protein content until a specific level of protein content is reached (Chapter 2). Once the protein content exceeds this limit, vitreosity has reached its maximum and remains constant. This knowledge can have great consequences in field management. Farmers often apply a late nitrogen fertilization to improve protein content and also vitreosity. Hadjichristodoulou (1979) reported an increasing vitreosity with increasing nitrogen application, which was not the case for lines with high level of glassiness. More information about the stability of vitreosity of a genotype could prevent farmers applying unnecessary fertilization treatments.

CHALLENGES IN SELECTION FOR FROST TOLERANCE

Efficient selection for frost tolerance is limited by the varying occurrence of frost stress across different years. Testing under field conditions in Central Europe is additionally complicated by snow coverage, isolating plants from extreme temperatures. Also problematic is the selection for frost susceptibility in early generations due to low seed availability. Frost nurseries in combination with extreme winters can cause large plant losses, which aggravated the seed availability. Alternative methods are required to permit an effective selection of frost tolerance to enhance winter survival in durum wheat. Artificial methods are time-consuming and labor-intensive. Also problematic is the comparison between results of controlled and non-controlled (field) experiments. The more manipulation takes place the more standardization in the results is generated, which is not useful for selection. The 'Weihenstephaner Auswinterungsanlage' enables to test the plants under all naturally occurring weather conditions, except for snow coverage (Chapter 2, Hoeser 1954). In the following the 'Weihenstephaner Auswinterungsanlage' will be referred to as semi-controlled test or SCT. Only a small amount of seeds is needed in the SCT. Frost tolerance is evaluated as percentage leaf damage after a frost period. A potential second evaluation is the recovery after winter, determining the amount of re-greening plants. In this thesis it was tested whether the SCT is suitable for durum wheat. Despite the relatedness of the genotypes a good differentiation for frost tolerance was observed (Chapter 3 & 4). This illustrates the genetic potential available in Central European material that can be exploited in breeding programs. Such a selection tool is, however, only useful when the gained information can be compared to traditional field data. Frost tolerance data from the SCT and the field were highly comparable and the SCT thus is a valuable tool for selection of frost tolerance and allows an evaluation in early generations. Nevertheless, the observed genotype-by-environment interactions require to use a multi-stage selection to avoid a misjudging in a single year. Further it is suggested to place the SCT directly on the ground, allowing the SCT more flexibility and reducing factors like direct frost stress on the roots.

One of the major requests by the pasta industry was a high quality level in winter durum. Breeders have the widespread opinion that it might not be possible to combine frost tolerance with quality due to negative correlation between those characteristics (pers. communication F. Lacoudre and R. Papa). Results from this thesis could not confirm the existence of negative associations. The observed correlations were small or close to zero, except for falling number (Chapter 4). Additionally, no negative effect of improved frost tolerance on yield was observed. This indicates that winter durum breeding in Central Europe, can be successful introducing stronger frost tolerance in high quality breeding material without lowering the yield potential in winter durum.

POPULATION STRUCTURE

Another way to increase the level of frost tolerance in winter durum is to use molecular markers as selection tool. In this thesis a genotyping-by-sequencing (GBS) approach was applied to analyze a highly diverse panel of winter durum, including a small group of spring durum, with worldwide origin (Chapter 5). The obtained silicoDiversity Arrays Technology (DArT) markers (presence-abscence) and single nucleotide polymorphism (SNP) markers, in the following referred to as markers, were used to determine the genetic relatedness as well as the population structure. A molecular characterization of the set assessed the genetic diversity as well as the extent of linkage disequilibrium (LD) to determine whether an association mapping is possible.

A high number of markers were obtained and showed an equal distribution across the chromosomes of the A and B genome. In the rather short breeding history of winter durum (Palamarchuk 2005) characteristics like winter hardiness were improved in a relatively short time, which could have resulted in a reduced genetic diversity in some genomic regions. However, such larger monomorphic regions were not identified, indicating no or only a low effect of a selection bottleneck. The LD decays under the population-specific threshold within approximately 2-5 cM, which is similar to spring durum (Somers *et al*. 2007). Bread wheat showed a slightly slower LD decay within 5-10 cM (Würschum *et al*. 2013). A reason for this could be the long breeding history resulting in a lower diversity in bread wheat. Another possibility could be the relatedness of the used bread wheat material, which were registered elite varieties and related experimental lines (Würschum *et al*. 2013). The combination of the observed extent of LD and the high number of polymorphic markers that densely cover the genome, make the winter durum set used in this thesis (Chapter 5) a promising platform for high-resolution association mapping (Myles *et al*. 2009; Würschum 2012). Association mapping allows to identify QTL for frost tolerance, to build the foundation for marker-assisted selection.

A principal coordinate analysis (PCoA), did not separate between the winter and the spring growth type (Chapter 5). Bread wheat and triticale in contrast showed a strong differentiation in a PCoA study and cluster analysis, respectively (Chao *et al*. 2010; Badea *et al*. 2011). Reasons for this could be the non-representative number of spring durum lines used in the thesis or the continuous crossing with spring durum in the creation of the winter durum founder lines. Furthermore, no major population structure was found in the present winter durum panel, but the cluster analysis demonstrates a grouping into eight clades corresponding to the origin of the genotypes. A similar grouping was also observed in spring durum (Maccaferri *et al*. 2005). Group 1-4 are predominantly old varieties from Eastern Europe, slightly separated into old Hungarian, Ukrainian, and Russian origin. They are characterized by a very good frost tolerance and low quality. The remaining groups are modern winter durum, known for a good quality, but showing a moderate winter survival. The modern lines separated into lines with an Austrian and German origin as well as lines from the breeding program of the State Plant Breeding Institute of Hohenheim. One group presents a mixture of young varieties from Austria, Germany, and Hungary. The German group included several elite varieties as well as spring durum lines which might be a result of constant crossing with spring durum to integrate their quality features into winter durum. Modern Hungarian lines having an excellent frost tolerance and adequate quality, seem to be more attractive for an integration in Central European breeding programs. The winter survival in Austrian or German lines could be further increased without dramatic effects on quality by conducting crosses with these modern Hungarian lines. First attempts in such a direction were already done, which is mirrored in the mixed group of Austrian, German, and Hungarian lines.

The identified groups are also useful for building up a hybrid breeding program in winter durum. A requirement for hybrid breeding is the availability of distinct heterotic groups (Gowda *et al*. 2010; Longin *et al*. 2012). One potential heterotic group could be built in the Eastern European Group as well as one of the elite German and Austrian material. These two groups are genetically distant, consequently the effect of hybrid breeding could be satisfactory. The poor quality of the Eastern European lines, however, could also have strong negative effects on the quality characteristics of the resulting hybrids. Breeders should carefully think about in which direction they want to push their breeding programs. Continuing with conventional line breeding allows further mixing between the groups, but will disrupt heterotic patterns that would be advantageous for hybrid breeding.

GENETIC ARCHITECTURE OF FROST TOLERANCE IN DURUM WHEAT

A good winter survival is determined by several characteristics. Farmers achieve positive effects by a good crop and field management (Knapp and Knapp 1978), but these practices have their limits in dealing with decreasing temperatures. Plants need an appropriate morphology and physiology to defend external stress. The growth stage has a strong influence on winter survival. Crops should not reach a too advanced growth stage to optimize the vernalization treatment and reduced surface for frost attacks (Brooking 1996; Rawson *et al*. 1998). For example, a growth apex generally placed lower in the plant is more advantageous, isolating the growth point from extreme temperatures (Levitt 1980)

Stable cell walls or a higher accumulation of non-freezing substances in the vacuole improve the winter survival on a cell level (Lamb 1987). A strengthened plant immune system, which is supported by resistances against snow mold, provides additional survival chances (Sãulescu and Braun 2001). The most important characteristic is to suppress premature plant development before the last frost stresses occur. When plants change into the generative phase too early, they risk dramatic damage on the spikelet which results in yield losses. A complex regulatory gene network is required to avoid such harm (Galiba *et al*. 2009). The *Frost resistance* (*Fr*) genes are responsible for increase survival during frost stress, and interact with *Vernalization* (*Vrn*) and *Response to Photoperiod* (*Ppd*) genes.

The previously described panel of winter durum (Chapter 5), was evaluated for frost tolerance using the SCT (Chapter 6). The approximately bimodal distribution of the phenotypic frost tolerance data suggested one major QTL controlling this trait. Conducting a genome-wide association study, a single peak was found on chromosome 5A. Significant markers were then corrected for collinearity, which resulted in two SNP markers with the same map position, explaining 82.2% (S2269949) and 8.5% (S1077313) of the genotypic variance. These two markers are suspected to flank the causal polymorphism and were therefore combined into a haploblock which explained 93.2% of the genotypic variance (Chapter 6). Chromosome 5A is well-known for *Vrn-A1* and *Fr-A2*, two genes located within a distance of 30 cM from each other (Sutka and Snape 1989; Vágújfalvi *et al*. 2003). Therefore, an additional a candidate-gene analysis for the *Fr-A2*, *Fr-B2*, and *Vrn-A1 locus* was conducted. Most polymorphisms of the used candidate genes were monomorphic, occurred at low frequency, or did not contribute to the proportion of genotypic variance for frost tolerance. The exception were *Fr-B2* and copy number variation (CNV) of *CBF-A14* at the *Fr-A2* locus. Strong associations with frost tolerance were found for *CBF-A14* CNV (Figure 5A), while *Fr-B2* showed rather weak correlation (Figure 5B).

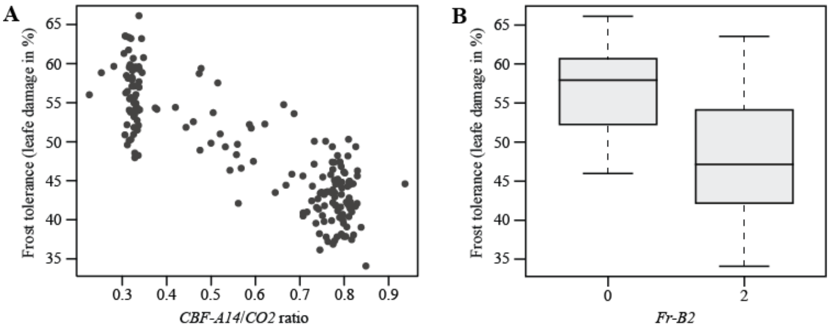

Figure 5 Association between frost tolerance (leaf damage in %) and (A) the signal ratio between CBF-A14 and the control gene TaCO2, and (B) Fr-B2.

After fitting the candidate loci into a model with the found SNP makers, they explained 4.2% (*Fr-B2*) and 91.6% (*Fr-A2*) of the genotypic variance, while the variance explained by the found markers strongly decreased to 0.1 and 3.2% (S2269949 and S1077313, respectively). The decrease of the explained proportion of genetic variance of the SNP markers indicate an association between the haploblock and *CBF-A14* CNV. Additionally, the significant correlation between the haploblock and *CBF-A14* CNV (r = -0.88, P <0.001) confirms the association. The results demonstrate that a major QTL is controlling frost tolerance, with minor contributions from variation at *Fr-B2*. Traits with few underlying major QTL are promising for marker assisted selection, providing a powerful selection tool for frost tolerance.

Frost tolerance increases in genotypes with a Continental European/West-Asian origin, while genotypes from South Europe were characterized by higher frost susceptibility and low winter survival. Further, the frequency of the frost tolerant allele of the haploblock was higher in Continental Europe (Chapter 6). Similar results were found for the signal ratio between *CBF-A14* and the control gene *TaCO2*. It can be assumed that the polymorphism was already under selection and has unconsciously been used to increase frost tolerance in durum wheat. *Fr-B2* followed a similar trend, but - in contrast to the previous mentioned marker - it was almost fixed in the Eastern European and West Asian germplasm. Copy number for *Vrn-A1* increased with a latitudinal gradient from south to north as well. Although *Vrn-A1* was not responsible for the regulation of frost tolerance, the results suggest that *Vrn-A1* CNV might be involved in adaptation (Chapter 6).

UPCOMING BREEDING CHALLENGES AND SELECTION STRATEGIES FOR WINTER DURUM BREEDING

Crop production can be improved by crop management, plant nutrition and protection. This is, however, just a dealing with exogenous factors and has its limits. Breeding on the other hand, improves the genotype. It is possible to improve traits by classical breeding at an early or a later stage in breeding programs (Allard 1999). This depends on the inheritance, heritability, and available evaluation methods for the trait. If measurements are time-consuming and the desired traits only exhibit moderate heritability, selection is normally done at a later stage in the breeding process. As mentioned earlier, the varying occurrence of frost, does not allow a selection for frost tolerance in every year. Also the seed availability in early generations can be problematic, which risks losses in frost nurseries. Selection tools like the SCT offer a platform for a relatively simple selection on frost tolerance in early generations. Nevertheless, developing new varieties using conventional breeding remains time-intensive and is high in costs. New techniques can help to speed up breeding programs or to save money.

The idea of hybrid breeding has already been introduced. Great research efforts are done in bread wheat (Longin *et al*. 2012). Durum wheat is only of minor importance on a global scale compared to bread wheat. Currently, it is rather unrealistic that breeding companies or research institutes cover the costs to develop commercial hybrids. Another option are double haploid (DH) lines (Konzak *et al*. 1987). The DH technique is a helpful tool to save time in a breeding program. Complex traits can be improved easily in early generations because homozygosity can be reached in one step. Considering winter hardiness as a complex trait, influenced by several characteristics (frost tolerance, cold adaptation, de-/hardening, etc.), the DH technology might be a helpful breeding method to screen the breeding material for this trait (Touraev *et al*. 2009). Costs, however, depend on the efficiency of the technology. The routine use of DH in commercial durum wheat variety development might be too expensive with regard on the global importance of winter durum. The application of marker-assisted selection to improve frost tolerance should be considered, since this trait is essential to survive extreme winter conditions and it seems to be controlled by only few major QTLs. Markers allow for a fast and efficient selection during early vegetative stages in plant breeding. Competitive allele specific PCR (KASP) is a fluorescence-based genotyping technology that offers a cost-effective, easily applicable and flexible marker assay (Semagn *et al.* 2014). The haploblock established in this thesis provides a good and reproducible predictive power for frost tolerance (Chapter 6). The two markers of the haploblock could be converted into KASP markers in order to enable a marker-assisted selection for frost tolerance and to facilitate successful winter hardiness improvement in durum wheat.

CONCLUSIONS AND OUTLOOK

Autumn-sown winter cereals are favored in Central Europe. The production of winter durum wheat instead of spring durum wheat would have several advantages, e.g. an increased yield performance. Central European winter durum has a wide range of quality available, which can compete with leading spring varieties, something that is necessary for acceptance in the pasta industry. With a special focus on the trait-trait interaction of grain protein content and vitreosity it was possible to identify a positive correlation between these traits. Vitreosity, however, increased only until a specific threshold in protein content was reached, above this threshold only protein content further increased while vitreosity kept constant. These findings can have huge influences on field management, especially on nitrogen fertilization. The ability to form vitreous kernels depends on the genetic potential to form the glassy character and to maintain it under different environmental factors. A novel trait ‘stability of vitreosity’ will support breeding programs to further enhance quality in durum wheat, the milling and pasta industry will benefit from these increased qualities. The ‘Weihenstephaner Auswinterungsanlage’, a semi-controlled test for frost tolerance testing, reveals the variation that can be used for winter durum breeding. Reliable and significant frost stress data becomes available in early generations with this selection tool. Increased yield performance of winter durum in comparison to spring durum demonstrates the attractiveness for farmers. Negative associations between frost tolerance and yield, or frost tolerance and quality could not be confirmed, increasing the interest of the pasta industry in winter durum. Winter durum production is promising for Central Europe and it is expected that the production area will increase further. The molecular characterization of the worldwide winter durum panel, showed the possibility to conduct high resolution association mapping. A major population structure does not exist in winter durum, but nevertheless, lines had the tendency to cluster in groups of the same origin. Such clusters can be useful for breeders. The clusters can be used to conduct targeted crosses to e.g. further improve frost tolerance in Central Europe material or allow the formation of heterotic groups. In the genome-wide association study, a major QTL for frost tolerance was identified. It could be shown that copy number variation of *CBF-A14* is responsible for variation in frost tolerance in durum wheat. SNP markers associated with *CBF-A14* CNV were converted to KASP markers to enable a marker-assisted selection in plant breeding.

8 SUMMARY

Durum wheat (*Triticum durum*) is a tetraploid wheat that is used for pasta and other semolina products. Quality standards for semolina requested by the pasta industry are very high. Different characteristics should come with the cereal as raw material for an optimal end product. Vitreosity, the glassy and amber quality feature of durum wheat kernels, is an indicator for high semolina yield. The complex protein-starch matrix of glassy kernels breaks the grain into the typical semolina granulate instead of flour during milling. Humid conditions, like late summer rains in Central Europe, have a huge effect on this characteristic, changing this matrix irreversibly. Such processes in the kernel are less understood and challenge plant breeders to find genotypes with improved vitreosity. A set of F_5 winter durum wheat lines (Chapter 2) was used to investigate the relationship between protein content and vitreosity as well as the impact of humidity on the stability of the trait. A method to evaluate the mealy part in kernels was improved and enabled to test for the influence of humidity on vitreosity. Furthermore, it was revealed that the vitreosity of a durum wheat kernel depends on the protein content up to a specific threshold as well as on the genotypic potential to form the complex endosperm matrix. The ability to maintain this kernel quality under humid conditions also highly depends on the genetics of a variety.

In the Mediterranean region, durum wheat is grown as autumn-sown spring type. The mild winters as well as rain during spring allow the plants to develop well, and the dry summers enable an early harvest in June. Durum wheat production in Central Europe, on the other hand, is confronted with harsh winters and recurring severe frosts. The lack of a sufficient frost tolerance in combination with high quality, forces farmers to use the spring type with a spring sowing. Growing winter durum instead of spring durum wheat, would allow an autumn sowing. Using the winter type in this growing area, could have several advantages like an increased yield and stability due to a prolonged growing time. Further, the constant soil coverage would prevent soil erosion and the growth vigor of winter durum has advantages against weeds. The success of winter durum breeding depends on frost tolerance as a key factor for varieties with excellent winter survival. Discontinuous occurrence of frosts across years and protective snow coverage, however, limit the phenotypic selection for this trait under field conditions. Greenhouses or climate chambers could be used as alternative to test under the necessary conditions, but those fully-controlled tests are time consuming and labor-intensive. The 'Weihenstephaner Auswinterungsanlage' are wooden boxes with movable glass lids used as a semi-controlled test. Plants are exposed to all seasonal conditions, including frost stresses, in this test, but they can be protected from snow coverage. While this method is already successfully used to test for frost tolerance in bread wheat, the application in durum wheat has not been evaluated yet. The frost tolerance scorings of winter durum elite lines (F_5 and F_6) based on the 'Weihenstephaner Auswinterungsanlage' were compared to the field evaluation (Chapter 3). It was demonstrated that this semi-controlled test produces reliable and highly

heritable (h^2 = 0.83-0.86) frost tolerance data. The correlation of those results compared with the field data (r = 0.71) suggests this semi-controlled test as an indirect selection platform. Since it is now possible to test cost-efficient at early stages for frost tolerance, the next challenge was to determine whether the kernel quality or the grain yield suffers from an increased frost tolerance. In a survey with F_5 winter durum elite lines, no negative association between frost tolerance and quality or other important agronomic traits could be found in European breeding material (Chapter 4).

In order to support classical plant breeding, which relies predominantly on phenotypic data and parental information, molecular markers can be taken into account. Molecular markers can provide an in-depth look into the genetic architecture of traits, enable the determination of the relatedness of genotypes, identify the genetic variation in a population, or can assess the effect of geographic selection preferences. Furthermore, it is possible to assist knowledge-based selection. This improves plant breeding programs on a genetic level. The population structure in spring durum has already been examined with molecular methods in several studies. Winter durum, on the other hand, was only analyzed as a small group as part of spring durum studies or in groups of landraces.

A highly diverse and unique panel of 170 winter durum and 14 spring durum lines was analyzed using a genotyping-by-sequencing (GBS) approach. A total of 30,611 markers, well distributed across the chromosomes, were obtained after filtering for marker quality. A principal coordinate analysis and a cluster analysis were applied. Together they revealed the absence of a major population structure (Chapter 5). The lines, however, grouped in a certain way, depending on their origin, associated with decreasing quality and increasing frost tolerance moving from South to Continental Europe. These groups allow breeders to conduct targeted crosses to further improve the frost tolerance in the Central European material. Another possibility is to build heterotic groups for hybrid breeding. The linkage disequilibrium (LD) decay was within 2-5 cM, indicating a high diversity in winter durum. The high marker density together with the extent of LD observed in this analysis allows to perform high-resolution association mapping in the present winter durum panel. The 30,611 markers and additional markers for candidate genes in frost tolerance were used to assess the genetic architecture of frost tolerance in durum wheat (Chapter 6). A major QTL was identified on chromosome 5A, likely being *Frost Resistance-A2* (*Fr-A2*). Additional analysis of copy number variation (CNV) of *CBF-A14* at *Fr-A2* support this conclusion. *CBF-A14* CNV explains about 90% of the proportion of genotypic variance. Two markers found in the QTL region were combined into a haploblock and enabled to capture the genetic variance of this QTL. Furthermore, the frequency of the QTL allele for frost tolerance shows a latitudinal gradient which is likely associated with winter conditions.

In summary, the selection tools for vitreosity and frost tolerance provided in this study create a platform for winter durum breeding to select for high quality genotypes with excellent winter survival utilizing phenotypic as well as genotypic information

9 ZUSAMMENFASSUNG

Durumweizen (*Triticum durum*) gehört zu den tetraploiden Weizen und wird für die Herstellung von Pasta und anderer Grießprodukte verwendet. Die geforderten Qualitätsstandards der Pasta-Industrie sind hierbei sehr hoch. Das Rohprodukt muss über verschiedenste Eigenschaften verfügen. Ein wichtiger Indikator für eine hohe Grießausbeute ist die Glasigkeit, sichtbar als typischer glasiger Bernsteincharakter des Durumkornes. Die komplexe Protein-Stärke Matrix des Endosperms veranlasst das glasige Korn während des Mahlprozesses in Gries anstelle von Mehl zu zerfallen. Feuchte Bedingungen, wie Spätsommerregen in Zentraleuropa, haben einen starken Einfluss auf diese Korneigenschaft und verändern hierbei irreversibel die Endospermmatrix. Über diese Vorgänge im Korn ist nur wenig bekannt, was die Selektion auf Glasigkeit erschwert. Daher wurden F_5 Winterdurumlinien verwendet (Kapitel 2), um die Zusammenhänge von Proteingehalt und Glasigkeit sowie den Einfluss von Feuchte auf die Glasigkeit zu untersuchen. Eine verbesserte Bestimmungsmethode der Glasigkeit ermöglichte es, die Veränderung der visuellen Struktur des Korns unter feuchten Bedingungen besser zu verstehen. Des Weiteren wurde festgestellt, dass die Ausprägung der Glasigkeit bis zu einem bestimmten Minimal-Proteingehalt des Kornes durch letzteren stark beeinflusst wird. Außerdem spielt das genetische Potenzial, glasige Körner zu bilden und diese auch stabil zu erhalten, eine wichtige Rolle.

Im Mittelmeerraum wird Durumweizen als Sommerform im Herbst gesät. Milde Winter und Frühjahrsregen erlauben eine starke Pflanzenentwicklung, und trockene Sommer ermöglichen eine frühe Juniernte. Die Hartweizenproduktion in Zentraleuropa ist jedoch mit starken Wintern und wiederkehrenden Frösten konfrontiert. Das Fehlen von Sorten, die eine ausreichende Frosttoleranz mit hoher Qualität kombinieren, zwingt die Landwirte zu einer Frühjahrsaussaat. Würde man jedoch Winterdurum anstelle von Sommerdurum anbauen, könnte eine Herbstaussaat verwendet werden. Vorteile wären zum Beispiel eine Ertragssteigerung, wie auch eine verbesserte Ertragsstabilität. Eine kontinuierliche Bodenbedeckung würde Bodenerosion vermeiden und der Wachstumsvorsprung der Winterform hätte Vorteile hinsichtlich Unkräutern. Die Frosttoleranz ist ein wichtiger Bestandteil für eine gute Überwinterung und spielt eine Schlüsselrolle für eine erfolgreiche Winterdurum-Züchtung. Das unregelmäßige Auftreten von Frost über die Jahre und isolierende Schneedecken, erschweren hierbei eine regelmäßige Selektion auf dieses Merkmal unter Feldbedingungen. Als Alternative könnten z.B. Klimakammern genutzt werden, solche Methoden sind aber zeitaufwändig und arbeitsintensiv. Die ‘Weihenstephaner Auswinterungsanlage’ sind im Feld aufgestellte Holzkisten mit einem bewegbaren Glasdach. Die Pflanzen werden hier allen natürlichen Wetterbedingungen ausgesetzt, unter anderem Froststress da das Dach die Bildung einer Schneedecke verhindert. Diese Methode wird bereits erfolgreich im Weichweizen eingesetzt, wurde aber bisher noch nicht für Durumweizen evaluiert. Frosttoleranzdaten für Winterdurum (F_5 und F_6 Linien) wurden mittels der

Auswinterungsanlage, wie auch auf dem Feld erhoben und verglichen (Kapitel 3). Es konnte gezeigt werden, dass die Auswinterungsanlage nachvollziehbare und hoch erbliche (h^2 = 0.83-0.86) Daten produziert, welche zudem mit den Feldergebnissen stark korrelierten (r = 0.71). Das macht die Auswinterungsanlage zu einer vielversprechenden Plattform für eine Selektion auf Frosttoleranz. Da es nun möglich ist, kostengünstig und zeitsparend bereits in frühen Generationen auf Frosttoleranz zu testen, war es wichtig, zu ermitteln, wie sich Frosttoleranz auf die Kornqualität und –ertrag auswirkt. In einer Studie mit F_5 Elite-Winterdurum konnte keine negative Assoziierung zwischen diesen Merkmalen festgestellt werden (Kapitel 4).

Klassische Pflanzenzüchtung basiert primär auf phänotypischen Daten und Informationen der Eltern. Mit molekularen Markern kann die Selektion tiefgreifender unterstützt werden. Molekulare Marker können einen Einblick in die genetische Architektur von Merkmalen geben, ermöglichen den Verwandtschaftsgrad zwischen Genotypen zu bestimmen, identifizieren die genetische Variation innerhalb einer Population oder beschreiben den Effekt von geografischen Selektionspräferenzen. Des Weiteren unterstützen sie eine wissensbasierte Selektion. Die Nutzen von molekularen Markern kann die Pflanzenzüchtung beschleunigen und präziser machen. Sommerdurum wurde bereits mit Markern in einigen Populationsstudien untersucht, wohingegen Winterdurum nur in kleinen Gruppen solcher Studien mituntersucht oder Gruppen von Landrassen verwendet wurden. Ein hoch diverses und einmaliges Set aus 170 Winterdurum und 14 Sommerdurum wurde mittels eines Genotyping-by-Sequencing Ansatzes untersucht. Nach einer Qualitätsanalyse ergaben sich daraus 30,611 Marker, welche gut über alle Chromosomen verteilt waren. Eine multidimensionale Skalierung und eine Clusteranalyse ergaben, dass es keine größere Populationsstruktur gibt (Kapitel 5). Die Linien gruppierten sich aber zu einem gewissen Grad anhand ihrer Herkunft, assoziiert mit einer sinkenden Qualität und einer steigenden Frosttoleranz von Südeuropa nach Kontinentaleuropa. Solche Cluster erlauben es Züchtern, gezielte Kreuzungen zwischen diesen Gruppen zu machen, um die Frosttoleranz im Zentraleuropäischen Material weiter zu verbessern. Eine andere Möglichkeit wäre es, diese Cluster beizubehalten, um daraus heterotische Gruppen für den Einsatz von Hybriden zu nutzen.

Das Kopplungsungleichgewicht (LD) fiel innerhalb von 2-5 cM unter den Schwellenwert, was eine breite genetische Varianz in Winterdurum signalisiert. Die hohe Markerdichte zusammen mit der Ausdehnung des LD erlaubt, eine hochauflösende Assoziationskartierung in diesem Winterdurum Set durchzuführen. Die 30,611 Marker, inklusive zusätzlicher Marker für Kandidaten-Gene der Frosttoleranz, wurden verwendet, um die genetische Architektur von Frosttoleranz innerhalb von Hartweizen zu analysieren (Kapitel 6). Ein neuer QTL wurde auf Chromosom 5A entdeckt und entspricht dem *Frost Resistance-A2* (*Fr-A2*). Weitere Analysen von *CBF-A14* am *Fr-A2* Lokus, welches in verschiedene Kopien vorkommt (CNV), unterstützen diese Annahme. *CBF-A14* CNV erklärt etwa 90% der genetischen Varianz. Zwei Marker die mit der Region des QTL assoziiert sind, wurden zu einem Haploblock zusammengefasst und ermöglichen es die genetische Varianz des QTL zu erfassen. Die

Frequenz des QTL-Allels für Frosttoleranz verteilte sich entlang der geographischen Herkunft der Genotypen.

Zusammenfassend kann festgestellt werden, dass die neu erarbeiteten Selektionsmethoden für Glasigkeit und Frosttoleranz in dieser Studie eine gute Basis für die Auswahl neuer Winterdurumlinien mit hoher Qualität und guter Winterhärte bilden. Hierbei kann auf phänotypische und genotypische Informationen zurückgegriffen werden.

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DECLARATION

Eidesstattliche Versicherung

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

1. Bei der eingereichten Dissertation zum Thema **Breeding winter durum wheat for Central Europe: Assessment of frost tolerance and quality on a phenotypic and genotypic level** handelt es sich um meine eigenständig erbrachte Leistung.

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

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