TaMFT-A1 IS ASSOCIATED WITH SEED GERMINATION SENSITIVE TO TEMPERATURE IN WINTER WHEAT

By

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Bachelor of Science in Biotechnology

Heilongjiang University

Harbin, Heilongjiang

2010

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2013

TaMFT-A1 IS ASSOCIATED WITH SEED GERMINATION SENSITIVE TO TEMPERATURE IN WINTER WHEAT

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ACKNOWLEDGEMENTS

I would first like to thank Oklahoma State University and the Department of Plant and Soil Sciences for supporting me in completing my master degree. I would also like to express the deepest appreciation to my advisor Dr.Liuling Yan for his guidance and support throughout my projects. I have learned many things of the research idea, bench work since I became Dr. Yan's student. He spent much time instructing me how to design experiment, how to analyze data and result. I am also grateful to thank Dr. Brett Carver and Dr. Million Tadege for spending time read this thesis and providing useful suggestions for it. I also would like to thank my laboratory co-workers, Dr. Genqiao Li, Ming Yu for their help on my research experiments.

This project was supported by the National Research Initiative Competitive Grants CAP project 2011-68002-30029 from the USDA National Institute of Food and Agriculture and the Oklahoma Wheat Research Foundation.

Name: LEI LEI

Date of Degree: JULY, 2013

Title of Study: *TaMFT-A1* IS ASSOCIATED WITH SEED GERMINATION SENSITIVE TO TEMPERATURE IN WINTER WHEAT

Major Field: PLANT AND SOIL SCIENCES

Abstract: The ability of seed to germinate under favorable environmental regimes is critical for seedling emergence, plant establishment, subsequence development and growth of adult plants. It is controlled by internal genetic factors and external environmental factors. Winter wheat in the southern Great Plains is often planted six weeks before the optimal planting date to produce more biomass for cattle grazing during the winter season. High germinability in this higher soil temperature environment is required for this specific management system. In this study, a major QTL for temperature-sensitive germination was mapped on the short arm of chromosome 3A (*QTsg.osu-3A*) in a RIL population generated from two winter wheat cultivars. Furthermore, *TaMFT-A1*, previously reported to regulate seed dormancy and pre-harvest sprouting in spring wheat cultivars, was mapped tightly associated with the peak of *QTsg.osu-3A*. However, allelic variation in *TaMFT-A1* between the two winter wheat cultivars differed from that was observed in spring wheat cultivars. There were 87 SNPs (single nucleotide polymorphisms) and 12 indels (insertions/deletions) in *TaMFT-A1* between the Jagger allele for high germination and the 2174 allele for low germination in the after-ripened seeds, in comparison with 2 SNPs between the two alleles for differential pre-harvest sprouting in spring wheat cultivars. The Jagger *TaMFT-A1* allele is a novel haplotype and appears extensively in winter wheat cultivars. *TaMFT-A1* transcript levels were up-regulated by high temperature but down-regulated by low temperature or seed storage time. These findings suggest that *TaMFT-A1* may invoke different mechanisms for controlling seed dormancy/germination among winter wheat cultivars.

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CHAPTER I

INTRODUCTION

Plant seed germinability, the ability of a viable seed to germinate under favorable environmental regimes, is critical to contribute to seedling performance important for plant establishment and subsequent development and growth of plants [1, 2, 3]. Seed germination is controlled by given internal factors, such as seed dormancy and hormones that delay or prevent germination through physiological mechanisms, and by external factors such as temperature, water, oxygen and light [4]. In plants, temperature is the most influential factor among external conditions controlling seed germinability [5].

The responses of seeds to temperature are complex. The optimum temperature to gain maximum seed germinability is approximately 20-25ºC. A low temperature (<4ºC) can break seed dormancy and promote seed germination [6], whereas a high temperature (>35ºC) has inhibitory effect on germination in wheat [7, 8]. Even after the dormancy is broken, the seeds may not be able to germinate under the high temperature, which confers an adaptive mechanism for plants to germinate with seasonal changes in different geographical areas [9, 10].

Winter wheat in the southern Great Plains is preferred to plant in the early of September or six weeks before the optimal planting date to produce more wheat biomass as forage for cattle grazing during the following winter season [11]. A high germinability of seed

in the presence of high temperature environment is required to adapt to the specific agronomic management system. However, some cultivars can germinate at the optimum temperature but cannot germinate at a high temperature, which is a biological phenomenon called high temperature germination sensitivity. The high temperature germination sensitivity has been reported in other plants, such as thermoinhibition in Arabidopsis [12], or thermodormancy in oat [13] and in lettuce [14].

The molecular mechanism underlying high temperature germination sensitivity may vary by plant species. In Arabidopsis, *FLC* was recently linked to the regulation of temperature-dependent germination; seeds with high-temperature thermodormancy exhibited high *FLC* expression during germination at low temperature [15, 16]. In lettuce, a quantitative trait locus (QTL) *Htg6.1* for thermodormancy (a failure to germinate when imbibed at temperatures above 25-30°C) was identified associated with *LsNCED4*, a gene in the ABA biosynthetic pathway [14]. Mutants exhibiting altered ethylene synthesis or sensitivity have been identified that also exhibited germination tolerance at high temperatures. However, little is known about the genetic basis and molecular mechanism underlying temperature sensitivity germination in wheat.

Genetic approaches have identified several genes that affect seed germination in wheat, but numerous studies on dormancy and germination of wheat seeds have been focused pre-harvest sprouting. More than 15 genetic loci have been reported to associate with this critical trait in a large range of wheat areas worldwide [17], and a QTL that appears to have stable and large effects on dormancy and germination is *QPhs.ocs-3A* on the short arm of chromosomes 3A [18]. In a recent study, *MFT-3A*, a homologue of *MOTHER OF FT* (*Flowering locus T*) and *TFT1* (*Terminal Flower 1*) genes, was mapped to chromosome 3A and associated with the seed dormancy at *QPhs.ocs-3A* in *T. aestivum*. *MFT-3A* is hereafter changed to *TaMFT-A1* which is consistent with wheat gene

terminology. *TaMFT-A1* was mapped based on a single nucleotide polymorphism in the promoter region [2]. The up-regulation of the *TaMFT-3A* transcriptional level was associated with strong dormancy of seed in spring wheat cultivar.

Winter wheat and spring wheat may not share common biological mechanisms controlling seed germination, because their seeds are produced under different environment regimes [11]. Spring wheat seed usually has no or weak dormancy that results in immediate germination even pre-harvest sprouting, whereas winter wheat usually has strong dormancy that prevents germination even for the seed that has been stored for several months [19,20,21,22]. The initial goal of this study aimed to identify genetic loci associated with the sensitivity of seed germination to temperature in winter wheat. After a major QTL for temperature-sensitive germination was mapped to the *TaMFT-A1* locus, this study was switched to analyses on allelic variation in *TaMFT-A1* and regulation of its expression by temperature winter wheat.

CHAPTER II

MATERIAL AND METHODS

Plant Materials

Two winter wheat cultivars, 'Jagger' and '2174' which were observed a large variation in seed germination in our annual nurseries. Based on field observations, Jagger has high seed germination, whereas 2174 has low seed germination. Jagger and 2174 were originally used to generate a population of recombinant inbred lines (RILs) segregating for stem elongation and winter dormancy release in winter wheat [23]; a total of 350 SSR markers were mapped in this population [24]. The two parental lines and their RIL population were tested seed germination at different temperatures in growth chambers with temperature-, photoperiod-, and moisture-controlled conditions.

A total of 121 wheat accessions were genotyped using a new *TaMFT-A1* marker developed in this study, including 34 varieties released in the southern Great Plains in recent years [25], 19 pairs of parental lines that were used to construct mapping populations in the WheatCAP applied genomics project [25], 56 Chinese spring wheat cultivars that were genotyped for *VRN2* genes in a recent study [26], 3 diploid wheat accessions of *T. uratu* (2n=2X =14, AA), and 9 tetraploid wheat accessions of *T. turgidum* (2n=4X=28, AABB). Table 2 summarized genetic materials that were used in this study.

Seed germination experiments

Seeds used for germination experiments were harvested from all RILs of the population grown in field in 2009 and 2010. After harvested, the seeds were stored at room temperature (20 to 22ºC) till the date of experiments. Fifty intact seeds of each RIL or parent were evenly placed on a Petri dish with the lower half of a pre-wetted germination paper, and three replicates for each line were performed. The dishes were incubated in the dark at three temperature regimes: 1) low temperature 4ºC for 1 day and then constant 24ºC for the optimum temperature, 2) constant 24ºC for the optimum temperature, 3) 35/27ºC for day/night simulating the seasonal temperatures at planting in the field. The germinated and ungerminated seeds were counted at 3, 5 and 7 days after planting. The criteria for germination were that either radical or shoot protruded out of seed coat.

Isolation of the complete *TaMFT-A1* gene from winter wheat

Primer MFT-F1M (5'-GGCGCCGACATCGAGTTGTGG-3') and MFT-A1R1 (5'-CATGCAAAGTGTGTGCGTATATATGTACC-3') were used to amplify the complete *TaMFT-A1* from each of the Jagger and 2174 alleles, including predicted 1 kb in the 5' upstream from the start code for translation and 400 bp in the 3' downstream from the stop code for translation, based on the sequences of Chinese Spring. Three nullisomic-tetrasomic (NT) lines, N3AT3B, N3BT3A, and NT3DT3A, were used to determine specialty of the primers to chromosome 3A. PCR reactions were performed at 94℃ for 3 minutes, following 40 cycles of 95℃ for 30 seconds, 57℃ for 30 seconds and 65℃ for 4 minute, with a final extension step at 65℃ for 10 minutes. PCR products were run on a 1% agarose gel. The amplified fragments were purified using Gel/PCR DNA Fragment Extraction kit (IBI), fused to TA vector using a pGEM-T vector System I kit, and transformed to DH5$^{\alpha}$ competent cell. The plasmid DNA from a single colony was extracted and sequenced.

Development of a PCR marker for *TaMFT-A1*

Two PCR markers were developed to map *TaMFT-A1*, which required the digestion of restriction enzymes or the distinguishing of a 3-bp indel between the alleles reported in previous studies [2]. In this study, a PCR marker was developed to map a 12-bp indel for variation between the Jagger and 2174 alleles. Primer MFT-A1F2 (5'-GAGCAAACATGTCCCGGTTCGTT-3') and MFT-A1R2 (5'-ATCACCATGCACACACATACATAAATCACC-3') were used to amplify the region containing 12 indel, and the expected size was 331 bp for the Jagger allele and 319 bp for the 2174 allele. PCR was performed at 95℃ for 3 minutes, following 40 cycles of 95℃ for 30 seconds, 57℃ for 30 seconds and 72℃ for 1 minute, with a final extension step at 72℃ for 10 minutes. PCR products were run on a 2% agarose gel.

Mapping of *TaMFT-A1*

A genetic map was constructed from a set of 350 simple sequence repeat (SSR) markers previously used to map a major QTL for stripe rust associated with *Yr17* in the Jagger x 2174 RIL population (Fang et al. 2011). Each of genetic linkage groups was analyzed for QTLs controlling seed germination characterized in this study. The mean seed germination rate for each line from different environments was analyzed to determine significant effects of a single marker on resistance using one-way analysis of variance (ANOVA).

Expression of *TaMFT-A1*

Primer QRT-MTF-F2 (5'-CCTCTACACCCTCGTGATGA-3') and QRT-TaMTF-R6 (5'-GCACCACCACCTCACCTTTA-3') that were used to test *MFT-3A* expression (Nakamura et al. 2011) were to amplify a cDNA fragment from *TaMFT-A1* of Jagger and 2174. Primers actin-F1 (5'-CTATGTTCCCGGGTATTGCT-3') and actin-R1 (5'-

AAGGGAGGCAAGAATCGAC-3') were used to amplify transcripts of *actin* as endogenous control. The *TaMFT-A1* transcripts were assessed using the SYBR Green PCR Master Mix (Applied Biosystems, Life Technologies) and the Applied Biosystems 7500 Real-Time PCR Systems. Total RNA was extracted using Trizol regents (Invitrogen). RNA samples were treated with Deoxyribonuclease I and first-strand cDNA was synthesized using a SuperScript™ II Reverse Transcriptase kit (Invitrogen). qRT-PCR was performed using a 7500 Real-Time PCR System (Applied Biosystems) and iQ™ SYBR Green Supermix kit (BIO-RAD).

CHAPTER III

RESULTS

Seed germination at the optimal temperature

Both Jagger and 2174 are winter wheat types. The mean germination rate at the optimal temperature (constant 25ºC) was between 30% for 2174 and 60% for Jagger when their seeds were harvested one week (Fig.1). When the time elapsed, the mean germination rate of the seeds stored at room temperature (20-25ºC) at the optimal temperature was significantly increased, reaching approximately 90-95% at 6 weeks after harvest for both of the tested cultivars. These results indicated that the two winter wheat cultivars had some dormancy, and the dormancy of all the seeds had almost completely disappeared by the test time. Jagger showed a significant higher germination rate than 2174; but the difference between the two cultivars was decreased when the time elapsed (Fig.1).

A QTL for seed germination on chromosome 3A

In order to find genomic regions causing the difference in seed germination between Jagger and 2174, the Jagger x 2174 RILs were tested at different temperature regimes (Table 1). The previously reported 350 SSR markers [24] were mapped in the population of Jagger x 2174 RILs. When the phenotypes and genotypes were incorporated, a QTL for seed germination at high temperature and normal temperature was consistently mapped on the distal end of the short arm of chromosome 3A, based on reference map of 5 SSR markers in hexaploid bread wheat [27], and this QTL for temperature sensitivity germination is thus referred to *QTsg.osu-3A* (Fig.2). This QTL did not have genetic effect on germination of seed treated with low temperature (Figures not shown), as almost all

seeds treated with low temperature germinated and no significant difference in seed germination rate among the RILs was observed. In order to confirm the physical location of *QTsg.osu-3A* on chromosome 3A, a gene encoding an ABC transporter that was known to locate on the short arm of chromosome 3A was also mapped in at the *QTsg.osu-3A* locus (Fig.2). *QTsg.osu-3A* spanned approximately 12 cM in genetic distance.

QTsg.osu-3A had LOD scores ranging from 2.6 to 6.2 accounted for 11.8-26.2% of the total phenotypic variation in seed germination tested at different years and different temperatures (Table 1). On average, *QTsg.osu-3A* explained 19.4% of the total phenotypic variation in the seed germination rate tested across years and temperatures, indicating that *QTsg.osu-3A* had a significant and consistent genetic effect on seed germination in winter wheat.

Allelic variation in *TaMFT-A1*

TaMFT-A1 is a gene that is co-localized with *QPhs.ocs-3A.1*, a seed dormancy QTL mapped in spring wheat [2]. In order to test if *TaMFT-A1* is associated with *QTsg.osu-3A* mapped in this study, different primers specific to *TaMFT-A1* were designed, based on sequence alignment of *TaMFT-A1* with other two homoeologous genes, *TaMFT-B1* and *TaMFT-D1*. *TaMFT-B1* and *TaMFT-D1* sequences were derived from wheat genome sequence database (http://www.cerealsdblished.uk.net/) using the fragment sequences originated from Chinese Spring. Surprisingly, many primers that were expected specific to *TaMFT-A1* worked for 2174 but not for Jagger, suggesting that Jagger might have a greatly diversified *TaMFT-A1* gene.

The whole gene of *TaMFT-A1* consisting of 4 exons and 3 introns from each of the Jagger and 2174 alleles was finally isolated using two primers which specificity to chromosome 3A was confirmed using Chinese Spring nulli-tetra lines of Chinese Spring

(Fig.3). The sequenced *TaMFT-A1* gene was 4,423 bp for the Jagger allele (GenBank accession: KF311059) and 4,330 bp for the 2174 allele (GenBank accession: KF311060). The Jagger *TaMFT-A1a* included 1,000 bp upstream from the start codon, 401 bp downstream from the stop codon, and 3,022 bp between the start codon and the stop codon; whereas the *TaMFT-A1b* allele included 1,012 bp upstream from the start codon, 401 bp downstream from the stop codon, and 2,917 bp between the start codon and the stop codon. The final sequence of the 2174 allele was exactly the same as AB571513 (Zen), except for a poly 'G' region, where 2174 had 2 more 'G' than Zen (Fig.4).

Overall, there was 96% identity between the Jagger allele and 2174 allele. A total of SNPs and 12 indels (insertions/deletions) with sequences from 1 to 20 bp were observed between the two alleles (Fig.4). Two SNPs occurred in exons, one that occurred in exon 1 but did not cause alternation of amino acid, and the other that occurred in exon 2 and resulted in the alternation of one amino acid between Arginine residue in Jagger and Lysine residue in 2174.

A PCR marker was developed to map *TaMFT-A1* in the Jagger x 2174 RILs using primers MFT-A1F2 and MFT-A1F2. The amplified region included three indels, the first one showing 20 bp more in Jagger than 2174, the second one showing 8 bp less in Jagger than 2174, and the third one showing 15 bp less in Jagger than 2174. Without digestion, the PCR products from the two alleles were well separated in a 2% agrose gel (Fig.5). *TaMFT-A1* was mapped under the center of *QTsg.osu-3A* found in the population of Jagger x 2174 RILs (Fig.2).

The effect of *TaMFT-A1* on seed germination

When the population was tested in 2009 (Fig.6A) and 2010 (Fig.6B), the averaged seed germination rate of the population was increased with the storage time. The trend was stable when seed was incubated at normal temperature and high temperature. At 15 days

after harvesting, the averaged seed germination rate of the population was 17.8% at high temperature, 51.6% at normal temperature, and 90% after the seed was treated with low temperature. These results indicated that the seed dormancy was broken by low temperature, but the seed germination was inhibited by high temperature, compared with the germination at normal temperature.

The Jagger x 2174 RILs were grouped into two types, one carrying the Jagger *TaMFT-A1a* allele and the other carrying the 2174 *TaMFT-A1b* allele. As shown in Fig.6, *TaMFT-A1a* promoted seed germination, or *TaMFT-A1b* inhibited seed germination. Genetic effects of *TaMFT-A1* on seed germination were reflected at significant levels when the seed germination was tested at 15 days after harvesting at normal temperature ($p<0.05$) and high temperature ($p<0.01$) in 2009, and at 45 days after harvesting at normal temperature ($p<0.05$) and high temperature ($p<0.01$) in 2010. The germination rate at the other testing times was consistently higher in the lines carrying the Jagger *TaMFT-A1a* allele than the lines carrying the 2174 *TaMFT-A1b* allele, but the differences were not significant in statistical analyses (Fig.6).

Regulation of *TaMFT-A1* expression by temperature and seed storage time

Using RT-PCR, the *TaMFT-A1* transcript levels in germinated seeds of Jagger and 2174 at normal temperature (25ºC) were determined (Fig.7A). At 2 weeks after harvesting, the Jagger *TaMFT-A1a* transcript level was 7.7, and the 2174 *TaMFT-A1b* transcript level was 29.5, which was approximately 3.8 folds of *TaMFT-A1a.* The *TaMFT-A1a* transcript level was significantly decreased to 3 by 4 weeks and 2.7 by 7 weeks after harvesting. The *TaMFT-A1b* transcript level was significantly decreased to 23 by 4 weeks and 11.7 by 7 weeks after harvesting. These results indicated that the *TaMFT-A1* transcript levels at the normal temperature were down-regulated by the seed storage time.

In order to investigate how *TaMFT-A1* was regulated by temperature, the *TaMFT-A1* transcript levels in 2174 seeds treated with three temperature regimes were tested: i) continuous normal temperature (NT, 25ºC), ii) low temperature (LT, 4ºC), and iii) high temperature (HT, 37ºC). As shown in Fig.7B, the *TaMFT-A1* transcript level in germinated seeds treated with low temperature was 2.4, which was 45.3% in seeds germinated at the normal temperature. The *TaMFT-A1* transcript level in germinated seeds treated with high temperature was 7.1, which was increased 34% compared with the seeds germinated at the normal temperature.

Diversity of *TaMFT-A1* among different plody wheat species

The primers MFT-A1F2 and MFT-A1R2 utilized for mapping were used to genotype cultivars/accessions of different plody wheat species. As summarized in Table S1, among 34 winter wheat varieties released in the southern Great Pains in recent years, one half of them were found to carry the Jagger *TaMFT-A1a* allele and the other half were found to carry the 2174 *TaMFT-A1b* allele. Among 19 pairs of parental lines that were used to construct mapping populations in the WheatCAP applied genomics project, 24 parental lines were found to carry the Jagger *TaMFT-A1a* allele and the remaining 14 parental lines were found to carry the 2174 *TaMFT-A1b* allele. Among 56 Chinese spring wheat cultivars/landrace, 20 were found to carry the Jagger *TaMFT-A1a* allele and the remaining 36 were found to carry the 2174 *TaMFT-A1b* allele. These results indicated that the *TaMFT-A1a* allele discovered in this study was not unique in Jagger, but it has been extensively utilized in many cultivars in different geographical areas.

The Jagger *TaMFT-A1a* allele is a novel haplotype, compared with the previously reported alleles, the Chinese Spring CS *TaMFT-A1* allele (AB571512) and the Zen *TaMFT-A1* (AB571513). In order to determine which allele is the ancestral type of *TaMFT-A1*, the primers MFT-A1F2 and MFT-A1R2 utilized for mapping were used to

genotype diploid and tetraploid wheat species. Among 9 tetraploid wheat accessions of *T. turgidum* tested, only 1 accession showed the same allele as Jagger and the other 8 accessions showed the same allele as 2174, suggesting that *TaMFT-A1* has been diversified at the tetraploid level. The complete sequence of *MFT-A1* from *T. turgidum* ssp. *durum* that was used to construct BAC library[28], the *MFT-A1* allele from the tetraploid wheat (GenBank accession: KF311061) showed 96% identity and 113 SNPs or indels compared to the Jagger *TaMFT-A1a* allele and 98% identity and and 50 SNPs or indels compared to the 2174 *TaMFT-A1b* allele. All of 3 diploid wheat accessions of *T. urartu* tested showed the same allele as Jagger. However, identity of 844 bp including partial exon 1, exons 2 and 3 from the *MFT-A1* of *T. urartu* (PI 428183) (GenBank accession: KF311062) was 94% to the Jagger *TaMFT-A1a*, 97% to the 2174 *TaMFT-A1b*, and 97% to the *T. durum MFT-A1*. The *MFT-A1* of *T. urartu* showed multiple SNPs or indels with the homologous genes in tetraploid and hexaploid wheat suggested that the *MFT-A1* gene has greatly diverged during the evolution from diploid through tetraploid to hexaploid wheat.

Table 1 A summary of genetic effects of *QTsg.osu-3A* on seed germination under various temperatures

Year	Temperature (°C)	Days after harvesting	LOD	Genetic effect (%)
2008	25°C	45	3.9	19.3
	37°C	45	3.6	18.7
2009	25°C	45	4.8	22.5
	37°C	45	6.1	25.5
2010	25°C	15	6.2	22.5
	37°C	15	2.7	12.5
	37°C	30	2.6	11.8
	37°C	45	4.8	22.5

Table 2 Wheat cultivars/accessions used for determining the frequency of the *TaMFT-A1* alleles

Allele	Source	Cultivar/line/landrace/accession
Jagger allele	2X wheat	*T. urartu* (PI 428183, PI 428180, PI428323)
		Turgidum (PI 352541)
	4X wheat	Yang9663, Huaimai19, Yannong578, Xinmai19, Ning0088, Ningmai11, Wanmai33, Hao9409, Yangmai16, Yangmai18, Xinmai18, Yanfu188, Suxu3, Lianmai1, Pin14, Yannong19, Jinan13, Baifengmai, Yannong21, Zhengmai004
	Chinese cultivars	
	CAP	UC 1110 (CA), CIMMYT-2 (PI 610750) (CA), IDO444 (ID), Zak (ID), Stephens (OR), OR 9900553 (OR), Finch (WA), Eltan (WA), Louise (WA), Panawawa (WA), GRN*5/ND614-A (MN), NY18/ Clark's Cream 40-1 (MN), Platte (CO), CO 940610 (CO), Jagger (OK, NE), Harry(KS), Heyne (KS), Wesley (KS), Weebill (TX), Pio 25R26 and Foster (NY), Cayuga (NY), USG 3209 (VA).
	SGP	Fuller, Jagger, OK Bullet, Santa Fe, Shocker, Endurance, Doans, Deliver, Jei 110, Guymon, Lakin, Tam 112, Neosho, Duster, Protection, Endurance, Ripper
2174 allele	4X wheat	*T. durum* (PI 366990, PI 191654, PI 384392), *T. turgidum* (PI 347135, PI 113393, PI14082), *T. dicoccum* (PI265004, PI 286061).
	Chinese cultivars	Sushenmai1, Yang9817, SuB, Yangmai158, Wanmai147, Yangmai11, Yang0188, Huamai0027, Yang05-334, Yang06-311, Yangmai7, Taikong5, Yangmai19, Yangmai17, Yangfumai1, Sushenmai1, Yangmai158, Yang06-135, Yang06-615, Yang05-311, Yang03-77, Yang06-385, Guandong107, Yang0135, Gaoyou503, Yangmai12, Hu438-15, Yangfymai2, Baiyingdong2, Baihuomai, Heixiaomai, Zixiaomai, Zheng99081, Wanmai18, Yangfu0082, Xumai26.
	CAP	Rio Blanco (ID), ID 0556 (ID), McNeal (MT), Thatcher (MT), TAM 105 (NE), KS01HW163-4 (KS), 2174 (OK), Jupeteco (TX), SS 550 (GA), PIONEER 26R46 (GA), P91193 (IN), P92201 (IN), Cayuga (NY), Jaypee (VA).
	SGP	OKField, Centerfield, Overleg, Jagalene, 2174, Tam 111, Cutter, Fannin, Danby, Trego, Ok 102, Tam 110, Above, Custer, Hatcher, Intrada, Overleg.

CAP: Coordinated Agriculture Project. SGP: Southern Great Plains.

Fig.1

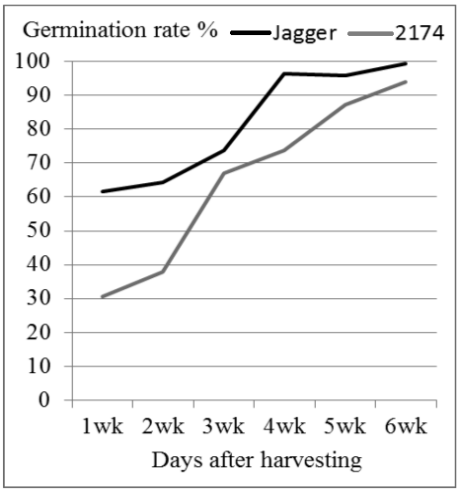

Fig.1 Germination rate of Jagger and 2714. The experiment was performed in 2011. Seed collected from a greenhouse was tested 1, 2, 3, 4, 5, and 6 weeks after harvesting in an incubator where constant temperature at 24 ºC was set up. The germination rate was an average of three replicates, and no standard error was calculated.

Fig.2

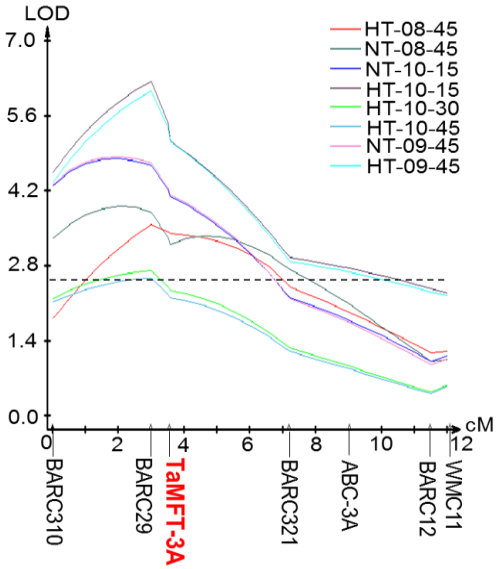

Fig.2 Chromosomal location and genetic effects of *QTsg.osu-3A* for seed germination. The QTLs were characterized at high temperature (HT) and normal temperature (NT) in years 2008, 2009, and 2010, when seed was harvested 15, 30, or 45 days. Germination rate was tested in the recombinant inbred lines (RILs) of the Jagger × 2174 population. Molecular markers along the chromosome are placed as centimorgans on the horizontal axis. The horizontal dotted line represents a common threshold value of 2.5 LOD.

Fig.3

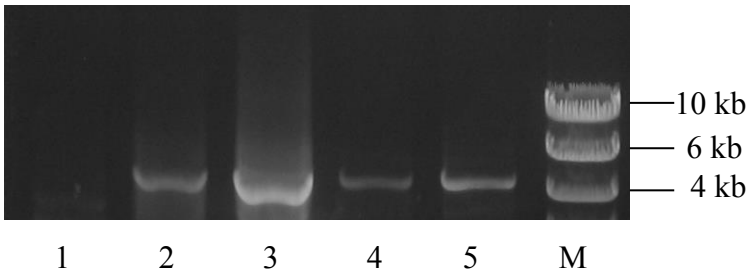

Fig.3 Specific amplification of *TaMFT-A1*. Primer MFT-F1M and MFT-A1R1 were used to amplify the complete *TaMFT-A1* from three nullisomic-tetrasomic (NT) Chinese Spring (CS) lines, N3AT3B (1), N3BT3A (2), NT3DT3A (3), as well as Jagger (4) and 2174 (5).

Fig.4

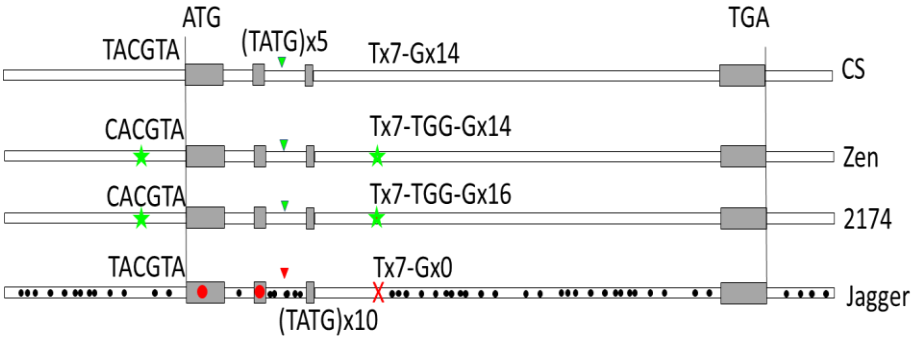

Fig.4 Diagram for mutated sites at the Jagger *TaMFT-A1* haplotype. Star symbol indicates positions of two reported polymorphisms in the CS allele for weak dormancy compared with the Zen allele for strong dormancy. Triangle star indicates the position of polymorphic site in intron 2 due to the presence of the poly 'G'. Dot symbol indicates mutation sites throughout the Jagger *TaMFT-A1* gene compared with the other three alleles.

Fig.5

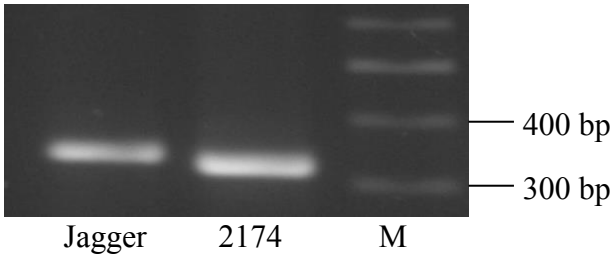

Fig.5 A PCR marker for *TaMFT-A1*. Primer MFT-A1F2 and MFT-A1R2 were used to amplify *TaMFT-A1* from Jagger (331 bp) and 2174 (319 bp). PCR products were directly run on a 1% agarose gel.

Fig.6

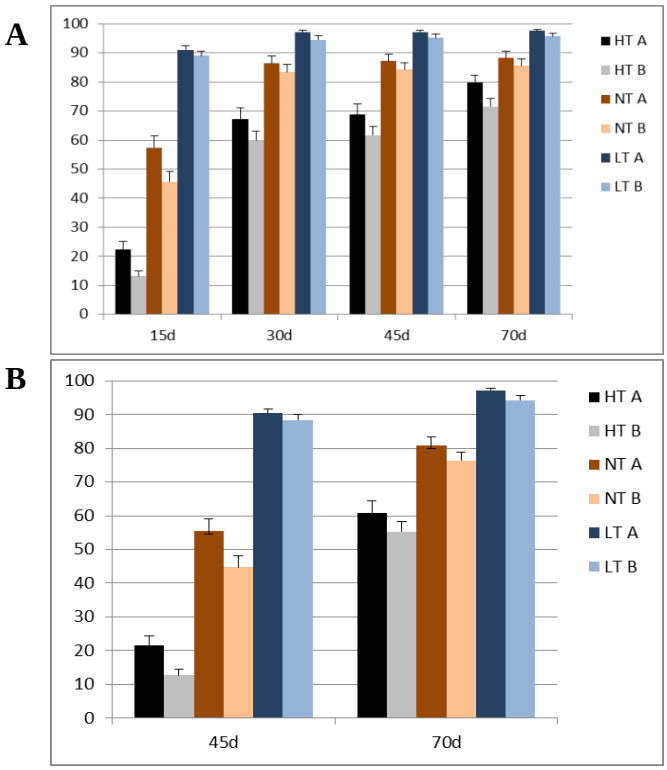

Fig.6 Genetic effect of *TaMFT-A1* on germination rate. The germination rate was averaged from each of the Jagger allele (A) or the 2174 allele (B) in the population (n=96) that were characterized at high temperature (HT) and normal temperature (NT) in 2009 (Fig.6A) and 2010 (Fig.6B), when seed was harvested 15, 30, 45, or 70 days. Bar indicates standard error.

Fig.7

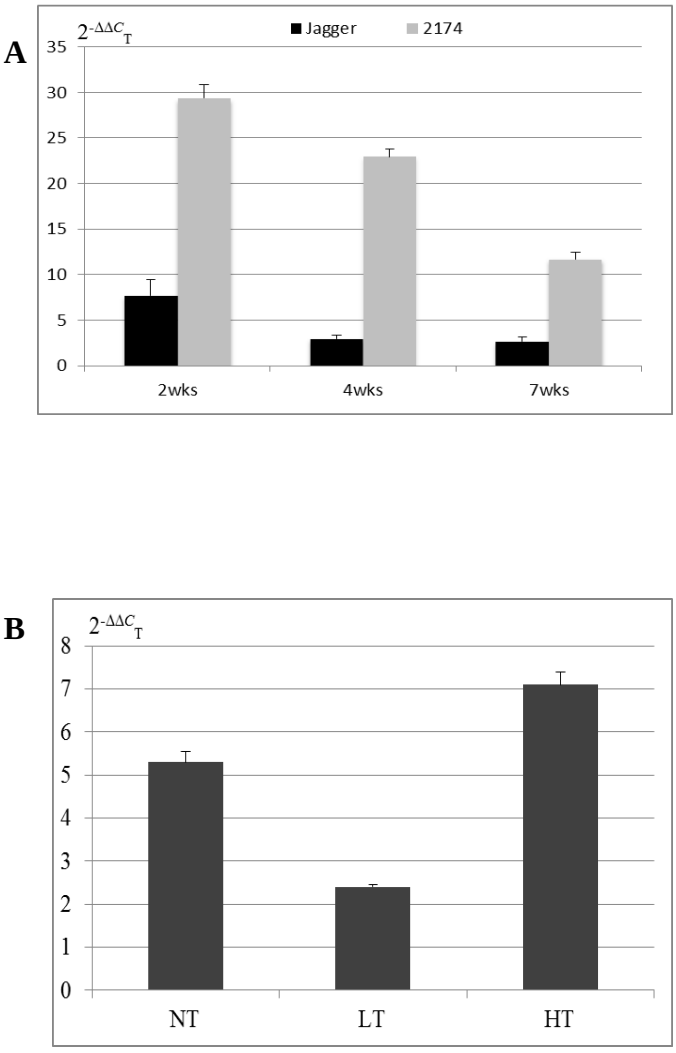

Fig.7 Expression profiles of *TaMFT-A1*. A) Transcript levels of *TaMFT-A1a* (the Jagger allele) and *TaMFT-A1b* (the 2174 allele) in the after-ripened seeds. RNA samples were collected from embryos of seeds at 2 weeks (2wks), 4 weeks (4wks), and 7 weeks (7wks) after harvest. B) Regulation of *TaMFT-A1* transcript levels by temperature. The RNA samples were collected from 2174 seeds that were treated with normal temperature (NT, 25℃), low temperature (LT, 4℃) for overnight, and high temperature (HT, 37℃)

for 5 days. Transcript levels are shown using the values calculated by the $2^{(-\Delta\Delta CT)}$ method, where CT is the threshold cycle, and *actin* was used as an endogenous control. The values represent mean expression levels (n=12), and the bar indicates standard error.

CHAPTER V

DISCUSSION

Previous studies on seed dormancy and germination in wheat have been focused on pre-harvest sprouting in spring wheat. More than 15 genetic loci have been reported that are responsible for this critical trait in a large range of wheat areas worldwide, including *QPhs.ocs-3A.1* on the short arm of chromosome 3A that repeatedly mapped using RILs derived from a cross between the highly dormant wheat cultivar Zenkoujikomugi (Zen) and the less dormant cultivar Chinese Spring (CS) [2,18,28,29]. *TaMFT-A1* was mapped co-segregated with a SSR marker *Xbarc310* on the short arm of chromosome 3A in a F_2 population derived from a cross between CS and CS (Zen3A) [2]. In this study, we mapped *QTsg.osu-3A* for seed germination in the population that was generated from a cross between two winter wheat cultivars and found that *QTsg.osu-3A* was tightly associated with *TaMFT-A1*. Liu et al. [1] also found that *Xbarc310* was associated with a major QTL for pre-harvest sprouting in the US hard winter (HWW) wheat cultivar 'Rio Blanco'. These studies have pointed to *TaMFT-A1* which may play an important role in moderation of seed dormancy/germination under various temperature conditions in spring and winter wheat cultivars. This may provide an

opportunity to compromise the efforts of *TaMFT-A1* on seed dormancy, pre-harvest sprouting, and high temperature germination sensitivity in wheat breeding.

One of the initial aims in this study was to explore the genetic loci involved in controlling high temperature germination sensitivity. The results indicated that differential germinability under high temperature between Jagger and 2174 was partly due to the *TaMFT-A1* locus. The dormancy rescues in the after-ripened seeds of winter wheat cultivars could be immediately released by low temperature, gradually released with storage time at normal temperature, and maintained at high temperature. The down-regulation of the *TaMFT-A1* transcript level by low temperature and up-regulation of *TaMFT-A1* transcript level by high temperature were consistent with the effects of the temperatures on seed germination in winter wheat. This study suggested that *TaMFT-A1* played a decisive role in the variation in seed germination in winter wheat cultivars when they were planted earlier to produce more biomass to graze the cattle in the southern Great Plains.

The *TaMFT-A1* gene is reported to promote or maintain seed dormancy in embryos matured at lower temperature (13°C) in comparison to favorable temperature (25°C) in wild type wheat, which is confirmed by the overexpression of *TaMFT-A1* preventing germination in transgenic wheat [2]. The previous study indicated a contrasting effect of the lower temperature on *TaMFT-A1* with this study that showed that this gene was down-regulated by low temperature. The difference is probably due to the seed state difference (seeds before physiological

maturity vs. after-ripened seeds). *MFT* in Arabidopsis was also reported to promote embryo growth during the germination of after-ripened seeds through negatively regulating ABA, as seeds having *mft* loss-of-function are hypersensitive to ABA [16]. Low temperature is usually used to promote seed germination in winter wheat research and production, though a mechanism involving this alternation is unknown. This study indicated that *TaMFT-A1* is a repressor in germination of the after-ripened seeds in winter wheat, and the repressor in the after-ripened seeds can be removed by low temperature.

Two polymorphic sites in *TaMFT-A1* exist between the CS allele for weak dormancy and the Zen allele for strong dormancy. One SNP is located 222 bp upstream from the initiation codon of *TaMFT-A1*, which is believed to be a cause of differential expressions due to the presence of a substitution of T in non-dormant CS with a C in the dormant Zen cultivar that occurs in the A-box motif, a bZIP transcription factor binding site [2]. The 2174 allele for the low seed germination was exactly the same sequence in the promoter as the Zen allele for the high seed dormancy, supporting that the polymorphic site is important in controlling *TaMFT-A1* expression. However, the Jagger *TaMFT-A1* allele that was found in this study for the high germination of the after-ripened seeds is very different in sequence from the CS *TaMFT-A1* allele that was identified for the low dormancy of the immature seeds. It is not known if the Jagger *TaMFT-A1* allele and the CS *TaMFT-A1* allele have different mechanisms in regulating seed dormancy and germination. If it is that case, the Jagger *TaMFT-A1* allele in this winter wheat can be introduced to spring wheat cultivars as to avoid pre-harvest

sprouting. Winter wheat cultivars have fewer problems with pre-harvest sprouting than spring wheat cultivars (Liu et al. 2008). No pre-harvest sprouting was observed in Jagger or other winter wheat cultivars derived from pedigrees of Jagger in nurseries for years [11].

MFT is a homologue of the phosphatidylethanolamine-binding proteins (PEBP) *FT* and *TFL1*, which have opposite roles in the promotion of flowering (30, 31, 32). *FT1* on chromosome 7B in the temperate crops showed the most robust induction of flowering under long days in wheat [33]. *FT-D* on chromosome 7D was found to affect physiological maturity in the same population as *TaMFT-A1* [34]. However, no significant genetic effect was observed for the *FT-D* gene to affect seed germination or for *TaMFT-A1* to affect physiological maturity, suggesting that the two members of the *FT* gene family have functionally diversified during the evolution of wheat.

The previous study suggested that the variation between CS and Zen was mutated during domestication of common wheat [2]. However, the substantial variation between the 2174 *TaMFT-A1b* allele and the Jagger *TaMFT-A1a* allele suggested that the two *TaMFT-A1* alleles should be originated from different ancestral genes in diploid and tetraploid wheat from 2174 or Zen. It has been repeatedly reported that common wheat cultivars have different tetraploid wheat donors [37]. These greatly diversified genes have facilitated developing PCR markers and their utilization in wheat molecular breeding.

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VITA

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