Exploiting genetic diversity in *Aegilops tauschii* to improve wheat flour quality under heat stress conditions

(高温ストレス条件下での小麦粉の品質改良に向けたタルホコムギが持つ遺伝的多様性の活用)

Ikram Elsadig Suliman Mohamed

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A Thesis Submitted to the United Graduate School of Agricultural Sciences, Tottori University in partial fulfillment of the requirements for the award of Doctoral of Philosophy (PhD) in Dryland Agriculture (Plant Molecular Breeding)

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DEDICATION

I dedicate this work to my father, who has given me and continues to give me every chance to find my way in life.

Ikram Elsadig
2022

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List of Abbreviation

HWW-GS	High-molecular-weight glutenin subunits
MSD	Multiple synthetic derivatives
GWAS	Genome wide association study
MTAS	Markers traits association

CHAPTER ONE

GENERAL INTRODUCTION

Genetic diversity in the population germplasm is crucial. Where is considered the best way to adapt to fluctuating environmental conditions by giving a chance for natural selection for desirable traits. Many major crops have a narrow genetic diversity, such as common wheat (*Triticum aestivum* L.), the most imperative food crop worldwide. It provides a quarter of the annual requirement of plant protein, dietary fiber, and carbohydrates (Shewry and Hey 2015). The narrow genetic diversity of common wheat is attributed to a bottleneck effect during the polyploid evolution of common wheat and intensive selection during the breeding process in recent decades (Kumar *et al.* 2019a).

In wheat, seed storage proteins are classified into two major components, monomeric gliadins, and polymeric glutenin. The gliadins are vicious, provide extensibility, and are stretchable, whereas glutenins give elasticity to bread dough (Payne 1987a). When gliadins and glutenins are mixed with water, they produce visco-elastic dough. These physical properties are associated with the functional traits of flour quality. For example, flour dough that exhibits appropriate gas-holding properties is required for bread making, whereas dough that exhibits weak gas-holding properties is necessary for making cookies and cakes. Therefore, the elasticity of the dough affects the quality and suitability of wheat flour to process into different end-products. Thus, among these two types of seed storage proteins, the high-molecular-weight glutenin subunits (HMW-GSs) of the glutenin have a significant impact on wheat flour quality. Because they are the primary factor that determines the gluten elasticity, and thus, they are essential for the bread-making process (Tatham *et al.* 1985; Shewry *et al.* 2003)

Although the HMW-GSs constitute about 10% of seeds storage proteins, about 80% of the variation in the Alveograph w value (which is a combined measure of dough strength and extensibility) can be attributed to variations in HMW-GSs composition and protein content (Payne *et al.* 1988). Therefore, the broadening in the variation of HMW-GS alleles potentially leads to increased varieties of options for wheat flour end-products.

The genes encoding for HMW-GSs are located on the long arms of chromosomes 1A, 1B, and 1D at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively (Payne *et al.* 1982; Payne 1987a). Several investigations and explorations were undertaken in common wheat to find HMW-GSs that have a good effect on dough strength and flour quality. The quality of wheat flour is greatly affected by alleles present on the *Glu-D1* locus (Payne *et al.* 1987; Kolster *et al.* 1991). Payne et al. (1987) invented the Glu-1 quality score system based on worldwide observations relating to correlations between HMW-GS patterns and wheat quality. It has been proved that subunit 5+10 at the *Glu-D1* locus has the highest positive effect on flour quality and the high Glu-1-score compared to subunit 2+12 and other subunits at the *Glu-1* locus. Subunit 5 contains an extra cysteine residue due to

replacing amino acid at position 118, which led to replacing a serine residue with cysteine at the beginning of the repetitive domain (Anderson *et al.* 1989). This extra cysteine in subunit 5 permits linkage via intermolecular disulfide bonds and the formation of larger insoluble polymers. The polymer size and complexity of the gluten protein in the mature grain and changes in the dough configuration phase are essential for bread making (Johansson *et al.* 2013). Thus, varieties that possessed subunit 5+10 at the *Glu-D1* locus exhibiting good elasticity and strong dough characteristics are desired for bread making (Payne *et al.* 1981; Redaelli *et al.* 1997). Therefore, it has been associated with good bread-making quality in commercial bread wheat cultivars grown in Canada (Bushuk 1998), Great Britain (Payne *et al.* 1987), Norway (Uhlen, 1990), the United States (Dong *et al.* 1991), Syria (Mir Ali *et al.* 1999), Germany (Wieser and Zimmermann 2000), and New Zealand (Luo *et al.* 2001).

However, the superior glutenin alleles are still restricted in the modern common wheat (Wang *et al.* 2012b). Also, the germplasm available to breeders is not diverse enough to facilitate the selection of superior alleles or lines.

Grain yield and grain protein content are crucial factors determining the economic value of common wheat. Attempts have been made by the breeding programs targeting to increase the grain protein content and, at the same time, maintain a high grain yield. However, the negative relationship between the grain protein content and the grain yield is still the primary constraint in developing lines that combine high yield and high protein content (good quality). Most studies have proven the negative relationship between grain protein content and grain yield(Löffler *et al.* 1983; Kibite and Evans 1984; Cox *et al.* 1985; Gauer *et al.* 1992; Marinciu *et al.* 2008; Giancaspro *et al.* 2019; Taheri *et al.* 2021). Thus, grain quality and high yield potential are still among the most critical goals for breeders to develop superior wheat cultivars. Besides that, the protein content, grain quality, and yield are substantially influenced by environmental changes, especially heat stress.

Heat stress is classified as one of the abiotic factors that have a tremendous adverse impact on wheat grain yield and quality worldwide. During the grain filling stage, high temperatures (>30–35°C) are reported to have a negative influence on bread dough strength ((Randall and Moss 1990), due to accompanying alterations in the composition of gluten proteins ((Daniel and Triboi 2000), and altering the ratio of gliadin to glutenin (Blumenthal et al., 1995). With rising temperatures and warnings of an increase in global warming over time, it is necessary to understand the impact of high temperatures on wheat quality and grain yield and, evaluate the different responses of genotypes to high temperatures are crucial. As the effect of high temperatures is not understood regarding some characteristics such as the effect of HMW-GSs on dough strength under high temperatures. Therefore, understanding the effect of heat stress on all these things is imperative in to identify resilient genetic resources/materials that combine both heat

tolerance and good end-use quality.

The studies conducted for identifying heat-tolerant genotypes was focused on evaluating the influence of heat stress on yield or yield-related traits without addressing the quality aspects (Reynolds et al., 1994; Mondal et al., 2015).

On the other hand, most studies on wheat quality, have been carried out with a small number of genotypes or under-controlled environments (Wrigley et al., 1994; Blumenthal et al., 1995; Stone et al., 1997; Spiertz et al., 2006).

Moreover, the genetic basis of the diversity resilience to wheat quality under heat stress has not been fully explored. Also, reports on genome-wide association studies for wheat quality traits and grain yield under continuous heat stress in the field are scarce.

The germplasm available to breeders is not diverse enough to select climate-resilient lines under heat-stressed conditions due to the narrow genetic diversity of the common wheat (Ogbonnaya et al., 2013). *Aegilops tauschii* (2n = 2x = 14, DD), the D genome donor of common wheat, is a valuable resource of genetic diversity for the endosperm proteins gliadin and glutenin and stress resilience (Lagudah and Halloran, 1988; Pflüger et al., 2001; Ogbonnaya et al., 2013; Tsujimoto et al., 2015; Cox et al., 2017; Kishii, 2019). Thus, to explore the genetic diversity of *Ae. tauschii* for wheat improvement, a multiple synthetic derivative (MSD) panel has been developed using 43 *Ae. tauschii* accessions covered the diversity of its natural habitat entire natural habitat (Sohail et al., 2012; Tsujimoto et al., 2015; Gorafi et al., 2018). These 43 *Ae. tauschii* accessions have been divided into three intraspecific lineages: TauL1, TauL2, and TauL3 (Matsuoka et al., 2013).

The objectives of chapter two were to i) explore and investigate the genetic diversity of HMW-GS from *Ae. tauschii* at the *Glu-D1* locus, and to ii) evaluate their expression and effects in the background of a common wheat cultivar regarding dough strength, protein content, and grain yield potential.

The objectives of chapter three to i) explore the effect of heat stress on flour quality and grain yield under moderate and continuous heat stress in the field, ii) identify MTAs associated with quality and grain yield traits under heat stress conditions, iii) identify stress resilience lines which combine both grain yield and good quality traits and iv) assess to which extent the *Ae. tauschii* diversity can be harnessed to improve wheat quality under heat stress conditions.

CHAPTER TWO

Enhancing wheat flour quality through introgression of high-molecular-weight glutenin subunits from *Aegilops tauschii* accessions

2.1 Introduction

Genetic diversity is essential for crop adaptation to diverse and fluctuating environmental conditions. The genetic diversity of common wheat (*Triticum aestivum* L.) has narrowed due to a bottleneck effect during the polyploid evolution of common wheat and intensive selection during the breeding process in recent decades. This narrow genetic diversity often restricts the improvement of many traits in wheat (Kumar *et al.* 2019a).

Grain yield and grain protein content are important factors affecting the economic value of common wheat. Many breeding programs aim to increase the grain protein content and simultaneously maintain a high grain yield. However, the well-documented negative relationship between grain protein content and grain yield is still a major challenge to producing lines that combine high yield and high protein content and hence good quality (Kibite and Evans 1984; Cox *et al.* 1985; Gauer *et al.* 1992; Delzer *et al.* 1995; Marinciu *et al.* 2008; Giancaspro *et al.* 2019; Taheri *et al.* 2021). In addition, the protein content and grain yield are strongly affected by environmental changes. One well-known example is that high temperatures after anthesis reduce grain yield because individual kernel weights are lower (Sofield *et al.* 1977; Tahir *et al.* 2006), and alter protein content and composition (Kolderup 1975; Tahir *et al.* 2006). Wheat quality is essentially determined by both the composition and the amount of glutenin and gliadin, the two major components of gluten. The polymeric glutenins, comprising high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GSs), are the main determinant of the unique dough elasticity of wheat flour (Tatham *et al.* 1985; Payne 1987a; Shewry *et al.* 2003). The genes encoding HMW-GS are *Glu-A1*, *Glu-B1*, and *Glu-D1* loci (Payne *et al.* 1982, Payne 1987a).The *Glu-D1* locus has the strongest effect, followed by the *Glu-B1* and *Glu-A1* loci (Yang *et al.* 2013). Although HMW-GS constitute about 10% of seed storage proteins, about 80% of the variation in the Alveograph *w* value (which is a combined measure of dough strength and extensibility) can be attributed to variations in HMW-GS composition and protein content (Payne *et al.* 1988). Therefore, broadening the variation of HMW-GS alleles would potentially lead to increased options for developing wheat flour used in a variety of end-products. Several investigations and explorations have been undertaken in common wheat to find HMW-GS that have significant effects on dough strength. It has been proved that subunit 5+10 at *Glu-D1* locus has the highest positive effect on dough strength (Payne 1987a) because the subunit 5 contains an extra cysteine residue at the beginning of the repetitive domain (Anderson *et al.* 1989). However, the number of excellent glutenin alleles is still limited in common wheat (Wang *et al.* 2012a). Also, the germplasm available to breeders is not diverse enough to facilitate the selection of superior lines.

Many genes from *Aegilops tauschii* have been successfully transferred to common wheat using synthetic hexaploid wheat (SHW) (Mujeeb-Kazi *et al.* 1996; Gill *et al.* 2008; Halloran *et al.* 2008; Ogbonnaya *et al.* 2013). The SHW has the same genome constitution as common wheat, so the chromosomes/genes introduced through crosses are stably transmitted to the offspring. *Ae. tauschii*, the D genome donor of common wheat, is a valuable resource of genetic diversity for the endosperm proteins gliadin and glutenin. Furthermore the SHW has a high yield potential compared to bread wheat (Lagudah and Halloran 1988; Pflüger *et al.* 2001; Elbashir *et al.* 2017a; Kumar *et al.* 2019a). Thus, *Ae. tauschii* can be used as a resource for increasing genetic variation and combining superior alleles for both grain yield and grain quality. However, expression of the genes that affect quality could be completely different when transferred into common wheat (Pflüger *et al.* 2001). Therefore, to evaluate the effects of these genes in the background of common wheat, a panel of multiple synthetic derivatives (MSD) has been developed using 43 *Ae. tauschii* accessions that represent the existing diversity in the entire natural habitat (Sohail *et al.* 2012; Tsujimoto *et al.* 2015; Gorafi *et al.* 2018). These 43 *Ae. tauschii* accessions (Table 1) have been classified into three intraspecific lineages: TauL1, TauL2, and TauL3 (Matsuoka *et al.* 2013). The MSD makes it a powerful platform to detect and quantify the effect of the *Ae. tauschii* and that is why several studies could detect the impact of the *A. tauschii* segments on heat, drought, and seed shape characteristics (Elhadi et al., 2021b; Itam et al., 2021)

The objectives of this study were to explore and investigate the genetic diversity of HMW-GS from *Ae. tauschii* at the *Glu-D1* locus, and to evaluate their expression and effects in the background of a common wheat cultivar regarding dough strength, protein content, and grain yield potential.

2.2 Materials and Methods

2.2.1 Plant materials

This study used BC_1F_5 seeds harvested from 392 BC_1F_4 MSD panel (Elbashir *et al.* 2017b), which was developed through crossing and backcrossing of the Japanese common wheat cultivar 'Norin 61' (hereafter referred to as N61) with 43 lines of SHW (Tsujimoto *et al.* 2015; Gorafi *et al.* 2018). The 43 lines of SHW were derived from crosses between 43 diverse genotypes of *Ae. tauschii* and *T. turgidum* var. *durum* cv. 'Langdon' (LDN) (Matsuoka and Nasuda 2004; Kajimura *et al.* 2011).
We detected HMW-GS in the 43 lines of SHW and subsequently used the data to confirm HMW-GS in the MSD panel. To identify the HMW-GS' alleles in the 392 MSD panel and the 43 lines of SHW, the recurrent parent N61 and LDN were used in each electrophoresis assay.

2.2.2 Experimental site, design, and cultural practices

The 392 BC_1F_4 MSD panel was grown in season 2015/2016 in the field of the Arid Land

Research Center, Tottori, Japan (35°32′N, 134°13′E, 11 m above sea level), where the soil contains 95% sand, 1.3% silt, and 3.7% clay (Fujiyama and Nagai 1989). The field experiment was arranged in an augmented randomized complete block design with eight blocks and four replicated checks, one of which was the recurrent parent N61. The plot size was one row with five plants spaced 0.2 m apart. Before sowing, three types of fertilizers were used: Kumiai Fukugo PKN 366 at a rate of 60 kg ha^{-1} (MC FERTICOM Co., Ltd., Japan), Hitachi Fukugo 1 at a rate of 40 kg/ha (HITACHI CHEMICAL INDUSTRIES Co., Ltd., Japan), and granular carbonated magnesium lime at a rate of 100 kg/ha (SHIMIZU INDUSTRIAL Co., Ltd., Japan). At the tillering stage, the fertilizer Koudokasei 444 (Mitsubishi Shoji Agri-Service Co., Japan) was used at a rate of 500 kg/ha.

2.2.3 Identification of HMW-GS composition

The composition of HMW-GS in the MSD panel was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method used by Tanaka et al. (2003). The process of staining and decolorizing the gel was done according to that used by Dyballa and Metzger (2009). The gel was scanned using an image scanner (ES-2200, Seiko Epson Co., Japan).

Since the BC_1F_5 seeds in the MSD panel might still be genetically segregated, we investigated the composition of HMW-GS in three grains per line. We considered the MSD lines to have HMW-GS introduced from the SHW if at least one out of the three tested seeds had the HMW-GS of SHW.

The HMW-GS alleles at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci were identified based on the numbering system of Payne and Lawrence (1983). The subunits derived from *Ae. tauschii* were followed by the superscript designation “t” to refer to their origin. The nomenclature of subunits derived from *Ae. tauschii* is tentative in this paper because it is only based on electrophoresis and has not been confirmed by DNA analysis.

2.2.4 Evaluation of flour quality

Grain yield (g) per plant was calculated from an average of five plants. Whole wheat flour was obtained by grinding 4 g from each line of the MSD panel using a UDY cyclone sample mill (UDY Co., USA) equipped with a 1-mm screen. The protein content of the samples was measured as a percentage of the total weight by near-infrared spectroscopy (NIR composition analyzer KJT-270, Kett Electric Laboratory Co. Ltd., Japan). To assess the gluten quantity and quality, we measured the SDS sedimentation volume in 1 g of flour, using the method according to Takata et al. (1999). The sedimentation volume is highly correlated with bread loaf volume (Axtord *et al.* 1979), where dough strength is the main factor. For lines that derived their HMW-GS from *Ae. tauschii*, specific sedimentation values, which are highly correlated with dough strength, were assessed as an index of gluten quality by dividing the SDS sedimentation volume (mL) by protein

content (%), because protein content is reported to be highly correlated with sedimentation volume (Moonen *et al.* 1982; Tanaka and Tsujimoto 2012).

2.2.5 Statistical analysis

Data were tested for normality and homogeneity of variance before analysis using the Shapiro–Wilk Test and Levene's Test, respectively. Analysis of variance (ANOVA) was performed for dough strength and protein content of the MSD lines using the GenStat Software program (18th edition). The least significant difference (0.05) was used for mean separation. Duncan's Multiple Range Test was used to compare the mean dough strength of the different HMW-GS combinations using SPSS Software (version 25.0.1). ANOVA for the field experiment was performed using Plant Breeding Tools v. 1.4 software (International Rice Research Institute, http://bbi.irri.org/products). Regression analysis was conducted using Microsoft Excel 2019.

2.3. Results

2.3.1 Identification and frequency of HMW-GS in the MSD panel

At *Glu-A1* locus, 307 lines possessed subunit 2* inherited from N61, and 85 lines had a null allele derived from the LDN genome in SHW (Table 2). At *Glu-B1* locus, 288 lines had the subunit pair 7+8 from N61, and 104 lines possessed the subunit pair 6+8 from LDN. As for the *Glu-D1* locus, 289 lines inherited the 2.2+12 subunit pair from N61, whereas 103 lines inherited their *Glu-D1* subunit pair from SHW harboring *Ae. tauschii*. Given the nature of the MSD development method, the expected segregation ratio was 75% from N61 and 25% from SHW. Our result for the frequency of HMW-GS pairs at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci in the MSD panel fitted to the Mendelian expected ratio of 3:1. This indicates that no special selection occurred for any of the alleles during the development of the MSD panel and that no identical alleles with that of N61 (2.2+12) have been found in *Ae. tauschii.*

In the 103 lines harboring HMW-GS at *Glu-D1* derived from *Ae. tauschii*, three subunits were x-type (2.1^t, 2^t, and 5^t) and three were y-type (10^t, 12^t, and 12.1^t) (Figure 1). Subunit 12.1^t had slightly smaller molecular weight and faster mobility than subunit 12 in N61, whereas subunit 2.1^t had slower mobility than subunit 2 in N61. The HMW-GS were found in the form of five different haplotypes, 2^t+10^t, 2^t+12^t, 5^t+10^t, 2.1^t+12^t, and 2^t +12.1^t. The most frequent pair of HMW-GS was 2^t+12^t (42 lines), followed by 5^t+10^t (30 lines) (Table 3). The subunit pair 2^t+12.1^t was found in 21 lines, 2^t+10^t in 9 lines, and 2.1^t+12^t in only one line.

In all, 16 combinations of HMW-GS at the three *Glu* loci were distinguished in the 103 MSD lines. The most frequent combination was 2*, 7+8, 2^t+12^t, which was observed in 25 lines, followed by 2*, 7+8, 2^t+12.1^t, and 2*, 7+8, 5^t+10^t which were observed in 18 and 17 lines, respectively (Table 3).

2.3.2 Relationship between HMW-GS in MSD lines and *Ae. tauschii* intraspecific lineages

The MSD lines used in this study originated from three lineages of *Ae. tauschii*. The 60 MSD lines from TauL2 *Ae. tauschii* contained all combinations of HMW-GS except 2.1^t+12^t (Figure 2). The most common subunit pair was 2^t+12^t, which was found in 27 MSD lines in TauL2; the subunit pair $2^t+12.1^t$ was exclusively found in 21 lines from TauL2.; and subunit pairs 5^t+10^t and 2^t+10^t were found in 7 and 5 lines, respectively. All 15 lines of the TauL3 lineage contained the subunit pair 2^t+12^t. In TauL1, the 28 lines possessed 3 pairs of HMW-GS (5^t+10^t, 2^t+10^t, and 2.1^t+12^t); the most common subunit pair was 5^t+10^t (23 lines), followed by the subunit pair 2^t+10^t (4 lines), and the subunit pair 2.1^t+12^t was found in only a single line from TauL1. TauL1 had no lines that possessed subunit pair 2^t+12^t, although it is the most abundant subunit in MSD lines (Table 3).

2.3.3 Evaluation of dough strength in the 103 MSD lines

Highly significant ($P < 0.001$) differences for dough strength were found among the 103 MSD lines that derived their HMW-GS from *Ae. tauschii* (Table 4 and Figure 3). Moreover, dough strength for MSD lines showed a normal distribution according to the Shapiro–Wilk normality test ($P < 0.05$). The variation in dough strength among MSD lines ranged from weak to strong (0.232–0.732 mL/%). In comparison with N61, 3.9% of MSD lines (4 lines, viz. MSD272, MSD363, MSD219, and MSD61) (Table S2) showed dough strength significantly higher than N61. A total of 42 MSD lines (40.8%) showed dough strength comparable with that of N61, whereas 55.3% of MSD lines (57 lines) showed significantly lower dough strength than N61.

2.3.4 Variation and evaluation in dough strength within the five HMW-GS haplotypes derived from *Ae. tauschii*

MSD lines with subunit pair 2^t+12^t exhibited the widest variation in dough strength (0.31–0.68 mL/%) followed by those that carried subunit pairs 5^t+10^t, $2^t+12.1^t$, and 2^t+10^t (Figure 4).

The mean dough strength of the recurrent parent N61 was significantly higher than the mean dough strength for lines possessing subunit pair 5^t+10^t which exhibited the lower dough strength average among all subunits derived from *Ae. tauschii*. The dough strength of the single line that carried subunit pair 2.1^t+12^t was notably higher than the means of the other HMW-GS pairs derived from *Ae. tauschii* ($2^t+12.1^t$, 2^t+10^t, 2^t+12^t, and 5^t+10^t), and was also higher than that of the recurrent parent N61 although this difference was not significant. There was variation inside each subunit pair where some lines were comparable, and others were significantly lower or higher than N61. For example, although the means dough strength for the subunits $2^t+12.1^t$ and 2^t+12^t was comparable to N61, there were two lines from both subunits that were significantly higher than N61

and 13 and 14 comparable, 6 and 24 lines were significantly lower than N61 respectively (Figure 4).

2.3.5 Impact of *Glu-D1* locus on dough strength

To explore the impact of the *Glu-D1* locus on dough strength and exclude the effects of *Glu-A1* and *Glu-B1* loci, we calculated the mean dough strength of the lines that had the same subunits at *Glu-A1* and *Glu-B1* loci but different subunits at *Glu-D1* locus (Figure 5). Accordingly, lines were divided into four groups: (i) Group 1 consisted of lines with subunit combination of 2* and 7+8 at *Glu-A* and *Glu-B* loci, respectively, and four subunit pairs at *Glu-D1* locus: 2^t+12.1^t, 2^t+12^t, 2^t+10^t, and 5^t+10^t; (ii) Group 2 comprised of lines with subunit combination of 2* and 6+8 at *Glu-A* and *Glu-B* loci, respectively, together with four subunit pairs at *Glu-D1* locus: 2^t+12.1^t, 2^t+12^t, 2^t+10^t, and 5^t+10^t; (iii) Group 3 consisted of lines showing null subunits and 7+8 at *Glu-A* and *Glu-B* loci, respectively, and five different subunit pairs at *Glu-D1* locus: 2.1^t+12^t, 2^t+12^t, 2^t+12.1^t, 2^t+10^t, and 5^t+10^t; (iv) Group 4 consisted of subunits null and 6+8 at *Glu-A* and *Glu-B* locus, respectively, and three different subunit pairs at *Glu-D1* locus: 2^t+12^t, 2^t+12.1^t, and 2^t+10^t (Figure 5).

We found that MSD lines with the same subunit combination at *Glu-A1* and *Glu-B1* loci showed a wide variation in their dough strength values when the subunit pair at *Glu-D1* was different. In Group 1, N61 and the MSD lines that had subunit pair 2^t+12.1^t at *Glu-D1* locus had significantly higher mean dough strength of than lines with subunit pairs 2^t+10^t and 5^t+10^t (Figure 5). In Group 2, no significant differences were observed in the dough strength of the MSD lines. In Group 3, N61 and two MSD lines that had subunit pairs 2.1^t+12^t and 2^t+10^t at *Glu-D1* locus had significantly higher mean dough strength than those with subunit pairs 2^t+12.1^t, 2^t+12^t, and 5^t+10^t. In Group 4, no significant differences were observed in the dough strength of the MSD lines, but all had significantly lower dough strength than that of N61. When results in Group 1 were considered in detail, two MSD lines with the subunit combination of 2*, 7+8, 2^t+12.1^t showed significantly higher dough strength than that of N61, whereas 12 lines were comparable, and 4 lines were significantly lower than N61 respectively. Interestingly, 2 lines out of 4 lines that were significantly lower than N61 were developed from the same *Ae. tauschii* accession of the superior lines (MSD272 and MSD219) of the same combination (2*, 7+8, 2^t+12.1^t). For the subunit combination of 2*, 7+8, 2^t+12^t, two lines were significantly higher than N61, 10 were comparable and 13 lines were significantly lower than N61 respectively. Also, out of the 13 lines that were significantly lower than N61 in this combination there was three and two lines were developed from the same *Ae. tauschii* accession of the superior lines MSD61 and MSD363 respectively. On the other hand, all lines with the subunit combinations of null, 7+8, 5^t+10^t and null, 7+8, 2^t+12^tshowed significantly lower dough strength than N61 (Figure 5).

2.3.6 Relationship between protein content and grain yield/plant for the MSD lines

To explore the impact of introgressions from *Ae. tauschii* on the yield and the quality, we performed a regression analysis for protein content and grain yield/plant (Figure 6). Results showed no relationship between the two traits ($r = 0.046$; $P < 0.6438$). The grain yield/plant in all MSD lines did not differ from N61 (according to the least significant difference of 0.05) (Table 3). For the protein content, 69 lines were significantly higher (Table 5), 20 lines were significantly lower, and 15 were comparable to N61. Thus, we separated the MSD lines into three categories (A), (B), and (C) based on significant differences in protein content.

2.4 Discussion

2.4.1 Identification and frequency of HMW-GS in the MSD panel

In this study, we reported a large variation in HMW-GS and flour quality in MSD lines derived from 43 *Ae. tauschii* accessions in the background of the Japanese wheat cultivar N61.

Our investigation at *Glu-D1* locus revealed five types of HMW-GS pairs derived from *Ae. tauschii*. Sixteen subunit combinations at *Glu-1* loci were distinguished in the 103 MSD lines. This result is considered representative of the wide diversity in *Ae. tauschii* across their natural habitat.

Our result on the frequency of HMW-GS at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci in the MSD panel (Table 2) indicated that the SHW genome was successfully introgressed into the recurrent parent (N61) through self-pollinating and backcrossing, and that no identical alleles with that of N61 (2.2+12) have been found in *Ae. tauschii*. The subunit 2.2 (x-type subunit) has not been observed in any previous study of *Ae. tauschii*, which supports the hypothesis of Payne et al. (1983) that this subunit arose within hexaploid wheat by a rare unequal crossing-over with another HMW-GS gene. Since there is a tight link between x-type and y-type subunits in bread wheat (Lawrence and Shepherd, 1980), there is no possibility that some lines can simultaneously derive subunit 12 from N61 (which is a y-type subunit) and x-type subunits from *Ae. tauschii*. This result agrees with Gorafi et al. (2018) who analyzed the crossing over status of MSD individuals and found the result consistent with the expected ratio after one backcross event.

In our study, we used SDS-PAGE analysis, the traditional standard for distinguishing HMW-GS; thus, the identification of the five subunits that derived from *Ae. tauschii* was based on previously reported electrophoresis mobility results. For instance, William et al. (1993), Rasheed et al. (2012), and Tariq et al. (2018) reported similar SDS-PAGE mobility of 5^t+10^t and 2^t+12^t derived from *Ae. tauschii* accessions. Yan et al. (2003) observed similar SDS-PAGE mobility of 2^t+10^t and 2^t+12^t derived from *Ae. tauschii* accessions. Similarly, the subunit pair 2.1^t+12^t has been identified according to its SDS-PAGE mobility (Pflüger *et al.* 2001; Rasheed *et al.* 2012; Tariq *et al.* 2018). Our finding was in agreement with Lagudah et al. (1987), who documented the presence of subunit pairs 5^t+10^t and 2^t+12^t, which were equivalent to the SDS-PAGE bands of common

wheat, and they also found subunit 2.1^t in conjunction with subunit 10.1^t. Gianibelli et al. (2001) reported for the first time the presence of subunit 2^t+12.1^t by SDS-PAGE in *Ae. tauschii* accessions. Our results contrasted with the findings of Delorean et al. (2021), who documented the absence of the 5+10 wheat haplotype in 273 sequenced *Ae. tauschii* accessions at both the molecular level and also by SDS-PAGE mobility.

The most frequent subunit pair in our study was 2^t+12^t (40.8%), which is the most abundant pair in common wheat as well (Payne 1987a), followed by subunit pair 5^t+10^t (29.1%) (Table 3). The same pattern of frequency for these two pairs (2^t+12^t and 5^t+10^t) has been observed in primary SHW derived from 52 *Ae. tauschii* accessions (Tariq *et al.* 2018). (Rasheed *et al.* 2012) reported a lower frequency for the subunit pair 2^t+12^t and a higher frequency for 5^t+10^t in 95 selected synthetic lines developed by the International Maize and Wheat Improvement Center. Subunit pairs 2^t+10^t and 2.1^t+12^t were found in 9 lines (8.7%) and only one line (1%), respectively. In contrast to our result, the subunit pair 2^t+10^t has been found at the higher frequency of 12.63% in 198 *Ae. tauschii* accessions (Yan *et al.* 2003), and 2.1^t+12^t has been reported in six accessions in a 92 accessions of *Ae. tauschii* accessions (Pflüger *et al.* 2001) and at a frequency of 16.8% in a 95 synthetic hexaploid accessions (Rasheed *et al.* 2012).

2.4.2 Relationship between HMW-GS in the MSD lines and *Ae. tauschii* intraspecific lineages

TauL2 exhibited the widest diversity at the *Glu-D1* locus, compared to TauL1 and TauL3. MSD lines that belonged to TauL2 contained all types of HMW-GS derived from *Ae. tauschii* except the subunit 2.1^t+12^t. All MSD lines belonging to TauL2 originated from Iran. This diversity at *Glu-D1* alleles in TauL2 matched well with literature that considers Iran to be the center of genetic variation of *Ae. tauschii* (Dudnikov and Goncharov 1993). Also, Delorean et al. (2021) evaluated the *Glu-D1* diversity relative to the geographic origin of *Ae. tauschii* accessions and found that the greatest concentration of haplotype diversity was located along the shores of the Caspian Sea in Iran. Similarly, Lagudah and Halloran (1988) reported that the northeastern region of Iran exhibited a wide diversity of the *Glu-D1* subunits. Therefore, identifying geographical areas where the progenitor species of existing SHW were collected would assist in guiding future collection missions (Ogbonnaya *et al.* 2013).

Subunit 12.1^t exclusively belongs to the *Ae. tauschii* genome and does not exist at the *Glu-D1* locus in common wheat (Tahernezhad *et al.* 2013). In our study, we found the subunit pair 2^t+12.1^t exclusive to TauL2, which might indicate that this subunit pair has a unique origin, but further studies are needed to confirm this. All lines that belonged to TauL3, which originated from Georgia, carried subunit pair 2^t+12^t and were genetically similar to TauL2 (Matsuoka *et al.* 2013). Delorean et al. (2021) studied gene-level phylogeny at *Glu-D1* for 273 sequenced *Ae. tauschii* accessions and showed that a unique group of *Glu-D1* alleles belonging to Lineage 3 accessions was found within a narrow clade with Lineage 2.

The most common 2^t+12^t subunit pair was not found in TauL1. This may indicate that, *Ae. tauschii* genotypes belonging to this TauL lineage may not have been involved in the evolution of common wheat. Indeed, it appears that TauL2 and TauL3 are closer to common wheat than TauL1 because they contain the most common HMW-GS allele prevalent in common wheat (2^t+12^t) (Matsuoka *et al.* 2013). Moreover, Delorean et al. (2021) demonstrated that the superior subunit pair 5+10 was found to be clustered very tightly with TauL3, whereas the wheat subunit pair 2+12 was found to be clustered with TauL2, indicating the contribution of these two lineages (TauL2 and TauL3) to the current wheat genome.

2.4.3 Evaluation of dough strength of the MSD lines

We evaluated the dough strength of the 103 MSD lines that derived their HMW-GS from *Ae. tauschii* to explore the effect of the wild gene in the background of N61. The significant genotypic differences ($P < 0.001$) observed in dough strength indicated high genetic diversity among the MSD lines. This variation has been attributed mainly to different introgression segments of *Ae. tauschii* in the MSD lines (Itam et al., 2021a). Itam et al. (2021b) and Elbashir et al. (2017a) also found high genetic diversity in the MSD lines for different traits and have attributed these variations to *Ae. tauschii*.

We calculated the mean dough strength for each of the five pairs of HMW-GS derived from *Ae. tauschii*. In our study, the subunit 2.1^t exhibited the strongest dough strength average in combination with subunit 12^t, and this pair was significantly higher than 5^t+10^t, 2^t+10^t, and 2^t+12^t. This indicates the positive impact of this subunit when combined with subunit 12^t; however, it has been reported to have a weak contribution to specific rheological characteristics when associated with subunit 10.1^t in SHW (Lagudah *et al.* 1987). The subunit pair $2^t+12.1^t$ also showed relatively strong dough strength. Two MSD lines possessing this subunit pair showed significantly stronger dough compared to N61, indicating the positive impact of this subunit pair on dough strength. To the best of our knowledge, this is the first time that the effect of subunit pair $2^t+12.1^t$ in *Ae. tauschii* on wheat quality has been studied in the background of a wheat cultivar.

Payne (1987) proved that the subunit pair 5+10 at *Glu-D1* locus has the highest positive effect on dough strength and a higher *Glu-1* score compared to 2+12. This explains its frequent association with dough characterized by stronger elasticity and superior end-use qualities for bread making. Results in our study showed that lines carrying subunit 5^t+10^t exhibited the lowest dough strength values among all subunits at *Glu-D1* and were significantly lower than N61. For the first time, the impact on dough strength of subunit pair 5^t+10^t inherited from *Ae. tauschii* in the background of a wheat cultivar is reported as poor. Previous studies documented that the subunit pair 5^t+10^t, which has mobility in SDS-PAGE typical to bands of 5+10 in wheat derived from *Ae. tauschii*, is associated with good bread-making quality (Lagudah *et al.* 1987; Hsam *et al.* 2001). We suggest that the decreased dough strength values in this study of lines carrying subunit pair 5^t+10^t could be due to the lack of the extra cysteine in 1Dx5. The absence of an additional

cysteine in subunit 1Dx5^t derived from *Ae. tauschii* has been reported in previous studies (Pflüger *et al.* 2001). It is also possible that the subunit pair (5^t+10^t) present in our materials might be a different pair of the same size and therefore with the same mobility in SDS-PAGE—as the subunits 5+10 in *T. aestivum*. (Delorean *et al.* 2021) used haplotype molecular sequence diversity and SDS-PAGE to explore whether the electrophoresis mobility would reflect the differences visible at the molecular level and observed that in some cases genes showed a difference in SDS-PAGE mobility, but the alleles looked identical at the molecular level. Similarly, haplotypes were seen to be different at the molecular level but identical in SDS-PAGE mobility. Interestingly, the presence of an *Ae. tauschii* haplotype identical to that of wheat subunit pair 2+12 and the absence, even by SDS-PAGE mobility, of the exact wheat 5+10 haplotype in the *Ae. tauschii* accession has been documented (Delorean *et al.* 2021). Likewise, the difference in basic isoelectric value between subunit 5^t+10^t derived from *Ae. tauschii* and the same subunit pair in common wheat has been confirmed by a two-dimensional method (isoelectric focusing with SDS-PAGE), although these subunit pairs had identical electrophoretic mobility (Lagudah and Halloran 1988). Mackie et al. (1996) reported that the subunits Dy10^t and Dy12^t from *Ae. tauschii* were more hydrophobic than those from *T. aestivum.* Yan et al. (2003) reported a difference in relative mobility by A-PAGE between subunits Dx5^t and Dy10^t from *Ae. tauschii* and those of common wheat, although they showed the same mobility under SDS-PAGE. Thus, the unexpectedly poor impact of subunit 5^t+10^t from *Ae. tauschii* on dough strength could be attributed to the lack of an extra cysteine or to difficulties in interpreting SDS-PAGE mobility. More confirmatory investigations are needed.

2.4.4 Impact of *Glu-D1* locus on dough strength

The same subunit may play varied roles on wheat quality in different pairs of HMW-GS (Zhao *et al.* 2020). Our result showed a wide variation even between the same HMW-GS pair. This variation in the same subunit pair, and even between sister lines with the same subunit pair, indicates that there might be other factors/genes that affect dough strength. It might be due to the different recombinant portions from SHW (introgressed segments) across the 21 chromosomes. The presence of different introgressed segments from SHW in MSD lines, including sister lines, has been documented (Itam *et al.* 2021a). These different genomic segments have been found to cause a variation in physio-agronomic traits between MSD lines (Itam *et al.* 2021a). In further study, a genome-wide association study will need to be performed to find factors/genes other than HMW-GS. Furthermore, LMW-GS and gliadin, which were not investigated in this study, are known to contribute markedly to flour quality, sometimes even more so than the HMW-GS (Pogna *et al.* 1982, Gupta *et al.* 1989). Therefore, revealing the allelic compositions of LMW-GS and gliadin is very important to better understand the observed variations in wheat quality.

Two lines (MSD61 and MSD363) (Table 5 and Figure 4) that carried 2^t+12^t exhibited good dough strength despite the fact that this subunit pair is frequently associated with

weak dough strength (Payne 1987a). This might be attributed to their high proportion of total HMW-GS at the *Glu-D1* locus, as has been reported earlier (Horvat *et al.* 2006). We observed that two lines that carried the null allele at the *Glu-A1* locus showed dough strength values higher than the recurrent parent N61. This suggested that the introgression of the *Glu-D1* locus from *Ae. tauschii* compensated for the negative impact of the null allele in these lines, where most of the studies reported a significant negative effect of null allele and its association with lower values of gluten strength (Ruiz and Carrillo, 1993; Raciti et al., 2003). Thus, those lines could be used in breeding programs to improve the quality and overcome the negative impact of the null allele.

2.4.5 Relationship between protein content and grain yield in the MSD lines

Although the negative relationship between grain protein content and grain yield is well-known (Kibite and Evans 1984; Cox *et al.* 1985; Gauer *et al.* 1992; Delzer *et al.* 1995; Marinciu *et al.* 2008; Giancaspro *et al.* 2019; Taheri *et al.* 2021), our findings showed no relationship between the two traits in MSD lines. Moreover, most of the MSD lines had higher or lower protein content with comparable grain yield values to the recurrent parent. This may indicate that the increase or decrease in protein content that occurred due to the introgression of the D genome is independent of the grain yield in MSD lines. Also, it may have increased the variation in protein content to such an extent that it counteracts the generally known negative relationship between protein content and grain yield. Although our finding is based on a homogenous grain yield and protein content of five independent plants evaluated for one season under optimum condition, it is very promising and pave the way for more detailed investigation and validation. The regression analysis used was powerful and allowed the classification of the MSD lines in different groups considering their protein content and grain yield. Some lines in group C had a clear good comparable grain yield and high protein content compared to N61, these lines could be a target for more detailed analysis and evaluation to elucidate the basis of the positive or no correlation between the grain yield and protein content especially that breaking the negative relationship between these traits is an important aspect for wheat breeding to increase the grain yield and maintain the quality characteristics. The identified MSD lines could provide a valuable genetic resource for enhancing the end-use quality without any loss in productivity.

2.5 Conclusion

This study found that the MSD lines derived all the allelic variations at *Glu-D1* locus that existed in their ancestor *Ae. tauschii* accessions. Five subunit pairs (2.1^t+12^t, 2^t+12.1^t, 2^t+12^t, 2^t+10^t, and 5^t+10^t) were identified with different frequency in 103 MSD lines. These subunit pairs may offer different options in breeding programs for different end-use products. The MSD lines also exhibited a wide variation in dough strength even in lines with the same HMW-GS composition, and even between sister lines with the same

HMW-GS composition. Since dough strength (elasticity) is a critical factor determining the end-use quality of wheat flour, the variation that the MSD lines showed on dough strength (from strong to weak) could be used in breeding programs for different purposes, not only for improving bread-making quality. We documented the poor impact of subunit pair 5^t+10^t from *Ae. tauschii* on dough strength in contrast to the well-documented positive impact of this subunit pair on dough strength. However, we found the subunit pair 2^t+12.1^t to have a positive impact on dough strength.

We identified four MSD lines that significantly enhanced the flour quality, MSD219, MSD363, MSD272, and MSD61, which carried two different alleles at the *Glu-D1* locus (2.1^t+12^t and 2^t+12^t) derived from *Ae. tauschii*. These lines are promising and could serve as a good source to improve wheat flour quality in the breeding programs. A total of 69 MSD lines were identified with comparable grain yield and significantly higher protein content than the recurrent parent N61. These MSD lines could be used in breeding programs to improve wheat quality without any concern about the deterioration in grain yields.

Table 2.1. The forty-three *Aegilops tauschii* accessions that used to develop the MSD population and their origins

Number	*Aegilops tauschii* accessions	Origins	lineages
1	AE1090	Kazakhstan	1
2	AE454	Georgia	3
3	AE929	Georgia	3
4	AT55	China	1
5	AT76	China	1
6	AT80	China	1
7	IG126387	Turkmenistan	1
8	IG131606	Kyrgyzstan	1
9	IG47259	Syria	1
10	IG48042	India	1
11	KU-20-10	Iran	2
12	KU-2039	Afghanistan	1
13	KU-2074	Iran	2
14	KU-2075	Iran	2
15	KU-2076	Iran	2
16	KU-2078	Iran	2
17	KU-2079	Iran	2
18	KU20-8	Iran	2
19	KU-2080	Iran	2
20	KU-2088	Iran	2
21	KU-20-9	Iran	2
22	KU-2090	Iran	2
23	KU-2091	Iran	2
24	KU-2092	Iran	2
25	KU-2093	Iran	2
26	KU-2096	Iran	2
27	KU-2097	Iran	2
28	KU-2098	Iran	2
29	KU-2103	Iran	2
30	KU-2105	Iran	2
31	KU-2109	Iran	2
32	KU-2124	Iran	2
33	KU-2126	Iran	2
34	KU-2132	Turkey	1
35	KU-2136	Turkey	1
36	KU-2155	Iran	2
37	KU-2156	Iran	2

38	KU-2158	Iran	2
39	KU-2159	Iran	2
40	KU-2829A	Georgia	3
41	PI476874	Afghanistan	1
42	PI499262	China	1
43	PI508262	China	1

Table 2.2. The segregation of HMW-GSs in each locus of MSD lines.

Locus	Origin of HMW-GS		Total	Expected ratio N61:SHW	$\chi 2$	P
	Number of N61 types	Number of SHW types				
Glu-A1	307 (78.3%)	85 (21.7%)	392	3:1	1.72	0.189693
Glu-B1	288 (73.5%)	104 (26.5%)	392	3:1	0.255	0.613576
Glu-D1	289 (73.7%)	103 (26.3%)	392	3:1	0.255	0.613576

HMW-GS, high-molecular-weight glutenin subunits; MSD, multiple synthetic derivative; N61, Norin 61; SHW, synthetic hexaploid wheat.

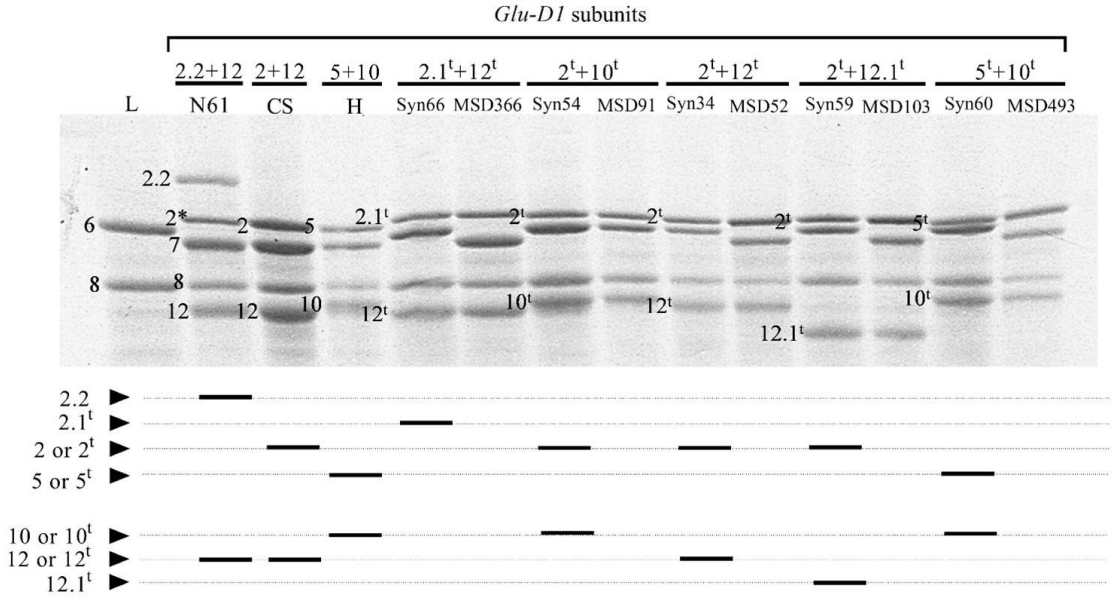

Figure 2.1. Expression of HMW-GS derived from Aegilops tauschii in (upper panel) SDS-PAGE and (lower panel) a schematic diagram. N61, Norin 61; L, Langdon;CS, Chinese spring; H, Haruhikari; Syn, synthetic hexaploid line; MSD, multiple synthetic derivatives.

Table 2.3. Different subunits pairs, combination, and gene frequencies of high-molecular-weight glutenin subunits (HMW-GSs) encoded at *Glu-A1*, *Glu-B1* and *Glu-D1* loci among the 103 and 289 MSD lines derived *Glu-D1* from Ae. tauschii and N61 respectively.

Subunits at *Glu-A1*	Subunits at *Glu-B1*	Number of MSD lines	Subunits at *Glu-D1*	Number of MSD lines	Frequency of HMW-GS combinations
2*	7+8	18	2^t+12.1^t	21 (20.4%)	17.5
Null	7+8	1			1
2*	6+8	1			1
Null	6+8	1			1
2*	7+8	7	2^t+10^t	9 (8.7%)	6.7
Null	7+8	1			1
2*	6+8	1			1
Null	6+8				
2*	7+8	17	5^t+10^t	30 (29.1%)	16.5
Null	7+8	5			4.8
2*	6+8	5			4.8
Null	6+8	3			3
2*	7+8	25	2^t+12^t	42 (40.8%)	24.3
Null	7+8	5			4.8
2*	6+8	11			10.6
Null	6+8	1			1
2*	7+8	1	2.1^t+12^t	1 (1%)	1
Null	7+8				
2*	6+8				
Null	6+8				
2*	7+8	166	2.2+12	289 (100)	57.5
Null	7+8	55			19
2*	6+8	44			15.2
Null	6+8	24			8.3

MSD, multiple synthetic derivatives; HMW-GS, high-molecular-weight glutenin subunits

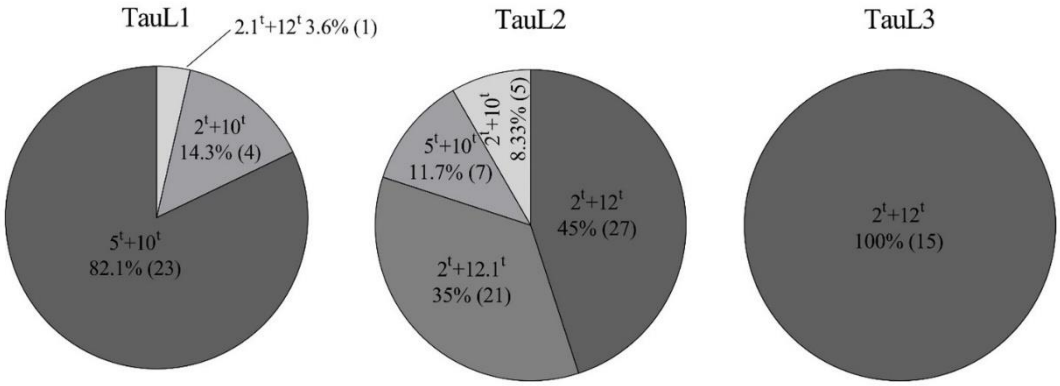

Figure 2.2. Frequency of the different subunit pairs derived from three lineages of *Ae. tauschii*: TauL1, TauL2, and TauL3. Numbers in parentheses refer to the number of MSD lines in which the subunit pair appeared.

Table 2.4. ANOVA and heritability for protein content (%), grain yield/plants (g) and dough strength (mL/%).

Traits	P-value	SED±	LSD	CV%	H^2
Protein content (%)	< 0.001	0.2443	0.4817	1.8	0.99
Grain yield/plants (g)	< 0.0107	14.9001	32.88	21.3	0.59
Dough strength (mL/%)	< 0.001	0.037	0.073	9.6	0.95

SED±, standard error of differences; LSD, least significant differences; CV%, coefficients of variation.

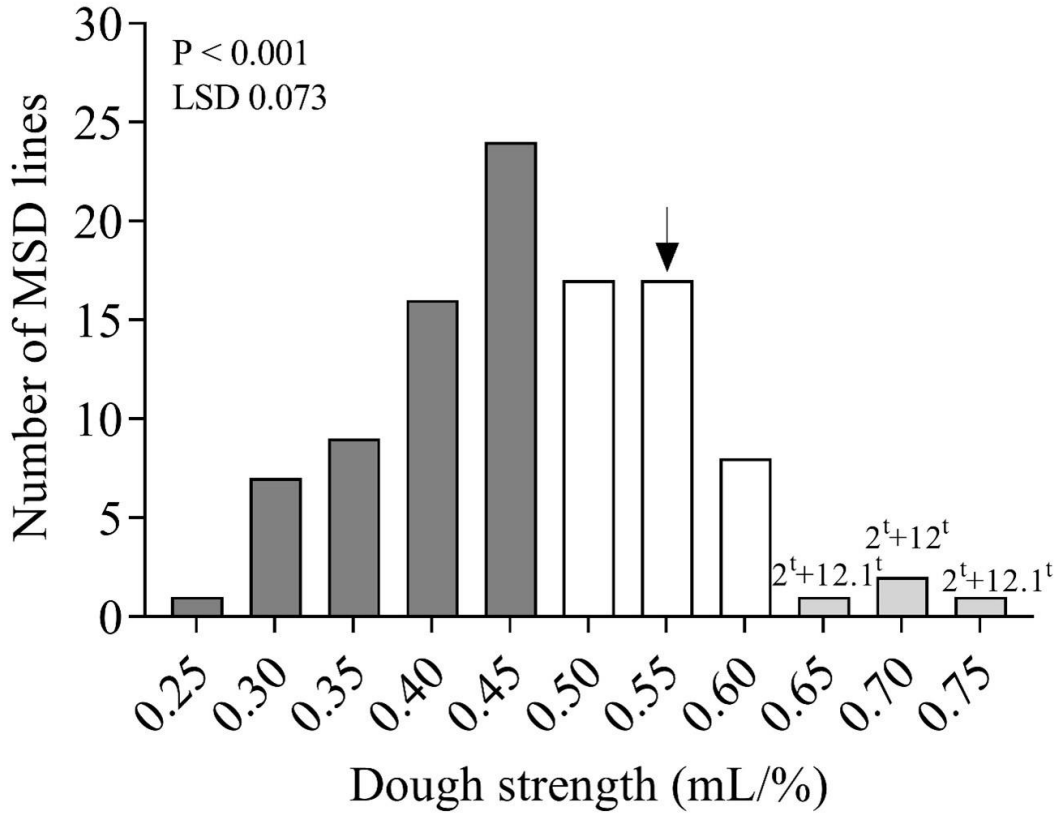

Figure 2.3. Distribution of the mean dough strength (mL/%) of 103 MSD lines that derived their HMW-GS from Ae. tauschii and the recurrent parent N61, shown by ablack arrow. Dark gray, white, and light gray columns indicate the number of lines in the MSD panel that had significantly lower, comparable, or higher dough strength than N61, respectively. Numbers and letters above the columns indicate the important subunit pairs in the MSD lines that had dough strength superior to N61. LSD stands for least significant difference.

Table 2.5. Means of dough strength (mL/%) and protein (%) content for most promising lines with their pedigree.

MSD ID	Protein content (%)	Dough strength (mL/%)	Pedigree	Origins	lineages
MSD100	16.594	0.467	Norin 61/T. durum cv. Langdon ×KU-2096//*Norin 61	Iran	2
MSD109	16.343	0.430	Norin 61/T. durum cv. Langdon ×KU-2039//*Norin 61	Afghanistan	1
MSD11	18.971	0.439	Norin 61/T. durum cv. Langdon ×KU-2039//*Norin 61	Afghanistan	1
MSD119	16.159	0.547	Norin 61/T. durum cv. Langdon ×AE454//*Norin 61	Georgia	3
MSD133	19.845	0.396	Norin 61/T. durum cv. Langdon ×PI508262//*Norin 61	China	1
MSD16	17.624	0.426	Norin 61/T. durum cv. Langdon ×AT80//*Norin 61	China	1
MSD165	18.816	0.454	Norin 61/T. durum cv. Langdon ×KU-2093//*Norin 61	Iran	2
MSD170	17.515	0.402	Norin 61/T. durum cv. Langdon ×KU-2039//*Norin 61	Afghanistan	1
MSD178	16.03	0.571	Norin 61/T. durum cv. Langdon ×KU-2156//*Norin 61	Iran	2
MSD203	16.545	0.540	Norin 61/T. durum cv. Langdon ×KU-2096//*Norin 61	Iran	2
MSD207	20.172	0.441	Norin 61/T. durum cv. Langdon ×IG131606//*Norin 61	Kyrgyzstan	1
MSD219	14.547	0.642	Norin 61/T. durum cv. Langdon ×KU-2097//*Norin 61	Iran	2
MSD227	16.169	0.517	Norin 61/T. durum cv. Langdon ×KU-2097//*Norin 61	Iran	2
MSD229	15.913	0.463	Norin 61/T. durum cv. Langdon ×KU-2090//*Norin 61	Iran	2
MSD234	16.745	0.512	Norin 61/T. durum cv. Langdon ×KU-2075//*Norin 61	Iran	2
MSD24	17.01	0.410	Norin 61/T. durum cv. Langdon ×KU-2109//*Norin 61	Iran	2
MSD242	16.205	0.475	Norin 61/T. durum cv. Langdon ×PI476874//*Norin 61	Afghanistan	1
MSD244	16.205	0.592	Norin 61/T. durum cv. Langdon ×AT80//*Norin 61	China	1
MSD254	18.709	0.601	Norin 61/T. durum cv. Langdon ×KU-2126//*Norin 61	Iran	2
MSD259	17.046	0.503	Norin 61/T. durum cv. Langdon ×KU-2105//*Norin 61	Iran	2
MSD265	15.932	0.594	Norin 61/T. durum cv. Langdon ×KU-2124//*Norin 61	Iran	2
MSD270	15.703	0.514	Norin 61/T. durum cv. Langdon ×KU-2132//*Norin 61	Turkey	1

MSD272	11.064	0.732	Norin 61/T. durum cv. Langdon ×KU-2092//*Norin 61	Iran	2
MSD274	16.961	0.495	Norin 61/T. durum cv. Langdon ×KU-2080//*Norin 61	Iran	2
MSD280	16.632	0.553	Norin 61/T. durum cv. Langdon ×KU-2105//*Norin 61	Iran	2
MSD285	18.393	0.312	Norin 61/T. durum cv. Langdon ×AE454//*Norin 61	Georgia	3
MSD296	18.855	0.490	Norin 61/T. durum cv. Langdon ×KU-2039//*Norin 61	Afghanistan	1
MSD320	16.058	0.495	Norin 61/T. durum cv. Langdon ×KU-2079//*Norin 61	Iran	2
MSD324	18.193	0.313	Norin 61/T. durum cv. Langdon ×KU-2109//*Norin 61	Iran	2
MSD325	15.541	0.453	Norin 61/T. durum cv. Langdon ×KU-2075//*Norin 61	Iran	2
MSD340	18.456	0.440	Norin 61/T. durum cv. Langdon ×AE929//*Norin 61	Georgia	3
MSD349	17.192	0.382	Norin 61/T. durum cv. Langdon ×KU-2098//*Norin 61	Iran	2
MSD355	16.581	0.387	Norin 61/T. durum cv. Langdon ×AE454//*Norin 61	Georgia	3
MSD362	16.003	0.452	Norin 61/T. durum cv. Langdon ×KU-2158//*Norin 61	Iran	2
MSD363	17.91	0.679	Norin 61/T. durum cv. Langdon ×AE454//*Norin 61	Georgia	3
MSD369	17.998	0.456	Norin 61/T. durum cv. Langdon ×KU-2093//*Norin 61	Iran	2
MSD37	19.685	0.376	Norin 61/T. durum cv. Langdon ×PI499262//*Norin 61	China	1
MSD371	21.781	0.306	Norin 61/T. durum cv. Langdon ×IG126387//*Norin 61	Turkmenistan	1
MSD386	19.382	0.301	Norin 61/T. durum cv. Langdon ×PI508262//*Norin 61	China	1
MSD390	15.898	0.442	Norin 61/T. durum cv. Langdon ×AE1090//*Norin 61	Kazakhstan	1
MSD395	19.459	0.414	Norin 61/T. durum cv. Langdon ×AE929//*Norin 61	Georgia	3
MSD401	15.76	0.523	Norin 61/T. durum cv. Langdon ×KU-2124//*Norin 61	Iran	2
MSD42	16.574	0.572	Norin 61/T. durum cv. Langdon ×AE929//*Norin 61	Georgia	3
MSD423	21.518	0.362	Norin 61/T. durum cv. Langdon ×KU-2079//*Norin 61	Iran	2
MSD424	19.082	0.367	Norin 61/T. durum cv. Langdon ×KU-2039//*Norin 61	Afghanistan	1
MSD426	16.306	0.525	Norin 61/T. durum cv. Langdon ×KU-2093//*Norin 61	Iran	2
MSD43	18.135	0.471	Norin 61/T. durum cv. Langdon ×KU-2096//*Norin 61	Iran	2
MSD440	18.728	0.415	Norin 61/T. durum cv. Langdon ×KU-2090//*Norin 61	Iran	2

MSD443	19.515	0.328	Norin 61/T. durum cv. Langdon ×IG126387//*Norin 61	Turkmenistan	1
MSD444	18.202	0.382	Norin 61/T. durum cv. Langdon ×KU-2156//*Norin 61	Iran	2
MSD446	19.821	0.404	Norin 61/T. durum cv. Langdon ×KU-2090//*Norin 61	Iran	2
MSD448	15.54	0.320	Norin 61/T. durum cv. Langdon ×AE454//*Norin 61	Georgia	3
MSD450	15.906	0.413	Norin 61/T. durum cv. Langdon ×KU-2097//*Norin 61	Iran	2
MSD46	19.75	0.459	Norin 61/T. durum cv. Langdon ×KU-2090//*Norin 61	Iran	2
MSD490	15.733	0.460	Norin 61/T. durum cv. Langdon ×KU-2092//*Norin 61	Iran	2
MSD493	17.198	0.516	Norin 61/T. durum cv. Langdon ×KU-2090//*Norin 61	Iran	2
MSD5	19.369	0.458	Norin 61/T. durum cv. Langdon ×IG48042//*Norin 61	India	1
MSD50	18.092	0.416	Norin 61/T. durum cv. Langdon ×KU-2098//*Norin 61	Iran	2
MSD500	18.914	0.393	Norin 61/T. durum cv. Langdon ×KU-2075//*Norin 61	Iran	2
MSD51	17.716	0.344	Norin 61/T. durum cv. Langdon ×KU-2124//*Norin 61	Iran	2
MSD52	18.214	0.465	Norin 61/T. durum cv. Langdon ×KU-2075//*Norin 61	Iran	2
MSD56	16.484	0.374	Norin 61/T. durum cv. Langdon ×KU-2124//*Norin 61	Iran	2
MSD57	18.478	0.503	Norin 61/T. durum cv. Langdon ×IG48042//*Norin 61	India	1
MSD6	15.653	0.554	Norin 61/T. durum cv. Langdon ×KU-2132//*Norin 61	Turkey	1
MSD61	16.379	0.682	Norin 61/T. durum cv. Langdon ×AE929//*Norin 61	Georgia	3
MSD78	18.928	0.313	Norin 61/T. durum cv. Langdon ×PI476874//*Norin 61	Afghanistan	1
MSD81	16.669	0.370	Norin 61/T. durum cv. Langdon ×KU-2124//*Norin 61	Iran	2
MSD83	18.09	0.440	Norin 61/T. durum cv. Langdon ×KU-2159//*Norin 61	Iran	2
MSD84	18.744	0.397	Norin 61/T. durum cv. Langdon ×IG131606//*Norin 61	Kyrgyzstan	1
MSD85	16.529	0.298	Norin 61/T. durum cv. Langdon ×KU-2074//*Norin 61	Iran	2
MSD93	20.658	0.232	Norin 61/T. durum cv. Langdon ×PI508262//*Norin 61	China	1

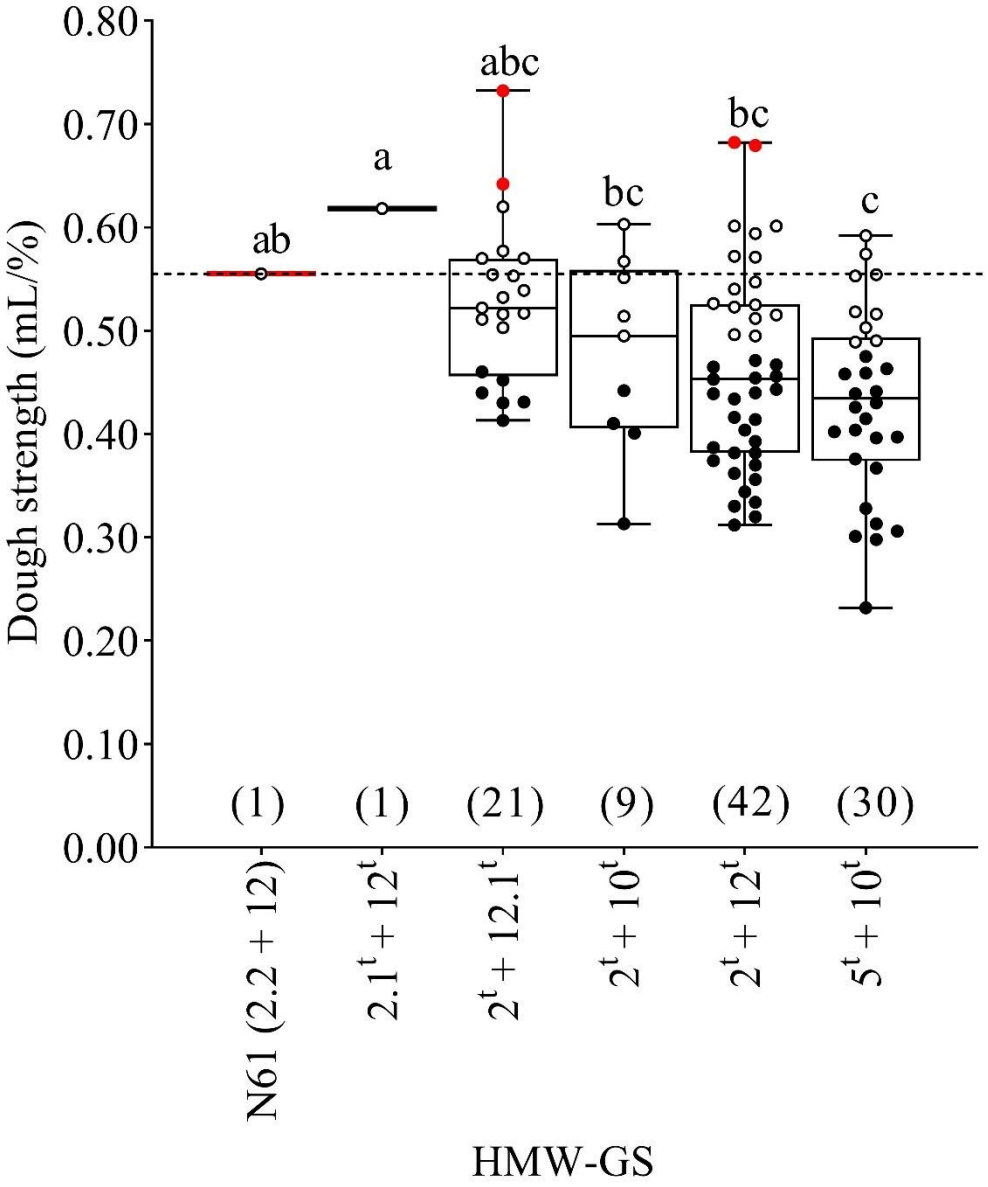

Figure 2.4. Boxplot for the dough strength (mL/%) as affected by the HMW-GS pairs derived from *Ae. tauschii* and the recurrent parent N61. Similar lower-case letters indicate that the means for HMW-GS pairs are not significantly different according to Duncan's Multiple Range Test at $P < 0.05$. A horizontal dashed line compares the N61 value with the values of other MSD lines. Red, white, and black dots indicate the MSD lines that were significantly higher, comparable, or lower than N61, respectively, according to the least significant difference (0.05). Numbers in parentheses refer to the number of MSD lines having each HMW-GS pair.

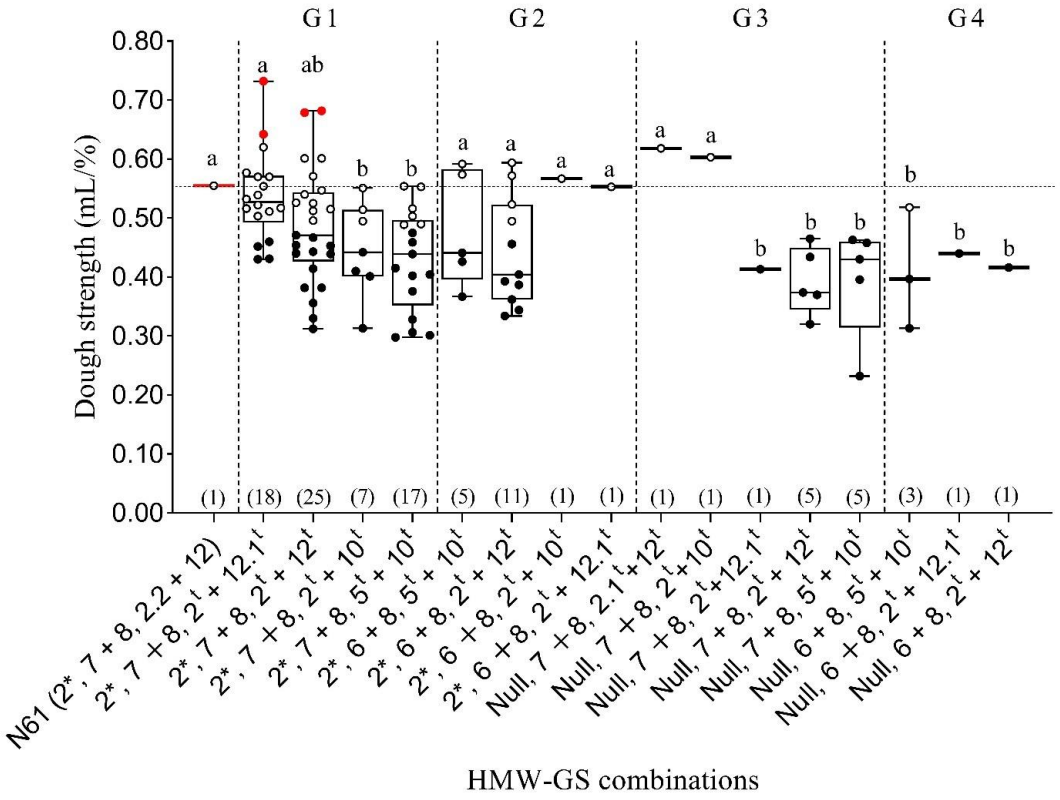

Figure 2.5. Boxplot of dough strength (mL/%) for different subunit combinations at three *Glu-1* loci in 103 MSD lines that derived their HMW-GS from *Ae. tauschii* and the recurrent parent N61. The horizontal dotted line compares the N61 value with those of MSD lines carrying different subunit combinations, which are separated by dashed vertical lines into groups G1–G4. Within a group, the same letter indicates that the means are not significantly different at the 5% probability level according to Duncan's Multiple Range Test. Red, white and black dots indicate the MSD lines that had significantly higher, comparable, or lower dough strength, respectively, than N61, according to least significant difference (0.05). Numbers in parentheses refer to the number of MSD lines having each HMW-GS combination.

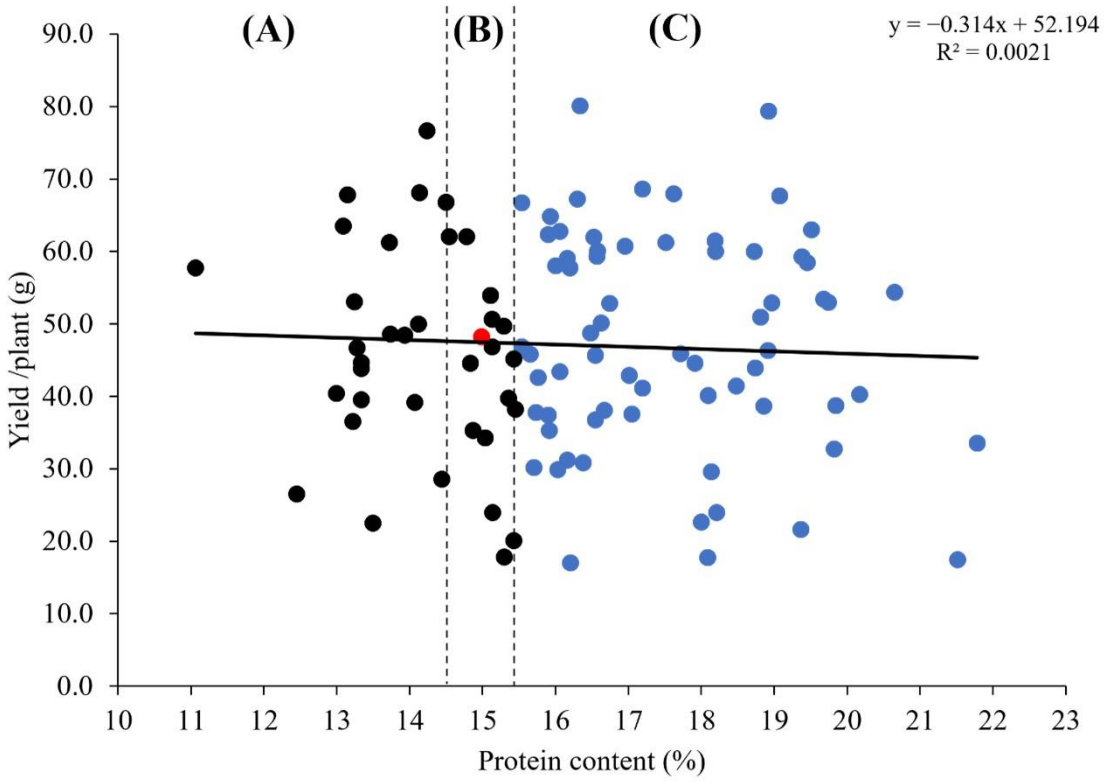

Figure 2.6. Regression analysis of the relationship between protein content (%) and grain yield/plant (g) for 103 MSD lines that derived their HMW-GS from *Ae. tauschii* and the recurrent parent N61, shown by a red dot. Vertical dotted lines classify results of comparable grain yield/plant into those with (A) a lower protein content than N61, (B) a comparable protein content than N61, and (C) a higher protein content than N61 (indicated with blue dots).

CHAPTER THREE

Identification of *Glu-D1* Alleles and Novel Marker–Trait Associations for Flour Quality and Grain Yield Traits under Heat-Stress Environments in Wheat Lines Derived from Diverse Accessions of *Aegilops tauschii*

3.1 Introduction

Heat stress is considered one of the most significant abiotic stress factors influencing wheat flour quality and grain yield. In the face of the increasing change in global climate, understanding and diagnosing the impact of high temperature on wheat flour quality and grain yield is necessary. Moreover, assessing differential genotypic responses is crucial for identifying resilient genetic resources that combine heat tolerance and good quality. The available literature focuses more on identifying heat-tolerant genotypes by examining the impact of heat stress on yield or yield-related traits without in-depth analysis of the quality aspects (Reynolds et al. 1994; Mondal et al. 2015). Moreover, most of the studies on wheat quality have been conducted under controlled environments or with a relatively small number of genotypes (Wrigley et al. 1994; Blumenthal et al. 1995; Stone et al. 1997; Spiertz et al. 2006). Furthermore, the genetic basis of the diversity resilience and genome-wide association studies for wheat quality under heat stress has yet to be fully explored. Wheat grain quality, a characteristic that affects food processing quality and nutritional value, is crucial for assessing the market potential and commercial value of new wheat varieties. One of the most important characteristics affecting wheat quality is the unique gluten protein. The gluten proteins, also called seed storage proteins (SSPs), are classified into monomeric gliadins and polymeric glutenin. The gliadin proteins are classified into four major types: α-, β-, γ-, and ω-gliadins, according to their electrophoretic mobility in acid conditions (Bushuk and Zillman 1978). The glutenins are classified into high molecular weight (HMW) and low molecular weight (LMW) types. The gliadins are vicious, provide extensibility, and are stretchable, whereas glutenins give elasticity to bread dough (Payne 1987b). When gliadins and glutenins are mixed with water, they produce visco-elastic dough. These physical properties are associated with the functional traits of flour quality. For example, flour dough that exhibits appropriate gas-holding properties is required for bread making, whereas dough that exhibits weak gas-holding properties is necessary for making cookies and cakes. Therefore, the technical properties of wheat flour are directly related to the gliadin: glutenin ratio in the flour. Thus, various food products can be made depending on the specific balance of functional properties of the dough (Wang et al. 2017). The high-molecular-weight glutenin subunits (HMW-GSs) have a significant impact on wheat flour quality because they constitute the primary factor determining gluten elasticity, thus, important for the bread-making process (Tatham et al. 1985; Shewry et al. 2003). The HMW-GSs represent about 10% of SSPs; however, almost 80% of the variation in the Alveograph baking strength (w) value can be attributed to variations in HMW-GS composition (Payne et al. 1988).

The genes encoding HMW-GSs are located on the long arms of chromosomes 1A, 1B, and 1D at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively (Payne *et al.* 1982; Payne 1987a). Alleles on the Glu-D1 locus were reported to greatly affect the wheat flour quality (Payne et al. 1987; Kolster et al. 1991). It has been demonstrated that HMW-GS 5+10 at Glu-D1 locus has the highest positive effect on flour quality than HMW-GS 2+12 and other subunits. Moreover, the HMW-GSs at *Glu-D1* derived from *Aegilops tauschii* have been reported to cause a wide variation in dough strength (Mohamed et al. 2022). These significant effects of HMW-GSs on dough strength and other flour quality characteristics have been evaluated in crops grown under normal conditions. However, it is not well understood whether the effect of HMW-GSs on dough strength and flour quality is similar under field conditions with continuous heat stress. Heat stress has been documented to increase the protein content (Corbellini et al. 1997; Stone and Nicolas 1998; Tahir et al. 2006; Tanaka et al. 2021). The high protein content is generally used as an indicator of strong dough strength and increased bread loaf volume. However, during grain filling, high temperatures (>30–35°C) have been reported to cause adverse impacts on bread dough strength (Randall and Moss 1990) as a result of the concomitant alterations in the composition of gluten proteins (Daniel and Triboi 2000) and increasing the gliadin : glutenin ratio (Blumenthal et al. 1995). Wheat quality is controlled by many genes and quantitative trait loci (QTLs), which are significantly influenced by environmental factors (Kulwal *et al.* 2005; Kumar *et al.* 2019b). Previous studies identified QTLs associated with GPC in almost all tetraploid and hexaploid wheat chromosomes (Blanco et al. 1996; Bogard et al. 2013). However, most previous studies documented more MTAs for the protein content on the B and A genomes than on the D genome. For example, Irina et al. (Leonova et al. 2022) identified eleven significant MTAs for mean protein content evaluated across six environments, of which nine were on chromosome 6A. Also, Liu et al. (Liu et al. 2019) detected QTLs for protein content on chromosomes 2B and 7B, while Prasad et al (Prasad et al. 2003) reported 13 QTLs on chromosomes 2A, 2B, 2D, 3D, 4A, 6B, 7A, and 7D. From the previous literature, the D genome was observed to have relatively lower contribution to the protein content, which may be attributed to the lack of diversity in the D genome in common wheat (Reif et al. 2005).

High temperature stress significantly restricts common wheat productivity in tropical and subtropical areas (Shokat et al. 2021). It causes morphological and physiological changes at all stages, resulting in considerable yield losses (Al-Khatib and Paulsen 1990; Tahir and Nakata 2005; Tahir et al. 2006; Tewolde et al. 2006). During flowering, high temperature decreases grain number; and after anthesis, a temperature above 34°C reduces yield potential (Ferris et al. 1998; Asseng et al. 2011; Lobell et al. 2012). It has been documented that the high temperature causes a massive drop in grain yield of up to 46.63 % (Modarresi et al. 2010). A report by Asseng et al. (Asseng et al. 2015) stated that an increase of 1°C reduces grain yield by 6 %. Also, temperature and yield analysis conducted in the world's hottest wheat-growing region (Iizumi et al. 2021) underscore the critical need of developing climate-resilient wheat cultivars. Identifying genetic loci for grain yield is essential for yield improvement through marker-assisted selection (MAS)

to develop resilient wheat cultivars. Many MTAs have been identified for grain yield on chromosomes 1A, 1B, 2A, 2D, 3A, 3B, 3D, 5A and 5B (Li et al. 2019), as well as on chromosomes 4B and 6B (Id et al. 2019). Under heat stress conditions, MTAs on chromosomes 4A, 6A, 5B, and 3B have been identified (Pinto and Reynolds 2010; Sukumaran et al. 2015; Tadesse et al. 2019; Suliman et al. 2021).

Sudan is characterized as the world's hottest wheat-growing environment (Iizumi et al. 2021). Heat stress is the main abiotic stress that reduces wheat productivity in Sudan. However, yields of up to 5-6 tons/ha have been achieved thanks to tremendous research efforts in collaboration with international research centers such as CIMMYT and ICARDA. However, the average yield in farmers' fields across the country still far exceeds (1.8-2.0 t/ha) what has been achieved in research for various reasons. Improving wheat productivity during the short season (100-110 days), heat-stressed conditions of Sudan is a significant challenge for wheat researchers and producers (Elahmadi 1995; Tahir et al. 2006). The methodology used to meet this challenge included evaluation of agronomic performance and stability of promising wheat genotypes in multi-environment trials across locations with heat-stress gradient from relatively cool northern Sudan (Dongola and Hudeiba) to hot central Sudan (Wad Medani and New Halfa). This methodology has led to identification of many high-yielding elite lines adapted to favorable irrigated areas as well as heat-stress environments(Tahir et al. 2020).

Climate-resilient wheat germplasm is becoming rare due to the narrow genetic diversity of the common wheat (Ogbonnaya et al. 2013; Singh et al. 2018). The wild relative, the D genome donor of common wheat, *Aegilops tauschii*, has been widely reported in stress resilience breeding to expand wheat genetic diversity (Ogbonnaya et al. 2013; Tsujimoto et al. 2015; Cox et al. 2017; Kishii 2019). Thus, to explore the genetic diversity of *Ae. tauschii* for wheat improvement, a platform of wheat multiple synthetic derivative (MSD) panel has been developed using 43 *Ae. tauschii* accessions (Tsujimoto et al. 2015; Gorafi et al. 2018). This MSD platform successfully enabled exploring the wide genetic diversity of heat stress-adaptive traits (Elbashir *et al.* 2017b; Gorafi *et al.* 2018), and showed high genetic variation in drought resilience-related traits (Itam et al. 2021a). Moreover, novel alleles and QTLs associated with resilience to combined heat and drought stress under natural field conditions were identified in MSD lines (Itam et al. 2021b). In addition, kernel weight and shape-related characteristics under heat and combined heat-drought stresses were explored (Elhadi et al. 2021b; a). Likewise, the MSD population showed a wide range of allelic diversity at the Glu-D1 locus and a considerable variation in dough strength due to different introgressed portions of *Ae. tauschii* (Mohamed et al. 2022). Thus, this population is expected to hold genes or resilience lines for improving wheat quality under heat stress.

With this expectation, we conducted this study using the MSD population to i) explore the effect of heat stress on flour quality and grain yield under moderate and continuous heat stress in the field, ii) identify marker-trait associations (MTAs) significantly associated with quality and grain yield traits under heat stress conditions, iii) identify stress resilience lines which combine both grain yield and good quality traits and iv)

assess to which extent the *Ae. tauschii* diversity can be harnessed to improve wheat quality under heat stress conditions.
We found that the presence of certain HMW-GS alleles at the *Glu-D1* locus derived from *Ae. tauschii*, was associated with relatively stable dough strength across environments ranging from optimum to severe heat-stressed conditions. We identified novel MTAs for grain yield and flour quality traits under heat stress environments in wheat lines derived from diverse accessions of *Aegilops tauschii*. In addition, we identified stress resilience lines which combine both grain yield and good quality traits.

3.2 Materials and Methods

3.2.1 Plant materials

This study used a multiple synthetic derivative (MSD) panel that was developed by crossing and backcrossing the Japanese common wheat cultivar 'Norin 61' (hereafter referred to as N61) with 43 synthetic hexaploid wheat (SHW) lines (Tsujimoto et al. 2015; Gorafi et al. 2018). The 43 SHW lines were developed by crosses between 43 diverse accessions of Ae. tauschii and T. turgidum var. durum cv. 'Langdon' (LDN) (Matsuoka and Nasuda 2004; Kajimura et al. 2011). The experiment consisted of 147 MSD lines (BC1F6 in season 2018/19 and BC1F7 in season 2019/20) in addition to three check cultivars. The three check cultivars included the recurrent parent, N61, and two adapted Sudanese cultivars (Imam and Goumria).

3.2.2The experimental sites and field management

The study was carried out in four environments located at three agro-ecological sites in Sudan (Fig. 1): Dongola Research Farm (DON), located in the Northern State (19°08′N, 30°27′E, 239 masl), Hudeiba Research Farm (HUD), located in the River Nile State (17°35′′N, 33°50′E, 409 masl),Wad Medani (MED) at Gezira Research Farm, Agricultural Research Corporation in the central clay plain of Gezira State (14°24′N, 29°33′E, 407 masl) The Soil texture at DON is sandy clay loam at 0–30 cm and silty clay loam at 30–60 cm with pH of 8.0 and low organic matter content (<5%). The soil of HUD is classified as a middle-terrace soil (Karu; pH 8) whereas the soil of MED is a heavy clay soil (pH 8.0–8.4) with low organic matter content (<5%) and low levels of nitrogen (380 ppm) and phosphorus. The experiment was conducted during the 2018/2019 season at MED (MED18/19) and during the 2019/2020 season at MED (MED19/20), HUD (HUD19/20) and DON (DON19/20). The Gezira Research Farm at MED has been classified as mega-environment 5B (ME5B) for wheat cultivation (Gbegbelegbe et al. 2017). The characteristics of each environment has been described in Elbashir et al. (2017). We considered DON as the optimum environment for wheat cultivation in Sudan, whereas HUD and MED were considered moderate and continuous heat-stressed environments, respectively. All experiments in the four environments were arranged in an alpha lattice design with two replications. Each line was sown in a plot consisting of

four rows, 1 m long and 0.2 m apart. Thus, the harvested area was 4 rows x 1 m long x 0.2 m between rows. At MED18/19, MED19/20 and HUD19/20, the sowing was done during the 4th week of November, whereas at DON19/20, the sowing was on the 3rd of December 2019.

The seeds were treated with the insecticide Gaucho (imidacloprid, 35% WP, Bayer Crop Science, USA) and the fungicide Raxil (tebuconazole) at 0.75 and 1.25 g/kg of seed, respectively, to control termites, aphids, and soil-borne diseases. The treated seeds were manually sown at the rate of 120 kg ha^{-1}. Superphosphate was applied by furrow placement before sowing at the rate of 43 kg ha–1 of P2O5. Two doses of nitrogen (86 kg N ha^{-1}) were applied in the form of urea; the first dose was at the three-leaf stage (second irrigation), and the second dose was at the tillering stage (fourth irrigation). The experiments were irrigated frequently every 10-12 days to avoid exposure to water stress. Hand weeding was done at least twice to keep the field free of weed infestation. It is worth mentioning that no serious diseases were reported in the three agro-ecological sites where the experiments were conducted.

Daily maximum and minimum temperature data during the two cropping seasons (2018/2019 and 2019/20) for the three agro-ecological sites were kindly provided by the Sudan Meteorological Authority.

3.2.3 Evaluation of flour quality

Five grams from each MSD line were milled using a UDY cyclone sample mill (UDY Co., USA) equipped with a 1-mm screen to get whole wheat flour. The protein content was measured as a percentage of the total weight by near-infrared spectroscopy (NIR composition analyzer KJT-270, Kett Electric Laboratory Co. Ltd., Japan). The SDS sedimentation volume (SDS-SV) was measured using the method of Takata et al. (Takata et al. 1999) to assess the gluten quantity and quality. As the sedimentation volume is highly correlated with dough strength and bread loaf volume (Axtord et al. 1979), the specific sedimentation values (which are highly correlated with dough strength) was calculated, as an index of gluten quality, by dividing the SDS sedimentation volume (mL) by protein content (%). The protein content is also reported to be highly correlated with sedimentation volume (Moonen et al. 1982; Tanaka and Tsujimoto 2012).

3.2. 4 Genome-wide association analysis (GWAS)

We performed GWAS using DArT-seq markers (Diversity Arrays Technology, Bruce, Australia https://www.diversityarrays.com) for 127 MSD lines and N61. A mixed linear model (MLM) was adopted, including the population structure and kinship matrix using TASSEL v. 5.2.66 software (Bradbury et al. 2007). A total of 19155 high-quality SNP markers with a call rate of 90% (10% missing data) and MAF (minor allele frequency) of > 0.05 were used in the analysis. Manhattan plots were created using (− log10) (P). The adjusted threshold of $P < 3 \text{ X } 10^{-3}$ was used to refer to the degree of association between each SNP marker and a trait, whereas R^2 referred to the variation explained by the

significantly associated markers. To draw the Manhattan plots and quantile-quantile plots, we used MLM product from TASSEL in R v. 4.0.3 with custom scripts in the developed GWAS package rMVP (Yin et al. 2021).

3.2.5 Candidate genes and gene expression

To identify candidate genes for dough strength, grain yield and relative performance indices, we selected the top MTAs that were identified for each trait, and we BLAST them against the International Wheat Genome Sequencing Consortium (IWGS) RefSeq V.1 chromosomes, using URGI with BLAST option (https://urgi.versailles. inra.fr/blast/). Then, we searched for the candidate genes with high confidence in the distance (±500 kbp) for the genome region. We used version 2.1 of IWGSC_Ref_seq to search for genes with high confidence. We used version 1.1 of IWGSC_Ref_Seq_Annotations along with EnsemblPlant (https://plants.ensembl.org) to identify the protein function.

We investigated the expression levels of all candidate genes that highly contributed to dough strength and compared them to the expression of the *Glu-D1* genes using the Wheat Expression Browser expVIP. This led to understanding the association between the candidate genes and dough strength.

3.2.6 Statistical analysis

Phenotypic data were subjected to analysis of variance separately for each environment, and then combined analyses were done. A total of 129 MSD lines, the data of which were commonly available in the four environments, were analyzed using the GenStat Software (18th edition). We used the least significant difference (LSD, 0.05) for genotype mean separation, and the Tukey test to compare the mean of each trait across all environments using SPSS Software (version 25.0.1). Broad-sense heritability (H^2) was calculated using Plant Breeding Tools v. 1.4 software (International Rice Research Institute, http://bbi.irri.org/products).

3.2.7 Relative performance

To compare the performance of the MSD lines under heat stress condition relative to the optimum environment, the relative performance (RP) was calculated considering DON19/20 as an unstressed (optimum) environment and both MED18/19, and MED19/20 as heat-stressed environments. The RP values for dough strength and grain yield for each line were calculated as:

$$\frac{\text{Phenotypic value of each line under heat stress environment}}{\text{Phenotypic value of each line under optimum environment}} * 100$$

Two RP values were calculated for each line: one for MED18/19 (RP1) and the other for MED19/20 (RP2).

3.3 Results

During the heading and grain filling stages, DON19/20 was the coolest, followed by HUD19/20 and MED19/20, whereas MED18/19 was the hottest (Fig. 2).

3.3.1. Protein content (%)

The environmental (E) and the genotypic (G) effects for protein content were significant, however, the G×E interaction effect was not significant (Table 1). Significant differences were found among the four environments in protein content of the MSD lines (Fig. 3a). The mean protein content under the optimum environment at DON19/20 was the lowest whereas the highest mean value was recorded at MED18/19. Compared to the coolest environment (DON19/20), the protein content increased by 11.0, 13.9 and 25.3% at HUD19/20, MED19/20 and MED18/19, respectively.

A wide range of variation was found in protein content within each environment. In the MSD lines, the protein contents ranged from 10.19-16.69% at DON19/20, from 12.21-18.28% at HUD19/20, from 13.21-18.06% at MED19/20, and from 13.62-20.5% at MED18/19. The protein contents of the check cultivars were comparable at MED18/19, DON19/20, and HUD19/20 (Table 1). The protein contents of Imam and Goumria slightly increased under the heat stress conditions, albeit not significantly different from the value obtained under normal condition. For N61, the protein content under the optimum conditions at DON19/20 was significantly higher than that under moderate heat stress condition at HUD19/20.

In comparison with N61, protein contents of 25 (17.6%), 40 (28.0%) and 69 (47.0%) MSD lines were significantly higher at DON19/20, MED19/20 and MED18/19, respectively (Table 2). On the other hand, protein contents of 11 (7.7%), 20 (15.0%) and 3 (2.1%) obtained from the MSD lines were significantly lower than those of N61 at DON19/20, HUD19/20, and MED19/20, respectively. The MSD lines MSD5, MSD24, MSD12 and MSD81 showed more than 25% increase in protein content across the moderate and continuous heat stress environments compared to DON19/20. On the other hand, protein content of some MSD lines (MSD160, MSD219 and MSD413) was relatively stable across the four environments.

3.3.2. Dough strength/(SSVs) (mL/%)

The separate as well as the combined analysis revealed a highly significant effects of the genotype and G×E on dough strength. The mean dough strength at MED19/20 was significantly higher than that at DON19/20, MED18/19, and HUD19/20 (Fig. 3b). The mean dough strength at HUD19/20 was significantly higher than that at DON19/20 and

was comparable with that of MED18/19. The MSD lines showed highly significant differences in dough strength ($p < 0.01$) at MED18/19, MED19/20, HUD19/20, and DON19/20 (Table 1). The dough strength of MSD lines ranged from 0.20 - 0.48 mL/% at DON19/20, from 0.18 - 0.54 mL/% at HUD19/20, from 0.13 - 0.62 mL/% at MED18/19, and from 0.20 - 0.65 mL/% at MED19/20 (Fig. 3b).

The dough strength values of the three checks were comparable at MED19/20, DON19/20, and HUD19/20. Under severe heat stress at MED18/19, N61 showed a significantly lower dough strength value than that of Imam. Imam showed the highest dough strength under continuous heat stress conditions at MED18/19 and MED19/20 among the check cultivars. Interestingly, the lowest dough strength value for Imam was found at the moderate heat stress (HUD19/20), where the highest protein content value was recorded (Table 1).

Compared to N61, dough strength of two lines (MSD65 and MSD159) was significantly higher at MED19/20. The MSD112 line maintained high dough strength at all environments (ranked among the top seven genotypes at the four environments). A number of MSD lines showed comparable dough strength values to N61 at the four locations. On the other hand, 94, 39, 72, and 37 MSD lines showed significantly lower dough strength than N61 at MED18/19, MED19/20, DON19/20, and HUD19/20, respectively (Table 2).

Next, we studied the effect of HMW-GSs at the *Glu-D1* locus on dough strength. Regardless of the HMW-GSs at the A and B genomes, the HMW-GS 2.2+12 derived from N61 had higher dough strength at MED19/20 than at other environments (Fig.3a). For the HMW-GSs derived from *Ae. tauschii*, 2^t+12^t, and 2^t+10^t showed higher dough strength under heat stress environments than under the normal environment (Fig.4b and c, respectively), whereas HMW-GSs 2.1^t+12^t, 2^t+12.1^t, and 5^t+10^t showed no significant effects under the normal and heat stress environments (Fig. 3d, e, and f). At the hot environment (MED19/20), lines possessing these three HMW-GSs, 2.1^t+12^t, 2^t+12.1^t and 5^t+10^t, showed slightly higher (albeit insignificant) dough strength than under optimum environment (DON19/20).

When we considered the three HMW-GSs in the A, B, and D genomes, MSD lines with the HMW-GSs combinations of 2*, 6+8, 2.2+12; 2*, 7+8, 2.2+12; 2*, 7+8, 2^t+12^t; 2*, 6+8, 2^t+10^t; and 2*, 6+8, 2^t+12^t showed higher dough strength at MED19/20 than at DON19/20. Meanwhile, the lines with the HMW-GSs combinations of 2*, 7+8, 2^t+12^t; 2*, 6+8, 2^t+10^t; and 2*, 6+8, 2^t+12^t derived from the D genome of *Ae. tauschii* had higher dough strength under continuous heat stress at MED18/19 than under the optimum condition at DON19/20 (Fig. 5).

The dough strength relative performance at MED18/19 (RP1.SSVs) ranged from 51.4 - 162% with that of N61 being 97%, whereas the RP at MED19/20 (RP2.SSVs) ranged from 74.1 - 202.7%, and N61 had a RP2 value of 104% (Table 2). The MSD lines with

RP.SSVs values above 100% were considered highly efficient in maintaining or possessing better dough strength. Accordingly, 78 MSD lines had higher RP1.SSVs values than that of N61. For RP2.SSVs, 118 MSD lines had higher values than that of N61. A total of 75 MSD lines had consistently higher RP1.SSVs and RP2.SSVs values than that of N61. Among these 75 lines, 35 MSD lines had the HMW-GSs composition 2*, 7+8, 2.2+12 of the recurrent parent N61. The remaining 40 MSD lines possessed HMW-GSs compositions 2*, 7+8, 2^t+12^t (12 lines); null, 7+8, 2.2+12 (8 lines); 2*, 6+8, 2.2+12 (5 lines); 2*, 7+8, 2^t+10^t (4 lines); 2*, 6+8, 2^t+12.1^t; 2*, 6+8, 2^t+12^t; and null, 6+8, 2.2+12 (2 lines for each); and null, 6+8, 2^t+10^t; 2*, 7+8, 5^t+10^t; null, 7+8, 5^t+10^t; and null, 6+8, 2^t+10^t (one line for each). It is worth mentioning that among the 75 lines that had consistently higher RP1.SSVs and RP2.SSVs values, 24 lines were comparable to N61 in their dough strength at DON19/20, and the others showed significantly lower values.

3.3.3 Grain yield (kg/ha)

The combined analysis revealed a highly significant G, E and G×E effects. Highly significant differences ($P < 0.001$) were found among the MSD lines for grain yield in all environments (Table 1). The grain yield significantly differed among the three environments (Fig. 3c). The reductions in grain yield at MED19/20 and MED18/19 were 30.1 and 39.1%, respectively, compared to DON19/20. Even within the same location, high temperatures at MED18/19 caused 12.7% reduction in grain yield compared to MED19/20.

The heat stress significantly decreased the grain yield of the two adaptive Sudanese cultivars Imam and Goumria, as well as N61. The decrease was consistent with increases in temperature and protein contents of the cultivars. Imam showed the highest grain yield value in all conditions and its protein contents were the lowest under all conditions. Goumria showed a significant decrease even between the two stressed environments (MED19/20 and MED18/19). Grain yield of N61 was comparable to that of the Sudanese cultivar under the two heat stress environments (MED18/19 and MED19/20), whereas it was significantly lower at DON19/20 (Table 1).

Under the optimum environment at DON19/20, three MSD lines (MSD53, MSD55, and MSD222) had significantly higher grain yield than N61, whereas eight MSD lines showed lower grain yield than that of N61 (Table 2). Under heat stress environment at MED19/20, nine MSD lines had significantly higher grain yield than N61, whereas 13 MSD lines showed lower grain yield than N61.

Under the continuous heat stress at MED18/19, 10 MSD lines had significantly higher grain yield than N61. Notably, the grain yield of MSD53 at MED18/19 was significantly higher than those of even the adaptive Sudanese cultivars, Imam and Goumria. Meanwhile, three MSD lines (MSD55, MSD77, and MSD205) showed significantly lower grain yield than N61 under heat stress conditions at MED18/19. The highest reduction percent in grain yield at MED18/19 was recorded for MSD55 and MSD205 (77.5 and 69.4%, respectively) compared to DON19/20. At MED19/20, the highest

reductions in grain yield were recorded for MSD427 (75.8%), MSD332 (73.3%) and MSD215 (70.2%) compared to DON19/20 (Table 2).
The relative performance values of grain yield at MED18/19 relative to DON19/20 (RP1) ranged from 22.5 - 201.2 % and that of N61 recorded 57.7 %. On the other hand, the RP value of grain yield at MED19/20 relative to that at DON19/20 (RP2) ranged from 24.2-170% and that of N61 recorded 71.0%. Seven MSD lines consistently had RP1 and RP2 values above 100% (Table 2). Among these lines, MSD024 and MSD026, also showed RP values for dough strength above 100%. On the other hand, 14 MSD lines showed RP1 and RP2 values less than 50%, whereas four MSD lines showed relatively stable RP values (RP1 and RP2 ranged from 90-110 %)
Among the 75 MSD lines with RP of dough strength consistently above 100 %, six lines showed RP for grain yield far better than that of the recurrent parent N61 (RP ranged from 90-130%). Five of these six MSD lines had the HMW-GSs composition of 2*, 7+8, 2.2+12. The other line had the HMW-GSs combination of Null, 7+8, 2.2+12 (Table 2).

MSD lines showed moderate broad sense heritability for protein content (0.68) and grain yield (0.58), while for dough strength had a high broad sense heritability estimate of 0.86 (Table 1).

3.3.4. Marker-trait association for Protein content

Across the four environments, we identified 43 MTAs significantly associated with the variation for protein content on 14 chromosomes (Table 3).
The highest number of MTAs (33 MTAs) for protein content were detected under the optimum environment at DON19/20, which explained 10 - 19% of the phenotypic variation in protein content (Fig. 6a and Table 3). It is noteworthy that 70% of them (23 MTAs) were on the D genome, of which 74% (17 MTAs) were collocated on chromosome 6D at 365.03 - 471.7 Mbp, which explained 10-19% of the phenotypic variation in protein content.
Under moderate heat stress condition at HUD19/20, we identified only one MTA on chromosome 3D that explained 11.8 % of the phenotypic variation (Fig. 5b). Under the continuous heat stress conditions at MED18/19 and MED19/20, we found four and five significant MTAs, respectively (Fig. 6c and d). At MED18/19, the MTAs explained 10-16% of the phenotypic variation, whereas, at MED19/20, they explained 9 - 17% (Table 3). We did not detect any stable marker for protein content across the environments. However, at DON19/20, the MTA on chromosome 4B (at 575.3 Mbp) was close to those MTAs on the same chromosome at MED18/19 and MED19/20 (at 654.1 and 478.9-533.3 Mbp, respectively).

3.3.5. Marker-trait association for dough strength (SSVs)

Under optimum conditions at DON19/20, we identified 5 MTAs on chromosomes 1A, 1D, 2B, 2D and 5B, explaining about 9.1-15.8% of the variation in dough strength (Fig.

6e and Table 4). Under moderate heat stress condition at HUD19/20, we identified 6 MTAs explaining 11.9-16.7% of the phenotypic variation (Fig. 5f). Of the six MTAs identified at HUD19/20, 4 MTAs collocated on chromosome 6D (12.4-17.9 Mbp), and the other 2 MTAs were located on chromosomes 2B and 4B (Fig. 5f and Table 4). A total of 35 and 61 MTAs explaining 9.4 - 20% and 9.6 - 48.5% were identified at MED18/19 and MED19/20, respectively. At MED18/19, several MTAs were collocated on chromosomes 1A (two at 12.72-12.74 Mbp and seven at 500.2-559 Mbp), 1D (two at 412.3-421.8 Mbp and one at 6.321 Mbp), 4D (five at 4.8-26.18 Mbp and three at 86.2-123 Mbp), 6A (two at 37.5-38.4 Mbp), 6D (three at 11.02-24.03 Mbp) and 7D (three at 191.1- 348.9 Mbp). At MED19/20, most of the 61 MTAs were collocated in specific chromosomes. Interestingly, some of these collocated markers' positions overlapped with the positions of the markers detected at MED18/19, for example, some MTAs on chromosomes 1A and 1D (Fig. 6g and h, and Table 4).

We detected 18 stable MTAs at MED18/19 and MED19/20 (Table 5). Out of these 18 MTAs, eight were on chromosome 1A (one at 97.9 Mbp and seven at 500-513 Mbp), two each were on chromosomes 1D (at 412.3-421.8 Mbp), 4D (at 4.8 and 123.0 Mbp), and 6A (at 37.5-39.4 Mbp), and one each on chromosomes 2A (at 697.3 Mbp), 4A (at 403.7 Mbp), 1B (at 559.0 Mbp) and 7D (at 245.7 Mbp). One of the stable markers (1055706|F|0-65) on chromosome 4D had a pleiotropic effect on grain yield in MED18/19 and RP2.SSVs. Although we did not detect stable markers under both optimum and heat stress conditions, a region on chromosome 1D consistently possessed MTAs under both conditions. Under optimum environment at DON19/20, an MTA on chromosome 1D (at 470.8 Mbp) was close to the MTAs on the same chromosome detected at MED18/19 and MED19/20 (at 412.3-421.8 Mbp and 410.5 - 431.3 Mbp, respectively) (Table 4). Similarly, the MTA on chromosome 2D at DON19/20 (at 607.9 Mbp) was close to MTAs on the same chromosome detected at MED18/19 and MED19/20 (at 637.7 and 588.5-613.17 Mbp, respectively) (Table 4).

3.3.6 Marker-trait association for grain yield

A total of 53 MTAs significantly associated with grain yield were identified across 12 chromosomes (2A, 2D, 3D, 4A, 4B, 4D, 5A, 5B, 6A, 7A, 7B, and 7D) at optimum and continuous heat stress environments (Table 6). We detected 27 MTAs for grain yield under optimum condition at DON19/20, explaining 9 – 20 % of the phenotypic variation (Fig. 6i). Out of the 27 MTAs, 52% (14 MTAs) were identified on the D genome, 30% (8 MTAs) on the A genome, and 19% (5 MTAs) on the B genome. A region containing eight MTAs on chromosome 4D located close to each other (at 22.2-29.5 Mbp) showed a strong association with grain yield and explained 9-20% of the phenotypic variation (Table 6). Similarly, regions on chromosomes 3D and 7B showed the same trend, with three MTAs located close to each other (at 12.45-19.069 Mbp), and (at 650.414-647.712 Mbp), respectively, showing a strong association with grain yield and accounted for 14 - 17%, and 10 - 11% of the phenotypic variation, respectively.

Under severe heat stress condition at MED18/19, we identified 9 MTAs, of which seven were located on chromosomes 2D (2 MTAs at 13.7 and 647.8 Mbp) and 4D (one at 10.4, two at 98.4-123.08, and three at 123.01 – 335.2 Mbp) (Fig. 6j and Table 6). The remaining two MTAs were located very close to each other on chromosome 4B at 657.37- 657.47 Mbp. The contribution of these nine MTAs to the observed phenotypic variation ranged from 9 - 14% (Table 6).

At MED19/20, 17 MTAs which explained 9-19% of the phenotypic variation were identified (Fig. 6k and Table 6). Thirteen MTAs (76%) were on the D genome, 3 MTAs were on the B genome, and one MTA was on the A genome. An MTA 3944774|F|0-68 on Chromosome 4D had the strongest association and explained 19% of the phenotypic variance, followed by MTAs on chromosome 3D (2259412|F|0-14) and on chromosome 7D (3947097|F|0-6) that explained 17 and 16% of the phenotypic variance, respectively.

Although no stable markers for grain yield were observed across all environments, several markers were collocated on the same chromosomal regions across the different environments. The MTAs on chromosome 7B at DON19/20 (at about 647-650 Mbp) overlapped with those on the same chromosome (at 639.2-650.4 Mbp) detected at MED19/20. The MTAs detected at MED18/19 on chromosome 4D were close to those detected at MED19/20 on the same chromosome. Similarly, the MTA on chromosome 6D at 474.5 Mbp detected at DON19/20, was close to that detected at 464.8 Mbp at MED19/20 (Table 6).

3.3.7. Marker-trait association for relative performance (RP) of dough strength and grain yield

We conducted GWAS using the RP to identify MTAs significantly associated with the stability of the dough strength and grain yield under heat stress conditions. For the dough strength, we detected 35 and 5 MTAs using RP1.SSVs and RP2.SSVs, respectively. For RP1.SSVs, 10 MTAs collocated on chromosome 4D (five at 6.8-33.8 Mbp, three at 99.3-152.1 Mbp, and two at 335.2-465.8 Mbp), explaining 13-19% of the phenotypic variation (Table 7). Similarly, eight MTAs collocated on chromosome 5A at 466.9-654.8 Mbp and explained 14-16% of the phenotypic variation. For RP2.SSVs, out of the five MTAs, four were consistent with RP1.SSVs and were considered as stable MTAs (one on chromosome 2B (at 42.9 Mbp), one on chromosome 4D (at 123.01 Mbp), and two on chromosome 6D (at 31.1 and 139.07 Mbp) (Table 7).

For the grain yield, we detected 6 MTAs using RP1 and 5 MTAs using RP2.GY with no consistent MTAs between both RP1 and RP2. In RP1.GY, the MTAs explained 11-17 % of the phenotypic variation, whereas in RP2 they explained 12-43% of the phenotypic variation (Table 7). The MTAs detected in RP1.GY on chromosome 2A at 4.1 Mbp was close to that detected for dough strength RP1.GY at 4.2 Mbp on the same chromosome. Likewise, the MTA detected in RP2 on chromosome 3D at 14.2 Mbp was close to that detected for dough strength RP1 at 19.6 Mbp on the same chromosome.

3.3.8. Concurrent/pleiotropic effect

We identified 9 MTAs that had a pleiotropic effect on grain yield, dough strength, and the RPs of grain yield, and dough strength in different environments (Table 5). Among these 9 MTAs, five were collocated on chromosome 4D; three at the distal part (at 23.4 - 25.7 Mbp) and two at 123.01 - 335.2 Mbp. These MTAs were significantly associated with grain yield, dough strength, and RP of dough strength, and could serve as potential markers in wheat molecular breeding for these traits. MTAs on chromosome 4D (1201923|F|0-38 and 1062681|F|0-26) had a pleiotropic effect on grain yield at DON19/20 and dough strength at MED18/19, which explained about 10.47-18.27% of the phenotypic variation An MTA on chromosome 4A (1042486|F|0-52) had a pleiotropic effect on grain yield at DON19/20 and dough strength at MED18/19, as well as on the RP1 for dough strength. Three MTAs on chromosome 4D (998809|F|0-7, 1055706|F|0-65, and 1051116|F|0-23) underlie both grain yield and dough strength under heat stress environment (MED18/19), as well as RP1 or RP2 for dough strength. Similarly, we identified 3 MTAs that control grain yield at DON19/20 and RP1 and RP2 for grain yield and RP1 for dough strength (Table 5). Although the number of the pleiotropic markers is only nine, several MTAs identified in this study collocated with other MTAs on the same chromosome regions that affect other traits.

3.3.9. Allele's contribution, candidate genes, and gene expression

To investigate the contributions of N61 and *Ae. tauschii* alleles to the heat stress tolerance in each HMW-GSs, the alleles of the RP1 and RP2 for dough strength were analyzed and explained in Figures 7 and 8. For RP1, the marker rs1092339 on chromosome 3D was associated with stability/heat stress tolerance of dough strength under heat stress conditions in lines harboring HMW-GS 2.2+12, and 2^t+12^t and SNP allele "C" from *Ae. tauschii* (Fig. 6a). The result was opposite in marker rs32025569 on chromosome 6D for the same HMW-GS (Fig. 7b). The marker rs1099989 on chromosome 4D was associated with decreased dough strength due to the heat stress in lines harboring HMW-GS 2^t+12^t and SNP allele "N" (Fig. 7c). The marker rs32025569 on chromosome 6D was associated with maintaining/stability of dough strength under heat stress in lines harboring HMW-GS 5^t+10^t and SNP allele "N" (Fig. 7b); however, the same subunit (5^t+10^t) also showed good heat tolerance (RP above 80%) when it was carrying SNP allele "C" from N61 and heterozygous SNP allele "C:T". The same trend was observed for subunit 2^t+2.1^t, where it showed a high relative performance (above 80%) when it carries SNP allele "T" from *Ae. tauschii* and SNP allele "C" from N61, as well as SNP allele "N". The marker rs1100384 on chromosome 1D was associated with heat tolerance of dough strength under heat stress conditions in lines harboring HMW-GS 2^t+12.1^t, 5^t+10^t and, 2^t+10^t, and both SNP allele "G" and "A" from N61 and *Ae. tauschii,* respectively (Fig. 7e).

For RP2, the marker rs1696915 on chromosome 2B was associated with maintaining dough strength under the heat stress for lines harboring HMW-GS 2.2+12, 2.1^t+12^t and 2^t+12.1^t and SNP allele "C: A" (Fig. 8a). However, the subunit 2^t+12.1^t also maintained dough strength when it carries the SNP allele "C" from N61 and SNP allele "N". The

marker rs986590 on chromosome 6D was associated with stabilizing dough strength under the heat stress for lines harboring HMW-GS 2.2+12 and 2^t+12^t and SNP alleles "N" (Fig. 8b). The same marker was associated with stabilizing dough strength under heat stress for lines harboring HMW-GS 2^t+12.1^t, 5^t+10^t, and 2^t+10^t regardless of the source of allele.

From the RP1 and RP2 results (Fig.7 and 8), we noticed that the three HMW-GSs (2.1^t+12^t 2^t+12.1^t, and 5^t+10^t) whether carrying the *Ae. tauschii* alleles or N61 alleles or even heterozygous alleles showed high heat tolerance (RP above 80%) in terms of maintaining high dough strength values under heat stress.

We searched for candidate genes associated with the significant markers. We targeted the markers with a high probability combined with high R^2. The resulting candidate genes are listed in Table 3. The markers rs1105119 and rs4262010 on chromosome 2B and 2D, respectively, were associated with dough strength under DON19/20 and encodes a MYB transcription factor and Cytochrome P450 protein, respectively (Table 8). The markers rs1201923, on chromosome 4D, and rs1240703 on chromosome 6D that were associated with grain yield and dough strength at DON19/20 and HUD19/20, respectively, encoded for glutamine synthase and high-affinity nitrate transporter genes, respectively. Under heat stress environments, most of the markers were associated with enhancing wheat heat stress tolerance. The marker rs1092278 on chromosome 1D associated with dough strength under heat stress encodes for Potassium transporter. Moreover, we found that the marker rs1100384 on chromosome 1D that was significantly associated with RP1 of dough strength, encodes protease inhibitor. Both markers rs1055706 on chromosome 4D and rs32025569 on chromosome 6D have a pleiotropic effect on RP1 and RP2 of dough strength and encode an NBS-LRR protein, and an F-box domain-containing protein, respectively (Table 8). The markers rs1668806|F|0-24 and rs3026863|F|0-12 on chromosome 4D and 2D significantly associated with dough strength and grain yield under heat stress condition encode for Protein kinase and Pentatricopeptide protein, respectively.

Using the expression data from expVIP databases (Borrill *et al.* 2016), the expression of the candidate genes was detected and compared to the *Glu-D1*gene expression (TraesCS1D02G317301) (Fig. 8a and b). The expression of *Glu-D1* was high on the seed parts such as endosperm, starchy endosperm, seed coat and aleurone (Fig. 8a). Similarly, the expression level was high during seed developmental stages such as milk and dough developing and repining stages (Fig. 9b). The two candidate genes, *TraesCS2B02G387800* on chromosome 2B and *TraesCS4D02G047400* on chromosome 4D, which were associated with dough strength and grain yield under optimum conditions, respectively, showed a high expression on the seed parts and developmental stage similar to *Glu-D1* gene. Pearson's correlation indicated a strong association between the expression of these candidate genes (*TraesCS2B02G387800*, and *TraesCS4D02G047400*), and *Glu-D1* gene expression (Fig. 9a and b). The candidate gene *TraesCS1D02G321000* on chromosome 1D showed high expression during stem

elongation and seed germination. The candidate gene *TraesCS1D20G159700* on chromosome 1D showed expression on floret parts, embryo, and at the booting stage (Fig. 8a). We noticed that the candidate gene *TraesCS4D02G136900* on chromosome 4D was expressed during all stages (Fig. 8a and b).

3.4 Discussion

This study evaluated the effect of heat stress on flour quality and grain yield under moderate and continuous heat stress in the field using a diverse panel of MSD lines derived from 43 *Ae. tauschii* accessions. The study clearly revealed the significant differential performance of the MSD lines in response to different thermal gradient used in this study as well as the differences observed among the testing environments for all different measured characters. We identified MTAs associated with quality traits and grain yield under heat stress conditions, as well as heat-stress resilient lines that combined heat stress tolerance with high grain yield and good end-use quality traits. Our results indicated that the D genome contributed strongly to grain yield and quality-related characteristics under all conditions, with a diverse range of D-genome markers associated with dough strength and grain yield.

3.4.1. Quality traits

Previously, we reported a wide variation in dough strength of MSD lines grown in a cool environment in Japan (Mohamed *et al.* 2022). In the current study, the MSD lines showed wide variation for dough strength under all conditions ranging from optimum to severe heat stress environments. However, we noticed that the variation in dough strength of the MSD lines was greater at higher temperatures. This variation reflects the wide genetic diversity in MSD lines in response to heat stress, which has been attributed to various introgression segments of *Ae. tauschii* in the MSD lines (Itam *et al.* 2021b). In our study, most of the significant markers for dough strength were identified on the D-genome confirming the wide diversity of the MSD Panel.

The protein content consistently increased with increases in temperature across all environments, especially during the grain filling period. However, the significantly increased protein content under heat stress was not associated with increase in dough strength. For instance, the mean protein content was highest in the hottest environment (MED18/19), while the mean dough strength was significantly lower than that at MED19/20. Nevertheless, the dough strength at MED19/20 was higher than that at DON19/20 and HUD19/20. This might be due to the fact that some MSD lines maintained high dough strength values at high temperatures. The increase in protein content and dough strength, in terms of SDS-SV under heat stress has been reported earlier under field condition (Tahir *et al.* 2006), which might have been due to the increase in both protein content and protein composition (glutenin and gliadin), in addition to the differences in the growth conditions. We observed that temperature above 30 °C at MED19/20 led to a significant increase in the dough strength, and a higher temperature at MED18/19 led to a significant decrease in the dough strength. Alvarado et al. (Alvarado *et al.* 2016)

reported that the same genotype grown in different environments had the same protein content; however, a significantly weak dough was observed at the site with higher mean maximum temperatures. These results signify that not only protein quantity but also its quality is influenced by heat stress. This could be explained by the fact that gluten components (gliadin and glutenin) do not aggregate synchronously, and thus the gliadin: glutenin ratio is affected. The gliadins are synthesized earlier, whereas glutenins tend to be synthesized later at the grain filling stage (Stone and Savin 1999; Shewry *et al.* 2009). This means that any factor that negatively affects or shortens the grain-filling period may change the gliadin: glutenin ratio, and thus negatively affect the dough strength and bread-making quality. In our study, the MSD lines grown at MED18/19 were exposed to an average temperature of 38.5 °C during the grain-filling period, which shortened the grain-filling period. This might be the reason behind the decrease in the dough strength due to the change in the gliadin: glutenin ratio. Although we did not measure the gliadin and glutenin contents directly, their consequent impact on dough strength can be expected.

Previous studies hypothesized that wheat varieties carrying the *Glu-D1d* (5+10) allele are largely more tolerant to heat stress-induced declines in dough quality (Blumenthal *et al.* 1995; Don *et al.* 2005; Irmak *et al.* 2008; Uthayakumaran *et al.* 2012; Tanaka *et al.* 2021). Our study observed the stable performance of lines possessing subunits $2.1^{t}+12^{t}$, $2^{t}+12.1^{t}$ and, $5^{t}+10^{t}$ derived from *Ae. tauschii*. The dough strength of lines carrying these subunits did not differ significantly across the contrasting environments suggesting that these subunits are associated with heat tolerance.

In this study, two MSD lines (MSD065 and MSD159) showed dough strength values superior to the recurrent parent (N61) at MED19/20. However, MSD159 was significantly lower than N61 at MED18/19. This might indicate that MSD159 was affected at temperatures above 35°C. On the other hand, MSD065 maintained better or comparable performance to that of N61 and the adaptive Sudanese cultivars in terms of dough strength across all environments. Thus, it could be used in breeding programs as a source to improve dough strength even under severe heat stress conditions.

3.4.2. Grain yield

The substantial impacts of high temperature on grain yield were clearly shown across all environments. Temperatures above 35 °C have been reported to be more destructive to wheat grain yield and quality (Blumenthal *et al.* 1991; Ciaffi *et al.* 1996). In our study, the high temperatures at MED18/19 led to about 40% reduction in grain yield compared to the normal condition at DON19/20, similar to what has been reported by Modarresi et al. (Modarresi *et al.* 2010). In this study, the reduction was significant even between the two heat stressed environments (MED18/19 and MED19/20).

Wardlaw et al. (Wardlaw *et al.* 1980) stated that the negative impact of temperature increases on the grain weight could be interpreted through the various behaviors of the carbon (C) and nitrogen (N) metabolisms. High temperatures during grain filling increase the daily flow of C and N through the grain but decrease the flow of C per degree day. Thus, the quantity of C in the grain is more influenced by the temperature than the

quantity of N. This explains the decrease in grain yield and the increase in protein content under heat stress.
Despite the significant decrease in grain yield, MSD lines with differential performances across environments were identified. Compared to N61, MSD053 showed significantly higher grain yield at DON19/20 and MED18/19 and was significantly higher than Imam at MED19/20. Two MSD lines (MSD135 and MSD181) gave significantly higher grain yield than N61 at MED18/19 and MED19/20.

3.4.3. Relative performance under heat stress conditions

Using the relative performance of each MSD line under the heat-stressed environments, the degree of heat tolerance was estimated for dough strength and grain yield. We identified 75 lines that consistently maintained RP values above 100% for dough strength, which were higher than that of the N61 and the adaptive Sudanese cultivar Imam. Likewise, we identified three MSD lines that consistently maintained RP values above 100% for grain yield. By comparing the results of RPs for dough strength and grain yield, we found that one MSD line (MSD024) that carried HMW-GS 2^t+10^t maintained comparable grain yield and dough strength to the recurrent parent with high RP values (above 100). On the other hand, the consistently low RP values of a number of MSD lines indicated the considerable negative effects of high temperatures on their performance in terms of grain yield and dough strength.
Considering the moderate to high broad sense heritability recorded for all the traits studied here, selection for these traits within the MSD population will be effective.

3.4.4. Marker-trait association for quality traits

We could not find stable or pleiotropic MTAs for protein content across the four environments, although the genotype x environment (GxE) interaction was insignificant. All the MTAs identified for the protein content were environment-specific. Consistent with our results, Suliman et al. (Suliman *et al.* 2021) could not observe stable MTAs for protein content across three environments. We observed the notable contribution of chromosome 6D at DON, where 17 MTAs lying between 365.039Mbp – 471.721Mbp explained 10.5-19% of phenotypic variance were found. Analysis of the gene distribution across A, B, and D genomes revealed the lowest number of gene loci on the D genome compared with the A and B genomes (Pfeifer *et al.* 2014). Leonova et al. (Leonova *et al.* 2022) detected 50 SNPs across ten chromosomes significantly associated with the grain protein content in six environments. Interestingly, some of the MTAs identified on chromosome 5D were very close to an MTA (rs 3025015|F|0-20 at 560.305Mbp) that we identified on chromosome 5D under optimum conditions at DON19/20. Additionally, one of these MTAs identified on chromosome 6D (467.9165Mbp) was very close to a group of 13 MTAs that identified on chromosome 6D (457.676 -471.721) under optimum conditions at DON19/20. The literature on the genetic mapping indicated that QTLs significantly associated with grain protein content were identified in almost all

chromosomes of tetraploid and hexaploid wheat (Blanco *et al.* 1996; Bogard *et al.* 2013; Kumar *et al.* 2018). We could identify MTAs in most of the chromosomes that were previously reported, thereby indicating the wide diversity inherent in the MSD genetic makeup.

In this study, we identified 89 MTAs across 17 chromosomes that were significantly associated with dough strength. Among them, 18 MTAs were stable for dough strength under heat-stressed environments (MED18/19 and MED19/20). These markers could be valuable for marker-assisted selection of flour quality under heat stress environments. Interestingly, we noticed that one of the stable markers (rs 7352852|F|0-19; 4.891Mbp) on chromosome 4D significantly associated with dough strength under heat stress conditions was close to the markers rs 5332499, rs4440031 and re 3946288 identified in MSD lines for hardness under heat and heat-drought stresses (Elhadi *et al.* 2021a). This indicates that this region may contribute to hardness and dough strength under heat stress conditions.

Although we could not detect stable markers under optimum and heat stress conditions, a region on chromosome 1D consistently possessed MTAs under control and heat stress conditions. These results indicate the contribution of other genes in chromosome 1D to dough strength. In this context, we identified different candidate genes associated with dough strength and RP.SSVs under different conditions. The marker rs1105119 on chromosome 2B was associated with dough strength under optimum conditions at DON19/20 and encodes a MYB transcription factor. The MYB family transcription factors have been reported to play key roles in response to drought stress and salinity (Liu *et al.* 2011; Qin *et al.* 2012; Zhao *et al.* 2018). Pearson correlation showed a strong association between this candidate gene and *Glu-D1* gene. Similar to *Glu-D1* gene, the expression of this gene was on seed parts at milk development, dough development, and repining stages. This confirms our GWAS results that identified this marker on chromosome 2B associated with dough strength under optimum conditions at DON19/20. Liu et al. (Liu *et al.* 2019) identified a candidate gene encoded by MYB transcription factor on chromosome 3B (94.94 cM) associated with protein content in wheat lines derived from wild emmer wheat. The marker rs4262010 on chromosome 2D that was associated with dough strength under optimum conditions at DON19/20 encodes a cytochrome P450 protein, which is the enzyme that can perform several types of oxidation-reduction reactions. Candidate genes encoding cytochrome P450 on chromosomes 2A, 2B, 5A, and 7B that were shown to be associated with protein content has been identified (Liu *et al.* 2019) . Likewise, the wheat cytochrome P450 was found to enhance resistance to deoxynivalenol and grain yield (Gunupuru *et al.* 2018). Although the identified candidate gene was associated with dough strength at optimum condition, and not with grain yield, a marker (3024386|F|0-37) identified for grain yield at the same environment was very close (599.99 Mbp) to this MTA on the same chromosome (Table 6). This confirms the association of cytochrome P450 with grain yield and reveals its association with dough strength. However, the expression of this gene was on floret, rachis and spikelet, and was not close to *Glu-D1* gene (Fig. 8a and b).

Under heat stress conditions at MED19/20, the marker rs1092278|F|0-29 on chromosome 1D was associated with dough strength and found to encode a potassium transporter, which is reported to play essential roles in plant growth and environmental adaptation and regulate potassium uptake in wheat (Cheng *et al.* 2018). It has been reported that the HMW-GS levels were regulated by foliar spraying of potassium fertilizer (Gu *et al.* 2021). Furthermore, the marker rs1055706|F|0-65 on chromosome 4D that was stable for dough strength under heat stress conditions and controls both grain yield and RP2.SSVs under heat stress conditions at MED18/19, MED19/20, respectively, was found to be associated with the candidate gene TraesCS4D02G136900 that encodes NBS-LRR. The TaRPM1 is a type of CC-NBS-LRR that positively regulates wheat response to high temperature. In wheat, Wang et al. (Wang *et al.* 2020) concluded that TaRPM1 positively regulates the high-temperature seedling-plant (HTSP) resistance to *Puccinia striiformis* f. sp. *tritici* (Pst) through the salicylic acid (SA) signaling pathway. These results might suggest that factors controlling dough strength stability are associated with plant defense against pathogens.

The marker rs32025569|F|0-33 on chromosome 6D was associated with RP1.SSVs and RP2.SSVs. The candidate gene for this marker encodes an F-box proteins that play crucial roles in abiotic stress responses, and have been reported to enhance heat stress tolerance in wheat by improving enzymatic antioxidants (Li *et al.* 2018). Thus, this result indicated their contribution to the heat stress tolerance in wheat and dough strength stability. On the other hand, a study on MSD panel identified F-box proteins on chromosome 6A associated with kernel weight and kernel diameter under optimum condition (Elhadi *et al.* 2021b).

Beside the identification of candidate genes, we could identify markers that control more than one trait under different conditions. Chromosome 4D showed the highest contribution of markers with pleiotropic effects. Similar to our results, a previous study involving MSD lines, showed chromosome 4D with the highest contribution of MTAs identified for hardness under optimum, heat and heat-drought conditions, as well as hardness heat index and hardness heat drought index (Elhadi *et al.* 2021a). Interestingly, the pleiotropic marker rs 998809|F|0-7 that controls both grain yield and dough strength under heat stress at MED18/19, overlapped with the marker rs1043872|F|0-49 that have been identified for hardness under heat stress condition in MSD lines (Elhadi *et al.* 2021a). These results indicate that the region on chromosome 4D harbors MTAs that control important quality traits and grain yield under heat stress conditions. Therefore, this region could be used in marker-assisted selection targeting these traits under heat stress conditions. Moreover, we found the pleiotropic marker 1079306|F|0-62 on chromosome 4D that controls grain yield under optimum condition and RP1.SSVs. Similarly, Itam et al. (2021b) (Itam *et al.* 2021b) found that the same marker (1079306|F|0-62) on chromosome 4D, controls plant height under heat and combined heat–drought stress in MSD lines.

We found MTAs with a pleiotropic effect on grain yield at DON19/20 and dough strength at MED18/19, as well as RP1.SSVs. Likewise, we found MTAs that control dough

strength at MED18/19 and MED19/20, grain yield at MED18/19 and DON19/20, as well as RP1.SSVs, RP2.SSVs, RP1.GY and RP2.GY. These MTAs could be utilized for marker-assisted selection targeting flour quality and grain yields and their stability under heat stress. To the best of our knowledge, this is the first report of MTAs controlling dough strength and grain yield under heat stress conditions. In our previous study involving the MSD lines (Mohamed *et al.* 2022), we reported no negative relationship between dough strength and grain yield under optimum conditions, indicating the suitability of the MSD in breeding high dough strength without a negative effect on grain yield. In our study, most of the markers identified for RP and pleiotropic markers were on chromosome 4D, confirming the contribution of this chromosome to heat stress tolerance. Thus, collaborative work/research of gene mining on chromosome 4D would facilitate the production of cultivars that combine different desired traits.

The few common MTAs for dough strength and grain yield may be due to the complexity of the MSD population, which have huge diversity resulting from the diverse D genome sources. After validation, these markers could be used in wheat molecular breeding for the identified traits under optimum and heat stress conditions.

Our results clearly showed the association of the different candidate genes with the identified markers for dough strength under heat and optimum conditions. Therefore, since most of the identified markers for dough strength were under heat stress, this may indicate that other genetic factors contribute to the dough strength especially under continuous heat stress environments. Thus, the cooperative expression of these MTAs under both heat stress and optimum conditions may contribute to wheat quality's stability and heat stress tolerance.

3.4.5. Marker-trait association for grain yield

Concerning grain yield and related traits, the D genome has been reported to possess the lowest number of loci, consistent with its relative lowest diversity (Azadi *et al.* 2015; Li *et al.* 2019). In our study, we identified 53 significant MTAs across all environments, with the highest contribution shown by MTAs being on the D-genome under optimum and heat stress conditions. This indicates that the diversity of hexaploid wheat was successfully increased by the introgression of the *Ae. tauschii's* D genome.

Previous studies identified MTAs on chromosomes 5A, 6A, 3B and 5B for grain yield under temperate and heat stress environments (Sukumaran *et al.* 2015). Moreover, Li et al. (Li *et al.* 2019) identified QTLs for grain yield on chromosomes 2D, 3D, and 5A. These markers overlapped with the markers identified here for grain yield on chromosome 2D and 3D under optimum and heat stress condition and chromosome 5A under optimum condition. We found the marker rs1201923|F|0-5 on chromosome 4D contributing to grain yield at DON19/20 (R^2 = 0.20). The associated candidate gene TraesCS4D02G047400 encodes glutamine synthetase, which regulates nitrogen metabolism in wheat (Németh *et al.* 2018). The Glutamine synthetase has been reported to play an essential role in nitrogen-use efficiency, uptake, and assimilation of nitrogen (Zhang *et al.* 2017). These findings indicate that these MTAs are associated with nitrogen-

use efficiency genes under the optimum environment. Interestingly, Elhadi et al.(Elhadi *et al.* 2021a), based on MSD lines, identified the same candidate gene (TraesCS4D02G047400) on chromosome 4D which was associated with hardness under heat, and combined heat-drought and hardness indexes. This result indicates a potential association between genes underlying hardness and grain yield, as well as with nitrogen-use efficiency genes in MSD lines under optimum and heat stress conditions. Although this candidate gene was associated with grain yield under optimum conditions, its expression was similar to the *Glu-D1* gene, which might suggest its potential contribution to dough strength. The position of this marker (encoding glutamine synthetase) lay on the same (exact) region (23.837 Mbp) of the marker (1201923|F|0-38) that was identified for dough strength under heat stress (MED18/19). Thus, this may explain its similar expression to the *Glu-D1* gene.
Under heat stress condition (MED18/18), the marker rs3026863|F|0-12 on chromosome 2D was found to control the grain yield. The candidate genes of this marker encode Pentatricopeptide (PPR) proteins, which have been reported to be important in regulating plant growth, development, cytoplasmic male sterility, stress responses, and seed development (Li *et al.* 2021). The expression of this gene was not close to *Glu-D1.*
Stable MTAs could not be spotted for grain yield across the four environments, indicating that the trait was significantly influenced by the environment and genotype x environment (G x E) interaction.

Generally, the identified MTAs in this study could be used to understand the genetic response to heat stress regarding quality traits and grain yield; thus, facilitating the introduction of desirable and stable alleles to develop resilient cultivars that combine both grain yield and end-use quality under heat stress using marker-assisted selection.

3.4.6. Allele's contribution

Allele's contribution of markers that were associated with heat tolerant and stability of dough strength showed that both N61 and *Ae. tauschii* alleles contributed either negatively or positively to the dough strength stability in each subunit. The absence of a clear relationship between the stability of dough strength in the lines with subunits 2.1^t+12^t, 2^t+12.1^t, and 5^t+10^t might be attributed genetically to the small number of lines carrying these subunits. Thus, more investigation is needed. However, we observed that irrespective of the allele originating from N61 or *Ae. tauschii*, the three subunits 2.1^t+12^t, 2^t+12.1^t, and 5^t+10^t showed high RP above 80%, which indicates that their heat tolerance or stability might be mainly due to their subunits at *Glu-D1* locus and not due to the identified alleles (N61 or *Ae. tauschii*). This observation is consistent with our phenotypic results.

The results of this study demonstrated the significant effects of integrating the D genome from diverse *Ae. tauschii* accessions into beard wheat genome. This is positively reflected

on identifying MSD lines that showed good and stable grain yield as well as good quality-related characteristics under moderate and sever heat stress environments.

3.5 Conclusions

With a hypothesis that *Ae. tauschii* genes could enhance bread wheat bread quality characteristics under heat stress conditions, in this study we explored the variation in dough strength and grain yield in a diverse population of MSD lines (harboring different *Ae. tauschii* introgressions) and conducted GWAS. We found considerable genetic variation for both traits and identified several MTAs, most of them on the D genome, under optimum, moderate, and continuous heat stress conditions. We identified one MSD line (MSD024) that maintained comparable grain yield and dough strength to the recurrent parent with high heat tolerance efficiency. We found that the presence of three HMW-GS alleles at the *Glu-D1* locus (2.1^t+12^t, 2^t+12.1^t, and 5^t+10^t) derived from *Ae. tauschii*, was significantly associated with relatively stable dough strength across four environments ranging from optimum to severe heat-stressed conditions. These alleles could be used for future improvement of wheat end-use quality characteristics under severe heat stress. We successfully identified several chromosomal regions affecting grain yield and dough strength, representing a potential target for MAS to improve both traits under optimum and heat stress conditions. We documented that chromosome 4D in MSD lines harbors promising regions/genes that control different traits under different conditions. Thus, after validation of these MTAs, the collaborative work/research of gene mining on chromosome 4D would facilitate the production of cultivars that combine desired traits. Also, we identified several candidate genes associated with dough strength and grain yield. This study represents one of the rare cases where a large population has been studied for grain yield and quality traits under field conditions with temperature gradients ranging from relatively optimum, moderate, and continuous heat stress. The study provided valuable germplasm lines and potential markers useful for further applications in wheat molecular breeding. Moreover, our results emphasized the importance of *Ae. tauschii* as a great genetic resource for wheat productivity and flour quality improvement in the face of the increasing climate change.

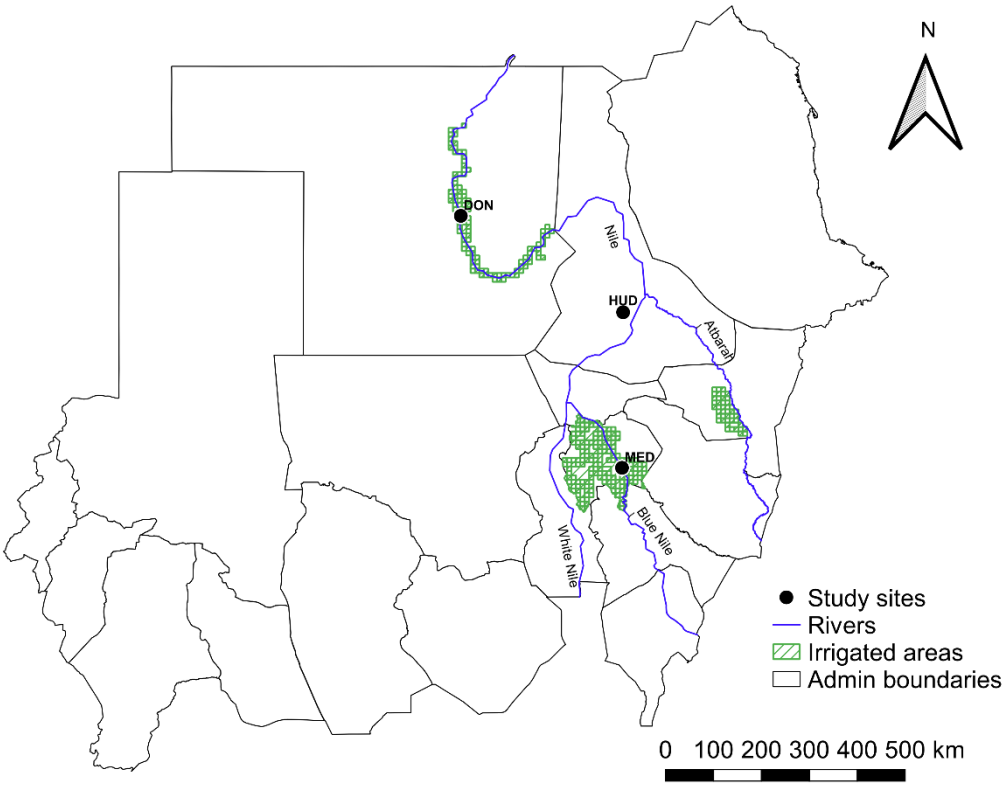

Figure 3.1. Trials sites in Sudan. DON, Dongola; HUD, Hudeiba; MED, WadMedani.

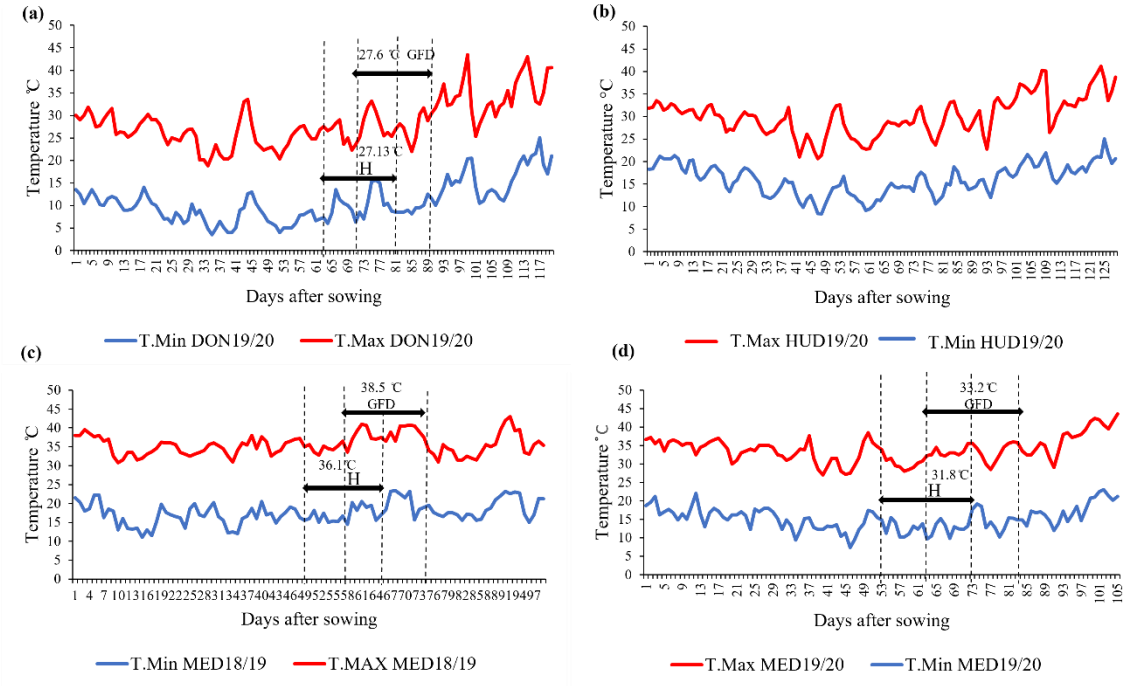

Figure 3.2. Daily maximum and minimum temperatures (T.Max and T.Min, respectively) during the wheat-cropping season at (a) DON19/20, (b) HUD19/20, (c) MED18/19, and (d) MED19/20. H: heading stage; GFD: grain-filling duration. The average maximum temperatures during the heading and grain-filling stages are shown at each location, except for HUD19/20 (no data).

Table 3.1. Effects of genotype (G), environment (E), and their interaction on dough strength, protein content, and grain yield of multiple synthetic derivative lines grown under optimum (DON19/20) and heat stress (HUD19/20, MED18/19, and MED19/2020) conditions.

	Protein content (%)					Dough strength (Ml/%)					Grain yield kg/ha^{-1}			
	DON19/20	HUD19/20	MED18/19	MED19/20	C	DON19/20	HUD19/20	MED18/19	MED19/20	C	DON19/20	MED18/19	MED19/20	C
G	<0.001	0.103	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
E	-	-		-	<0.001	-	-	-	-	<0.001	-	-	-	<0.001
GXE	-	-		-	0.56	-	-	-	-	<0.001	-	-	-	<0.001
Mean	13.6	15.1	17	15.5	15.5	0.30	0.32	0.32	0.38	0.33	4485	2733	3133	3433
Range	10.2-16.3	12.2-18.3	14.4-20.5	13.2-18.1	13.8-17.6	0.20-0.48	0.18-0.47	0.15-0.45	0.20-0.53	0.20-0.44	1942-7218	1030-5084	1150-4764	2154-5395
CV%	4.2	7.4	4.9	3.5	8.5	15.5	12.7	8.9	10.0	14.0	21.34	22.21	18.76	25.2
LSD	1.25	2.58	1.63	1.12	2.85	0.104	0.093	0.056	0.079	0.101	1850	1175.4	1132	1711
SE±	0.57	1.12	0.83	0.54	1.31	0.047	0.041	0.028	0.038	0.046	934.5	593.8	571.9	870.4
h^2	-	-	-	-	0.68	-	-	-	-	0.86	-	-	-	0.58
Goumria	14.6	15.8	17.0	16.1	15.9	0.33	0.37	0.42	0.42	0.39	6438	2188	4166	4258
Imam	12.9	15.6	14.6	13.2	14.2	0.39	0.35	0.45	0.47	0.41	6969	3594	4387	4965
Norin 61	13.5	16.4	15.5	14.8	15.1	0.41	0.38	0.39	0.43	0.40	4579	2636	3277	3494

C: indicate combined analysis; G, the main genotypes effect at each environment; E, environment main effect; G × E, genotype-by-environment interaction; h2 heritability estimate; SE±, standard error of differences; LSD, least significant difference; CV%, coefficient of variation.

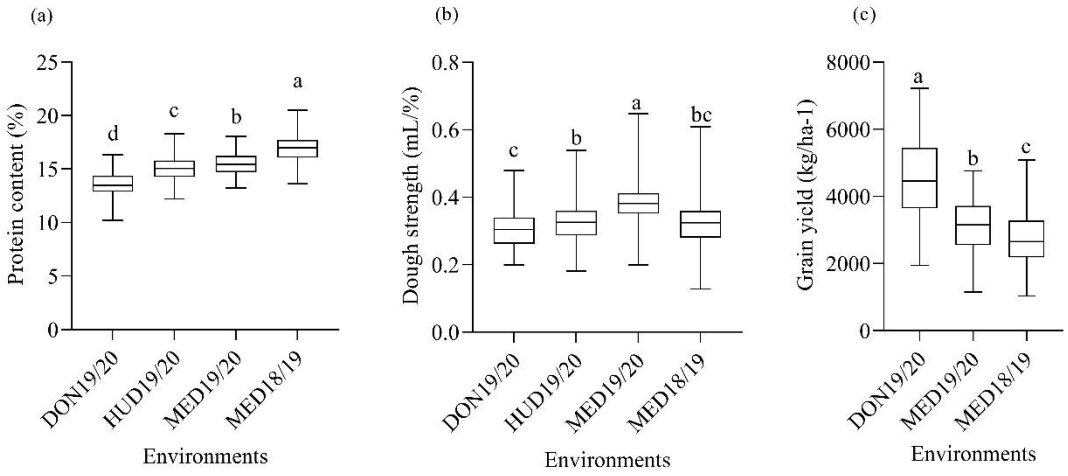

Figure 3.3. Boxplot of (a) mean protein content (b) dough strength and (c) grain yield of multiple synthetic derivative lines grown under optimum (DON19/20), moderate heat stress (HUD19/20) and continuous heat stress (MED18/19 and MED19/2020) conditions. Boxplots with similar lower-case letters indicate that the means for the trait are not significantly different according to the Tukey Test at $P < 0.05$.

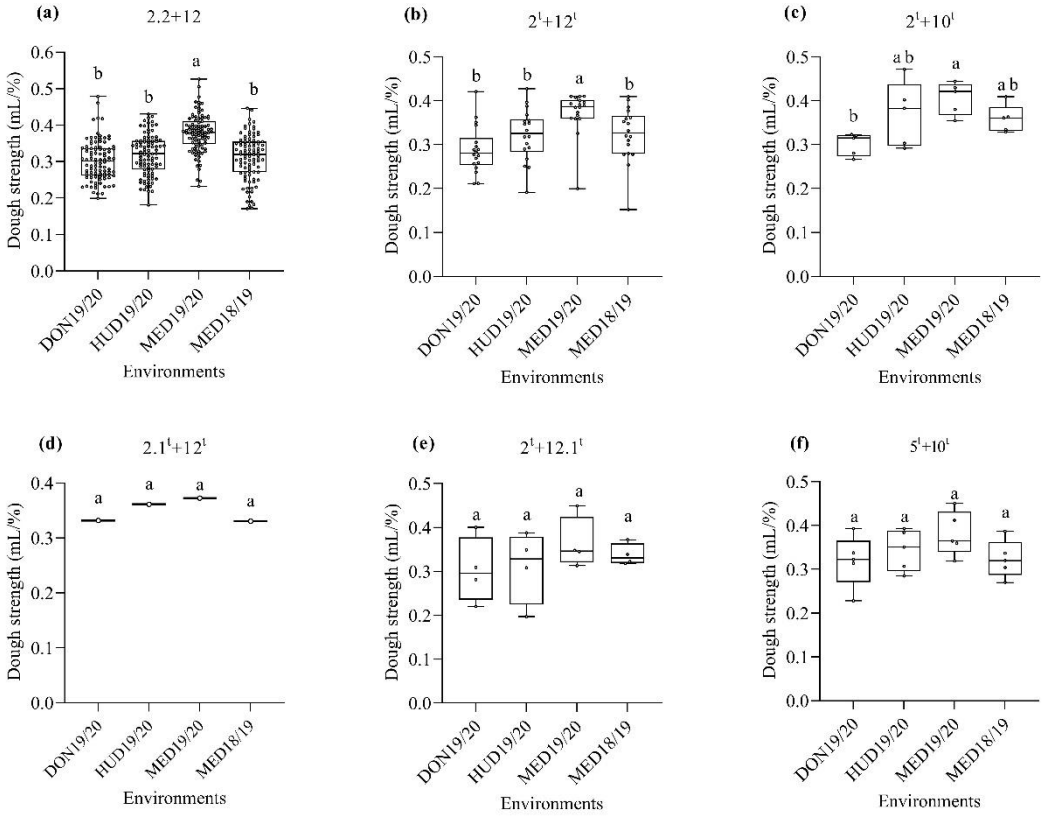

Figure 3.4. Boxplot for the dough strength (mL/%) as affected by the HMW-GS at *Glu-D1* locus across the four environments. (a), subunits 2.2+12; (b), subunit 2^t+12^t; (c), subunit 2^t+10^t; (d), subunit 2.1^t+12^t; (e), subunit 2^t+12.1^t; (f), subunit 5^t+10^t. Similar lower-case letters indicate that the means for HMW-GS pairs are not significantly different according to Tukey Test at $P < 0.05$.

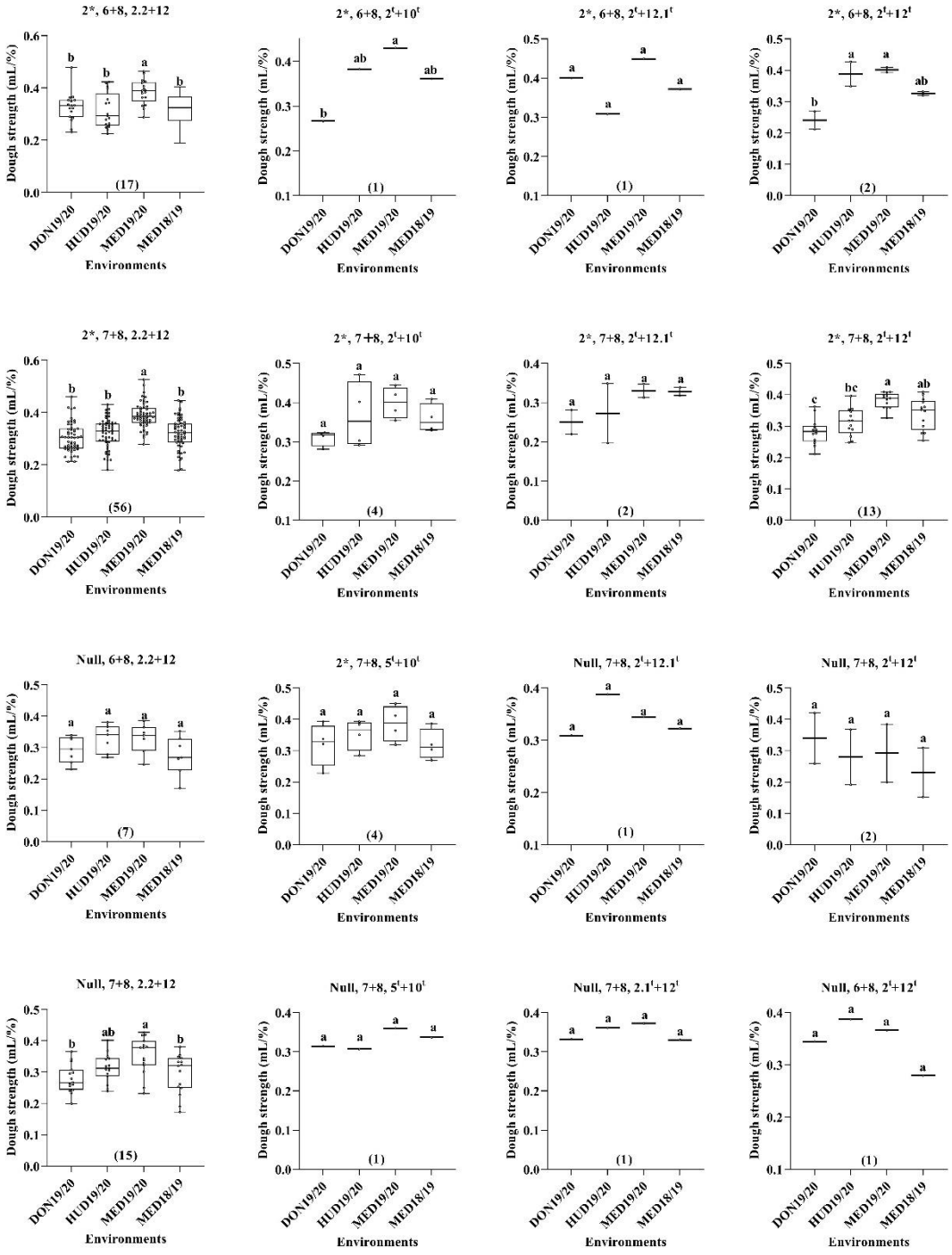

Figure 3.5. Box plot for the dough strength (mL/%) as affected by the HMW-GS combination across the four environments. Numbers in parentheses indicate the number of lines. Similar lower-case letters indicate that the means for the HMW-GS pairs are not significantly different according to the Tukey test at $p < 0.05$.

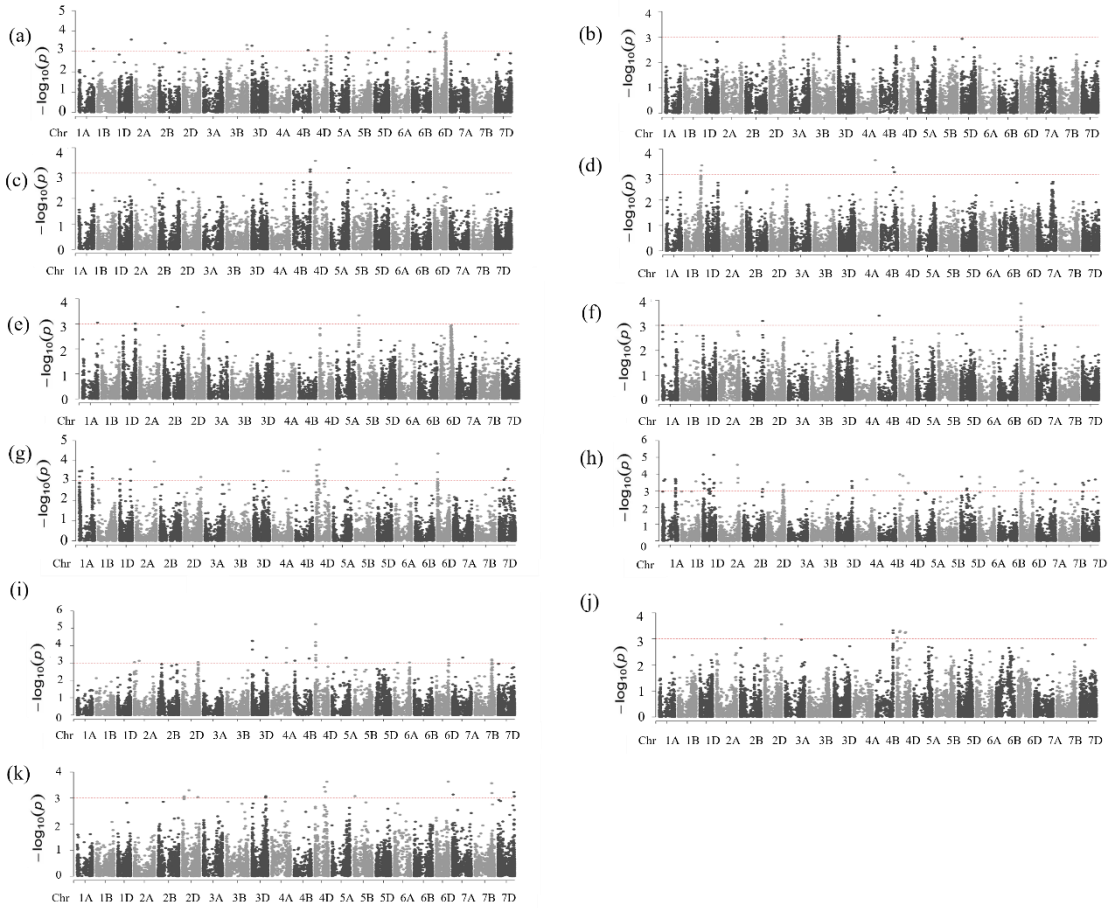

Figure 3.6. Manhattan plots for protein content (a–d) at DON19/29, HUD19/20, MED18/19, and MED19/20, respectively, for dough strength, (e–h), at DON19/29, HUD19/20, MED18/19 and MED19/20, respectively, and for grain yield (i–k) at DON19/20, MED18/19, and MED19/20, respectively.

Table 3.2. The predicted mean of protein content, dough strength, and grain yield and relative performance (RP) for dough strength (SSVs) and grain yield for MSD lines for MSD lines and the checks cultivars across the four environments in Sudan.

Genotypes	*Glu-A1*	*Glu-B*	*Glu-D*	RP1. SSVs	RP2.SSVs	RP1.GY	RP2.GY	Protein content (%)				Dough strength (mL/%)				Grain yield (kg/ha-1)		
								DON19/20	HUD19/20	MED18/19	MED19/20	DON19/20	HUD19/20	MED18/19	MED19/20	DON19/20	MED18/19	MED19/20
MSD005	Null	7+8	5^{t}+10^{t}	107.3	114.7	46.1	86.5	10.2	14.3	15.4	14.5	0.31	0.31	0.34	0.36	5088	2346	4403
MSD006	2*	7+8	5^{t}+10^{t}	98.5	114.8	95.7	93.4	12.6	15.8	16.0	14.4	0.39	0.38	0.39	0.45	3594	3438	3356
MSD007	2*	7+8	2.2+12	55.4	93.4	38.9	44.1	13.3	13.8	17.4	14.8	0.42	0.36	0.23	0.39	5545	2159	2448
MSD008	2*	7+8	2.2+12	89.0	111.8	180.6	149.1	13.1	13.6	17.6	16.3	0.36	0.37	0.32	0.40	1968	3555	2935
MSD012	2*	7+8	2.2+12	120.5	144.7	45.0	55.1	11.9	15.2	16.9	15.0	0.26	0.31	0.31	0.38	5842	2630	3217
MSD017	2*	7+8	2.2+12	91.9	118.3	117.8	107.5	13.1	16.6	17.8	14.7	0.38	0.39	0.35	0.45	3795	4471	4080
MSD018	2*	7+8	2.2+12	122.6	140.8	72.5	65.8	13.6	14.9	18.1	15.5	0.27	0.30	0.34	0.39	4205	3050	2768
MSD019	2*	7+8	2.2+12	89.3	148.6	69.6	71.2	13.4	16.0	16.6	14.3	0.25	0.29	0.22	0.37	4683	3260	3336
MSD022	2*	7+8	2.2+12	99.1	131.6	44.1	44.0	13.9	15.9	19.3	15.9	0.29	0.34	0.29	0.38	5812	2563	2556
MSD024	2*	7+8	2^{t}+10^{t}	101.7	109.6	123.0	129.1	13.0	16.9	18.4	18.0	0.32	0.40	0.33	0.35	2859	3517	3690
MSD026	2*	7+8	2.2+12	101.1	129.7	101.0	112.3	13.5	14.5	16.7	15.4	0.27	0.24	0.27	0.35	3901	3940	4380
MSD031	2*	7+8	2.2+12	134.7	154.7	62.3	55.3	12.9	14.3	15.8	15.0	0.31	0.35	0.42	0.48	4129	2572	2282
MSD032	2*	6+8	2.2+12	67.0	117.9	40.8	57.5	13.5	15.1	18.5	15.2	0.32	0.34	0.22	0.38	3832	1564	2205
MSD041	Null	7+8	2.2+12	90.9	105.4	44.5	54.0	12.0	16.0	16.5	13.8	0.37	0.34	0.33	0.39	5982	2659	3229
MSD043	2*	7+8	2^{t}+12^{t}	76.7	109.2	83.8	109.1	13.2	15.6	18.8	15.6	0.36	0.35	0.28	0.40	2312	1937	2522
MSD044	2*	7+8	2.2+12	129.0	150.0	33.5	50.7	13.2	15.3	16.2	14.3	0.28	0.29	0.35	0.41	6156	2063	3123
MSD050	Null	6+8	2^{t}+12^{t}	81.2	106.4	50.0	63.5	13.8	15.5	19.5	15.9	0.34	0.39	0.28	0.37	3250	1625	2063
MSD052	Null	7+8	2^{t}+12^{t}	73.5	91.5	54.6	77.7	14.7	15.9	17.4	15.4	0.42	0.37	0.31	0.38	3686	2011	2864
MSD053	2*	7+8	2.2+12	91.6	121.9	70.4	53.7	13.4	15.9	17.1	14.7	0.27	0.34	0.24	0.33	7218	5084	3878
MSD055	2*	7+8	2.2+12	80.6	120.2	22.5	44.8	14.3	16.8	18.2	15.6	0.27	0.29	0.22	0.32	6467	1456	2899
MSD061	2*	7+8	2^{t}+12^{t}	107.0	151.8	43.5	41.9	13.2	16.5	18.6	16.3	0.24	0.35	0.25	0.36	5531	2406	2316
MSD062	Null	7+8	2.2+12	142.4	161.9	56.2	112.4	12.7	14.4	16.9	15.6	0.23	0.31	0.33	0.38	3281	1844	3687
MSD065	2*	7+8	2.2+12	139.7	166.6	80.7	60.9	13.6	14.7	16.4	14.8	0.32	0.22	0.44	0.53	3137	2277	2678
MSD066	2*	7+8	2.2+12	76.9	107.1	72.6	85.4	15.9	15.2	17.8	16.5	0.36	0.28	0.28	0.39	3605	1379	3599
MSD081	Null	7+8	2^{t}+12^{t}	58.9	77.1	37.3	65.7	13.0	18.0	19.4	16.3	0.26	0.19	0.15	0.20	4239	1583	2785

MSD091	2*	6+8	2^t+10^t	135.6	161.4	87.0	77.8	12.8	15.7	16.9	14.5	0.27	0.38	0.36	0.43	3844	3344	2989
MSD092	Null	6+8	2.2+12	80.8	106.2	73.9	56.4	15.7	16.2	17.7	17.6	0.33	0.38	0.26	0.35	3365	2488	1899
MSD095	2*	6+8	2.2+12	89.3	112.7	73.9	96.5	12.5	14.3	17.0	13.8	0.32	0.26	0.29	0.36	3719	2750	3590
MSD106	2*	7+8	5^t+10^t	118.1	139.7	46.6	51.6	14.5	16.7	17.6	16.7	0.23	0.35	0.27	0.32	4637	2162	2394
MSD108	2*	7+8	2^t+12^t	124.1	140.2	54.1	68.6	11.6	13.0	16.2	14.5	0.26	0.32	0.32	0.36	5462	2955	3748
MSD112	2*	7+8	2.2+12	106.8	110.1	72.9	83.8	12.0	14.1	15.6	14.6	0.42	0.43	0.45	0.46	4531	3301	3795
MSD114	2*	6+8	2.2+12	115.5	130.5	90.3	67.8	11.2	13.4	16.0	15.3	0.35	0.41	0.40	0.46	4844	4375	3283
MSD117	Null	7+8	2.2+12	93.9	111.6	70.7	76.6	15.0	15.8	16.2	13.6	0.34	0.29	0.32	0.38	5467	3867	4185
MSD119	2*	7+8	2^t+12^t	145.0	147.8	43.3	59.2	13.7	15.3	15.9	15.3	0.28	0.27	0.40	0.41	5344	2313	3166
MSD121	2*	7+8	2.2+12	112.5	138.7	62.1	55.9	12.5	16.6	16.6	15.5	0.26	0.34	0.29	0.36	2969	1844	1660
MSD122	2*	6+8	2.2+12	118.2	134.8	59.8	60.6	15.8	15.5	17.9	17.0	0.29	0.36	0.34	0.39	5735	3431	3475
MSD123	Null	6+8	2.2+12	89.8	114.3	201.2	128.5	13.3	14.7	17.0	14.5	0.25	0.27	0.23	0.29	1942	3907	2495
MSD128	Null	7+8	2.2+12	116.5	135.0	70.7	63.0	12.3	13.7	16.1	14.0	0.30	0.34	0.34	0.40	4580	3237	2887
MSD130	2*	7+8	2.2+12	105.0	111.1	42.5	53.7	13.6	15.9	16.5	15.7	0.34	0.36	0.36	0.38	4187	1781	2248
MSD131				115.5	150.7	51.4	69.1	13.9	14.0	18.7	15.8	0.23	0.23	0.27	0.35	4844	2488	3346
MSD135	2*	7+8	2.2+12	148.6	169.0	69.5	82.0	13.9	15.2	14.4	15.7	0.26	0.31	0.38	0.43	5607	3895	4599
MSD141	2*	7+8	2.2+12	112.0	112.9	59.5	70.1	14.1	15.3	15.7	15.7	0.32	0.33	0.36	0.36	4516	2687	3165
MSD143	2*	6+8	2.2+12	122.1	127.9	66.7	49.3	14.3	15.4	17.3	15.3	0.31	0.40	0.38	0.40	5062	3375	2498
MSD145	2*	7+8	2^t+10^t	129.7	133.5	79.4	112.1	12.2	15.4	16.0	14.3	0.32	0.47	0.41	0.42	4250	3375	4764
MSD147	2*	7+8	2.2+12	152.8	202.7	95.7	105.8	15.0	13.8	16.9	13.8	0.23	0.18	0.35	0.46	3662	3505	3875
MSD148	2*	7+8	5^t+10^t	99.3	113.1	55.3	66.6	14.3	14.8	17.1	16.1	0.32	0.28	0.32	0.36	3844	2125	2560
MSD159	2*	7+8	2.2+12	84.9	151.5	48.8	66.5	12.7	14.5	18.1	14.8	0.33	0.40	0.28	0.51	5000	2438	3327
MSD160	2*	7+8	2.2+12	114.9	128.9	73.7	85.6	15.1	15.7	16.0	15.3	0.33	0.29	0.38	0.42	4454	3281	3814
MSD162	2*	6+8	2.2+12	57.1	86.5	55.0	74.0	13.9	15.0	16.1	16.2	0.33	0.28	0.19	0.29	4064	2234	3009
MSD163	Null	7+8	2.2+12	105.7	113.0	39.7	82.1	12.0	15.1	17.4	16.1	0.33	0.37	0.35	0.38	5510	2186	4521
MSD165	2*	7+8	2^t+12^t	124.3	138.1	67.5	75.9	11.5	14.1	16.6	15.4	0.28	0.32	0.35	0.39	4812	3250	3654
MSD169	2*	7+8	2.2+12	115.0	122.5	61.6	51.9	13.3	14.6	14.9	15.0	0.32	0.32	0.37	0.40	4822	2969	2505
MSD177	2*	7+8	2.2+12	89.2	115.8	86.4	83.5	13.3	14.3	19.2	18.1	0.27	0.23	0.24	0.32	3219	2781	2689
MSD178	2*	7+8	2^t+12^t	130.3	137.9	34.7	45.9	12.9	12.6	16.4	15.8	0.30	0.30	0.39	0.41	5406	1875	2482

MSD181	2*	6+8	2.2+12	91.2	122.1	157.4	170.1	14.2	17.7	16.8	13.8	0.33	0.25	0.30	0.41	2719	4281	4624
MSD186	2*	7+8	2.2+12	112.1	136.1	114.4	91.5	13.5	15.4	17.2	16.9	0.26	0.30	0.30	0.36	3250	3719	2974
MSD187	2*	7+8	2.2+12	128.2	134.5	89.8	78.3	14.6	13.4	15.4	15.3	0.31	0.36	0.39	0.41	3969	3563	3107
MSD189	Null	7+8	2.2+12	145.1	174.1	96.8	123.3	14.4	15.1	16.6	14.7	0.24	0.40	0.35	0.42	3042	2945	3750
MSD190	Null	7+8	2.2+12	102.3	95.2	67.9	82.5	12.7	15.1	17.2	17.5	0.24	0.24	0.25	0.23	4477	3042	3692
MSD192	2*	7+8	2.2+12	128.0	162.3	74.3	96.9	13.5	15.1	16.4	13.2	0.23	0.32	0.30	0.38	4125	3063	3999
MSD195	2*	7+8	2.2+12	151.3	180.3	52.3	69.8	13.8	15.5	16.0	14.6	0.21	0.38	0.32	0.38	5017	2625	3501
MSD205	2*	7+8	2.2+12	133.5	171.7	30.6	76.4	14.7	15.0	17.1	14.2	0.23	0.33	0.31	0.40	3369	1030	2573
MSD215	2*	7+8	2.2+12	111.1	117.6	42.4	29.8	13.0	16.2	16.2	15.1	0.36	0.39	0.40	0.42	6188	2625	1846
MSD217	2*	7+8	2.2+12	70.9	84.7	101.1	78.3	13.0	14.1	18.0	16.6	0.46	0.33	0.33	0.39	2875	2906	2252
MSD222	2*	7+8	2.2+12	93.6	117.8	38.2	41.8	15.3	15.0	18.6	16.3	0.36	0.40	0.34	0.43	6875	2625	2874
MSD226	Null	7+8	2.2+12	100.4	115.3	56.1	83.5	13.4	14.3	17.6	16.3	0.26	0.32	0.26	0.43	4620	2593	3856
MSD241	2*	7+8	2.2+12	95.9	122.9	61.3	38.1	13.3	14.2	16.9	15.9	0.32	0.41	0.31	0.39	5471	3351	2087
MSD247	2*	7+8	2.2+12	104.9	106.7	36.1	84.4	12.3	14.6	15.7	15.0	0.29	0.24	0.30	0.31	4841	1748	4084
MSD249	2*	7+8	2.2+12	102.4	115.5	53.5	58.9	13.3	14.9	16.6	13.8	0.34	0.29	0.34	0.39	5822	3116	3428
MSD250	Null	6+8	2.2+12	96.8	113.5	76.0	87.9	12.4	17.5	16.9	15.3	0.34	0.35	0.33	0.39	4470	3398	3929
MSD254	2*	7+8	2^t+12^t	141.7	134.5	62.0	62.9	11.8	12.2	15.5	16.4	0.29	0.35	0.41	0.39	5039	3124	3169
MSD255	2*	7+8	2.2+12	59.0	91.2	65.5	99.1	13.7	14.0	17.4	16.6	0.42	0.35	0.25	0.38	2851	1866	2824
MSD257	2*	7+8	2.2+12	104.1	152.4	90.0	115.7	15.1	13.8	17.3	16.4	0.26	0.29	0.27	0.40	2500	2250	2893
MSD265	2*	6+8	2^t+12^t	151.0	185.6	64.7	94.0	12.8	15.0	18.3	14.3	0.21	0.43	0.32	0.39	4250	2750	3997
MSD270	2*	7+8	2^t+10^t	104.4	118.7	41.8	46.8	12.8	15.8	16.6	15.7	0.32	0.29	0.33	0.38	5448	2275	2547
MSD274	2*	7+8	2^t+10^t	129.4	158.4	51.5	52.4	14.7	13.7	17.7	14.1	0.28	0.30	0.36	0.44	6125	3156	3211
MSD275	2*	7+8	2.2+12	152.0	164.0	48.8	87.5	15.4	14.2	17.6	14.9	0.27	0.37	0.41	0.44	4448	2170	3893
MSD278	2*	6+8	2.2+12	65.7	76.0	40.0	54.5	13.2	14.6	18.0	15.6	0.48	0.34	0.31	0.36	5156	2063	2812
MSD280	2*	6+8	2^t+12.1^t	92.9	112.2	50.3	53.4	13.6	15.0	16.0	15.7	0.40	0.31	0.37	0.45	5780	2910	3089
MSD284	2*	7+8	2.2+12	69.3	96.6	51.1	49.9	14.0	15.1	17.2	15.4	0.36	0.36	0.25	0.35	4528	2315	2260
MSD289	2*	6+8	2.2+12	97.0	116.2	80.7	91.3	15.7	14.7	17.7	16.3	0.37	0.22	0.35	0.42	3841	3101	3507
MSD296	2*	7+8	5^t+10^t	90.1	122.0	97.9	96.2	13.5	15.2	17.8	15.2	0.34	0.39	0.30	0.41	2972	2911	2859
MSD298	2*	7+8	2.2+12	104.1	119.9	68.3	56.6	13.5	13.2	16.7	15.7	0.31	0.26	0.32	0.37	4953	3382	2803
MSD301	Null	7+8	2.2+12	95.1	125.2	145.8	131.0	13.4	15.0	15.9	16.4	0.20	0.25	0.19	0.25	2250	3281	2947

MSD307	2*	7+8	2.2+12	119.6	139.5	47.3	49.6	14.1	14.6	16.3	15.8	0.30	0.36	0.36	0.42	6321	2992	3133
MSD311	2*	6+8	2.2+12	91.9	131.1	84.4	47.4	13.5	14.3	16.7	15.2	0.35	0.42	0.33	0.46	3406	2875	1616
MSD313	2*	7+8	2.2+12	54.0	110.2	72.1	72.9	16.3	16.2	20.5	14.6	0.34	0.33	0.18	0.37	3685	2656	2686
MSD317	2*	7+8	2.2+12	120.8	120.0	56.9	75.9	13.8	13.5	15.9	14.8	0.31	0.22	0.37	0.37	6094	3469	4623
MSD325	2*	7+8	2t+12t	131.7	154.3	62.5	125.6	15.0	14.7	17.7	15.3	0.21	0.29	0.28	0.33	3104	1939	3899
MSD330	Null	7+8	2.2+12	126.3	134.3	39.6	56.3	12.5	12.4	15.7	14.9	0.26	0.31	0.32	0.34	5433	2154	3058
MSD332	2*	6+8	2.2+12	87.7	138.5	41.3	26.7	13.3	12.8	16.3	16.5	0.23	0.29	0.20	0.32	4312	1781	1150
MSD335	2*	7+8	2.2+12	107.4	98.0	40.0	90.0	14.0	15.7	17.5	15.3	0.37	0.35	0.40	0.36	5044	2020	4541
MSD340	2*	7+8	2^t+12^t	130.8	133.6	48.9	44.3	12.9	14.4	15.7	15.8	0.29	0.25	0.38	0.39	6334	3097	2804
MSD342	2*	7+8	2^t+12^t	117.6	134.0	73.9	81.0	13.3	17.6	18.5	16.1	0.31	0.33	0.36	0.41	4187	3094	3392
MSD343	Null	7+8	2.2+12	82.1	106.5	44.6	53.1	13.6	14.9	19.8	16.7	0.31	0.29	0.25	0.33	4062	1813	2157
MSD345	2*	7+8	2.2+12	111.3	146.6	48.9	51.3	13.3	15.8	17.7	16.3	0.27	0.28	0.30	0.40	4128	2019	2117
MSD346	2*	6+8	2.2+12	134.9	151.8	41.3	47.4	14.6	15.8	16.7	15.6	0.28	0.24	0.37	0.42	5375	2219	2548
MSD360	2*	7+8	2.2+12	119.0	155.9	100.4	95.3	12.9	15.6	17.5	15.4	0.26	0.27	0.31	0.40	3085	3096	2941
MSD361	2*	7+8	2.2+12	127.8	147.1	76.6	82.9	13.2	18.3	16.1	14.7	0.31	0.41	0.39	0.45	4459	3416	3698
MSD363	2*	7+8	2^t+12^t	126.4	130.3	73.6	80.4	13.2	15.3	17.0	16.9	0.28	0.25	0.35	0.36	4375	3219	3518
MSD366	Null	7+8	2.1^t+12^t	99.6	112.2	62.0	89.9	15.5	17.5	17.3	16.8	0.33	0.36	0.33	0.37	3531	2188	3176
MSD368	2*	7+8	2.2+12	104.8	125.7	56.4	86.4	14.5	14.5	17.6	15.6	0.28	0.33	0.29	0.35	4371	2466	3775
MSD370	2*	7+8	2.2+12	89.2	113.6	42.3	41.0	12.5	16.1	18.9	15.4	0.37	0.32	0.33	0.42	5469	2313	2243
MSD377	2*	7+8	2.2+12	162.1	158.1	68.7	66.3	13.7	14.3	15.8	16.2	0.21	0.36	0.34	0.34	3924	2695	2603
MSD378	2*	6+8	2^t+12.1^t	153.7	142.1	43.9	62.3	13.8	13.5	15.2	14.6	0.22	0.20	0.34	0.31	6406	2813	3989
MSD383	2*	7+8	2.2+12	137.0	152.0	81.7	58.4	14.6	17.1	17.2	16.0	0.26	0.34	0.35	0.39	5469	4469	3194
MSD384	2*	7+8	2.2+12	155.8	161.1	68.4	70.3	12.7	14.9	16.8	15.3	0.23	0.22	0.36	0.37	4617	3157	3246
MSD389	Null	7+8	2.2+12	81.4	115.7	67.4	72.6	13.7	17.1	20.4	16.7	0.28	0.26	0.23	0.32	2875	1937	2087
MSD392	2*	7+8	2.2+12	119.8	155.0	56.4	75.2	14.8	15.2	18.1	15.2	0.28	0.25	0.34	0.44	3930	2215	2956
MSD394	Null	7+8	2.2+12	115.7	128.7	52.8	58.7	14.7	16.5	17.1	17.3	0.26	0.35	0.30	0.34	3053	1613	1792
MSD395	2*	7+8	2^t+12^t	121.4	161.6	62.5	42.9	14.4	14.7	17.0	16.1	0.25	0.29	0.30	0.40	5487	3427	2353
MSD397	Null	6+8	2.2+12	51.4	74.1	52.3	85.3	15.1	16.4	18.9	16.9	0.33	0.37	0.17	0.25	2926	1529	2495
MSD401	2*	6+8	2^t+12^t	123.4	152.6	47.2	73.9	12.8	15.2	16.3	13.6	0.27	0.35	0.33	0.41	6193	2923	4575
MSD404	2*	7+8	2.2+12	99.3	106.4	59.9	76.4	12.8	14.2	15.1	15.4	0.31	0.35	0.31	0.33	4906	2938	3747

MSD406	Null	6+8	2.2+12	152.8	158.3	130.3	67.2	12.6	14.9	15.0	15.5	0.23	0.32	0.35	0.36	2687	3500	1805
MSD410	2*	7+8	2.2+12	99.2	110.9	38.6	69.5	13.3	14.2	16.9	14.0	0.33	0.35	0.33	0.37	5187	2000	3604
MSD413	2*	6+8	2.2+12	153.4	159.3	58.1	77.7	15.0	13.9	15.8	15.6	0.24	0.26	0.37	0.38	5812	3375	4517
MSD414	Nulll	6+8	2.2+12	112.2	120.6	61.3	71.5	14.3	13.3	16.7	15.4	0.27	0.28	0.31	0.33	4281	2625	3060
MSD426	2*	7+8	2^t+12^t	103.0	106.4	33.1	58.0	14.1	14.0	17.5	16.9	0.35	0.40	0.36	0.37	5742	1902	3333
MSD427	Null	7+8	2.2+12	142.7	156.7	61.2	24.2	11.8	13.5	15.7	15.5	0.27	0.32	0.38	0.42	5419	3318	1313
MSD434	2*	7+8	2.2+12	78.1	102.2	59.4	88.5	14.7	15.1	18.7	17.0	0.37	0.30	0.29	0.37	3883	2307	3438
MSD437	2*	7+8	2.2+12	83.2	129.4	52.2	79.8	12.6	15.3	18.0	15.6	0.22	0.25	0.18	0.28	4250	2219	3391
MSD450	2*	6+8	2^t+12.1^t	104.4	111.7	79.6	63.8	13.4	14.3	16.0	14.3	0.31	0.39	0.32	0.34	5812	4625	3706
MSD453	Null	6+8	2.2+12	90.8	114.9	85.0	120.6	14.7	15.5	17.8	15.6	0.30	0.34	0.27	0.34	3125	2656	3768
MSD455	Null	7+8	2.2+12	57.8	100.9	57.9	69.6	14.7	16.6	17.4	16.9	0.30	0.31	0.17	0.30	2969	1719	2066
MSD470	2*	6+8	2.2+12	93.9	121.3	85.2	83.3	14.7	16.5	17.3	15.7	0.35	0.42	0.33	0.43	2633	2242	2192
MSD487	2*	6+8	2.2+12	107.8	98.3	43.4	41.9	14.9	13.5	16.1	16.9	0.34	0.30	0.36	0.33	5250	2281	2199
MSD490	2*	7+8	2^t+12.1^t	113.2	123.8	68.8	113.1	13.5	14.9	16.8	15.1	0.28	0.35	0.32	0.35	4019	2764	4545
MSD496	2*	6+8	2.2+12	95.1	116.8	46.2	62.8	13.6	16.1	18.5	14.5	0.29	0.25	0.27	0.33	5332	2462	3351
Norin 61	2*	7+8	2.2+12	96.9	104.4	57.6	71.6	13.5	16.4	15.5	14.8	0.41	0.38	0.39	0.42	4579	2636	3277
Gomeria				125.5	128.8	34.0	64.7	14.6	15.8	17.0	16.1	0.33	0.37	0.42	0.43	6438	2188	4166
Imam	2*	7	5+10	115.1	119.1	51.6	63.0	12.9	15.6	14.6	13.2	0.39	0.35	0.45	0.47	6969	3594	4387
LSD								1.25	2.58	1.63	1.12	0.104	0.093	0.056	0.079	1850	1175.4	1132

LSD; Least Significant Differenc.

Table 3.3. Significant marker trait associations (MTAs) of protein content in multiple synthetic derivative lines grown under optimum (DON), heat stress (HUD, MED18/19, and MED19/20) conditions.

Chromosome	Environment	Marker	Position (Mbp)	P-value	Marker R^2
1A		1090200|F|0-54	565.686	0.0007	0.12559
1D		1218763|F|0-58	477.750	0.0003	0.1783
2B		4404537|F|0-7	241.895	0.0004	0.10954
3B		2277447|F|0-52	793.689	0.0005	0.13416
3B		5578277|F|0-31	819.233	0.0008	0.13065
3D		3533467|F|0-6	5.869	0.0005	0.18975
4B		12774702|F|0-12	575.371	0.0009	0.12613
4D		1210190|F|0-40	482.750	0.0010	0.12392
4D		1102377|F|0-53	501.020	0.0002	0.1692
4D		1697009|F|0-48	484.797	0.0005	0.14133
5D		3025015|F|0-20	560.305	0.0005	0.13317
6A		2292627|F|0-44	2.979	0.0002	0.15333
6A		3949089|F|0-17	601.607	0.0007	0.10035
6A		1238507|F|0-46	604.807	0.0001	0.16948
6B		3949223|F|0-26	92.208	0.0004	0.1096
6B		4910209|F|0-55	681.083	0.0001	0.13529
6D	DON19/20	1204187|F|0-32	365.039	0.0002	0.16271
6D		1667511|F|0-27	422.674	0.0006	0.11362
6D		6035492|F|0-7	428.104	0.0004	0.12905
6D		3944135|F|0-19	436.220	0.0002	0.12461
6D		1079661|F|0-14	457.676	0.0008	0.10514
6D		1395181|F|0-10	459.209	0.0003	0.11284
6D		1230858|F|0-52	459.880	0.0004	0.14241
6D		1236548|F|0-37	461.921	0.0004	0.12118
6D		2251812|F|0-21	462.262	0.0008	0.12493
6D		1243336|F|0-10	462.388	0.0002	0.12111
6D		29430285|F|0-14	462.620	0.0006	0.11176
6D		1159716|F|0-12	462.921	0.0004	0.14195
6D		1101947|F|0-7	462.926	0.0003	0.14623
6D		2253433|F|0-16	465.194	0.0001	0.18954
6D		988515|F|0-46	465.238	0.0005	0.1477
6D		1049628|F|0-25	465.639	0.0007	0.12835
6D		1079427|F|0-37	471.721	0.0008	0.12881
3D	HUD19/20	1005794|F|0-41	24.030	0.0009	0.11886
5A	MED18/19	1219555|F|0-59	702.795	0.0006	0.1015
4B		1126325|F|0-68	657.467	0.0007	0.10287

4B		2265275|F|0-65	654.189	0.0009	0.10587
4D		4396222|F|0-27	52.156	0.0003	0.1612
4A		1152147|F|0-46	650.634	0.0003	0.15897
1B		3533326|F|0-23	618.711	0.0007	0.09956
1B	MED19/20	1215020|F|0-54	648.966	0.0004	0.16224
4B		1093714|F|0-5	478.990	0.0005	0.17434
4B		1004646|F|0-68	533.343	0.0008	0.10595

Table 3.4. Significant marker trait associations (MTAs) of SSVs in multiple synthetic derivative lines grown under optimum (DON), heat stress (HUD, MED18/19, and MED19/20) conditions.

Chromosome	Environment	Marker	Position (Mbp)	P-value	Marker R^2
1A	DON19/20	1221552|F|0-16	579.369	0.0009	0.09249
1D		3947374|F|0-23	470.884	0.0010	0.09083
2B		1105119|F|0-22	555.199	0.0002	0.15788
2D		4262010|F|0-9	607.965	0.0003	0.14146
5B		1117502|F|0-38	11.592	0.0005	0.13283
2B	HUD19/20	9766407|F|0-34	750.742	0.0007	0.12701
4B		2266629|F|0-15	14.441	0.0004	0.11933
6D		1124525|F|0-6	12.476	0.0006	0.13278
6D		3947355|F|0-21	12.874	0.0005	0.15456
6D		1240703|F|0-26	14.399	0.0001	0.13831
6D		1012529|F|0-35	17.952	0.0001	0.16746
1A	MED18/19	1696485|F|0-33	12.727	0.0003	0.16031
1A		2257989|F|0-53	12.745	0.0006	0.13778
1A		3953635|F|0-16	97.919	0.0003	0.16925
1A		1204551|F|0-57	500.253	0.0002	0.13907
1A		1094315|F|0-45	506.846	0.0008	0.09787
1A		3959168|F|0-15	508.265	0.0008	0.09787
1A		1210578|F|0-9	510.293	0.0007	0.0997
1A		3947627|F|0-33	510.911	0.0007	0.13115
1A		4910833|F|0-62	511.070	0.0008	0.09787
1A		2303774|F|0-6	513.884	0.0004	0.1212
1B		996849|F|0-11	559.059	0.0008	0.09787
1D		3026990|F|0-29	6.321	0.0008	0.097
1D		1092278|F|0-29	412.338	0.0003	0.14894
1D		1696345|F|0-38	421.872	0.0010	0.12097
2A		994055|F|0-66	697.354	0.0001	0.18719
2D		5325441|F|0-23	637.784	0.0006	0.13144
4A		3953635|F|0-25	403.756	0.0003	0.16925
4A		1042486|F|0-52	577.563	0.0003	0.14108
4D		7352852|F|0-19	4.891	0.0003	0.11665
4D		1093709|F|0-18	11.685	0.0002	0.12524
4D		1062681|F|0-26	23.470	0.0006	0.13149
4D		1201923|F|0-38	23.837	0.0006	0.10471
4D		1668806|F|0-24	26.187	0.0010	0.19815
4D		1105795|F|0-60	86.287	0.0002	0.15578
4D		998809|F|0-7	98.475	0.0009	0.09491
4D		1055706|F|0-65	123.018	0.0000	0.18889

4D		1051116|F|0-23	335.228	0.0010	0.09455
6A		3534425|F|0-23	37.593	0.0001	0.13946
6A		3940208|F|0-6	39.432	0.0005	0.12304
6D		1109704|F|0-7	11.024	0.0004	0.1449
6D		1127210|F|0-57	11.938	0.0008	0.14172
6D		4991566|F|0-11	24.039	0.0000	0.19061
7D		1067632|F|0-46	191.168	0.0009	0.09678
7D		1091824|F|0-36	245.790	0.0007	0.09947
7D		39604095|F|0-8	348.928	0.0003	0.13958
1A		5582345|F|0-50	51.304	0.0002	0.17134
1A		3953635|F|0-16	97.919	0.0002	0.16139
1A		1204551|F|0-57	500.253	0.0002	0.14026
1A		999238|F|0-52	506.650	0.0009	0.09604
1A		1094315|F|0-45	506.846	0.0003	0.11566
1A		2280040|F|0-40	508.209	0.0002	0.12485
1A		3959168|F|0-15	508.265	0.0003	0.11566
1A		2276262|F|0-38	508.465	0.0007	0.1069
1A		1210578|F|0-9	510.293	0.0002	0.12012
1A		3947627|F|0-33	510.911	0.0004	0.14158
1A		4910833|F|0-62	511.070	0.0003	0.11566
1A		2303774|F|0-6	513.884	0.0004	0.1147
1B		996849|F|0-11	559.059	0.0003	0.11566
1D		1044989|F|0-40	4.726	0.0003	0.16402
1D		3021838|F|0-28	9.276	0.0001	0.21037
1D		1139100|F|0-57	46.893	0.0007	0.10474
1D	MED19/20	1026708|F|0-15	50.436	0.0004	0.14171
1D		1105608|F|0-24	202.295	0.0008	0.09662
1D		1100869|F|0-63	202.333	0.0008	0.09662
1D		18732944|F|0-17	228.202	0.0010	0.09933
1D		1055623|F|0-39	258.602	0.0002	0.12133
1D		1103630|F|0-50	260.292	0.0007	0.12687
1D		2249789|F|0-29	410.542	0.0007	0.14197
1D		1092278|F|0-29	412.338	0.0000	0.22316
1D		1696345|F|0-38	421.872	0.0004	0.13836
1D		2249431|F|0-44	431.398	0.0003	0.15356
2A		12766663|F|0-29	55.441	0.0003	0.14099
2A		994055|F|0-66	697.354	0.0000	0.20257
2A		3934339|F|0-28	710.375	0.0003	0.16159
2A		7352382|F|0-13	726.411	0.0002	0.12454
2B		1298690|F|0-27	751.391	0.0008	0.15876
2D		1060080|F|0-45	0.370	0.0003	0.24668
2D		1233281|F|0-20	588.530	0.0004	0.10766

2D	1246237|F|0-31	605.656	0.0010	0.13628
2D	1094311|F|0-58	612.620	0.0004	0.18422
2D	9724888|F|0-45	613.170	0.0009	0.12823
3A	3954380|F|0-34	743.470	0.0003	0.14313
3D	977377|F|0-66	576.903	0.0003	0.17539
3D	1127175|F|0-44	588.017	0.0007	0.13573
3D	3026388|F|0-8	588.760	0.0005	0.14128
4A	3953635|F|0-25	403.756	0.0002	0.16139
4D	7352852|F|0-19	4.891	0.0001	0.13635
4D	1055706|F|0-65	123.018	0.0001	0.15888
4D	1325403|F|0-22	337.646	0.0003	0.15568
5D	1107051|F|0-13	41.926	0.0001	0.20085
5D	12852245|F|0-49	237.425	0.0009	0.10453
5D	1093066|F|0-14	246.134	0.0007	0.13158
6A	3534425|F|0-23	37.593	0.0001	0.13696
6A	3940208|F|0-6	39.432	0.0004	0.12194
6A	1091880|F|0-17	614.767	0.0006	0.15945
6D	4989686|F|0-55	10.614	0.0001	0.19785
6D	1210420|F|0-50	10.842	0.0004	0.1678
6D	1091018|F|0-40	66.147	0.0001	0.17728
6D	3943157|F|0-24	131.417	0.0003	0.15892
6D	39692052|F|0-53	461.921	0.0002	0.13992
7A	4009968|F|0-27	42.052	0.0003	0.2178
7A	1076268|F|0-6	720.645	0.0004	0.13857
7D	1101906|F|0-53	17.851	0.0003	0.48459
7D	1060822|F|0-46	61.304	0.0004	0.15081
7D	1091824|F|0-36	245.790	0.0002	0.11825
7D	4910759|F|0-15	504.154	0.0002	0.16243

Table 3.5. Stable and pleiotropic MTAs of dough strength (SSVs), relative performance of dough strength (SSVs.RP), grain yield (GY) and relative performance of grain yield (GY.RP) in multiple synthetic derivative lines grown under optimum (DON), heat (HUD, MED18/19, and MED19/20) conditions.

			Environments				
Marker	Chr	Pos	DON19/20	HUD19/20	MED18/19	MED19/20	R^2
1204551|F|0-57	1A	500.253			(SSVs)	(SSVs)	11.2-14.0
1094315|F|0-45	1A	506.846			(SSVs)	(SSVs)	9.8-11.6
3959168|F|0-15	1A	508.265			(SSVs)	(SSVs)	9.8-11.6
1210578|F|0-9	1A	510.293			(SSVs)	(SSVs)	9.9-12.0
3947627|F|0-33	1A	510.911			(SSVs)	(SSVs)	12.7-14.1
4910833|F|0-62	1A	511.070			(SSVs)	(SSVs)	9.8-11.6
2303774|F|0-6	1A	513.884			(SSVs)	(SSVs)	10.9-12.1
3953635|F|0-16	1A	97.919			(SSVs)	(SSVs)	15.1-17.3
996849|F|0-11	1B	559.059			(SSVs)	(SSVs)	9.8-11.6
1092278|F|0-29	1D	412.338			(SSVs)	(SSVs)	12.7-22.3
1696345|F|0-38	1D	421.872			(SSVs)	(SSVs)	12.1-13.8
994055|F|0-66	2A	697.354			(SSVs)	(SSVs)	17.2-20.2
1088439|F|0-52	3D	14.283	(GY)			(RP2.GY)	13.2-15.4
1042486|F|0-52	4A	577.563	(GY)		(SSVs), (RP1.SSVs)		13.1-15.5
3953635|F|0-25	4A	403.756			(SSVs)	(SSVs)	15.1-17.3
1134011|F|0-55	4B	585.751	(GY)		(GY.RP1)		12.0-13.1
1201923|F|0-38	4D	23.837	(GY)		(SSVs)		10.5-18.3
1062681|F|0-26	4D	23.470	(GY)		(SSVs)		13.1-17.6
1051116|F|0-23	4D	335.228			(SSVs), (GY), (RP1.SSVs)		9.4-10.2

1055706|F|0-65	4D	123.018		(SSVs), (GY)	(SSVs), (RP2.SSVs)	13.2-18.9
7352852|F|0-19	4D	4.891		(SSVs)	(SSVs)	10.0-13.6
1079306|F|0-62	4D	25.702	(GY)	(RP1.SSVs)		13.1
998809|F|0-7	4D	98.475		(SSVs), (GY)		9.5-10.2
3534425|F|0-23	6A	37.593		(SSVs)	(SSVs)	10.4-13.9
3940208|F|0-6	6A	39.432		(SSVs)	(SSVs)	12.2-12.3
1091824|F|0-36	7D	245.790		(SSVs)	(SSVs)	9.9-11.8

Ch, chromosome; Pos, Position; R^2, the variation explained by the significantly associated markers

Table 3.6. Significant marker trait associations (MTAs) of grain yield in multiple synthetic derivative lines grown under optimum (DON), heat stress (MED18/19, and MED19/20) conditions.

Chromosome	Environment	Marker	Position (Mbp)	P-value	Marker R^2
2A		1230957|F|0-42	8.394	0.0009	0.12031
2A		1251114|F|0-17	190.128	0.0007	0.12673
2D		3024386|F|0-37	599.992	0.0009	0.12744
3D		39692882|F|0-49	12.453	0.0001	0.16953
3D		1088439|F|0-52	14.283	0.0002	0.15437
3D		1220362|F|0-45	19.069	0.0001	0.142
3D		1117705|F|0-46	552.481	0.0005	0.10904
4A		3946186|F|0-33	567.757	0.0009	0.11937
4A		1042486|F|0-52	577.563	0.0001	0.15544
4B		3958247|F|0-58	37.990	0.0007	0.15115
4B		1134011|F|0-55	585.751	0.0005	0.13086
4D		1102857|F|0-12	22.246	0.0001	0.14029
4D		1062681|F|0-26	23.470	0.0001	0.1762
4D	DON19/20	1201923|F|0-38	23.837	0.0000	0.1827
4D		1201923|F|0-5	23.837	0.0001	0.20238
4D		1079306|F|0-62	25.702	0.0005	0.13073
4D		1220977|F|0-14	29.489	0.0007	0.10004
4D		1001438|F|0-46	29.513	0.0003	0.14223
4D		2259608|F|0-44	29.513	0.0007	0.09856
5A		3064466|F|0-20	555.952	0.0005	0.13083
6A		1148078|F|0-44	119.062	0.0010	0.09259
6A		1157517|F|0-28	582.710	0.0009	0.09364
6D		5410740|F|0-17	474.592	0.0006	0.13399
7A		993056|F|0-39	402.087	0.0005	0.13929
7B		1202710|F|0-8	647.712	0.0006	0.10932
7B		1092427|F|0-46	650.414	0.0007	0.09866
7B		4009342|F|0-5	650.470	0.0010	0.09704
2D		1118828|F|0-21	13.746	0.0010	0.13211
2D		3026863|F|0-12	647.814	0.0003	0.1428
4B		1091993|F|0-59	657.378	0.0005	0.14117
4B		2245409|F|0-68	657.473	0.0006	0.12878
4D	MEDS18/19	7151394|F|0-9	10.424	0.0009	0.09547
4D		998809|F|0-7	98.475	0.0006	0.10255
4D		1055706|F|0-65	123.018	0.0005	0.13194
4D		1090714|F|0-63	318.616	0.0006	0.11157
4D		1051116|F|0-23	335.228	0.0006	0.10246
2D	MEDS19/20	6036213|F|0-36	54.176	0.0009	0.11976

2D	1031825|F|0-33	55.127	0.0010	0.11887
2D	2260995|F|0-27	57.885	0.0009	0.12763
2D	1071677|F|0-10	247.423	0.0005	0.13309
2D	4262013|F|0-11	587.055	0.0009	0.11965
3D	1125840|F|0-24	526.094	0.0010	0.09242
3D	2259412|F|0-14	529.339	0.0009	0.17021
4D	3944774|F|0-68	359.709	0.0004	0.18788
4D	12772973|F|0-60	399.627	0.0006	0.1338
4D	1130841|F|0-26	458.871	0.0002	0.11923
5B	1305083|F|0-47	37.594	0.0008	0.12168
6D	2242142|F|0-27	464.802	0.0002	0.14517
7A	7352373|F|0-57	31.834	0.0007	0.12785
7B	1135269|F|0-10	639.250	0.0003	0.15301
7B	1234181|F|0-13	650.454	0.0006	0.13833
7D	1220524|F|0-7	606.041	0.0006	0.13316
7D	3947097|F|0-6	627.056	0.0009	0.15935

Table 3.7. Significant marker-trait associations (MTAs) of dough strength relative performances (DS.RP) and grain yield relative performances (GY.RP) in multiple synthetic derivative lines grown under optimum (DON), heat stress (MED18/19, and MED19/20) conditions.

Chromosome	Trait	Marker	Position (Mbp)	P-value	Marker R^2
1D	RP1.SSVs	1100384|F|0-67	224.729	0.0006	0.1344
2A		1228734|F|0-53	4.227	0.0008	0.12322
2B		1696915|F|0-34	42.957	0.0005	0.13405
2B		1136141|F|0-39	707.236	0.0006	0.13073
3D		2252258|F|0-61	19.660	0.0008	0.12502
3D		1135824|F|0-39	352.498	0.0003	0.14119
3D		1092339|F|0-34	363.905	0.0001	0.16405
3D		1031016|F|0-13	490.223	0.0005	0.1342
4A		1042486|F|0-52	577.563	0.0002	0.14605
4D		1099989|F|0-65	6.898	0.0001	0.16105
4D		2257344|F|0-47	11.526	0.0006	0.18975
4D		7350081|F|0-38	12.205	0.0008	0.12757
4D		1079306|F|0-62	25.702	0.0005	0.13119
4D		3020940|F|0-6	32.872	0.0003	0.1399
4D		15328360|F|0-66	99.392	0.0002	0.15663
4D		1055706|F|0-65	123.018	0.0000	0.18704
4D		1043872|F|0-49	152.159	0.0004	0.13817
4D		1051116|F|0-23	335.228	0.0002	0.14635
4D		12419821|F|0-22	465.802	0.0008	0.13971
5A		1088387|F|0-9	466.940	0.0003	0.15433
5A		4909518|F|0-22	467.808	0.0002	0.14715
5A		1263136|F|0-17	470.518	0.0002	0.15866
5A		1094259|F|0-17	470.519	0.0002	0.1502
5A		1089823|F|0-19	476.151	0.0003	0.14556
5A		10983977|F|0-25	477.573	0.0003	0.14478
5A		5360863|F|0-14	478.516	0.0002	0.14916
5A		2259275|F|0-15	654.815	0.0005	0.16397
5B		5324316|F|0-61	477.754	0.0006	0.13642
5D		2296391|F|0-32	525.942	0.0006	0.14131
6D		32025569|F|0-33	31.129	0.0001	0.1967
6D		986590|F|0-56	139.073	0.0002	0.15699
6D		2248917|F|0-16	381.346	0.0005	0.13695
2B	RP2.SSVs	1696915|F|0-34	42.957	0.0008	0.12496
4D		1102535|F|0-53	12.166	0.0003	0.10993
4D		1055706|F|0-65	123.018	0.0006	0.12468
6D		32025569|F|0-33	31.129	0.0007	0.14331
6D		986590|F|0-56	139.073	0.0009	0.12781
2A	RP1.GY	1400644|F|0-12	4.149	0.0008	0.12259

2B		1696881|F|0-26	714.137	0.0002	0.17258
3B		3222374|F|0-61	832.163	0.0004	0.14219
4B		1134011|F|0-55	585.751	0.0009	0.11985
5B		3570130|F|0-6	43.065	0.0005	0.12684
5B		986240|F|0-45	47.662	0.0005	0.12684
4A		3064762|F|0-7	694.681	0.0010	0.15832
6B		1204245|F|0-5	712.286	0.0008	0.12997
2D	RP2.GY	3956433|F|0-28	34.042	0.0002	0.42876
3D		1088439|F|0-52	14.283	0.0004	0.13183
7D		3960185|F|0-17	59.005	0.0008	0.12326

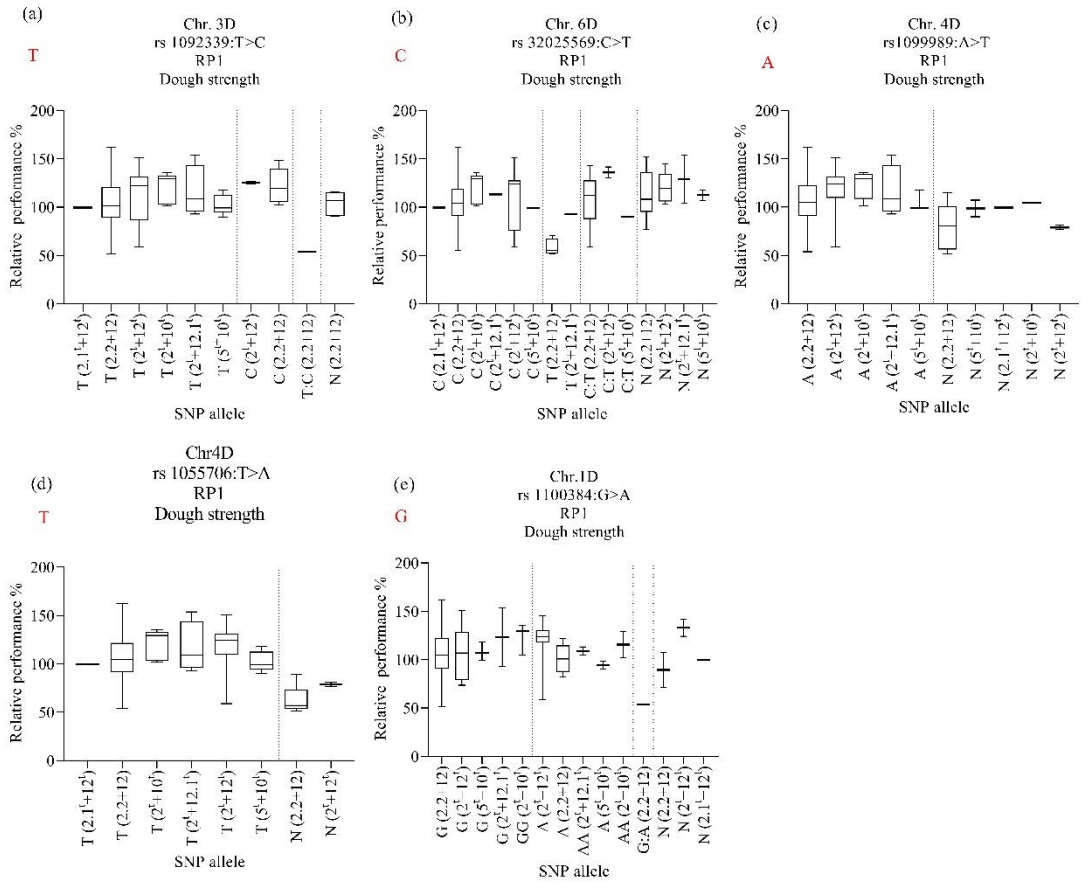

Figure 3.7. Effect of selected marker–trait associations (a–e) on relative performance of dough strength (PR1) in lines with different HMW-GS in MSD lines grown under heat stress conditions. A, adenine; C, cytosine; T, thymine; G, guanine; N, unknown. Alleles in red refer to those of the backcross parent of the population, 'Norin 61'.

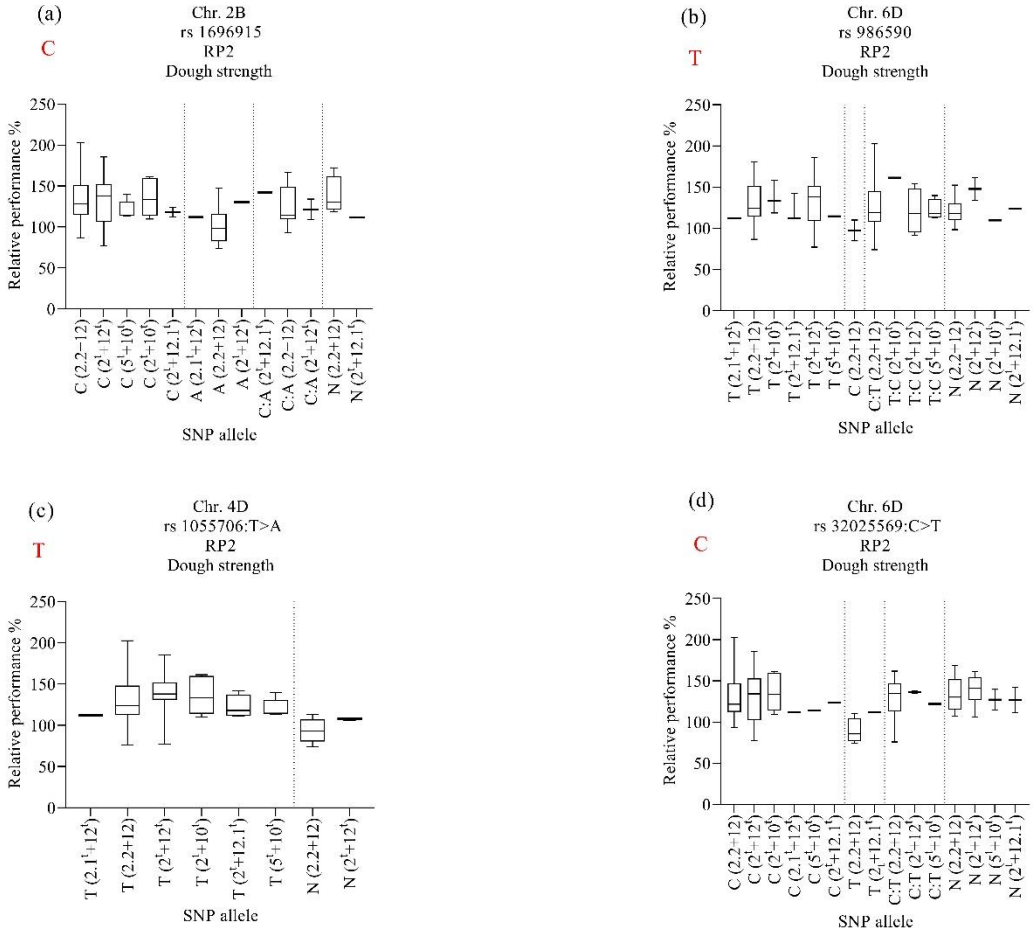

Figure 3.8. Effect of selected marker–trait associations (a–d) on relative performance of dough strength (PR2) in lines with different HMW-GS in MSD lines grown under heat stress. A, adenine; C, cytosine; T, thymine; G, guanine; N, unknown. Alleles in red refer to those of the backcross parent of the population, 'Norin 61'.

Table 3.8. Candidate genes for strong marker-trait association identified for grain yield, dough strength and relative performance calculated for dough strength under different environments

Marker	Environment	Trait	Ch.	R^2	Gene	Protein	Function
1105119|F|0-22	DON19/20	SSVs	2B	0.15	*TraesCS2B02G387800*	MYB transcription factor	Drought stress response in wheat
4262010|F|0-9	DON19/20	SSVs	2D	0.14	*TraesCS2D02G521800*	Cytochrome P450 family protein	Enhanced biotic stress resistance and grain development in wheat
1201923|F|0-5	DON19/20	GY	4D	0.20	*TraesCS4D02G047400*	Glutamine synthase	Regulate nitrogen metabolism in wheat
1240703|F|0-26	HUD19/20	SSVs	6D	0.13	*TraesCS6D02G035600*	High affinity nitrate transporter	Improved nitrogen uptake, root growth and grain yield in wheat
1668806|F|0-24	MED18/19	SSVs	4D	0.19	*TraesCS4D02G048800*	Protein kinase	Regulates plant development and stress tolerance in wheat
3026863|F|0-12	MED18/19	GY	2D	0.14	*TraesCS2D02G588900*	Pentatricopeptide repeat-containing family protein	Regulates plant growth, development, cytoplasmic male sterility, stress responses, and seed development

1092278|F|0-29	MED19/20 MED18/19	SSVs		1D	0.22	*TraesCS1D02G321000*	F-box family protein	Enhanced tolerance to the oxidative stress in wheat
						TraesCS1D02G320200	Potassium transporter	Regulates potassium uptake in wheat. HMW-GL levels were regulated by potsassium avilability
1055706|F|0-65	MED18/19	SSVs	GY	4D	0.18	*TraesCS4D02G136900*	NBS-LRR disease resistance protein, putative, expressed	*TaRPM1* is a type of CC-NBS-LRR positively regulate wheat to high temperature
	MED19/20	SSVs RP2.SSVs						
32025569|F|0-33		RP1.SSVs RP2.SSVs		6D	0.19	*TraesCS6D02G056300*	F-box and associated interaction domains-containing protein TE	Enhance heat stress tolerance in wheat through improve enzymatic antioxidant
1100384|F|0-67		RP1.SSVs		1D	0.13	*TraesCS1D02G159700*	Protease inhibitor/seed storage/lipid transfer family protein	Seed storage protein regulate elasticity and extensibility of dough that determine the processing qualities of various end-products

SSVs, specific sedimentation values (dough strength); RP.SSVs, relative performance for dough strength ; GY, grain yield; Ch.; chromosome.

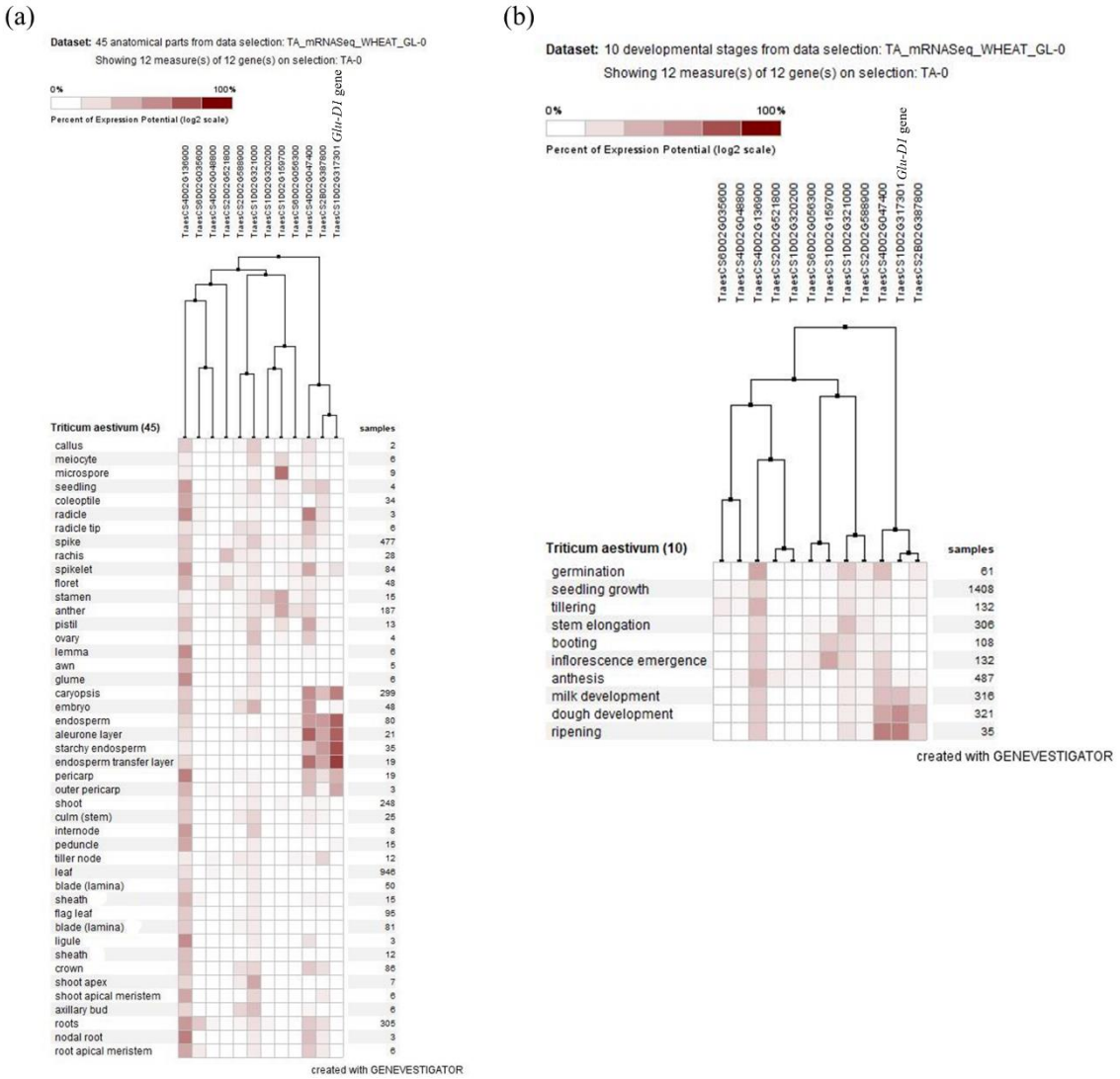

Figure 3.9. The expression of the candidate genes compared to the *Glu-D1* gene expression TraesCS1D02G317301) in different anatomical parts (a) and at different developmental stages (b).

CHAPTER FOUR
SUMMARY OF THE THESIS IN ENGLISH

Wheat grain quality, a characteristic that affects food processing quality and nutritional value, is crucial for assessing new wheat varieties' market potential and commercial value. One of the most important characteristics that affect the quality of wheat is the unique gluten protein, which gives viscoelastic properties that are harnessed to process wheat dough into different products likes: bread, noodles, pasta, and other food products. The gluten proteins are seeds storage proteins, divided into monomeric gliadins and polymeric glutenins. Gliadin proteins are classified into the four major types, α-, β-, γ-, and ω-gliadins, according to their electrophoretic mobility in acid conditions. Glutenins are classified into high molecular weight (HMW) and low molecular weight (LMW) types. The high-molecular-weight glutenin subunits (HMW-GSs) of the glutenin constitute about 10% of seeds storage proteins. However, they are the decisive factor in wheat flour quality to be processed into different products. This is due to the fact that they are the major factor that determines the gluten elasticity, and thus, they are essential for the bread-making process. Therefore, the expansion in the diversity of HMW-GS alleles possibly leads to increased varieties of choices for wheat flour end-products.

The genes encoding for HMW-GSs are located on the long arms of chromosomes 1A, 1B, and 1D at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively. The literatures stated that alleles at *Glu-D1* locus have a significantly strong effect on dough strength and documented the positive and the negative impact of allele 5+10 and 2+12 on dough strength in common wheat respectively. However, the limitation of allele at *Glu-D1* locus due to the narrow diversity in the common wheat is well documented.

Breeding to obtain a wheat variety combine high yields and good quality is very difficult due to the inverse relationship between quality traits (mainly protein content) and grain yield. This relationship depends on several factors, including genotype, source-sink interactions, and environmental factors such as heat stress.

Heat stress is one of the most important abiotic factors that negatively affect both grain yield and flour quality all over the world, especially if the crop is exposed to heat stress during the grain-filling period. It was documented the huge reduction in yield and decrease in the dough strength if the wheat was exposed to heat during this period.

Therefore, breeding for heat stress-resilience wheat genotypes that combine high yield and good quality is crucial to counteracting or adapting to global warming that is expected to increase severely over time.

Thus, to breed for a heat stress-resilience wheat genotypes, it's essential to understand and determine the impact of high temperature on wheat flour quality and grain yield, understand the genetic basis of the diversity resilience to wheat quality, and evaluate differential genotypic responses.

The studies that evaluated the effect of heat and identified climate-resilient wheat genotypes either used very few genotypes or the investigation was under a controlled environment or only identified climate-resilient genotypes considering grain yield and

grain-related traits without addressing the wheat quality aspects.
Since the wheat quality (dough strength) have been documented in several studies to be greatly affected by the HMW-GSs, these studies have been done under normal condition and the effect of HMW-GSs on dough strength under heat stress condition is unclear.
Since both the superior HMW-GS alleles and climate-resilient wheat germplasm is becoming rare due to the narrow genetic diversity of the common wheat, we used in this study (chapter two and three) a diverse panel of wheat multiple synthetic derivatives (MSD). The MSD panel has been developed using 43 *Aegilops tauschii* accessions that represent the existing diversity in the entire natural habitat.

In chapter two, we aimed to explore allelic variation of HMW-GS at the *Glu-D1* locus from *Ae. tauschii* in 392 MSD lines. We aimed to evaluate the impact of the allele at the *Glu-D1* locus from *Ae. tauschii* on dough strength which was under normal condition in Japan to identify good flour quality lines. We also aimed to know the relationship between protein content and grain yield to identify lines that could combine good quality and high grain yield. We observed broad diversity for alleles at the *Glu-D1* locus, reflecting the influence of the different chromosomal inserted segments from *Ae. tauschii*. These alleles at the *Glu-D1* locus may confer different choices in breeding programs for different end-use products. We documented a large variation in dough strength even between lines with the same HMW-GS composition. We document an adverse effect of allele 5^t+10^t and a relatively positive effect of allele 2^t+12.1^t from *Ae. tauschii* on dough strength. We identified four superior lines that improved the flour quality; MSD272, MSD363, MSD219, and MSD61 which carried two different alleles at the *Glu-D1* locus (2.1^t+12^t and 2^t+12^t) derived from *Ae. tauschii.* These lines are promising and could serve as a good source to improve wheat flour quality in the breeding programs. The regression between grain yield and protein content for MSD lines revealed no correlation between the two traits. We could identify MSD lines maintained comparable yields and high protein content compared to the backcrossed parent 'Norin 61'. These MSD lines are promising and could serve as a good source to improve wheat flour quality without any concern about the deterioration in grain yields.

In chapter three, we studied the effect of heat stress on flour quality and grain yield under moderate and continuous heat stress in the field condition in Sudan in 129 MSD lines. We aimed identify heat-stress resilience lines which combine both grain yield and good quality traits. We studied the effect of HMW-GS alleles on flour quality under heat stress to identify subunits that has stable performance or could maintain good dough strength under both optimum and heat stress condition. We performed genome wide association study on a panel of 127 MSD lines, to identify marker-trait associations (MTAs) associated with quality traits and grain yield under heat stress conditions that can be used to enhance both grain yield and flour quality under heat stress conditions. We also aimed to evaluate to which extent the *Ae. tauschii* diversity can be utilized to improve wheat

quality under heat stress conditions.

The MSD lines exhibited noticeable genetic variation for quality traits and grain yield under heat stress conditions. We identified two lines, MSD159 and MSD65 showed superior performance to the recurrent parent Norin 61 regards dough strength under heat-stressed and severe heat-stressed environments respectively. Thus, those lines could be used in breeding programs to improve dough strength under heat stress and even in severe heat stress environments. We Identified three HMW-GS alleles at the *Glu-D1* locus (2.1^t+12^t, 2^t+12.1^t, and 5^t+10^t) derived from *Ae. tauschii*, that showed no significant difference in their dough strength across four environments ranging from optimum to severe heat-stressed conditions. These alleles could be used in applications for future improvement of end-use qualities targeting wheat under severe heat stress. We could identify several MSD lines that showed grain yield higher than the recurrent parent Norin 61 under heat stress condition. Thus, the identified lines could be used as a source to improve grain yield under heat stress environments.

We identified 251 markers traits association, the majority of them on the D genome, confirming the power of the MSD panel as a platform for mining and exploring the genes of *Ae. tauschii.* We identified stable markers for dough strength under heat stress conditions, which simultaneously control grain yield under heat stress or optimum conditions. The identified lines, stable and pleiotropic markers explored in this study, are considered a good resource to develop resilient wheat cultivars that combine both good flour quality and grain yield under stress conditions using marker-assisted selection.

Overall, both studies showed that the wheat wild relative (*Ae. tauschii*) is an inexhaustible resource for gene mining to improve common wheat.

SUMMARY OF THE THESIS IN JAPANESE

コムギ穀粒の品質は、食品加工の品質や栄養価に影響する特性であり、新しいコムギ品種の市場性や商品価値を評価する上で極めて重要である。コムギの品質に影響を与える最も重要な特性の一つは、コムギに特有のグルテンタンパク質である。このタンパク質は、小麦生地をパン、麺、パスタなどのさまざまな製品に加工する際に利用される粘弾性特性を与えている。グルテンタンパク質は種子貯蔵タンパク質であり、単量体のグリアジンと多量体のグルテニンに分けられる。グリアジンは、酸性条件下での電気泳動移動度により、α-、β-、γ-、ω-グリアジンの4種類に大別される。グルテニンは、高分子量（HMW）型と低分子量（LMW）型に分類される。高分子量グルテニンサブユニット（HMW-GS）は、種子貯蔵タンパク質の10％程度を占める。しかし、このサブユニットは、様々な製品に加工される小麦粉の品質を決定付ける要因となっている。これは、グルテンの弾力性を決定する主要因であるためで、製パン工程に不可欠である。したがって、HMW-GS対立遺伝子の多様性の拡大は、小麦粉の最終製品の選択肢の多様性を高めることにつながる可能性がある。

HMW-GSをコードする遺伝子は、染色体1A、1B、1D長腕の*Glu-A1*、*Glu-B1*、*Glu-D1*座にそれぞれ座乗する。*Glu-D1*座の対立遺伝子は生地強度に有意に強い影響を与えることが文献に記載されており、パンコムギでは対立遺伝子5+10と2+12がそれぞれ生地強度に正と負の影響を与えることが記載されている。しかし、コムギの多様性が小さいため、*Glu-D1*座の対立遺伝子数が少ないことはよく知られている。

品質形質（主にタンパク質含量）と収量が逆相関するため、高い収量と良好な品質を兼ね備えた小麦品種を得るための育種は非常に困難である。この関係は、遺伝子型、ソース・シンク相互作用、高温ストレスなどの環境要因を含むいくつかの要因に依存する。高温ストレスは、世界中で穀物収量と小麦粉の品質の両方に悪影響を及ぼす最も大きな生物的要因の一つであり、特に、コムギが登熟期に高温ストレスにさらされた場合、収量が大幅に減少し、小麦粉の生地強度が低下することが報告されている。したがって、高収量と高品質を両立する高温ストレス耐性コムギの育種は、時間とともに深刻になると予想される地球温暖化への対策や適応に極めて重要である。さらに、高温ストレスに強いコムギを育種するためには、高温が小麦粉の品質や穀物収量に与える影響を理解・判定し、コムギの品質に対する多様な耐性の遺伝的基盤を理解し、遺伝子型の反応の差を評価することが不可欠である。

高温の影響を評価し、気候変動に強いコムギを同定したこれまでの研究は、使用した遺伝子型が非常に少ないか、制御された環境下での調査であるか、コムギの品質面には触れずに、収量と穀粒関連形質のみを考慮して気候変動に強い遺伝子型を同定したものである。コムギの品質（生地強度）はHMW-GSによって大きく影響を受けることがいくつかの研究で報告されているが、これらの研究は通常条件下で行われており、高温ストレス条件下でのHMW-GSの生地強度への影響は不明である。

パンコムギの遺伝的多様性が狭いために、その中では優れたHMW-GS対立遺伝子や気候変動に強いコムギ遺伝資源は少ないので、本研究（第2章および第3章）では、多重合成コムギ派生集団（MSD）パネルを用いた。MSDパネルは、自然界全体に存在する多様性を代表する43の*Aegilops tauschii*のアクセッションを用いて開発された。

第2章では、*Ae. tauschii*由来の*Glu-D1*座におけるHMW-GSの対立遺伝子変異を392のMSD系統で探索することを目的とした。また、*Ae. tauschii*由来の*Glu-D1*座の対立遺伝子が、日本の通常条件下での生地強度に与える影響を評価し、高品質な小麦粉が作れる系統の同定を目指した。 また、タンパク質含量と収量との関係を把握し、高品質な小麦粉と高い収量を両立できる系統を同定することを目的とした。その結果、*Glu-D1*座の対立遺伝子には、*Ae. tauschii*とは異なる染色体挿入断片の影響を反映して、幅広い多様性が観察された。これらの*Glu-D1*座の対立遺伝子は、異なる最終加工用途の品質向上を目指した育種プログラムにおいて、それぞれに対応できる可能性がある。私たちは、同じHMW-GS構成を持つ系統間であっても、生地強度に大きなばらつきがあることを見出した。*Ae. tauschii*に由来するHMW-GS対の5^t+10^tは生地強度を弱くし、2^t+12.1^tは比較的強くすることが分かった。また、小麦粉の生地強度を向上させる4つの優良系統を同定した。それらMSD272、MSD363、MSD219、MSD61は*Ae. tauschii*由来の*Glu-D1*遺伝子座の異なる2対のHMW-GS（2.1^t+12^tと2^t+12^t）を保有していた。これらの系統は有望であり、育種プログラムにおいて小麦粉の品質を向上させるための良い材料となる可能性がある。MSD系統について、穀物収量とタンパク質含量の回帰を行ったところ、両形質の間に相関は見られなかった。私たちは、MSD系統の遺伝的背景である農林61号と比較して、同等の収量と高いタンパク質含量を維持するMSD系統を同定することができた。これらのMSD系統は有望であり、穀物収量の低下を心配することなく小麦粉の品質を向上させるための良い材料となる可能性がある。

第3章では，スーダンの圃場条件下で，129系統のMSDを用い，高温ストレスおよび厳しい高温ストレスが小麦粉品質および穀物収量に及ぼす影響について検討した。私たちは、穀物収量と優良品質形質の両方を兼ね備えた高温ストレス耐性系統を同定することを目的とした。HMW-GS対立遺伝子が高温ストレス下の小麦粉品質に及ぼす影響を調べ、通常条件と高温ストレス条件の両方で安定した性能を示し、良好な生地強度を維持するサブユニットを同定した。また、高温ストレス条件下における小麦粉の品質および収量に関連するマーカー-形質連関（MTA）を同定するため、MSD127系統のパネルに対してゲノムワイド関連解析を実施した。また、*Ae. tauschii*の多様性が高温ストレス条件下での小麦の品質向上にどの程度利用できるかを評価することを目的とした。MSD系統は、高温ストレス条件下での品質形質および穀物収量について顕著な遺伝的変異を示した。その結果、MSD159およびMSD65の2系統が、それぞれ高温ストレス環境下および厳しい高温ストレス環境下で、生地強度に関して遺伝的背景の農林61号よりも強いことを見いだした。このことから、これらの系統は、高温ストレス下

および厳しい高温ストレス環境下でも生地強度を維持するための育種プログラムに利用できる可能性がある。 *Ae. tauschii* 由来の *Glu-D1* 座における3対のHMW-GS（2.1^t+12^t, 2^t+12.1^t, 5^t+10^t）を同定し、通常から厳しい高温ストレス環境までの4環境において、その生地強度に有意差は認められなかった。これらの対立遺伝子は、将来、厳しい高温ストレス下でコムギを対象とした最終加工用途の品質改良に応用できる可能性がある。また、高温ストレス条件下で遺伝的背景の農林61号よりも高い収量を示すMSD系統を複数同定することができた。このように、同定された系統は、高温ストレス環境下での穀物収量を向上させるためのソースとして利用できる可能性がある。251のマーカーを同定したが、その大部分はDゲノム上にあり、MSDパネルが*Ae. tauschii*の遺伝子探索のためのプラットフォームとして有効であることを確認した。私たちは、高温ストレス条件下での生地強度に関するマーカーを同定し、同時に高温ストレスまたは通常条件下での穀物収量を制御するマーカーを同定した。本研究で探索したマーカーおよび同定された系統は、高温ストレス条件下でも良好な小麦粉品質と穀物収量の両方を兼ね備えた品種を開発するための良いリソースとなると考えられる。

両研究の結果、*Ae. tauschii*はコムギ改良のための遺伝子探索において豊富な資源を持っていることが示された。

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Mohamed, I. E. S., Oe, H., Kamal, N. M., Mustafa, H. M., Gorafi, Y. S. A., Tahir, I. S. A., Tsujimoto, H. and Tanaka, H. 2022 Enhancing Wheat Flour Quality Through Introgression of High-Molecular-Weight Glutenin Subunits From *Aegilops tauschii* Accessions. Frontiers in Sustainable Food Systems 6: 887795. doi.org/10.3389/fsufs.2022.887795 (Chapter Two).

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