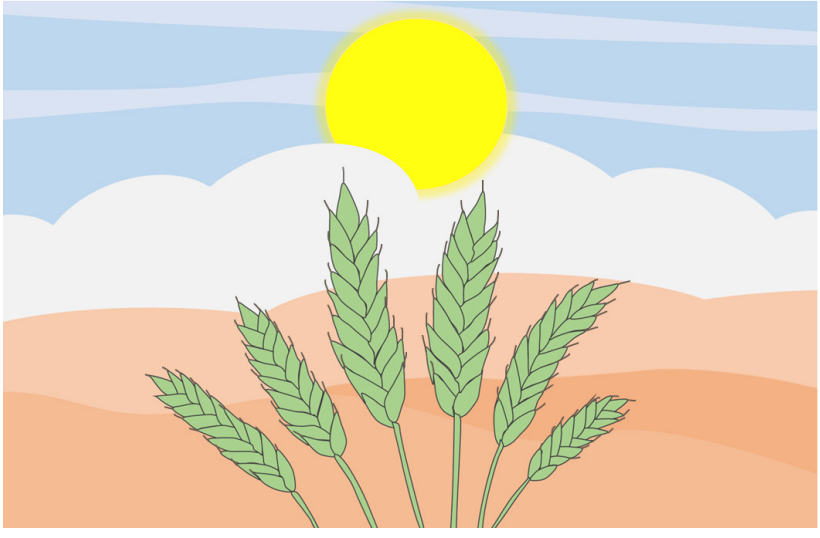
Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2023:26
Faculty of Landscape Architecture, Horticulture and Crop Production Science

Wheat quality under a climate spell

A focus on protein, physico-chemical and growth characteristics evaluated using innovatively combined approaches

Sbatie Lama



Wheat quality under a climate spell

A focus on protein, physico-chemical and growth characteristics evaluated using innovatively combined approaches

Sbatie Lama

Faculty of Landscape Architecture, Horticulture and Crop Production Sciences

Department of Plant Breeding

Alnarp

SLU

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

DOCTORAL THESIS

Alnarp 2023

Acta Universitatis Agriculturae Sueciae
2023:26

Cover: Some wheat spikes with an imaginary field, clouds, sun and sky in the background. Illustration by Sbatie Lama.

ISSN 1652-6880
ISBN (print version) 978-91-8046-104-7
ISBN (electronic version) 978-91-8046-105-4
https://doi.org/10.54612/a.7kcg0aghdm
© 2023 Sbatie Lama, https://orcid.org/10.54612/a.7kcg0aghdm
Swedish University of Agricultural Sciences, Department of Plant Breeding, Alnarp, Sweden
The summary chapter of this thesis is licensed under CC BY NC ND 4.0, other licences or copyright may apply to illustrations and attached articles.
Print: SLU Service/Repro, Alnarp 2023

Wheat quality under a climate spell

A focus on protein, physico-chemical and growth characteristics evaluated using innovatively combined approaches

Abstract

The quality of bread is largely determined by the gluten protein concentration and composition, both greatly influenced by environmental factors such as heat and drought. Future climate in Sweden is expected to fluctuate severely, affecting gluten proteins and the production of bread wheat, as well as future availability of food. The thesis aimed to enhance knowledge of the effect of varying climates on the gluten protein quality in Swedish wheat and to evaluate new methods for yield and gluten protein screening in order to assist in future wheat breeding programs. In this thesis, plant growth-yield traits and gluten protein quality in flour and dough were studied in Swedish wheat of varying genetic backgrounds and imported varieties, all grown in diverse environments in Sweden.

Red-green-blue (RGB) imaging and analytical chromatography tools, such as size exclusion high performance liquid chromatography (SE-HPLC) and mass spectrometry (LC-MS/MS), as well as near infrared spectroscopy (NIR) were used to study wheat plants and flour materials. A mixograph was used to prepare dough in this study. Robust flour sedimentation methods, such as swelling index of glutenins (SIG) and solvent retention capacity (SRC), were used to examine the gluten protein characteristics of wheat flour from varying growing environments and were compared to industrial flour screening methods.

The results show that the combined heat-drought stresses negatively affected biomass, yield and thousand-kernel weight (TKW) in the wheat studied. During extreme heat and prolonged drought, higher amounts of large polymeric gluten proteins (%UPP) were observed in the spring wheat flours in both field and controlled growth environments. Total extractable gluten protein (TOTE) was higher in the wheat genotypes grown in the cool climate in the field and combined heat-drought stress in the greenhouse. No difference in optimum dough mixing time in wheat from different years was observed. Dough mixing time, together with the gluten protein parameters (%UPP and TOTE) could be promising traits for gluten stability evaluation in varying climates. RGB imaging in combination with SE-HPLC can be useful in screening stable wheat genotypes for yield and gluten quality in varying climates. A combination of robust small-scale sedimentation tests to assess wheat flour suitability for bread-making, SIG in diluted lactic acid, SRC and SE-HPLC can be effectively used for efficient screening of wheat resilient to climate change. The new set of combined methods that include plant imaging, flour sedimentation, analytical chromatography and NIR, is of the greatest interest for both breeding and bread-baking industries to evaluate wheat in a changing climate.

Keywords: bread wheat, gluten polymers, drought, heat, sedimentation, SE-HPLC, mixing quality, gluten quality stability, robust screening.

Vetekvalite i föränderligt klimat

Sammanfattning

Bakningskvalitet hos vete bestäms till stor del av proteinhalt och sammansättning av proteiner av gluten som påverkas mycket av miljöfaktorer som värme och torka. Klimatet i Sverige bli mer fluktuerande i framtiden och med avseende på bakningskvalitet få en påverkan på glutenproteiner i synnerhet och mjöl- och brödproduktion i allmänhet vilket i förlängningen kan påverka vår livsmedelssäkerhet. Syftet med denna avhandling är att öka kunskapen om hur varierande klimat påverkar vetets glutenproteiner, samt att utvärdera och utveckla metoder för effektiv screening av dessa glutenproteiner. I avhandlingen undersöks svenska vetelinjer och utländska vetesorter som odlats under olika väderförhållanden, med avseende på avkastningsegenskaper och glutenproteinets kvalitet i både mjöl och deg med koppling till brödkvalite.

Växtmaterialet har analyserats med kombinerade flera analytiska metoder som RGB-imaging, kromatografi (size exclusion-high performance, SE-HPLC), masspektrometri (LC-MS/MS) och infraröd spektroskopi (NIR). En mixograf användes för att bereda degen och glutenprotein egenskaper samt degens egenskaper har utvärderades. Funktionella egenskaper av mjöl och brödvolym utvärderades i samband med användning av industriellt vetemjöl utvärderingsmetoder. Robusta sedimentation baserade analyser som utvärdera index av svällande gluteniner (SIG) och en lösning undanhållande kapacitet (SRC) användes för att utvärdera glutenproteinernas sammansättning i mjöl av vete odlat under olika väderförhållanden och jämfördes med industriella mjölscreeningsmetoder.

Resultaten visar att en kombination av värme och torka har en negativ effekt på biomassa, skörd och tusenkornvikt (TKW) hos vete. Under extrem värme och långvarig torka observerades högre mängder polymeriska proteiner (%UPP) i vårvetemjöl både odlat i fält och i växthus. Totalt extraherbara gluten proteiner (TOTE) var högre i de vetelinjer som odlades i det svala klimatet i fältet och i kombinerad värme- och torkstress i växthuset. Ingen skillnad i optimal degblandningstid av vetet odlat under olika klimat observerades. Optimal degblandningstid med glutenprotein parametrar (%UPP och TOTE) är lovande kriterium för att utvärdera glutenstabilitet i varierande klimat. RGB imaging och SE-HPLC kan bli ett verktyg att utvärdera stabilitet med fokus på avkastningen och glutenkvalitet hos vete under varierande väderförhållanden. En kombination av robusta, småskaliga sedimentationsanalyser (SIG och SRC) samt SE-HPLC kan användas för utvärdering av glutenkvalitet och stabilitet. En ny grupp av metoder som inkludera växtbilder, sedimentation, kromatografi och NIR kan vara av stor intresse för både förädlings-, kvarn- och brödbakningsindustrin för att utvärdera vete i varierande framtidens klimat.

Nyckelord: Brödvete, glutenpolymerer, torka, värme, sedimentering, SE-HPLC, blandningskvalitet, glutenkvalitetsstabilitet, robust screening.

Dedication

To my father Basanta Bahadur Lama and my sister Anne Lama.

“There are two tragedies in life. One is to lose your heart’s desire. The other is to gain it.”
George Bernard Shaw

Contents

List of publications 11

Abbreviations 13

1. Introduction 15

2. Background 17
   - 2.1 Wheat production in a changing climate 17
   - 2.2 What is wheat quality in a changing climate? 19
   - 2.3 Wheat protein: Gluten 21
   - 2.4 Climate change and wheat quality 23
     - 2.4.1 Impact of heat and drought on the quantity and composition of gluten protein 23
   - 2.5 Processing of wheat 25
     - 2.5.1 Dough mixing and baking 25
   - 2.6 Breeding prospects for wheat to mitigate climate change 27
   - 2.7 Methods for gluten protein evaluation under climate change 28
     - 2.7.1 Gluten protein composition using SE-HPLC 29
     - 2.7.2 Protein secondary structure analysis using NIR spectroscopy 29
     - 2.7.3 Flour sedimentation using SIG and SRC 30
     - 2.7.4 Gluten protein analysis using LC-MS/MS 31
   - 2.8 High throughput plant phenotyping 31

3. Thesis objectives 33

4. Materials and Methods 35
   - 4.1 Plant materials 35
   - 4.2 Growing characteristics (2017-2020) 35
   - 4.3 Protein analysis 37
     - 4.3.1 Gluten protein composition from SE-HPLC (papers I-V) 37
     - 4.3.2 NIR spectroscopy (paper I and IV) 38
   - 4.4 Dough mixing using a mixograph (paper II) 38
   - 4.5 Phenotyping using RGB imaging (paper III) 39

4.6 Sedimentation based physicochemical tests of flour (paper IV) . 39
4.6.1 SDS-SRC 39
4.6.2 SIG 40
4.7 LC-MS/MS (paper V) 40
4.8 Bread-baking 41

5. Result and discussion 43
5.1 Yield and agronomic trait stability under extreme growing environments (Paper III) 43
5.2 Changes in gluten protein quantity and quality in excessive climates (Papers I-IV) 45
5.2.1 Total protein concentration and gluten protein content .. 45
5.2.2 Gluten protein polymerization and de-polymerization 46
5.3 Influence of excessive growing environments on dough processing (Paper II) and baking quality 48
5.3.1 Dough processing traits for quality evaluation 48
5.3.2 Bread volume 50
5.4 Optimizing robust methods for analyzing wheat quality and agronomic traits under varying climates (paper III and IV) 52
5.4.1 Small-scale tests for screening gluten quality (Paper IV) 52
5.4.2 RGB imaging to evaluate agronomic traits and plant resistance under stress (paper III) 53
5.5 Can LC-MS/MS be a suitable tool for gluten protein quality evaluation in a varying climate? (paper V) 55

6. Conclusions 57

7. Future perspectives 59

References 61

Popular science summary 77

Populärvetenskaplig sammanfattning 79

Acknowledgements 81

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Sbatie Lama*, Pernilla Vallenback, Stephen A. Hall, Marina Kuzmenkova and Ramune Kuktaite (2022) Prolonged heat and drought vs cool climate on the Swedish spring wheat breeding lines: Impact on the gluten protein quality and grain micro-structure. *Food and Energy Security*. 11, 1-17.

II. Sbatie Lama*, Pernilla Vallenback, Stephen A. Hall, Marina Kuzmenkova and Ramune Kuktaite (2022) Striving for stability in the dough mixing quality of spring wheat under the influence of prolonged heat and drought. *Plants*. 11, 2662.

III. Sbatie Lama†*, Fernanda Leiva†, Pernilla Vallenback, Aakash Chawade and Ramune Kuktaite (2023) Heat, drought and combined stress impact on yield, phenotypic and gluten protein traits: Capturing stability of spring wheat in excessive environments (submitted).

IV. Sbatie Lama, Rikard Westbom, Jörgen Hansson, Aakash Chawade, Ramune Kuktaite* (2023) A new insight into multi-target assessment of wheat quality in a varying climate by the sedimentation methods, SE-HPLC and NIR techniques (manuscript).

V. Sbatie Lama†, Faraz Muneer†, Antoine HP America, Ramune Kuktaite* (2023) Search for potential markers to evaluate the climate stress impact on the bread-making quality in wheat by the use of proteomics approach (manuscript)

*Corresponding author and † equal contribution.
Papers I and II are reproduced with the permission of the publishers.

The contribution of Sbatie Lama to the papers included in this thesis was as follows:

I. Conducted laboratory analysis together with MK, did data analysis and wrote the final version of the manuscript together with the co-authors.

II. Conducted laboratory analysis together with MK, did data analysis and wrote the final version of the manuscript together with the co-authors.

III. Performed the greenhouse work, performed laboratory work of protein analysis, measured phenotypic traits, analysed the data obtained in the laboratory and phenotypic traits data, wrote the final version of the manuscript together with FL and co-authors.

IV. Conducted laboratory analysis and data analysis, and wrote the final version of the manuscript together with the co-authors.

V. Planned the experiment with the co-authors, performed laboratory work and the majority of the data analysis, wrote the materials and methods section and contributed to the final version of the manuscript.

Abbreviations

ANOVA	Analysis of variance
BV	Bread volume
dpa	Days after anthesis
FP%	Flour protein concentration
g	Gram
GP%	Grain protein concentration
HMW-GS	High molecular weight glutenin subunit
HTPP	High throughput plant phenotyping
LMW-GS	Low molecular weight glutenin subunit
NIR	Near-infrared reflectance spectroscopy
LA	Lactic acid
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LMP	Large monomeric proteins
LPP	Large polymeric proteins
%LUPP	Percentage of large SDS-unextractable polymeric proteins in total large polymeric proteins
%LUMP	Percentage of large SDS-unextractable monomeric proteins in the total large monomeric proteins
Mon/pol	Monomer *vs.* polymer ratio
PCA	Principal component analysis
RGB	Red, green, blue
SDS	Sodium dodecyl sulfate
SE-HPLC	Size exclusion-High performance liquid chromatography
SIG	Swelling index of glutenins
SMP	Small monomeric proteins
SPP	Small polymeric proteins

SRC	Solvent retention capacity
TKW	Thousand kernel weight
TMP	Total monomeric proteins
TOTE	Total SDS-extractable proteins
TOTU	Total SDS-unextractable proteins
TPP	Total polymeric proteins
%UPP	Percentage of SDS-unextractable polymeric proteins in total polymeric proteins

1. Introduction

Global temperature is getting warmer and more unpredictable. Higher temperatures and changes in rainfall patterns as a result of climate change are affecting vegetation worldwide (Sun et al., 2022). Several other climate parameters, such as rising concentration of carbon dioxide (CO_2), and changing surface solar radiation, also have significant impacts on crop production and development (Xiao et al., 2018). This is expected to lead to reduced yields of wheat in many regions in the world, including Europe (Osman et al., 2022).

Wheat is the third most produced cereal in the world (FAO, 2023). It contributes approximately 20% of the world's food and 21% of total human protein consumption (Shiferaw et al., 2013). In Sweden, during the period from 1990–2021, the consumption of bread and confectionery increased by 46% (76.1 kg per person in 2021) (Swedish Agency for Agriculture, 2023a). Thus, wheat-based food products are very important for Swedish food security. Like most countries in Europe, Sweden has experienced heat stress, combined with severe drought in 2018, which has significantly decreased wheat production (1.6 million tonnes), making it the lowest production since 1999 (FAO, 2023). Summer droughts are forecast to become more frequent in Sweden in the future (Grusson et al., 2021) and are expected to affect wheat production and, ultimately, food security.

Protein in wheat is one of the most important factors that determines the end-use quality of wheat-based food products. Around 80% of the protein in wheat is from gluten (Johansson et al., 2020). The unique properties of gluten, such as its ability to form large polymeric networks and the elasticity of dough, makes wheat suitable for baking (Johansson et al., 2013). The content and composition of gluten proteins are strongly influenced by environmental factors such as drought, heat and rain (Malik et al., 2013). Variations in gluten quality in wheat affects the dough mixing and quality of baked food products. A potential solution to this problem is the development

of wheat varieties adapted to climate change which will be influenced less by a changing environment. Greater understanding of the effects of climatic factors on overall wheat yield and gluten quality would help in this development.

Aside from improving our understanding of how gluten is affected by the environment, there is also a substantial need for methods for the extensive testing and efficient selection of wheat breeding lines. Wheat quality assessments related to bread baking quality include a number of different tests of dough rheology and baking. These tests are time consuming, labor intensive and expensive, and require relatively large amounts of flour. Therefore, in this thesis focus was given to: i) expanding the understanding of how genotypes interact with the environment in terms of yield, various agronomic traits, and flour, dough and bread quality (paper I, II and III) and ii) optimizing various wheat quality screening methods linked to protein quality (paper III, IV and V).

2. Background

2.1 Wheat production in a changing climate

Wheat (*Triticum aestivum*) is the third most produced cereal in the world (771 million tonnes (mln.t.)) after maize (1219 mln.t.) and rice (787 mln.t.) (FAO, 2021). The annual harvest of wheat contributes to global food security, serving as a staple food for approximately 40% of the global population (University of Western Australia, 2023).

Wheat is grown in different seasons in all agricultural regions of the world. China, India, the United States, Russia, France and Ukraine were among the main wheat producing countries in the world in 2021 (FAO 2023, Figure 1). However, Russia's invasion of Ukraine and the on-going war since February 2022 has created a threat to the production and export of wheat from Ukraine to other countries. The war will have a long-term effect on the overall production of wheat-based products and the global supply of wheat, with a subsequent negative effect on food security.

In Sweden, wheat is one of the main cereal crops. It is grown on around 48% of the area used for cereals and contributes 55% of total cereal production (Swedish Agency for Agriculture, 2023b). In 1961, total wheat production was 0.8 mln.t., increasing to 3 mln.t. by 2021 in Sweden.Since 1988 every year (except 1996 and 2010) yearly mean temperature were 0.3-3 ^{o}C warmer than the yearly mean temperature for 1961-1990 (Swedish Meteorological and Hydrological Institute, 2023). By the end of the century, the average annual temperature will be 2-6 ºC higher than the mean temperature for 1961-1990 (Swedish Meteorological and Hydrological Institute, 2023). More extreme and unpredictable weather patterns are expected to pose a threat to wheat production in Sweden and worldwide.

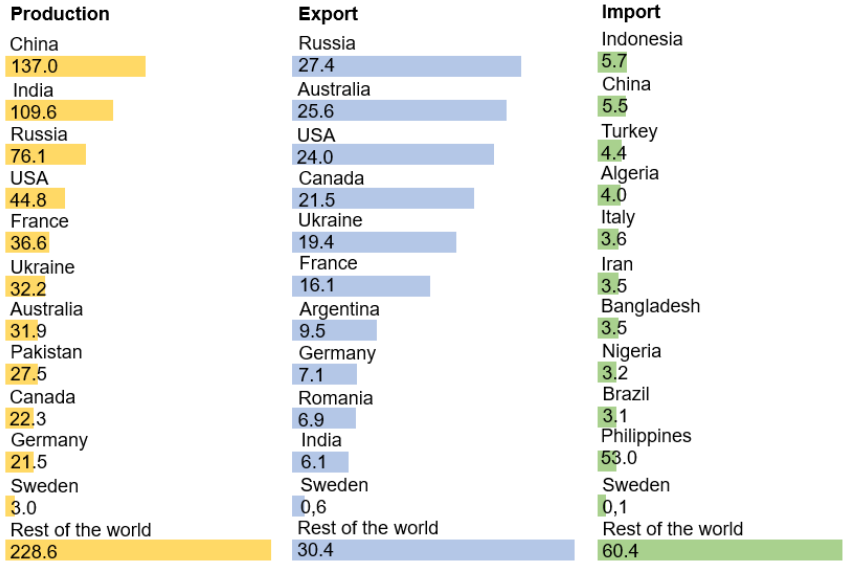


Figure 1. World's top-10 wheat producers, exporters and importers in 2021 (million tonnes). Data source: FAO 2023

Heat and drought are some of the consequences of climate change that can severely affect wheat production. High temperature affects the photosynthesis by decreasing the chlorophyll content and damaging the photosystem II (Pradhan et al., 2012). It was found that, in general, with a 1 ºC rise in temperature, the production of winter and spring wheat decreased by 3.7 % and 7.5 %, respectively, in North America (Zhang et al., 2022a). Another similar study indicated that wheat yield can be reduced by 18.4% and 28.5% under a 2 °C and 3 °C rise of temperature, respectively, in South Africa (Shew et al., 2020). If the greenhouse gas concentration goes up to 8.5 (CO_2 concentration 2050 ppm), wheat production is projected to decline by 9.4% by 2050 in China (Xie et al., 2020). Drought is one of the main abiotic stresses that can reduce wheat production severely (Nezhad et al., 2011). Similar to heat, drought also affects the yield by reducing the photosynthetic activity of the plants. However, unlike heat, drought limits access to carbon dioxide in the air through stomatal closure (Farooq et al., 2009). Drought at the beginning of the heading stage in wheat plants can reduce yield by 25-37% in the greenhouse (Lan et al., 2022). Drought after anthesis can reduce yield by 26%, while drought during the entire growing period could reduce yield by 84% (Wan et al., 2022). In a similar study, mild drought after anthesis

reduced wheat yields by 30%, while prolonged mild drought during anthesis and grain filling reduced the grain yields by 58-92% (Farooq et al., 2014).
The effect of combined heat and drought is even more severe on wheat production. Compared to separate heat or drought stress, combined stress can severely reduce the chlorophyll content (Pradhan et al., 2012) and stomatal conductance (Shah and Paulsen, 2003).
One of the most effective ways to ensure production of wheat in climate change is to develop new cultivars adapted to the changing environment (Pequeno et al., 2021).

2.2 What is wheat quality in a changing climate?

Wheat yield, grain hardness, protein concentration, gluten quantity, starch quality and color of the wheat flour are some of the characteristics frequently evaluated by breeding companies and other stakeholders. The definition of wheat quality varies depending on the viewpoints of those measuring it. For example, wheat quality for the farmers is high grain yield with low input (e.g., fertilizer), but for the millers it is higher amounts of flour after milling of the wheat grains with minimum energy input. Wheat quality for the bakers refers to the flour's suitability for baking different products, like buns, biscuits or pizza (Guzman et al., 2016a). The breeders and the consumers are positioned at the beginning and the end of the value-chain of edible wheat-based products. For the breeder the aim is to develop wheat cultivars that integrates as many characteristics as possible to meet the demand of the farmers, millers, bakers and consumers. On the other hand, consumers define wheat quality in many different ways, starting from the appearance of the product, to taste and nutritional value. At each section of the value-chain, quality is related to gaining maximum output with as minimal input as possible (Figure 2) (Guzmán et al., 2022).

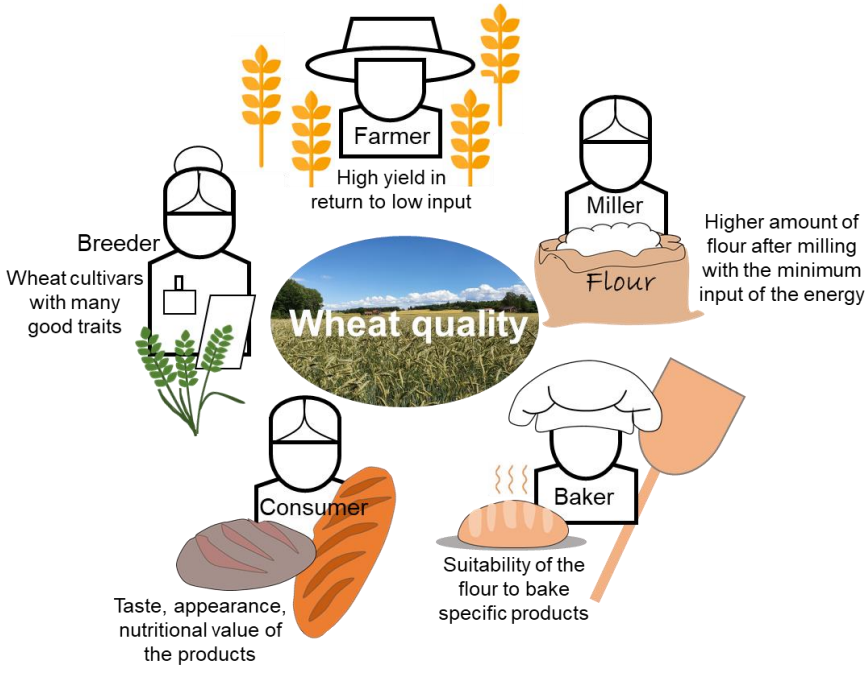


Figure 2. A simplified diagram describing wheat quality.
Diagram based on Guzman et al., 2016a; 2022.

Wheat is one of the most versatile crops and can be transformed into many different food products. Based on the end products, wheat quality can be divided into two parts: 1) Technological quality (milling, processing and end-use quality) and 2) Nutritional quality (protein and starch levels) (Johansson et al., 2020). Aside from being an important component of nutritional quality, protein is also an important component for determining the technological quality of wheat. Wheat grain contain 7-20% proteins, 1.5–2% lipids, 52-75% carbohydrates and minor amounts of vitamins and minerals (less than 1%). The molecular size distribution of the major wheat protein, gluten, largely determines bread quality (Johansson et al., 2020). Therefore, in order to determine bread quality, the most commonly used methods are the use of mixographs, alveographs and glutographs, mainly focusing on evaluating gluten quality (Guzman et al., 2022).

In Sweden, wheat is largely divided into two groups based on the growing seasons (e.g., winter and spring wheat). Winter wheat is known for having a higher yield potential (~7.1 tons/ha) than spring wheat (~4.8 tons/ha) (Swedish Agency for Agriculture, 2023c). Whereas, protein concentration is

slightly lower in the winter wheat (8-12%) in comparison to the spring wheat (10-17%), which makes spring wheat more suitable for baking bread (Koppel and Ingver, 2008). In Sweden, the main use for spring wheat flour is as an addition to flour from winter wheat, mainly to increase the protein concentration and to improve the baking quality properties of the flour. Winter wheat is also used as fodder and for ethanol production (Lantmännen, 2023).

2.3 Wheat protein: Gluten

A large part of wheat protein, around 80%, is comprised of the proteins that form gluten (Guzman et al., 2022). Gluten proteins are intrinsically disordered proteins that form extensive aggregated networks in wheat seeds (Markgren, 2022). Gluten protein is stored as a nutrient (i.e. nitrogen, amino acids) and an energy source of wheat seedling during germination (Wieser et al., 2023). The synthesis of gluten proteins occurs between 10 to 42 dpa (days post anthesis) (Shewry et al., 2009). Gluten proteins are stabilized by the disulphide bonds and inter-chain hydrogen bonds. The regions where gluten proteins form hydrogen bonds with water create *loops,* and the regions where gluten proteins form inter-chain hydrogen bonds with other gluten proteins form *trains* (Figure 3). This model was proposed by Belton in 1998 to explain gluten elasticity.

During desiccation, due to the dehydration of the water, the number of hydrogen bonds between the proteins is reduced, which significantly increases the proportion of polymeric glutenins (Shewry et al., 2009). Thus, more *train* regions form in the protein (Figure 3), which could lead to rearrangement of inter-chain disulphide bonds, leading to an increase in the proportion of polymeric glutenins (Shewry et al., 2009). In heavy rain or moist conditions, reduced amounts of polymeric proteins can be observed, and it is hypothesized to be due to the activation of the thioredoxin enzyme, which is involved in the reduction of disulphide bonds in glutenin polymers (Koga et al., 2020).

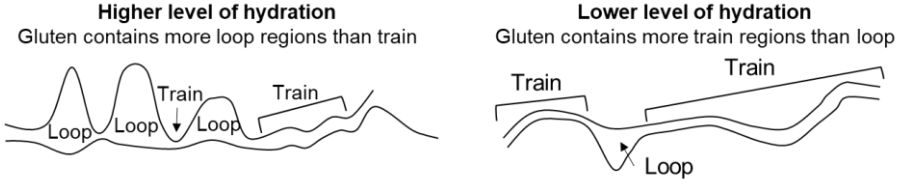


Figure 3. The “loop and train” model of gluten with higher and lower levels of hydration. Figure based on Belton, 1999.

According to the Osborne classification, gluten proteins are divided into monomeric gliadins (responsible for around 60% of the total gluten) and polymeric glutenins (around 40% of the total gluten). Gliadins are soluble in aqueous alcohols, whereas glutenins are soluble in aqueous alcohols only after reduction of their disulfide bonds (Schmid et al., 2016). Polymeric glutenins are formed through the aggregation of storage proteins within the lumen of endoplasmic reticulum (Tosi et al., 2011). LMW-GS and gliadins are formed from the protein bodies produced on the endoplasmic reticulam; they are transported via the golgi body into the vacuole and stored until seed germination (Tosi et al., 2009; Tosi et al., 2011). Production of the folding assistant proteins, such as foldases and chaperon, might be behind the cross-link formations (Markgren, 2022) and, thus, the formation of polymeric proteins in wheat. The effect of different growing conditions on the production of foldases and chaperon have not been studied before, they could be interesting to study from the perspective of climate change.

The α- and γ-gliadin were detected 3 days after anthesis (Mazzeo et al., 2017). Most of the gluten proteins accumulated rapidly between 11 and 21 dpa, with a minor further increase up to 30 dpa (Mazzeo et al., 2017).

Based on molecular weight, gliadins are divided into ω-gliadins, α/β-gliadins and γ-gliadins (Woychik et al., 1961). Disulphide bonds are present as intra-chain crosslinks in all of them (Wieser, 2007). Gliadins mainly contribute to viscosity and extensibility by working as plasticizers of the dough (Guzman et al., 2022). Glutenins are subdivided into high molecular weight glutenins (HMW-Gs, ~70–100 kDa) and low molecular weight glutenins (LMW-Gs, ~30–50 kDa). Glutenins are aggregated proteins with cysteine groups positioned at the end and in the middle of the protein sequence (Shewry et al., 2003; Anderson et al., 2012; Schmid et al., 2017). These cysteines enable intermolecular disulfide bonds, creating a vast range in molecular weight. Glutenins are responsible for the cohesive and elastic properties of the dough (Guzman et al., 2022).

2.4 Climate change and wheat quality

2.4.1 Impact of heat and drought on the quantity and composition of gluten protein

Wheat grain quality, an important determinant for the economy of the farmers, millers and bakers, is as important as yield in the improvement of wheat genotypes in stressed environments. Unfavorable weather conditions, such as heat and drought, reduce plant development time, which increases the size of the gluten proteins (Johansson et al., 2013). Drought and heat stresses cause protein polymerization during grain filling, which affects the overall wheat quality (Labuschagne et al., 2021). On the other hand, favorable weather conditions promote plant growth and development and extend plant maturation time, which can lead to higher starch accumulation in the wheat plant (Malik, 2009). Usually, starch and protein concentrations are negatively correlated. The effect of heat, drought and combined heat-drought effects on the gluten polymerization and dough quality is discussed in the coming paragraphs.

2.2.2.1. Heat

High temperatures, i.e. those up to 30^{o}C during the grain-filling period, result in a faster maturation of the wheat grain, which leads to less starch accumulation and higher protein concentration and a low glutenin/gliadin ratio (Randall and Moss, 1990). Studies done in the controlled environment showed that a temperature increase from 18^{o}C to 23^{o}C can increase the amounts of D-type LMW-GS and decrease the amount of B-type LMW-GS (Koga et al., 2016). Similarly, at higher temperatures (23^{o}C and 34^{o}C), higher amounts of ω-gliadins and lower amounts of γ- gliadin were observed (Daniel and Triboi, 2000; Koga et al., 2016). The concentration of HMW-GS was reported to increase by 84% under heat stress (35ºC/22ºC day/night) compared to the control environment (Zhao et al., 2022). Heat stress (>32-36^{o}C), particularly during the later part of grain filling, reduces protein polymerization and, thus, results in reduced dough strength and increased extensibility (Blumenthal et al., 1991; Blumenthal et al., 1998; Uhlen et al., 1998; Guzmán et al., 2016b). A reduced glutenin to gliadin ratio might be a possible reason behind the reduced strength and increased extensibility in the dough. Additionally, the production of heat shock proteins may affect

glutenin polymerization, which could lead to lower dough strength (Blumenthal et al., 1998).
Temperature has been found to have a larger effect on polymerization in flour with weaker glutens (Koga et al., 2016). Similarly, wheat varieties with HMW-GSs 2+12 showed higher variability under heat stress (Blumenthal et al., 1995a; Panozzo and Eagles, 2000). However, heat stress effects on gluten protein concentration and composition may vary among the genotypes (Blumenthal et al., 1995b; Singh et al., 2012).

2.2.2.2. Drought

The impact of drought stress on wheat quality has been evaluated to a lesser degree than that of heat stress (Johansson et al., 2020). Drought has similar effects as heat as it reduces the grain maturation period and starch accumulation, and increases grain protein concentration (Dupont and Altenbach, 2003). During droughts, the photosynthetic ability and efficiency of biochemical processes of the plants are reduced, which in turn decrease plant growth and yield (Ali, 2019). Reduction of starch during drought is due to the inactivation of starch synthase enzymes and in vivo crystallization of amylopectine within the amyloplast (Tester et al., 1995). Irrigation during the heading stage of wheat can reduce the positive effects of drought on protein concentration, which indicates how the heading stage is very sensitive to drought stress (Lorite et al., 2023). Zhao et al. (2009) reported that, at the post-anthesis stage, higher amounts of total protein and a higher ratio of glutenin/gliadin were observed at a 45% soil water level compared with a soil water level of 65% or 85% (Zhao et al., 2009). Similarly, a significant increase in glutenins, HMW-GS and a ratio HMW-GS/LMW-GS were observed in the water deficit conditions (Flagella et al., 2010; Phakela et al., 2021). Increased concentrations of large polymeric proteins were observed under severe drought conditions (Leiva et al., 2021; Olckers et al., 2022). Overall drought stress seemed to favor the polymeric protein content, which positively affects dough mixing and baking quality; for example, higher values related to the bread baking characteristics, such as bread volume and dough mixing time, were observed during both moderate and severe drought conditions (Olckers et al., 2022).

2.2.2.3. Combined heat and drought stresses

Combined heat and drought is reported to reduce the grain maturation period more than the individual treatments (Dupont and Altenbach, 2003). Thus, a combination of heat and drought affects plant growth and development to a higher extent than the individual stresses (Zahra et al., 2022). It also affects the gluten quality and total protein concentration (Balla et al., 2011). Compared to the wheat grown under control environments, protein concentration and gluten percentage increased by 65.6% and 32.5%, respectively, under combined heat and drought stress (Sattar et al., 2020). Compared with the number of studies done on the effects of heat and drought on the gluten quality, there is still a lack of studies done on the combined heat-drought effect.

2.5 Processing of wheat

The quality attributes that are the most important for wheat processing are gluten protein content and composition, which determine dough strength and extensibility, and overall baking behavior (Henry and Wrigley, 2018).

2.5.1 Dough mixing and baking

When wheat flour is mechanically mixed with water, it forms a unique continuous viscoelastic network of gluten, which makes it suitable for producing different food products (Li et al., 2015). During the dough development process, the mechanical energy (shear and tensile forces) facilitate the entanglement coupling of glutenin molecules which contributes to the strength and elasticity of dough (Singh and MacRitchie, 2001). During the dough development, the gliadins regulate dough viscosity by interacting with the gluten network (Kuktaite et al., 2004). Both the high molecular weight (HMW) and the low molecular weight (LMW) of glutenin subunits and gliadins are intrinsic to gluten network formation (Bonilla et al., 2019). Due to the smaller molecular size and absence of intermolecular disulfide bonds gliadins have higher mobility, thus are distributed faster in the samples upon mixing compared to the glutenins. Therefore, in the beginning of the dough mixing procedure, higher amounts of gliadins (17.6%) can be found in the dough compared to the HMW-GS (4.4%) and LMW-GS (3.4%) (Bonilla et al., 2019). By the time the dough is optimally mixed, HMW-GS and LMW-GS

produce more network-like structures and the gliadins are found to be evenly distributed throughout the dough. Due to the higher molecular weight and increase in cysteine residues, HMW-GSs have lower mobility and form a higher density of intermolecular disulfide bonds than LMW-GSs. Upon further mixing of the dough, HMW-GS and LMW-GS dissociate from the network and form aggregates. Since LMW-GS has a lower molecular weight and cysteine residues, they tend to dissociate earlier than the HMW-GS (Bonilla et al., 2019). Improvements to the kneading procedure must begin with the correct management of key parameters such as mixing time and the addition of water to the dough (Cappelli et al., 2020). When water is added to the dough, it strongly affects the mechanical behavior of the dough (Belton, 2005). For example, more water in the dough increases the free water outside the starch granule (Bosmans et al., 2012), leading to higher amounts and increased mobility of the protons in the dough (Doona and Baik, 2007; Parenti et al., 2021). This also reduces the firmness and elasticity of the dough. Whole meal flour requires a greater amount of water than refined flour. Higher amounts of fibre fractions are present in the whole mean flour, which is assumed to negatively affect the gluten network formation in the dough (Hemdane et al., 2017).

During the proofing stage (the resting stage for the dough), yeast breaks down and the starch turns into simple sugars. This releases carbon dioxide which expands the gluten network (Figure 4). The dough with a higher gluten network density has more cells (bubbles) in the dough. Higher extensibility of the dough allows the cells to enlarge even more and trap more gas. Depending on the strength of the gluten network, the dough can retain the gas without collapsing (Ortolan and Steel, 2017; Guzmán et al., 2022).

Figure 4. Dough from spring wheat flour (sieved) proofed with form (left) and without form (right).

Flour with an optimum extensibility and elasticity (gliadin-glutenin ratio) provides good bread quality (Barak et al., 2012). During heating (baking), the proteins denature, starch gelatinizes and new permanent cross-links (disulphide bonds) form between the glutenin polymers (Ortolan & Steel 2017). At 45ºC, the gluten networks are no longer elastic and start to solidify. After baking is finished, and upon cooling, a firm but light-textured loaf of bread is formed (Figure 5).

Figure 5. Wheat flour breads baked with form (left) and without form (right).

2.6 Breeding prospects for wheat to mitigate climate change

Abiotic stresses imposed by climate change have a negative impact on wheat productivity and this effect will continue to increase. One effective way to ensure production and quality of wheat is through breeding varieties adapted to climate change (FAO, 2022). Adaptation to climate change can be improved through short-term strategies, such as changing the crop management practices, or long-term strategies, such as developing varieties through introgression of stress resistance alleles into the breeding populations (Ortiz et al., 2008). Adapting appropriate strategies can increase the wheat production by 15% in the future (Challinor et al., 2014). A range of plant and grain characteristics are measured to select superior genotypes in order to improve abiotic resistance and adaptation (Guzman et al., 2022). Some important target traits against abiotic stresses are early crop vigor, reduced plant height, reduced days to anthesis and better root architecture compared to plants grown without stresses (Hernandez-Ochoa et al., 2019; Johansson et al., 2023). However, performance of the traits can vary between environments for different genotypes, which can make it difficult to

identify superior lines (Johansson et al., 2020). The knowledge of how cultivars respond to different environment is needed to select the best parental lines. Thus, for successful breeding to generate adaptive wheat genotypes, a multi-environmental evaluation of the desired traits and selection of genotypes with wide adaptability across environments is needed (Manès et al., 2012; Johansson et al., 2020). New prospects for breeding abiotic-tolerant crops have emerged as a result of the rapid development of molecular markers targeted at specific traits and technologies that enable comparisons of large phenotypic and genotypic data sets (Johansson et al., 2023). This allows marker-assisted selection (MAS) to be carried out to identify specific Quantitative Trait Loci (QTLs) related to the traits under investigation (Merchuk-Ovnat et al., 2016). For example, QTLs from wild emmer wheat, introgressed via MAS has enhanced drought resistance in durum and bread wheat cultivars (Merchuk-Ovnat et al., 2016).
Genomic selection (GS) is a technique where thousands of DNA markers covering the whole genome are analyzed in one go. It outperforms MAS when the trait in question can be considered quantitative and governed by more than a few genes. Both are based on the linkage disequilibrium between the QTL/genes and DNA markers (Madhusudhana, 2019). GS is a widely used method in the prediction of complex traits such as yield. However, there are only a few published examples of the use of GS to predict baking quality traits (Battenfield et al., 2016; Guzman et al., 2016a; Michel et al., 2018). Highly complex baking traits, such as dough rheology and bread volume, were shown to be predicted with a high degree of confidence by GS (Guzman et al., 2016a).

2.7 Methods for gluten protein evaluation under climate change

There are too many genes involved in gluten and milling quality to effectively select genotypes for better alleles. Thus, in order to improve wheat varieties for breeding programs, some degree of empirical phenotyping for quality is needed (Guzman et al., 2022).
Both breeding and the food processing industry demand robust, fast, low-cost wheat quality screening methods that can be conducted using a small amount of flour materials. In order to do the range of classical tests, such as farinogram, alveograph, extensograph and bread baking, to evaluate flour quality, up to 5 kg of wheat grains are required. Classical tests are time

consuming, expensive, laborious and sometimes lack accuracy, meaning that accurate high-throughput assessment of baking quality is difficult. Therefore, there is a need to develop small-scale reliable tests for assessing wheat flour quality for breadmaking. Here, different types of tests; SE-HPLC, NIR, SIG and SRC, and LC-MS/MS are briefly described.

2.7.1 Gluten protein composition using SE-HPLC

Size exclusion high performance liquid chromatography (SE-HPLC) is a well known gluten quality test for the wheat, which requires a small amount of flour (around 16). Gluten proteins from the wheat grown in different environments are extracted using SDS-phosphate buffers and sonication, and are separated according to the molecular sizes of proteins using SE-HPLC (Lama et al., 2022; Statkevičiūtė et al., 2022). The genotypes with higher polymeric protein (%UPP) in the flour showed extended protein networks compared with the genotypes with lower polymeric protein in the flour (Hussain et al., 2012). Thus, positive correlation of polymeric proteins and baking performance was observed in different studies (Singh et al., 1990; Labuschagne and Aucamp, 2004). The amount of total SDS-extractable proteins (TOTE) provides information about the protein concentration of the flour (Johansson et al., 2004; Malik et al., 2013).

2.7.2 Protein secondary structure analysis using NIR spectroscopy

Near-infrared reflectance spectroscopy is a rapid, efficient and nondestructive method suited for assessing the quality of wheat flour (Zhang et al., 2022b). NIR spectroscopy-based techniques are routinely used in agriculture and in the food industry to analyze both grain and flour quality (Porep et al., 2015; Cozzolino, 2021). The NIR instrument emits wavelengths across the whole sample, which is reflected back to the instrument (transmitted in the case of NIT (near-infrared transmittance)) in the form of the electromagnetic spectra (400–2500 nm) (Zhang et al., 2022b). Reflectance bands from fundamental vibrations of chemical bonds (C-H, N-H, O-H, S-H, C-C and C=O) are observed in the electromagnetic spectra (400–2500 nm) (Junior et al., 2020). Two NIR regions (1120-1350 and 1600-1850 nm) correspond to the protein content (Currà et al., 2022; Moraru et al., 2022). Previous studies showed positive relationships between the NIR spectra and gluten polymers (Scholz et al., 2007) and glutomatic/wet gluten (Sorvaniemi et al., 1993; Golea et al., 2023).

2.7.3 Flour sedimentation using SIG and SRC

Swelling index of glutenin (SIG) is based on the Zeleny test, i.e. a sedimentation test (Axford et al., 1979). The advantage of SIG is that a smaller amount of flour is used (40 mg). According to Wang and Kovacs (2002), SIG is mainly done using three different solvents: SDS (sodium dodecyl sulfate) in lactic acid, SDS in diluted lactic acid and SDS. Depending on the availability and size of the thermo-shaker and centrifuge used, 24-48 samples can be analyzed at the same time, which means that the average time spent per sample is less than one minute.

In SIG the lactic acid concentration is higher than in the SDS sedimentation test, which gives a clearer supernatant, making it easier to observe sedimentation boundaries (Wang and Kovacs, 2002). Another major advantage of the SIG test over the SDS sedimentation test is that SIG has a high positive correlation coefficient with insoluble glutenin content (Wang and Kovacs, 2002). SIG has been found to be the best predictor of gluten strength across different environments compared to SDS-sedimentation and SRC (solvent retention capacity) (Guzmán et al., 2016c).

Solvent retention capacity (SRC) is a relatively fast test for measuring quality of wheat flour. In SRC, different solvents can measure the swelling behavior of different flour components, such as gluten strength, damaged starch and arabinoxylans, individually. 5% w/w lactic acid (LA) in water, 5% w/w sodium carbonate (Na_2CO_3) in water, 50% w/w sucrose in water are used to measure glutenin, damaged starch and for arabinoxylans, respectively (Guttieri et al., 2002; Walker et al., 2008; Kweon et al., 2011). Among all the solvents the 5% lactic acid SRC has been found to be the best for assessing bread quality parameters (Walker et al., 2008; Kweon et al., 2011). Wheat genotype (Guttieri et al., 2002) and interaction between genotype and environment has been found to significantly affect SRC tests (Walker et al., 2008; Svec et al., 2012).

The SRC test is generally used for assessing the quality of soft wheat flour products like cookies and crackers (Xiao et al., 2006). However, SRC test has the potential for assessing the quality of hard flour with high accuracy (Xiao et al., 2006). However, all these tests were done on soft wheat; the effect on hard wheat should be further investigated.

2.7.4 Gluten protein analysis using LC-MS/MS

Polymerization of gluten protein is a result of the interchain disulfide bonds between HMW-GSs and LMW-GSs. Differences in the composition of polymeric proteins are due to the variation in formation of disulfide bonds (Johansson et al., 2013). Genotypes, environmental factors (i.e., temperature, precipitation and level of nitrogen fertilizers) and G x E can contribute to variations in the gluten polymerization.
Proteomic techniques have the advantage of providing information on individual proteins from a complex mixture of proteins. This can help to further the understanding of how different proteins are expressed during environmental stresses (Sancho et al., 2008; Irar et al., 2010). Quite a few studies have investigated the effect of environmental variations on the composition of polymeric proteins using proteomics mass spectrometry (MS)-based methods (Dupont et al., 2006; Yang et al., 2011; Altenbach, 2012). Combined MS and 2 D electrophoresis identified drought and heat stress responsive proteins and up- and down-regulation of several gliadins and LMW-GSs (Yang et al., 2011). Previous studies showed a higher expression of ω-type gliadins due to water stress (Hurkman et al., 2013; Giuliani et al., 2015; De Santis et al., 2017). Environmental factors, such as temperature and nitrogen fertilization were found to highly affect expression of ω-gliadin (Hurkman et al., 2013; Wan et al., 2013).

2.8 High throughput plant phenotyping

High-throughput plant phenotyping (HTTP) is a fast and non-destructive approach to monitoring and measuring multiple physiological traits related to plant growth and yield in different biotic or abiotic stresses (Pabuayon et al., 2019). Physiological traits related to yield are commonly assessed manually (for example, by weighing the biomass and measuring the plant height). However, these methods can sometimes be subjective, time-consuming, and laborious (Dhondt et al., 2013).
There is growing interest in conducting HTPP using remote sensing approaches. For ground-based phenotyping platforms, different remote-sensing devices, such as multispectral, hyperspectral, fluorescence and thermal sensors, are commonly used (Araus et al., 2018). The main difference between multispectral and hyperspectral data is the number of bands in the light spectrum, i.e., from 5-10 bands up to hundreds, respectively (Sara et al., 2021). Multispectral and hyperspectral monitoring

techniques are very promising tools for studying plants under abiotic stresses. For example, the photochemical reflectance index (PRI) is a multispectral monitoring process that can detect moderate to severe heat stress or late-stage heat stress of wheat plants (Cao et al., 2019). Multispectral imaging showed a great potential when estimating the stomatal conductance of winter wheat grown under water stress (Zhou et al., 2021).

Handheld sensors are mostly used to estimate plant chlorophyll concentration, maximum quantum efficiency and normalized difference vegetation index (NDVI) (Leiva et al., 2021; Lan et al., 2022). NVDI has been found to correlate well with drought stress in previous studies (Kumar et al., 2020). Under drought conditions, flag-leaf area measured by hand sensor showed a significant relationship with the root biomass (Lan et al., 2022).

When phenotyping large number of plants at a time, for both aerial and ground-based platforms, imaging technique such as using digital red–green–blue (RGB) cameras can be used (Araus et al., 2018). In fact, most of the current low-cost approaches to crop phenotyping are based on exploitation of the possibilities opened by RGB imaging (Araus et al., 2018). Low-cost digital RGB cameras can easily estimate plant shoot biomass, development, and growth rate which have been shown to be a suitable tool to map the plant responses under heat and drought (Blum et al., 1997; Humplík et al., 2015).

3. Thesis objectives

The overall objective of this thesis is to improve understanding of gluten protein quality in flour, dough and bread in the wheat grown in varying climate and to evaluate new robust tools for wheat quality and yield screening to assist in the breeding of climate resilient Swedish wheat.

Specific objectives were to:

- Investigate the effect of excessive growing environments on the gluten proteins in the flour and dough, and in the dough mixing quality of Swedish spring wheat breeding lines.
- Evaluate the impact of individual and combined heat-drought stresses on the phenotypic traits, yield and the gluten proteins in the spring wheat genotypes grown in the biotron.
- Assess the robust sedimentation, SE-HPLC and NIR methods of evaluating the bread-making quality in a varying climate.
- Explore the use of LC-MS/MS to assess the climate stress impact on the polymeric gluten proteins in bread wheat.

4. Materials and Methods

4.1 Plant materials

For paper I, 294 Swedish spring wheat genotypes were used. The wheat genotypes were grown by Lantmännen in field trials in 2017 and 2018 (55°55′N and 13°07′E) in Svalöv, Sweden. Based on gluten protein parameters (%UPP, TOTE, TOTU, %LUPP, and Mon/pol measured by SE-HPLC) of 294 genotypes, 56 spring wheat genotypes were selected and used for the mixing study in paper II.

For paper III, eight spring wheat genotypes were selected based on the gluten strength (%UPP) in the flour. They were grown under three different abiotic stresses, drought, heat and heat-drought. The detailed description of the experimental design is provided in manuscript III.

Based on the subunits of the gluten protein, two wheat spring wheat genotypes, Diskett (5+10 and 2*) and Bumble (5+10 and 1) from the years 2017, 2018 and 2019 were selected for the proteomics study in paper V. These genotypes were grown in the same location in Svalöv (55°55′N and 13°07′E) during the studied years in paper V.

For Paper IV, 13 winter wheat varieties and 1 spring wheat variety were grown in 2019 and 2020 in Kävlinge (55.79’’N, 13.20’’E) by Lilla Harrie Valskvarn were used.

4.2 Growing characteristics (2017-2020)

Wheat breeding lines and varieties grown over 4 years (2017, 2018, 2019 and 2020) are used in this thesis. The temperature (°C) and precipitation (mm) data for these years is shown in Figure 6 and Figure 7. The

temperature graph shows that the biggest varitions between the years were during February, March, May, June and July (Figure 6). The lowest differences between the years were observed from August-September. Overall, highest average temperature was observed in 2018 from April-May and in July and in 2019 in January, February, June, August, October and November (Figure 6).

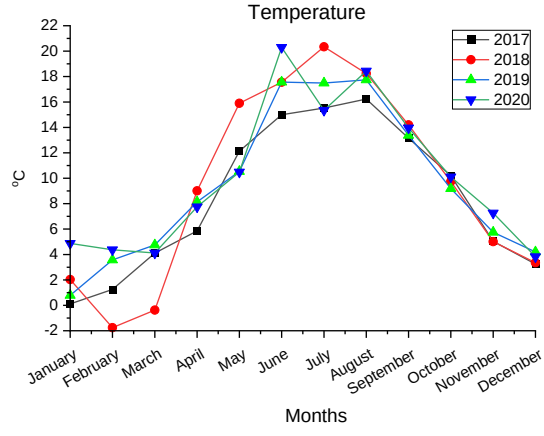


Figure 6. Average monthly temperature (ºC) between 2017-2020 in Svalöv, Sweden.

Considerably higher variations in precipitaion between the years were observed (Figure 7) compared to temperature (Figure 6). The highest rate of precipitations were observed in 2017 from June-December (Figure 7). The year 2019 showed three times more precipitation in March compared to the other 3 years. The lowest precipitations was observed from June-July and from Septerber-October in 2018 (Figure 7).

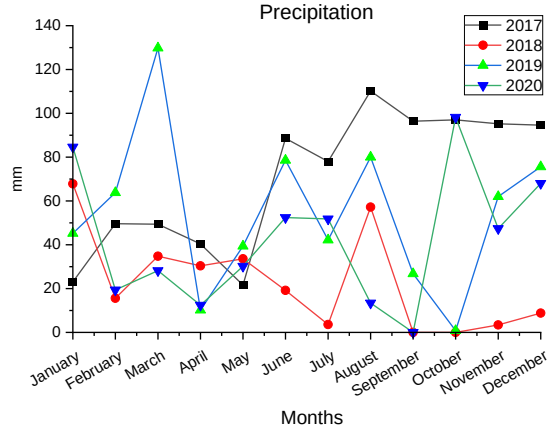


Figure 7. Total monthly precipitation (mm) between 2017-2020 in Svalöv, Sweden.

4.3 Protein analysis

4.3.1 Gluten protein composition from SE-HPLC (papers I-V)

The amount and size distribution of gluten proteins were analyzed using SE-HPLC according to a two-steps SDS-extraction procedure (Gupta *et al.*, 1993) in paper I and with minor modifications in paper II, III and IV. The extracted proteins from the first and the second steps are called SDS-extractable and SDS-unextractable proteins, respectively (Figure 8). The extracted protein from each steps are divided into four areas, indicating LPP (large polymeric protein), SPP (small polymeric proteins), LMP (large monomeric protein) and SMP (small monomeric protein) (paper I). The proportion of total SDS-extractable proteins (TOTE), total SDS-unextractable proteins (TOTU), percentage of un-extractable polymeric protein in total polymeric protein (%UPP), percentage of large unextractable polymeric protein in total large polymeric proteins (%LUPP), percentage of large unextractable monomeric protein in total large monomeric proteins (%LUMP), and the ratio of monomeric and polymeric proteins (Mon/pol) were calculated from these areas. The formulas to calculate these parameters are given in paper I.

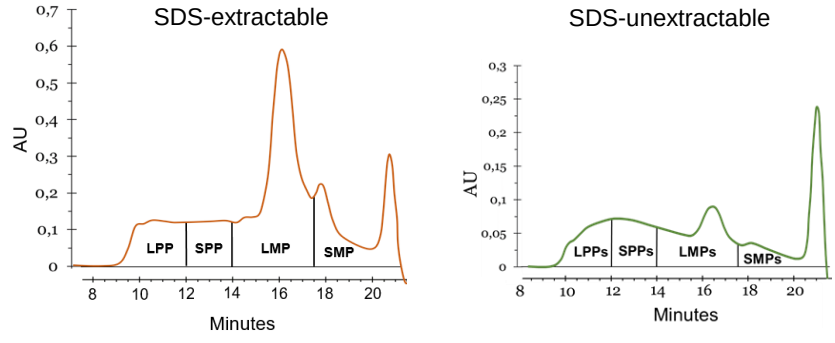


Figure 8. SE-HPLC chromatograms for the SDS-extractable (protein extracted after the 1st extraction step) and the SDS-unextractable (protein extracted after the 2nd extraction step) gluten proteins from Diskett in 2017.
The LPP and LPPs indicate large polymeric proteins; SPP and SPPs indicate small polymeric proteins, LMP and LMPs indicate large monomeric proteins and, SMP and SMPs indicate small monomeric proteins.

4.3.2 NIR spectroscopy (paper I and IV)

Near-infrared reflectance (NIR) spectroscopy was used to determine the grain protein concentration (GP%) of 294 genotypes grown in 2017 (paper I). Near-infrared transmission (NIT) spectroscopy was used to determine GP% of 282 genotypes from 2018 (paper I) and the flour protein concentration (FP%) of 109 genotypes from the wheat genotypes grown in 2017 and 2018 (paper I). NIR and NIT analysis was performed at Lantmännen, Svalöv, Sweden. The FP% of 14 genotypes from 2019 and 2020 used in paper IV was measured using NIT by Lilla Harrie Valskvarn, Kävlinge, Sweden.

4.4 Dough mixing using a mixograph (paper II)

Ten g of whole meal flour was used for each sample for the dough mixing study using a mixograph (Bohlin Reologi AB, Lund, Sweden) at 26°C. The amount of water added varied based on the FP% and moisture content of the flour. The flour was mixed with water for 10 min (overmixed) in order to determine the optimum mixing time (the highest mixing resistance) (Figure 9). In addition, two replicates of the flour samples were mixed for the defined

optimum mixing time (Figure 9). The dough samples were used for SE-HPLC analysis. Mixing parameters were obtained from the mixing curve (paper II).

Figure 9. Images showing over-mixed dough (left) and optimally mixed dough (right) from 10g of whole meal flour using mixrograph.

4.5 Phenotyping using RGB imaging (paper III)

Digital biomass of wheat was assessed through RGB imaging using two DSLR cameras placed on top and on the side of the plants. A canon EOS 1300D camera with an 18–55 mm kit lens was used for RGB imaging. For both cameras, focal length was set at 18 mm and ISO 1600. The images were obtained in a JPEG format using a resolution of 3456 x 2304 pixels for top projection and 5184 x 3456 pixels for side projection. From the images, the digital biomass of each plant was automatically extracted using Easy Leaf software (Easlon and Bloom, 2014). Detailed description of the camera setup and image processing can be found in manuscript III.

4.6 Sedimentation based physicochemical tests of flour (paper IV)

Two different physicochemical tests were used in this study, SDS-SRC and SIG. These tests are briefly described below.

4.6.1 SDS-SRC

For SDS-SRC sedimentation, the analysis was performed according to Seabourn et al. (2012) with some modifications. One g of whole meal flour was mixed with 5 ml of 0.47% lactic acid solution and 20 ml of 1.25% SDS.

The mixture was shaken and centrifuged for 2 minutes at 2900 x g. The supernatant liquid was poured off and centrifuged again for 1 minute. The tube was weighted and the SDS-SRC value was calculated as a percentage of the initial flour weight. This test was repeated 5 times (paper IV).

4.6.2 SIG

The swelling index of glutenin was conducted according to Wang and Kovacs (2002) with 40 mg of whole meal flour in three solvents, 1) SDS-lactic acid (SIG-SDS-LA), 2) dilute lactic acid (SIG-diluted LA) and 3) SDS-phosphate buffer (SIG-SDS-PB) (paper IV).

For SIG-SDS-LA, flour was hydrated in 0.6 ml of distilled water for 20 minutes at 25°C, followed by the addition of 0.6 ml of SDS-lactic acid stock solution. The mixture was hydrated again for 20 minutes at 25°C. Afterwards, the sample was centrifuged at 300 x g for 5 minutes. The supernatant liquid was poured off and the tube weight was determined (paper IV). For SIG-diluted LA, 0.8 of distilled water and 0.4 ml isopropanol-LA solution was added to the flour. The sample was hydrated for 10 minutes at 25°C before and after adding the isopropanol-LA solution. The sample was centrifuged at 100 x g for 5 minutes. The procedure for SIG-SDS-PB was the same as SIG-SDS-LA, except a solution of 0.05M Na_2HPO_4 containing 0.5 % SDS (pH 6.9) was used instead of the SDS-lactic solution. Centrifugation was done for 5 minutes at 1000 x g speed (Paper IV)

4.7 LC-MS/MS (paper V)

In order to do the proteomic study, gluten protein was extracted from 50±0.1 mg flour using 1.4 ml extraction buffer of 0.05mM Na_2HPO_4 containing 0.5 % SDS (pH 6.9). The extracted protein was separated using SE-HPLC for 30 minutes. The polymeric protein fraction was collected after 8.3 to 13.5 minutes in an eppendorf tube for 20 runs and the collected protein fraction was further used for LC-MS/MS. All the proteins were collected in triplicate for each genotype from each year. Detailed descriptions of the protein extraction and polymeric protein collection are provided in paper V. Further information regarding the sample preparation for LC-MS/MS is provided in paper V.

4.8 Bread-baking

The baking was done according to the standard protocol used at Lantmännen, Svalöv, Sweden. According to the protocol, 200 g of sieved flour was weighed and mixed with butter in the farinogram for 2 minutes. Afterwards, yeast diluted in an ascorbic acid, sugar and salt solution was added to the butter mixed flour. Extra flour was added within the first 2 minutes in order to reach the 400 BU line indicating the right consistency. All the ingredients were mixed with flour for 5 minutes. After 5 minutes, the dough was kept at 29^{o}C for 1 hour. After 1 hour the dough was divided into 3 parts, each part containing 100 g of dough. Two parts were proofed in separate rectangular forms and the 3rd part was proofed without a form (Figure 5), proofing continued for 80 minutes at 37^{o}C at 90% humidity. Baking was done at 225^{o}C for 20 minutes. Bread volume was measured using laser topography and a bread volume meter at Lantmännen, Svalöv (Figure 10).

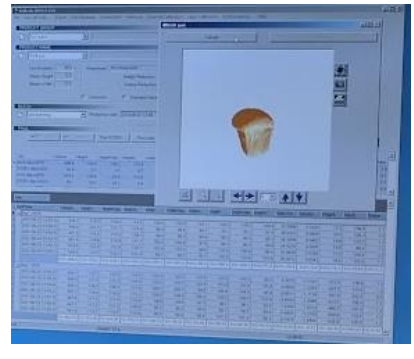


Figure 10. Image of bread volume being measured with a laser topography and a bread volume meter.
This image was taken at the laboratory of the Lantmännen, Svalöv.

5. Result and discussion

5.1 Yield and agronomic trait stability under extreme growing environments (Paper III)

Heat and drought stress was reported to damage the thylakoid membranes and reduce the electron transfer efficiency in wheat (Urban et al., 2018; Ru et al., 2023). Thus, heat and drought stress significantly interrupt the rate of photosynthesis, which becomes more intense under combined stress conditions (Ru et al., 2023). Furthermore, the activity of the key starch synthesis enzymes decreased under stress (Lu et al., 2019) and activity of starch degrading enzymes, such as α-amylase, increases (Hurkman and Wood, 2011), decreasing the overall starch synthesis in the grain. All these factors effectively contribute to yield reduction in wheat under stress. Similarly to previous studies (Hurkman et al., 2003; Balla et al., 2011; Prasad et al., 2011; Qaseem et al., 2019; Lorite et al., 2023), we observed lower yield under combined heat and drought stress in both field (paper I) and greenhouse (paper III) conditions.

Drought is known to be the most important factor for yield reduction (Lan et al., 2022) and to be more important than heat stress (Webber et al., 2018). However, depending on the intensity and timing of the stresses, these effects (either heat and drought) can be even more damaging (Qaseem et al., 2019; Ru et al., 2023). In our study, no clear difference between the single effect of heat and drought stresses on grain yield and yield related components, such as grain number and TKW, were observed (Table 1). Time after heading has been reported as being a crucial factor in terms of yield reduction from both heat and drought stresses (Balla et al., 2011; Poggi et al., 2023); this is due to most of the carbohydrates in the wheat grain being accumulated from photosynthesis after anthesis (Poggi et al., 2023). This is

caused by reduction of the phenological phases (vegetative and grain-filling periods), thus less time is available for photosynthesis and the translocation of assimilates (i.e. ion, amino acids) to form grains (Poggi et al., 2023).
A slight reduction of spike numbers and spike width was observed under stresses in our study (Table 1). Early drought stress has been reported to reduce spike development and, subsequently, the overall yield (Mohammadi et al., 2018). In our study, the reduction of spike development (weight of the spikes and width) seems to be more connected with either heat or combined stresses than drought stress alone (Table 1).

Table 1: Phenotypic and yield related traits of 8 spring wheat genotypes grown in the greenhouse.

Traits	Control	Drought	Heat	Combined
Biomass (g)	73.92 (±2.95)	72.04 (±1.25)	74.09 (±3.39)	50.51 (±1.64)
Weight of the spike (g)	33.25 (±2.01)	32.91 (±1.03)	29.42 (±1.64)	19.35 (±0.88)
Spike number	12.66 (±0.48)	12.44 (±0.43)	12.00 (±0.53)	11.25 (±0.49)
Spike length (mm)	101.24 (±1.97)	101.00(±2.52)	107.09 (±2.37)	105.11 (±2.05)
Spike width (mm)	18.33 (±0.18)	18.45 (±0.16)	16.17 (±0.37)	14.67 (±0.26)
Grain number	542.38 (±29.61)	519.59 (±24.09)	519.69 (±35.91)	387.84 (±33.08)
TKW (g)	37.7 (±1.06)	40.27 (±1.21)	39.27 (±0.91)	35.33 (±2.20)
Grain yield (g/plant)	6.8 (±0.38)	6.92 (±0.21)	7.07 (±0.45)	4.39 (±0.24)

Table based on the data included in paper III.
The values in the parenthesis show a standard error for the technical replicates in the field and biological replicates in the greenhouse.

GGE (genotype-by-environment interaction) biplot analysis is a widely used method to study the stability of yield and quality of wheat grown in multiple locations and years (Bishwas et al., 2021; Bosi et al., 2022; Mulugeta et al., 2022). This analysis is based on a PCA where the effects of the genotype (G) and environmental interaction (GXE) are taken into account (Gupta et al., 2022). In order to evaluate the stability of the genotypes grown under several stress conditions, we conducted GGE biplot analysis (paper III). We identified genotypes, i.e. Happy and SW1, that showed a stability yield and thousand-kernel weight (TKW) (paper III). However, for further confirmation of the stability, these genotypes should be grown in both field and controlled environments.

5.2 Changes in gluten protein quantity and quality in excessive climates (Papers I-IV)

5.2.1 Total protein concentration and gluten protein content

Environmental variations affect the grain protein concentration (GP%) composition to a great extent (Johansson et al., 2013). In our experiments, we found higher TOTE and GP% in the higher temperature in the greenhouse (paper III) and in the field (paper IV), which is supported by the previous studies (Rharrabti et al., 2003; Malik et al., 2011). Environmental factors, such as heat and drought, affect crop growth and development time, which in turn affects protein accumulations and polymerizations (Altenbach, 2012; Johansson et al., 2013; Johansson et al., 2020). In the study from 2007, Habash et al. showed that the shorter the time of anthesis, the more protein was observed in the grain. Cooler temperatures increase the time until plant maturation, which aids the carbohydrate assimilation and dilutes the protein concentration in the grain (Johansson et al., 2013; Johansson et al., 2020). Consequently, higher temperatures give higher grain protein concentrations. However, in our filed experiment for spring wheat (paper I), we found lower amounts of TOTE and GP% under combined heat-drought in the majority of the genotypes (86%). The reason behind this may be that under acute heat and drought, the availability of nitrogen was limited and thus the TOTE and GP% were lower. A previous study also showed that the availability of nitrogen is more important for protein polymerization than the temperature (Kuktaite et al., 2004). Increased nitrogen has been found to enhance the total amount of all protein components containing gliadins and glutenins (Johansson et al., 2004). The genes involved in controlling the GP% are also found to be related to nitrogen assimilation and transportation (Habash et al., 2007), thus nitrogen plays an important role in the controlling of GP%.

The effects of combined heat and drought stress on TOTE were higher than the individual effects of heat or drought (Dupont and Altenbach, 2003). In the greenhouse study, we observed the highest TOTE under combined heat and drought stress (paper III), which is supported by the study done by Balla et al. (2011). Additionally, in Balla's study, a higher effect on grain protein concentration (GP%) was observed from drought than from heat. In contrast, we observed a greater effect from heat stress than the drought stress on the GP% (paper III), which may be due to the mild effect of drought in our experiment.

The extractability of the gluten protein character (TOTE) depends on the strength of the dough. For example, when optimally mixed, dough made from weak biscuit flour showed greater amounts of extractable proteins than the dough made from strong bread flour (Kuktaite et al., 2004). In the same study, higher TOTE in the dough was observed compared with the TOTE in the flour (Kuktaite et al., 2004). Similarly, in our study, TOTE in the dough was found to be almost 1.5 times more than the TOTE in the flour (Figure 11). The mechanical energy used to mix the flour into dough weakens the non-covalent bonds (hydrogen, ionic and hydrophobic bonds) (Iwaki et al., 2020) and renders the protein more easily extractable. De-polymerisation of glutenin macro polymers during the dough mixing (Skerritt et al., 1999; Aussenac et al., 2001) could be another possible reason for the greater extractability of proteins in the dough.

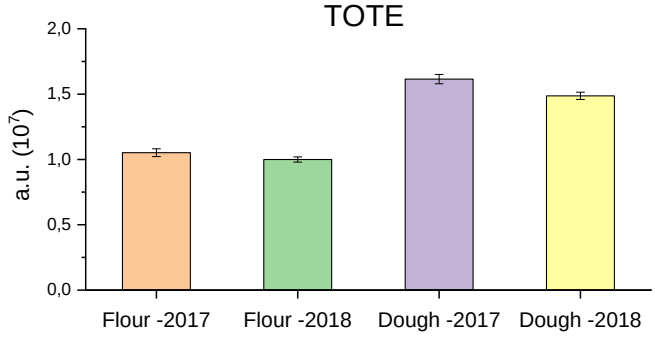


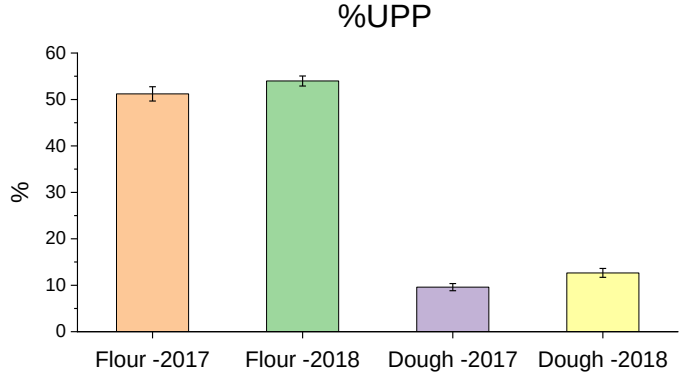
Figure 11. Average TOTE in the flour and dough in 2017 and 2018. Figures based on the data included in paper I and II.

5.2.2 Gluten protein polymerization and de-polymerization

Higher temperatures (up to 30^{o}C) during the grain-filling period lead to increased accumulation of the gluten proteins (Randall and Moss, 1990). High temperatures (>30^{o}C) during grain filling were also found to be associated with changes in the composition of gluten proteins (Daniel and Triboi, 2000; Hurkman et al., 2013) and a decrease in the glutenin to gliadin ratio (Cuniberti, 2000). In our study, polymeric protein and big monomeric protein accumulations (%UPP and %LUMP) were greatly affected by the combined heat and drought stress on the wheat grown in the field (paper I) and in the greenhouse (paper III).

Formation of the polymeric proteins mainly occurs during the latter half of the grain-filling period (Panozzo et al., 2001; Daniel and Triboï, 2002), thus the temperature during this period is crucial for polymeric protein accumulation. In our greenhouse experiment, we applied heat stress at the beginning of heading stage, which led to increased amounts of %UPP in the flour (paper III). This indicates that the temperature stress during the heading stage is crucial for gluten polymerization. However, the differences regarding the expected impact can vary between the greenhouse and field experiments. In addition to heat stress, the formation of the %UPP fraction is also related with water loss from the grain (Aussenac and Carceller, 2000). During the grain maturation period, the water loss from the grain took place gradually (Salgó and Gergely, 2001). Heat or drought stress can lead to rapid desiccation of the grains. Rapid desiccation contributed to faster glutenin polymer accumulation than gradual desiccation (Koga et al., 2020). In contrast, our study on the winter wheat grown in the field showed higher %UPP during the wet season (paper IV).

Previous studies have shown higher amounts of %UPP and %LUMP in dough than in flour (Kuktaite et al., 2004), which may be due to the cross-links between the tyrosine residues of HMW-GS and the disulphide bonds between the cystein residues (Shewry and Tatham, 1997; Tilley et al., 2001). However, in our study, considerably lower amounts of %UPP and %LUMP were observed upon mixing (Figure 12, paper II), which is due to the de-polymerization of protein aggregates during mixing; this is supported by previous studies (Aussenac et al., 2001; Iwaki et al., 2020).



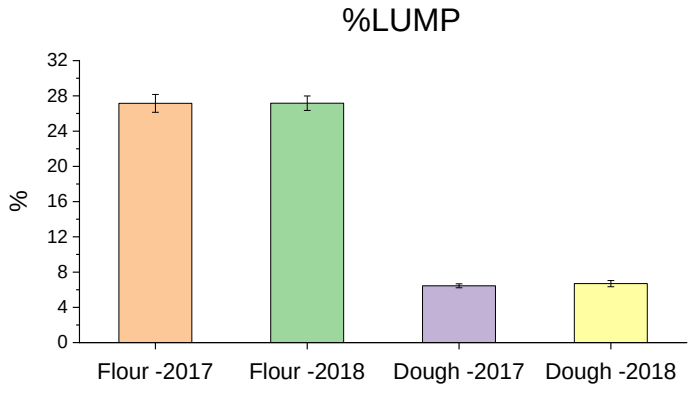


Figure 12. %UPP and %LUMP in the flour and dough in 2017 and 2018. Figures based on the data included in paper I and II.

5.3 Influence of excessive growing environments on dough processing (Paper II) and baking quality

5.3.1 Dough processing traits for quality evaluation

The polymeric proteins (%UPP) in the flour are positively correlated with gluten strength (Kuktaite et al., 2000). Gluten strength is an important indicator for, and is positively related to, bread volume (Cho et al., 2018). Studies done in the field have shown mean temperatures up to 30ºC positively correlating with gluten strength (UPP%) and bread-making quality, with any further temperature increase negatively correlating with the bread-making quality due to the production of heat-stress proteins (Randall and Moss, 1990; Johansson et al., 2002). Similarly, temperatures higher than 30ºC during grain filling had a negative effect on dough strength (Randall and Moss, 1990; Blumenthal et al., 1991) due to the changes in the gluten protein composition and reduction in the glutenin to gliadin ratio (Cuniberti, 2000; Daniel and Triboi, 2000; Hurkman et al., 2013).

Dough mixing time is an indicator of wheat flour strength (Boyacioglu and D'appolonia, 1994). Thus, gluten polymers control the dough mixing time (Hussain et al., 2012; Labuschagne and Moloi, 2015). Environmental factors like heat stress have been found to result in a weakening of the dough properties in both bread wheat and durum wheat (Cuniberti, 2000; Guzmán et al., 2016b; Magallanes-López et al., 2017). However, we didn't observe

any influence from different years on dough mixing time (paper II), which strengthens the explanation that this parameter is mainly genetically controlled (Ames et al., 1999; Li et al., 2013; Guzmán et al., 2016b). Water absorption of the dough is positively correlated with the FP% (Li et al., 2020). In support of this statement, we also observed a positive correlation between water absorption and total monomeric gluten protein (TMP) and TOTE (strong indicator of FP%) (paper II). Our study showed that water absorption is strongly influenced by the growing environments/years (paper II). Additionally, 88% genotypes from 2017 showed higher water absorption rates than the genotypes from 2018 (data from paper II), which is due to the higher protein concentration in the genotypes from 2017 (paper I).
Measuring stability in terms of wheat quality is challenging since there is a large number of quality criteria one can take into consideration. Genotypes may be more stable for criteria like GP% and dough stability time if they are less stable for quality parameters like TKW and Zeleny sedimentation volume (Koppel and Ingver, 2010; Mut et al., 2010). Furthermore, stability variation from flour to dough means genotypes with stable flour quality might not show stability in dough quality. The interaction of GxE on wheat quality makes the task of screening for stability even more difficult (Johansson et al., 2020).

It is not known how the stability of gluten quality affects the stability of dough processing quality. In order to explore the effect of gluten quality on dough processing, we conducted a PCA analysis of 294 genotypes from 2017 and 2018 using 5 gluten parameters: TOTE, TOTU, %UPP, %LUPP and Mon/pol (Figure 13). From the PCA analysis, the distance between PC1 and PC2 for each genotype was measured and divided into 3 groups; Stable (PCA distance is 0.17-1.42), intermediate stable (PCA distance is 1.45-2.57) and unstable (PCA distance is 2.58-9.10). Overall, genotypes in the stable group showed slightly better stability in peak time and water absorption compared with the unstable group (paper II).

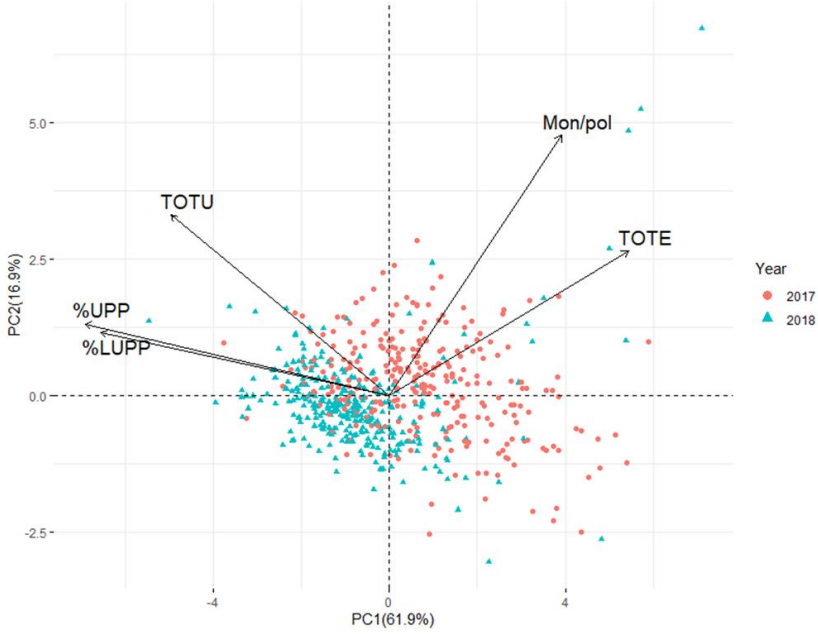


Figure 13: PCA plot of 294 spring wheat genotypes grown in 2017 and 2018. Figures based on the data included in paper I.

5.3.2 Bread volume

A baking test is the best method for assessing the baking qualities of wheat flour (Frauenlob et al., 2017). In many countries, the bread volume (BV) is included in trials in order for wheat varieties to be formally registered (Michel et al., 2017). It is common knowledge that BV is strongly associated with protein content and increases as protein level increases. A positive correlation exists between glutenin proteins and bread volume (Cho et al., 2018). The elements of a bread loaf that are typically evaluated to measure the quality of wheat breads are: bread volume, bread height, crumb density, crust and crumb color, crumb moisture, and relative crumb elasticity (Frauenlob et al., 2017; Cappelli et al., 2020; Alzuwaid et al., 2021).
The majority of the genotypes (70%) from 2018 showed slightly higher bread volume than the genotypes from 2017 (Figure 14). Previous studies have shown that changes in the amount and size distribution of polymeric protein (%UPP) in mature grains leads to differences in bread-making quality (Gupta et al., 1993; Johansson et al., 2003; Kuktaite et al., 2004). In spite of the

considerably higher %UPP observed in 2018 compared to 2017 (paper I), no significant difference in the bread volume was observed during these two years (Figure 14).

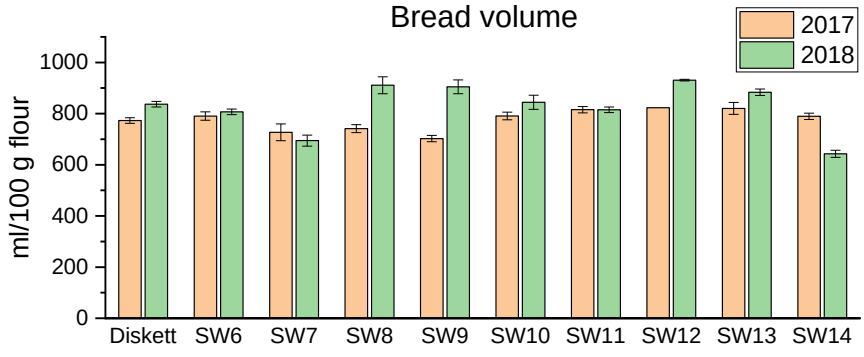


Figure 14. Volume of bread baked with form for 10 spring wheat genotypes grown in 2017 and 2018. The bars represent standard error.

In order to understand the factors affecting bread volume, a Pearson's correlation was conducted (Figure 15). Gluten strength is known to play an important role in determining bread volume (Johansson et al., 2002), which is negatively correlated to the protein % (Rharrabti et al., 2003). Similarly, in our study, we observed a negative correlation between the gluten strength (%UPP) and protein % (TOTE) (Figure 15). TOTE and Mon/pol showed a positive correlation with bread volume, whereas %UPP and %LUMP showed negative correlations. The genotypes with higher gluten stregth required mixing for a longer period of time in order to develop the gluten network. The standard dough mixing time (5 minutes) for baking breads used in this study might not be long enough for the genotypes with high gluten strengths, which could be the reason for the negative correlation between the %UPP and bread volumes.

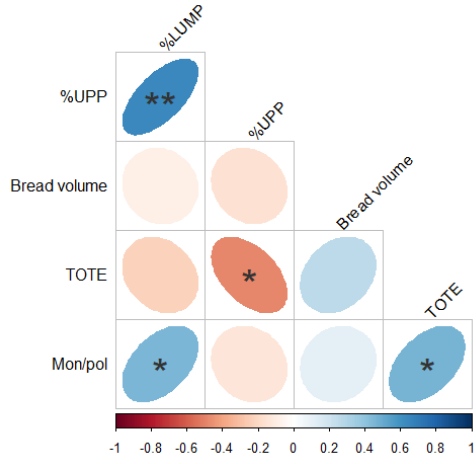


Figure 15. Pearson's correlation between the bread volume, TOTE, %UPP, %LUMP and Mon/pol for the 10 spring wheats grown in 2017 and 2018.

5.4 Optimizing robust methods for analyzing wheat quality and agronomic traits under varying climates (paper III and IV)

5.4.1 Small-scale tests for screening gluten quality (Paper IV)

Early generations in the breeding programs produced larger populations with lower amounts of grain, which do not allow the breeder to conduct time consuming quality analysis, such as dough mixing (e.g., using a farinograph and extensograph) and baking. A wide range of quality data in the early generation allows breeders to efficiently discard non-desirable wheat (Henry and Wrigley, 2018). Thus, the breeding companies desire simple, non-labor intensive, small-scale and fast tests in comparison to the time consuming, industrial quality tests used to screen wheat population in the early generations of the breeding programs (Guzmán et al., 2022).

In our study, we used a number of small-scale tests (SDS-SRC and SIG in different solvents) which required relatively low amounts of flour (40 mg to 1 g) and short testing times (around 1 minute per sample) (paper IV). Additionally, we also used SE-HPLC, which commonly uses a rather low amount of flour (16.5 mg). However, conducting the analysis required additional infrastructure, making this is not as simple as SDS-SRC and SIG tests. SIG- diluted LA and SDS-SRC showed significant correlation with

several of the farinograph and extensograph parameters (paper IV), including dough stability, development time, dough degradation time and extensibility.

In order to understand how small-scale tests (SIG and SDS-SRC) are related to SE-HPLC parameters (TOTE and %UPP), Pearson's correlation tests were conducted (Figure 16). SIG-diluted LA showed a significantly positive correlation with TOTE (protein concentration) in 2018-2019 ($p<0.05$) ,which is supported by the previous study (Labuschagne et al., 2021). Additionally, SDS-SRC showed a significant correlation with %UPP ($p<0.01$) in both years (Figure 16). Considering the overall correlation results, it can be concluded that SDS-SRC, SIG-diluted LA and SE-HPLC parameters such as %UPP and TOTE are best for use in the quality analysis of wheat flour (paper IV).

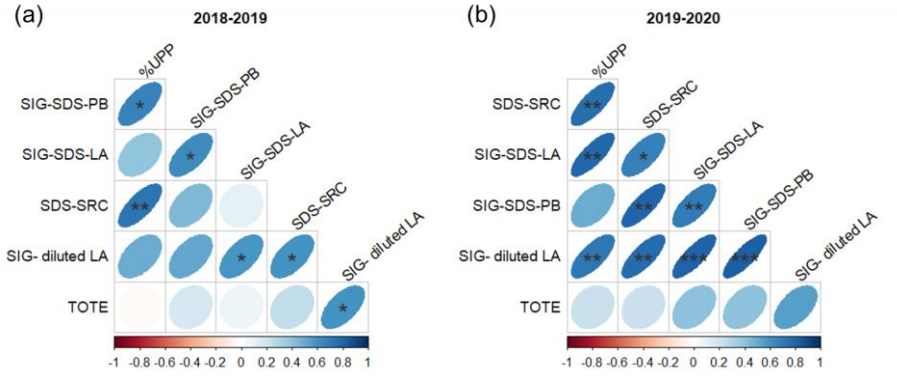


Figure 16. Pearson's correlation of the small-scale sedimentation (SIG and SDS-SRC) tests for the 13 winter wheat grown in (a) 2018-2019 and (b) 2019-2020. Figures based on the data included in paper IV.

5.4.2 RGB imaging to evaluate agronomic traits and plant resistance under stress (paper III)

Digital RGB cameras are inexpensive and able to estimate plant growth and development rate quickly and accurately in stressed environments, such as those under heat and drought (Blum et al., 1997; Humplík et al., 2015). Chlorophyll in the plants absorbs red light (Khan et al., 2018), thus heathy plants reflect less red light due to the higher amount of chlorophyll. This shows a high vegetative index, whereas plants under stress show a low vegetative index (Khan et al., 2018). Similarly, RGB imaging can also be

used to evaluate wheat quality characteristics such as nitrogen content in the green part of the plants (Li et al., 2022). In our study, a significant reduction of the digital biomass was observed for all stress parameters (paper III). The largest reduction of digital biomass was observed in plants grown under combined heat-drought condition compared with the plants grown under control condition (Figure 17). Similarly, previous studies have also shown significantly lower digital biomass for plants under drought stress during the heading period (Leiva et al., 2021) and under combined heat-drought period (Abdelhakim et al., 2021).

Two genotypes, Happy and Bumble, showed the least difference in digital biomass between control and combined stress conditions (Figure 17). These two genotypes also showed higher stability in TKW comparing with the other genotypes in GGE biplots analysis (paper III). Additionally, digital biomass under stress positively and significantly correlates with yield related traits (e.g., TKW, grain yield and biomass) under control and stress conditions (paper III). This indicates RGB imaging can be a useful tool to evaluate stability of the genotypes in terms of yield related traits such as grain yield, TKW and biomass under stress conditions.

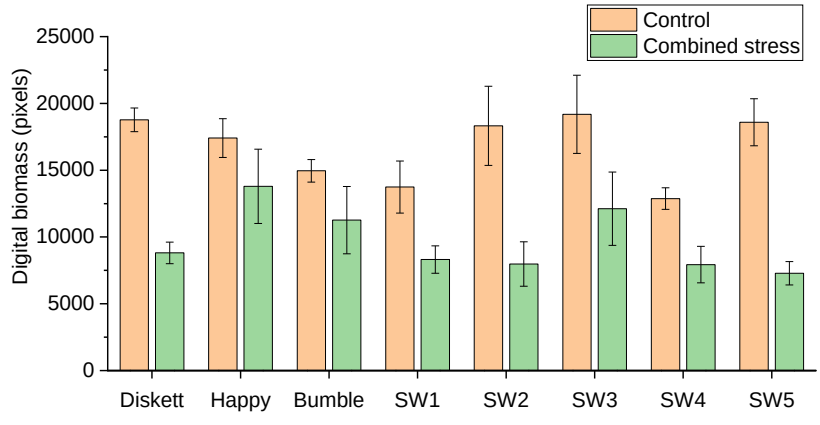


Figure 17. Digital biomass determined by RGB imaging (in pixels) of the eight spring wheat genotypes grown under control and after five days of induced combined heat-drought stress.

5.5 Can LC-MS/MS be a suitable tool for gluten protein quality evaluation in a varying climate? (paper V)

Higher amounts of polymers in flour are reported to be positively correlated with the rheological properties of wheat dough (Li et al., 2016) and bread volume (Johansson et al., 2001; Johansson et al., 2013). Polymerization of wheat proteins is largely affected by environmental variations, such as temperature and precipitation. However, we still lack information of which specific peptides in the polymers are affected by environment.

The polymeric fraction of wheat gluten proteins mostly contains HMW-GS and LMW-GS subunits, whereas the majority of the gliadins are found in the monomeric fractions (Kuktaite et al., 2004; Vensel et al., 2014). In contrast, our LC-MS/MS analysis of polymeric fractions showed only 11-13% of HMW-GS and LMW-GS in the polymeric fractions (paper V). A large percentage of uncharacterized proteins (~23%) and gliadins (~8%) was observed in both of the studied genotypes in the growing years.

Principal component analysis (PCA) results indicated a clear difference in collective protein expression between the genotypes (Diskett and Bumble) (paper V). The expression of a number of HMW-GSs and LMW-GSs (A0A0X9BSF8, V9P769, Q7Y075R9X, VC9K7WV92, Q0GQX1, Q00M55 and F6M7E1) showed a clear difference between the genotypes (paper v), which might be a reason for the overall difference in collective protein expression.

In terms of temperature and precipitation, 2017 and 2018 were hugely different during their spring wheat growing seasons, April-September (Figure 6 and Figure 7). In spite of the differences in the growing season, we did not observe a clear difference in collective protein expression between 2017 and 2018, or between either year and 2019 (paper V). However, Diskett showed slightly less variation in protein expression between the years, whereas more variations were observed in Bumble, indicating this genotype's higher sensitivity to the different growing conditions. In terms of peptide expression, we identified many peptides of HMW-GS, LMW-GS and gliadins that showed differential expression between the years (paper V). The expressions of these peptides showed us how they are affected by varying environments.

Proteomic results further showed that two peptides belonging to a HMW-GS protein, A0A0X9BSF8 (gene Glu-Ax), were highly expressed in the year 2019 in Diskett, whereas, four other peptides belonging to the same protein were poorly expressed or missing in the same year in Bumble (paper V). Diskett contains HMW-GS Ax2*, whereas Bumble contains Ax1. The

difference in these alleles (Ax2* and Ax1) in Diskett and Bumble might be the reason for the different expressions of peptides linked to the same protein under the same growing conditions.

Overall, our proteomics study conducted by LC-MS/MS identified peptides from polymeric gluten proteins that showed differential variations in composition and expression between the years. This indicates LC-MS/MS could be a suitable tool to assess the gluten protein quality of wheat growing in varying climates.

6. Conclusions

Based on the results, the following conclusions were drawn:

- The combined heat and drought stress had the strongest effect and significantly decreased grain yield, TKW and biomass in the studied spring wheat genotypes.
- The combined heat and drought increased gluten protein polymerization and induced both the formation of large gluten polymers (LPPs, %UPP, and %LUPP) and large monomers (%LUMP) in wheat grown in both field and greenhouse environments.
- The cool climate increased the amount of monomeric gluten proteins (LMP and SMP) and the protein concentration (TOTE) in the wheat compared to the wheat grown in the field under excessive heat and drought periods.
- The excessive prolonged heat and drought did not affect dough mixing parameters, such as the buildup and mixing time (expressed as peak time and time 1-2).
- No effect from varying weather on dough mixing time and buildup was observed, suggesting their potential use for dough stability evaluation. Screening for the wheat genotypes based on mixing time, TOTE and %UPP can be helpful in the future to evaluate dough stability in a changing climate.
- SE-HPLC is a promising analytical tool that showed a good evaluation of the protein concentration (TOTE) and gluten strength (%UPP) in wheat flour and dough from spring and winter wheat grown in diverse environments. These gluten parameters showed significant correlations with the industrial parameters of flour (dough stability, extensibility and total gluten) determined by farinograph, extensograph and glutograph.

- Small-scale sedimentation tests, SIG in diluted LA and SDS-SRC, showed a significantly positive correlation with the values from farinograph, extensograph and the gluten protein parameters (TOTE and %UPP) studied by SE-HPLC.
- With the use of LC-MS/MS it was possible to differentiate the polymeric gluten protein compositions and peptide expressions in the spring wheat varieties with similar HMW-GSs and grown in varying climates.

7. Future perspectives

Based on the results, the following future perspectives are recommended:

- SE-HPLC was used to capture the variations in gluten protein parameters for a large population of the breeding lines. This indicates that the SE-HPLC can be used for future breeding programs to generate stable wheat varieties in varying climates.
- Fast, small-scale sedimentation tests (i.e., SIG and SDS-SRC) used to analyze wheat flour quality showed a good correlation with SE-HPLC tests and industrial flour quality analyzing tests, such as farinograph and extensograph tests. Therefore, SIG and SDS-SRC, in combination with SE-HPLC tests, could be used further to evaluate breeding lines in the early generations of breeding programs when the amount of flour material is limited. Additionally, SE-HPLC and small-scale sedimentation tests are highly recommended for the milling industry.
- Phenotypic traits analyzed using RGB imaging in combination with the flour quality analysis by SE-HPLC was shown to be an effective combination to study the stability of wheat genotypes in terms of yield and flour quality. This combination for studying the stability of wheat genotypes could be of potential interest for both the breeding and milling industries.
- LC-MS/MS is a potential tool to study climate variation impacts on gluten proteins, although sample preparation procedures should also be further explored.
- Increasing levels of CO_2 seems to have a positive effect on wheat production, while also having a negative effect on grain protein concentration. Studying the combined effect of CO_2 and heat-drought on gluten proteins would give a better understanding of future climate change impacts on overall bread quality.

References

Abdelhakim, L.O.A., Rosenqvist, E., Wollenweber, B., Spyroglou, I., Ottosen, C.-O., and Panzarová, K. (2021). Investigating combined drought- and heat stress effects in wheat under controlled conditions by dynamic image-based phenotyping. *Agronomy* 11(2)**,** 364.

Ali, O.A.M. (2019). "Wheat responses and tolerance to drought stress," in *Wheat production in changing environments: responses, adaptation and tolerance*, eds. M. Hasanuzzaman, K. Nahar & M.A. Hossain. (Singapore: Springer Singapore), 129-138.

Altenbach, S.B. (2012). New insights into the effects of high temperature, drought and post-anthesis fertilizer on wheat grain development. *Journal of Cereal Science* 56(1)**,** 39-50. doi: https://doi.org/10.1016/j.jcs.2011.12.012.

Alzuwaid, N.T., Pleming, D., Fellows, C.M., and Sissons, M. (2021). Fortification of durum wheat spaghetti and common wheat bread with wheat bran protein concentrate-impacts on nutrition and technological properties. *Food chemistry* 334**,** 127497. doi: 10.1016/j.foodchem.2020.127497.

Ames, N.P., Clarke, J.M., Marchylo, B.A., Dexter, J.E., and Woods, S.M. (1999). Effect of environment and genotype on durum wheat gluten strength and pasta viscoelasticity. *Cereal Chem.* 76(4)**,** 582-586. doi: https://doi.org/10.1094/CCHEM.1999.76.4.582.

Anderson, O.D., Dong, L., Huo, N., and Gu, Y.Q. (2012). A new class of wheat gliadin genes and proteins. *PloS one* 7(12)**,** e52139. doi: 10.1371/journal.pone.0052139.

Araus, J.L., Kefauver, S.C., Zaman-Allah, M., Olsen, M.S., and Cairns, J.E. (2018). Translating High-Throughput Phenotyping into Genetic Gain. *Trends in Plant Science* 23(5)**,** 451-466. doi: 10.1016/j.tplants.2018.02.001.

Aussenac, T., Carceller, J.-L., and Kleiber, D. (2001). Changes in SDS solubility of glutenin polymers during dough mixing and resting. *Cereal Chem.* 78(1)**,** 39-45. doi: 10.1094/cchem.2001.78.1.39.

Aussenac, T., and Carceller, J.L. (2000). SDS-unextractable glutenin polymer formation in wheat kernels. *Spec. Publ. - R. Soc. Chem.* 261**,** 475-479.

Axford, D.W.E., McDermott, E.E., and Redman, D.G. (1979). Note on the sodium dodecyl sulfate test of breadmaking quality: comparison with Pelshenke and Zeleny tests. *Cereal Chemistry* (6)**,** 582-584.

Balla, K., Rakszegi, M., Li, Z., Bekes, F., Bencze, S., and Veisz, O. (2011). Quality of winter wheat in relation to heat and drought shock after anthesis. *Czech J. Food Sci.* 29**,** 117-128. doi: 10.17221/227/2010-CJFS.

Battenfield, S.D., Guzman, C., Gaynor, R.C., Singh, R.P., Pena, R.J., Dreisigacker, S., et al. (2016). Genomic Selection for Processing and End-Use Quality

Traits in the CIMMYT Spring Bread Wheat Breeding Program. *Plant Genome* 9(2). doi: 10.3835/plantgenome2016.01.0005.

Belton, P.S. (1999). On the elasticity of wheat gluten. *J. Cereal Sci.* 29:103–07.

Belton, P.S. (2005). New approaches to study the molecular basis of the mechanical properties of gluten. *J. Cereal Sci.* 41(2)**,** 203-211. doi: 10.1016/j.jcs.2004.06.003.

Bishwas, K.C., Poudel, M.R., and Regmi, D. (2021). AMMI and GGE biplot analysis of yield of different elite wheat line under terminal heat stress and irrigated environments. *Heliyon* 7(6)**,** e07206. doi: 10.1016/j.heliyon.2021.e07206.

Blum, A., Sullivan, C.Y., and Nguyen, H.T. (1997). The effect of plant size on wheat response to agents of drought stress. II. Water deficit, heat and ABA. *Funct. Plant Biol.* 24(1)**,** 43-48. doi: https://doi.org/10.1071/PP96023.

Blumenthal, C., Gras, P.W., Bekes, F., Barlow, E.W.R., and Wrigley, C.W. (1995a). Possible role for the Glu-D1 locus with respect to tolerance to dough-quality change after heat stress. *Cereal chemistry.* 72(1)**,** 135-136.

Blumenthal, C., Bekes, F., Gras, P., Barlow, E., and Wrigley, C. (1995b). Identification of wheat genotypes tolerant to the effects of heat stress on grain quality. *Cereal Chem.* 72(6)**,** 539-544.

Blumenthal, C., Stone, P., Gras, P., Bekes, F., Clarke, B., Barlow, E., et al. (1998). Heat-Shock Protein 70 and Dough-Quality Changes Resulting from Heat Stress During Grain Filling in Wheat. *Cereal Chemistry* 75**,** 43-50. doi: 10.1094/CCHEM.1998.75.1.43.

Blumenthal, C.S., Batey, I.L., Bekes, F., Wrigley, C.W., and Barlow, E.W.R. (1991). Seasonal changes in wheat-grain quality associated with high temperatures during grain filling. *Aust. J. Agric. Res* 42(1)**,** 21-30. doi: 10.1071/AR9910021.

Bonilla, J.C., Schaber, J.A., Bhunia, A.K., and Kokini, J.L. (2019). Mixing dynamics and molecular interactions of HMW glutenins, LMW glutenins, and gliadins analyzed by fluorescent co-localization and protein network quantification. *J. Cereal Sci.* 89. doi: 10.1016/j.jcs.2019.102792.

Bosi, S., Negri, L., Fakaros, A., Oliveti, G., Whittaker, A., and Dinelli, G. (2022). GGE Biplot Analysis to Explore the Adaption Potential of Italian Common Wheat Genotypes. *Sustainability* 14(2)**,** 897.

Bosmans, G.M., Lagrain, B., Deleu, L.J., Fierens, E., Hills, B.P., and Delcour, J.A. (2012). Assignments of proton populations in dough and bread using NMR relaxometry of starch, gluten, and flour model systems. *J. Agric. Food. Chem.* 60(21)**,** 5461-5470. doi: 10.1021/jf3008508.

Boyacioglu, M., and D'appolonia, B. (1994). Characterization and utilization of durum wheat for breadmaking. I. Comparison of chemical, rheological, and baking properties between bread wheat flours and durum wheat flours. *Cereal Chem.* 71(1)**,** 21-27.

Cao, Z., Yao, X., Liu, H., Liu, B., Cheng, T., Tian, Y., et al. (2019). Comparison of the abilities of vegetation indices and photosynthetic parameters to detect

heat stress in wheat. *Agric. For. Meteorol.* 265**,** 121-136. doi: https://doi.org/10.1016/j.agrformet.2018.11.009.

Cappelli, A., Bettaccini, L., and Cini, E. (2020). The kneading process: A systematic review of the effects on dough rheology and resulting bread characteristics, including improvement strategies. *Trends Food Sci. Technol.* 104**,** 91-101. doi: 10.1016/j.tifs.2020.08.008.

Challinor, A.J., Watson, J., Lobell, D.B., Howden, S.M., Smith, D.R., and Chhetri, N. (2014). A meta-analysis of crop yield under climate change and adaptation. *Nat. Clim. Change* 4(4)**,** 287-291. doi: 10.1038/nclimate2153.

Cho, K., Kang, C.-S., Kang, T.-G., Cho, K.-M., and Park, C. (2018). Influence of different nitrogen application on flour properties, gluten properties by HPLC and end-use quality of Korean wheat. *Journal of Integrative Agriculture* 17**,** 982-993. doi: 10.1016/S2095-3119(18)61920-3.

Cozzolino, D. (2021). The Ability of Near Infrared (NIR) Spectroscopy to Predict Functional Properties in Foods: Challenges and Opportunities. *Molecules* 26(22). doi: 10.3390/molecules26226981.

Cuniberti, M. (Year). "Caloric stress and their effect in the argentine wheat quality", in: *11th Cereal and Bread Congress. Queensland, Australia*), 144.

Currà, A., Gasbarrone, R., Bonifazi, G., Serranti, S., Fattapposta, F., Trompetto, C., et al. (2022). Near-infrared transflectance spectroscopy discriminates solutions containing two commercial formulations of botulinum toxin type a diluted at recommended volumes for clinical reconstitution. *Biosensors* 12(4). doi: 10.3390/bios12040216.

Daniel, C., and Triboi, E. (2000). Effects of temperature and nitrogen nutrition on the grain composition of winter wheat: effects on gliadin content and composition. *J. Cereal Sci.* 32(1)**,** 45-56. doi: 10.1006/jcrs.2000.0313.

Daniel, C., and Triboï, E. (2002). Changes in wheat protein aggregation during grain development: effects of temperatures and water stress. *European Journal of Agronomy* 16(1)**,** 1-12. doi: https://doi.org/10.1016/S1161-0301(01)00114-9.

De Santis, M.A., Giuliani, M.M., Giuzio, L., De Vita, P., Lovegrove, A., Shewry, P.R., et al. (2017). Differences in gluten protein composition between old and modern durum wheat genotypes in relation to 20th century breeding in Italy. *European Journal of Agronomy* 87**,** 19-29. doi: 10.1016/j.eja.2017.04.003.

Dhondt, S., Wuyts, N., and Inzé, D. (2013). Cell to whole-plant phenotyping: the best is yet to come. *Trends Plant Sci.* 18(8)**,** 428-439. doi: https://doi.org/10.1016/j.tplants.2013.04.008.

Doona, C.J., and Baik, M.-Y. (2007). Molecular mobility in model dough systems studied by time-domain nuclear magnetic resonance spectroscopy. *J. Cereal Sci.* 45(3)**,** 257-262. doi: 10.1016/j.jcs.2006.07.015.

Dupont, F.M., and Altenbach, S. (2003). Molecular and biochemical impacts of environmental factors on wheat grain development and protein yield. *J. Cereal Sci.* 38**,** 133-146. doi: 10.1016/S0733-5210(03)00030-4.

Dupont, F.M., Hurkman, W.J., Vensel, W.H., Tanaka, C., Kothari, K.M., Chung, O.K., et al. (2006). Protein accumulation and composition in wheat grains: Effects of mineral nutrients and high temperature. *European journal of agronomy* 25**,** 96-107.

Easlon, H.M., and Bloom, A.J. (2014). Easy Leaf Area: Automated digital image analysis for rapid and accurate measurement of leaf area. *Appl. Plant Sci.* 2(7). doi: 10.3732/apps.1400033.

Farooq, M., Hussain, M., and Siddique, K.H.M. (2014). Drought stress in wheat during flowering and grain-filling periods. *CRC Crit. Rev. Plant Sci.* 33(4)**,** 331-349. doi: 10.1080/07352689.2014.875291.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., and Basra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev.* 29(1)**,** 185-212. doi: 10.1051/agro:2008021.

Flagella, Z., Giuliani, M., Giuzio, L., Volpi, C., and Masci, S. (2010). Influence of water deficit on durum wheat storage protein composition and technological quality. *Eur. J Agron.* 33**,** 197-207. doi: 10.1016/j.eja.2010.05.006.

Food and Agriculture Organization of the United Nations (FAO). (2023). *Food and agriculture statistics (FAOSTAT).* http://www.fao.org/statistics/databases/en/ [accessed on 2 March 2023].

Frauenlob, J., Nava, M., D'Amico, S., Grausgruber, H., Lucisano, M., and Schoenlechner, R. (2017). A new micro-baking method for determination of crumb firmness properties in fresh bread and bread made from frozen dough / Entwicklung eines Mikrobackversuches zur Evaluierung der Krumeneigenschaften von frischen Broten und Broten aus vorgegarten Tiefkühlteiglingen. *Die Bodenkultur: Journal of Land Management, Food and Environment* 68. doi: 10.1515/boku-2017-0003.

Giuliani, M.M., Palermo, C., De Santis, M.A., Mentana, A., Pompa, M., Giuzio, L., et al. (2015). Differential Expression of Durum Wheat Gluten Proteome under Water Stress during Grain Filling. *Journal of agricultural and food chemistry* 63(29)**,** 6501. doi: 10.1021/acs.jafc.5b01635.

Golea, C.M., Codină, G.G., and Oroian, M. (2023). Prediction of wheat flours composition using fourier transform infrared spectrometry (FT-IR). *Food Control* 143**,** 109318. doi: https://doi.org/10.1016/j.foodcont.2022.109318.

Grusson, Y., Wesström, I., and Joel, A. (2021). Impact of climate change on Swedish agriculture: Growing season rain deficit and irrigation need. *Agric. Water Manag.* 251. doi: 10.1016/j.agwat.2021.106858.

Gupta, R.B., Khan, K., and Macritchie, F. (1993). Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. *J. Cereal Sci.* 18**,** 23–41. doi: 10.1006/jcrs.1993.1031.

Gupta, V., Kumar, M., Singh, V., Chaudhary, L., Yashveer, S., Sheoran, R., et al. (2022). Genotype by environment interaction analysis for grain yield of

wheat (Triticum aestivum (L.) em.Thell) genotypes. *Agriculture* 12(7)**,** 1002.

Guttieri, M.J., McLean, R., Lanning, S.P., Talbert, L.E., and Souza, E.J. (2002). Assessing environmental influences on solvent retention capacities of two soft white spring wheat cultivars. *Cereal Chem.* 79(6)**,** 880-884. doi: https://doi.org/10.1094/CCHEM.2002.79.6.880.

Guzman, C., Pena, R.J., Singh, R., Autrique, E., Dreisigacker, S., Crossa, J., et al. (2016a). Wheat quality improvement at CIMMYT and the use of genomic selection on it. *Applied and Translational Genomics* 11**,** 3-8. doi: 10.1016/j.atg.2016.10.004.

Guzmán, C., Autrique, J.E., Mondal, S., Singh, R.P., Govindan, V., Morales-Dorantes, A., et al. (2016b). Response to drought and heat stress on wheat quality, with special emphasis on bread-making quality, in durum wheat. *Field Crops Res.* 186**,** 157-165. doi: 10.1016/j.fcr.2015.12.002.

Guzmán, C., Mondal, S., Govindan, V., Autrique, J.E., Posadas-Romano, G., Cervantes, F., et al. (2016c). Use of rapid tests to predict quality traits of CIMMYT bread wheat genotypes grown under different environments. *LWT - Food Science and Technology* 69**,** 327-333. doi: 10.1016/j.lwt.2016.01.068.

Guzmán, C., Ibba, M.I., Álvarez, J.B., Sissons, M., and Morris, C. (2022). "Wheat quality," in *Wheat Improvement: Food Security in a Changing Climate*. Springer International Publishing Cham), 177-193.

Habash, D.Z., Bernard, S., Schondelmaier, J., Weyen, J., and Quarrie, S.A. (2007). The genetics of nitrogen use in hexaploid wheat: N utilisation, development and yield. *Theor. Appl. Genet.* 114(3)**,** 403-419. doi: 10.1007/s00122-006-0429-5.

Hemdane, S., Jacobs, P.J., Bosmans, G.M., Verspreet, J., Delcour, J.A., and Courtin, C.M. (2017). Study on the effects of wheat bran incorporation on water mobility and biopolymer behavior during bread making and storage using time-domain (1)H NMR relaxometry. *Food Chem.* 236**,** 76-86. doi: 10.1016/j.foodchem.2017.01.039.

Henry, R.J., and Wrigley, C.W. (2018). Towards a genetic road map of wheat-processing quality. *Journal of Cereal Science* 79**,** 516-517. doi: 10.1016/j.jcs.2017.12.010.

Hernandez-Ochoa, I.M., Luz Pequeno, D.N., Reynolds, M., Babar, M.A., Sonder, K., Milan, A.M., et al. (2019). Adapting irrigated and rainfed wheat to climate change in semi-arid environments: Management, breeding options and land use change. *Eur. J. Agron.* 109. doi: 10.1016/j.eja.2019.125915.

Humplík, J.F., Lazár, D., Husičková, A., and Spíchal, L. (2015). Automated phenotyping of plant shoots using imaging methods for analysis of plant stress responses – a review. *Plant Methods* 11(1)**,** 29. doi: 10.1186/s13007-015-0072-8.

Hurkman, W., Tanaka, C., Vensel, W., Thilmony, R., and Altenbach, S. (2013). Comparative proteomic analysis of the effect of temperature and fertilizer

on gliadin and glutenin accumulation in the developing endosperm and flour from Triticum aestivum L. cv. Butte 86. *Proteome science* 11**,** 8. doi: 10.1186/1477-5956-11-8.

Hurkman, W.J., McCue, K.F., Altenbach, S.B., Korn, A., Tanaka, C.K., Kothari, K.M., et al. (2003). Effect of temperature on expression of genes encoding enzymes for starch biosynthesis in developing wheat endosperm. *Plant Science* 164(5)**,** 873-881. doi: https://doi.org/10.1016/S0168-9452(03)00076-1.

Hurkman, W.J., and Wood, D.F. (2011). High temperature during grain fill alters the morphology of protein and starch deposits in the starchy endosperm cells of developing wheat (Triticum aestivum L.) grain. *Journal of Agricultural and Food Chemistry* 59(9)**,** 4938-4946.

Hussain, A., Larsson, H., Kuktaite, R., Prieto-Linde, M.L., and Johansson, E. (2012). Towards the understanding of bread-making quality in organically grown wheat: Dough mixing behaviour, protein polymerisation and structural properties. *J. Cereal Sci.* 56(3)**,** 659-666. doi: https://doi.org/10.1016/j.jcs.2012.07.016.

Irar, S., Brini, F., Goday, A., Masmoudi, K., and Pagès, M. (2010). Proteomic analysis of wheat embryos with 2-DE and liquid-phase chromatography (ProteomeLab PF-2D) — A wider perspective of the proteome. *Journal of Proteomics* 73(9)**,** 1707-1721. doi: 10.1016/j.jprot.2010.05.003.

Iwaki, S., Aono, S., Hayakawa, K., Fu, B.X., and Otobe, C. (2020). Changes in protein non-covalent bonds and aggregate size during dough formation. *Foods* 9(11). doi: 10.3390/foods9111643.

Johansson, E., Branlard, G., Cuniberti, M., Flagella, Z., Hüsken, A., Nurit, E., et al. (2020). "Genotypic and environmental effects on wheat technological and nutritional quality," in *Wheat quality for improving processing and human health*. Springer), 171-204.

Johansson, E., Kuktaite, R., Labuschagne, M., Lama, S., Lan, Y., Nakimbugwe, D., et al. (2023). "Adaptation to abiotic stress factors and their effects on cereal and pseudocereal grain quality," in *[In the press]*.).

Johansson, E., Malik, A.H., Hussain, A., Rasheed, F., Newson, W.R., Plivelic, T., et al. (2013). Wheat Gluten Polymer Structures: The Impact of Genotype, Environment, and Processing on Their Functionality in Various Applications. *Cereal Chemistry* 90(4)**,** 367-376. doi: doi:10.1094/CCHEM-08-12-0105-FI.

Johansson, E., Nilsson, H., Mazhar, H., Skerritt, J., MacRitchie, F., and Svensson, G. (2002). Seasonal effects on storage proteins and gluten strength in four Swedish wheat cultivars. *J. Sci. Food Agric.* 82**,** 1305-1311.

Johansson, E., Prieto-Linde, M.L., and Svensson, G. (2004). Influence of nitrogen application rate and timing on grain protein composition and gluten strength in Swedish wheat cultivars. *Journal of Plant Nutrition and Soil Science* 167(3)**,** 345-350. doi: https://doi.org/10.1002/jpln.200320332.

Johansson, E., Prieto-Linde, M.L., Svensson, G., and Jnsson, J. (2003). Influences of cultivar, cultivation year and fertilizer rate on amount of protein groups and amount and size distribution of mono- and polymeric proteins in wheat. *The Journal of Agricultural Science* 140(3)**,** 275-284. doi: 10.1017/S0021859603003162.

Johansson, E., Prieto-Linde, M.L., and Jönsson, J.Ö. (2001). Effects of Wheat Cultivar and Nitrogen Application on Storage Protein Composition and Breadmaking Quality. *Cereal Chemistry* 78(1)**,** 19-25. doi: 10.1094/CCHEM.2001.78.1.19.

Junior, S.B., Mastelini, S.M., Barbon, A.P.A.C., Barbin, D.F., Calvini, R., Lopes, J.F., et al. (2020). Multi-target prediction of wheat flour quality parameters with near infrared spectroscopy. *Inf. Process. Agric.* 7(2)**,** 342-354. doi: https://doi.org/10.1016/j.inpa.2019.07.001.

Khan, Z., Rahimi-Eichi, V., Haefele, S., Garnett, T., and Miklavcic, S.J. (2018). Estimation of vegetation indices for high-throughput phenotyping of wheat using aerial imaging. *Plant Methods* 14(1)**,** 20. doi: 10.1186/s13007-018-0287-6.

Koga, S., Aamot, H.U., Uhlen, A.K., Seehusen, T., Veiseth-Kent, E., Hofgaard, I.S., et al. (2020). Environmental factors associated with glutenin polymer assembly during grain maturation. *Journal of Cereal Science* 91**,** 102865. doi: https://doi.org/10.1016/j.jcs.2019.102865.

Koga, S., Böcker, U., Moldestad, A., Tosi, P., Shewry, P.R., Mosleth, E.F., et al. (2016). Influence of temperature during grain filling on gluten viscoelastic properties and gluten protein composition. *Journal of the Science of Food and Agriculture* 96(1)**,** 122-130. doi: 10.1002/jsfa.7068.

Koppel, R., and Ingver, A. (2008). A comparison of the yield and quality traits of winter and spring wheat. *Latvian Journal of Agronomy* 11**,** 83-89.

Koppel, R., and Ingver, A. (2010). Stability and predictability of baking quality of winter wheat. *Agronomy research* 8**,** 637-644.

Kuktaite, R., Johansson, E., and Juodeikiene, G. (2000). Composition and concentration of proteins in Lithuanian wheat cultivars: relationships with bread-making quality. *Cereal Res. Commun.* 28(1)**,** 195-202. doi: 10.1007/BF03543593.

Kuktaite, R., Larsson, H., and Johansson, E. (2004). Variation in protein composition of wheat flour and its relationship to dough mixing behaviour. *J Cereal Sci.* 40(1)**,** 31-39. doi: https://doi.org/10.1016/j.jcs.2004.04.007.

Kweon, M., Slade, L., and Levine, H. (2011). Solvent Retention Capacity (SRC) Testing of Wheat Flour: Principles and Value in Predicting Flour Functionality in Different Wheat-Based Food Processes and in Wheat Breeding—A Review. *Cereal Chemistry* 88(6)**,** 537-552. doi: 10.1094/CCHEM-07-11-0092.

Labuschagne, M., and Aucamp, U. (2004). The use of size exclusion high performance liquid chromatography (SE-HPLC) for wheat quality

prediction in South Africa. *S. Afr. J. Plant Soil* 21**,** 8-12. doi: 10.1080/02571862.2004.10635015.

Labuschagne, M., Guzman, C., Phakela, K., Wentzel, B., and van Biljon, A. (2021). Solvent retention capacity and gluten protein composition of durum wheat flour as influenced by drought and heat stress. *Plants* 10(5). doi: 10.3390/plants10051000.

Labuschagne, M.T., and Moloi, M.J. (2015). The influence of abiotic stress conditions on dough mixing characteristics of two hard red spring wheat cultivars. *J. Cereal Sci.* 63**,** 134-139. doi: 10.1016/j.jcs.2015.03.007.

Lama, S., Vallenback, P., Hall, S.A., Kuzmenkova, M., and Kuktaite, R. (2022). Prolonged heat and drought versus cool climate on the Swedish spring wheat breeding lines: Impact on the gluten protein quality and grain microstructure. *Food Energy Secur.* 11(2)**,** 1-17. doi: 10.1002/fes3.376.

Lan, Y., Chawade, A., Kuktaite, R., and Johansson, E. (2022). Climate change impact on wheat performance - Effects on vigour, plant traits and yield from early and late drought stress in diverse lines. *Int. J. Mol. Sci.* 23(6)**,** 1-17. doi: 10.3390/ijms23063333.

Lantmännen (2023). *Vete.* https://www.lantmannen.se/bra-mat/fyra-sadesslag/vete/ [accessed on 5 March 2023].

Leiva, F., Vallenback, P., Ekblad, T., Johansson, E., and Chawade, A. (2021). Phenocave: An automated, standalone, and affordable phenotyping system for controlled growth conditions. *Plants* 10(9)**,** 1817.

Li, C., Dhital, S., Gilbert, R.G., and Gidley, M.J. (2020). High-amylose wheat starch: Structural basis for water absorption and pasting properties. *Carbohydr. Polym.* 245**,** 116557.

Li, R., Wang, D., Zhu, B., Liu, T., Sun, C., and Zhang, Z. (2022). Estimation of nitrogen content in wheat using indices derived from RGB and thermal infrared imaging. *Field Crops Res.* 289**,** 108735. doi: https://doi.org/10.1016/j.fcr.2022.108735.

Li, X., Lv, Y., Chen, Y., and Chen, J. (2016). A study on the relationship between rheological properties of wheat flour, gluten structure, and dumpling wrapper quality. *Int. J. Food Prop.* 19. doi: 10.1080/10942912.2014.951894.

Li, Y.-F., Wu, Y., Hernandez-Espinosa, N., and Peña, R.J. (2013). Heat and drought stress on durum wheat: Responses of genotypes, yield, and quality parameters. *J. Cereal Sci.* 57(3)**,** 398-404. doi: https://doi.org/10.1016/j.jcs.2013.01.005.

Li, Y.F., Wu, Y., Hernandez-Espinosa, N., and Peña, R.J. (2015). Comparing small-scale testing methods for predicting wheat gluten strength across environments. *Cereal Chem.* 92(3)**,** 231-235. doi: https://doi.org/10.1094/CCHEM-07-14-0157-R.

Lorite, I.J., Castilla, A., Cabezas, J.M., Alza, J., Santos, C., Porras, R., et al. (2023). Analyzing the impact of extreme heat events and drought on wheat yield and protein concentration, and adaptation strategies using long-term

cultivar trials under semi-arid conditions. *Agric. For. Meteorol.* 329. doi: 10.1016/j.agrformet.2022.109279.

Lu, H., Hu, Y., Wang, C., Liu, W., Ma, G., Han, Q., et al. (2019). Effects of high temperature and drought stress on the expression of gene encoding enzymes and the activity of key enzymes involved in starch biosynthesis in wheat grains. *Front. Plant Sci.* 10**,** 1414. doi: 10.3389/fpls.2019.01414.

Madhusudhana, R. (2019). "Marker-assisted breeding in sorghum," in *Breeding Sorghum for Diverse End Uses*.), 93-114.

Magallanes-López, A.M., Ammar, K., Morales-Dorantes, A., González-Santoyo, H., Crossa, J., and Guzmán, C. (2017). Grain quality traits of commercial durum wheat varieties and their relationships with drought stress and glutenins composition. *Journal of Cereal Science* 75**,** 1-9. doi: 10.1016/j.jcs.2017.03.005.

Malik, A.H. (2009). Nutrient uptake, transport and translocation in cereals: influences of environmental and farming conditions.

Malik, A.H., Kuktaite, R., and Johansson, E. (2013). Combined effect of genetic and environmental factors on the accumulation of proteins in the wheat grain and their relationship to bread-making quality. *J. Cereal Sci.* 57(2)**,** 170-174. doi: 10.1016/j.jcs.2012.09.017.

Malik, A.H., Prieto-Linde, M.L., Kuktaite, R., Andersson, A., and Johansson, E. (2011). Individual and interactive effects of cultivar maturation time, nitrogen regime and temperature level on accumulation of wheat grain proteins. *J. Sci. Food Agric.* 91(12)**,** 2192-2200. doi: 10.1002/jsfa.4439.

Manès, Y., Gomez, H.F., Puhl, L., Reynolds, M., Braun, H.J., and Trethowan, R. (2012). Genetic yield gains of the CIMMYT international semi-arid wheat yield trials from 1994 to 2010. *Crop Sci.* 52(4)**,** 1543-1552. doi: 10.2135/cropsci2011.10.0574.

Markgren, J. (2022). Aggregation of gluten proteins-from wheat seed biology to hydrogels: scientific modelling based primarily on Monte-Carlo and HPLC methods.

Mazzeo, M.F., Di Stasio, L., D'Ambrosio, C., Arena, S., Scaloni, A., Corneti, S., et al. (2017). Identification of early represented gluten proteins during durum wheat grain development. *J. Agric. Food Chem.* 65(15)**,** 3242-3250. doi: 10.1021/acs.jafc.7b00571.

Merchuk-Ovnat, L., Barak, V., Fahima, T., Ordon, F., Lidzbarsky, G.A., Krugman, T., et al. (2016). Ancestral QTL alleles from wild emmer wheat improve drought resistance and productivity in modern wheat cultivars. *Front. Plant Sci.* 7**,** 452. doi: 10.3389/fpls.2016.00452.

Michel, S., Gallee, M., Löschenberger, F., Buerstmayr, H., and Kummer, C. (2017). Improving the baking quality of bread wheat using rapid tests and genomics: The prediction of dough rheological parameters by gluten peak indices and genomic selection models. *Journal of Cereal Science* 77**,** 24-34. doi: 10.1016/j.jcs.2017.07.012.

Michel, S., Kummer, C., Gallee, M., Hellinger, J., Ametz, C., Akgöl, B., et al. (2018). Improving the baking quality of bread wheat by genomic selection in early generations. *Theor Appl Genet* 131(2)**,** 477-493. doi: 10.1007/s00122-017-2998-x.

Mohammadi, R., Etminan, A., and Shoshtari, L.I.A. (2018). Agro-physiological characterization of durum wheat genotypes under drought conditions. *Exp. Agric.* 55(3)**,** 484-499. doi: 10.1017/s0014479718000133.

Moraru, A., Oancea, A., Ghiurea, M., Tritean, N., and Oancea, F. (2022). Closing the Loop in an Efficient Manner—The Feed-Forward Control Solution. *Chem. Proc.* 7(1)**,** 27.

Mulugeta, B., Tesfaye, K., Geleta, M., Johansson, E., Hailesilassie, T., Hammenhag, C., et al. (2022). Multivariate analyses of Ethiopian durum wheat revealed stable and high yielding genotypes. *Plos one* 17(8)**,** e0273008.

Mut, Z., Aydin, N., Bayramoglu, H., and Ozcan, H. (2010). Stability of some quality traits in bread wheat (Triticum aestivum) genotypes. *Journal of environmental biology / Academy of Environmental Biology, India* 31**,** 489-495.

Nezhad, K.Z., Weber, W.E., Röder, M.S., Sharma, S., Lohwasser, U., Meyer, R.C., et al. (2011). QTL analysis for thousand-grain weight under terminal drought stress in bread wheat (Triticum aestivum L.). *Euphytica* 186(1)**,** 127-138. doi: 10.1007/s10681-011-0559-y.

Olckers, S.-L., Osthoff, G., Guzmán, C., Wentzel, B., van Biljon, A., and Labuschagne, M. (2022). Drought and heat stress effects on gluten protein composition and its relation to bread-making quality in wheat. *J. Cereal Sci.* 108. doi: 10.1016/j.jcs.2022.103562.

Ortolan, F., and Steel, C.J. (2017). Protein characteristics that affect the quality of vital wheat gluten to be used in baking: A review. *Compr. Rev. Food Sci. Food Saf.* 16(3)**,** 369-381. doi: 10.1111/1541-4337.12259.

Osman, R., Ata-Ul-Karim, S.T., Tahir, M.N., Ishaque, W., and Xu, M. (2022). Multi-model ensembles for assessing the impact of future climate change on rainfed wheat productivity under various cultivars and nitrogen levels. *Eur. J. Agron.* 139**,** 126554. doi: https://doi.org/10.1016/j.eja.2022.126554.

Pabuayon, I.L.B., Sun, Y., Guo, W., and Ritchie, G.L. (2019). High-throughput phenotyping in cotton: a review. *J. Cotton Res.* 2(1). doi: 10.1186/s42397-019-0035-0.

Panozzo, J., Eagles, H., and Wootton, M. (2001). Changes in protein composition during grain development. *Crop and Pasture Science* 52**,** 485-493. doi: 10.1071/AR00101.

Panozzo, J.F., and Eagles, H.A. (2000). Cultivar and environmental effects on quality characters in wheat. II. Protein. *Australian Journal of Agricultural Research* 51(5)**,** 629-636.

Parenti, O., Guerrini, L., Zanoni, B., Marchini, M., Tuccio, M.G., and Carini, E. (2021). Use of the (1)H NMR technique to describe the kneading step of

wholewheat dough: The effect of kneading time and total water content. *Food Chem.* 338**,** 128120. doi: 10.1016/j.foodchem.2020.128120.

Pequeno, D.N.L., Hernández-Ochoa, I.M., Reynolds, M., Sonder, K., MoleroMilan, A., Robertson, R.D., et al. (2021). Climate impact and adaptation to heat and drought stress of regional and global wheat production. *Environ. Res. Lett.* 16(5). doi: 10.1088/1748-9326/abd970.

Phakela, K., van Biljon, A., Wentzel, B., Guzman, C., and Labuschagne, M. (2021). Gluten protein response to heat and drought stress in durum wheat as measured by reverse phase-high performance liquid chromatography. *J. Cereal Sci.* 100**,** 103267.

Poggi, G.M., Corneti, S., Aloisi, I., and Ventura, F. (2023). Environment-oriented selection criteria to overcome controversies in breeding for drought resistance in wheat. *J. Plant Physiol.* 280**,** 153895. doi: 10.1016/j.jplph.2022.153895.

Porep, J.U., Kammerer, D.R., and Carle, R. (2015). On-line application of near infrared (NIR) spectroscopy in food production. *Trends Food Sci. Technol.* 46(2, Part A)**,** 211-230. doi: https://doi.org/10.1016/j.tifs.2015.10.002.

Pradhan, G.P., Prasad, P.V.V., Fritz, A.K., Kirkham, M.B., and Gill, B.S. (2012). Effects of drought and high temperature stress on synthetic hexaploid wheat. *Funct. Plant Biol.* 39(3)**,** 190-198. doi: 10.1071/FP11245.

Prasad, P.V.V., Pisipati, S.R., Momčilović, I., and Ristic, Z. (2011). Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. *J. Agron. Crop Sci.* 197(6)**,** 430-441. doi: 10.1111/j.1439-037X.2011.00477.x.

Qaseem, M.F., Qureshi, R., and Shaheen, H. (2019). Effects of pre-anthesis drought, heat and their combination on the growth, yield and physiology of diverse wheat (Triticum aestivum L.) genotypes varying in sensitivity to heat and drought stress. *Sci. Rep.* 9(1)**,** 6955. doi: 10.1038/s41598-019-43477-z.

Randall, P., and Moss, H. (1990). Some effects of temperature regime during grain filling on wheat quality. *Australian Journal of Agricultural Research* 41(4)**,** 603. doi: 10.1071/AR9900603.

Rharrabti, Y., Villegas, D., Royo, C., Martos-Núñez, V., and García del Moral, L.F. (2003). Durum wheat quality in Mediterranean environments: II. Influence of climatic variables and relationships between quality parameters. *Field Crops Research* 80(2)**,** 133-140. doi: https://doi.org/10.1016/S0378-4290(02)00177-6.

Ru, C., Hu, X., Chen, D., Wang, W., Zhen, J., and Song, T. (2023). Individual and combined effects of heat and drought and subsequent recovery on winter wheat (Triticum aestivum L.) photosynthesis, nitrogen metabolism, cell osmoregulation, and yield formation. *Plant Physiol. Biochem.* 196**,** 222-235. doi: 10.1016/j.plaphy.2023.01.038.

Salgó, A., and Gergely, S. (Year). "Detection of wheat maturation process by NIR", in: *Wheat in a Global Environment: Proceedings of the 6th International*

Wheat Conference, 5–9 June 2000, Budapest, Hungary: Springer), 297-303.

Sancho, A.I., Gillabert, M., Tapp, H., Shewry, P.R., Skeggs, P.K., and Mills, E.N.C. (2008). Effect of environmental stress during grain filling on the soluble proteome of wheat (Triticum aestivum) dough liquor. *Journal of agricultural and food chemistry* 56(13)**,** 5386. doi: 10.1021/jf800209b.

Sara, D., Mandava, A.K., Kumar, A., Duela, S., and Jude, A. (2021). Hyperspectral and multispectral image fusion techniques for high resolution applications: a review. *Earth Sci. Inform.* 14(4)**,** 1685-1705. doi: 10.1007/s12145-021-00621-6.

Sattar, A., Sher, A., Ijaz, M., Ullah, M.S., Ahmad, N., and Umar, U.U.-D. (2020). Individual and combined effect of terminal drought and heat stress on allometric growth, grain yield and quality of bread wheat. *Pak. J. Bot.* 52(2). doi: 10.30848/pjb2020-2(5).

Schmid, M., Wieser, H., and Koehler, P. (2016). Isolation and Characterization of High-Molecular-Weight (HMW) Gliadins from Wheat Flour. *Cereal Chemistry* 93(6)**,** 536-542. doi: 10.1094/CCHEM-04-16-0078-R.

Schmid, M., Wieser, H., and Koehler, P. (2017). Disulphide structure of high-molecular-weight (HMW-) gliadins as affected by terminators. *Journal of cereal science* 78**,** 66-74. doi: 10.1016/j.jcs.2016.12.007.

Scholz, É., Prieto-Linde, M.L., Gergely, S., Salgó, A., and Johansson, E. (2007). Possibilities of using near infrared reflectance/transmittance spectroscopy for determination of polymeric protein in wheat. *J. Sci. Food Agric.* 87(8)**,** 1523-1532. doi: https://doi.org/10.1002/jsfa.2878.

Shah, N.H., and Paulsen, G.M. (2003). Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. *Plant Soil* 257(1)**,** 219-226. doi: 10.1023/A:1026237816578.

Shew, A.M., Tack, J.B., Nalley, L.L., and Chaminuka, P. (2020). Yield reduction under climate warming varies among wheat cultivars in South Africa. Nat. Commun. 11(1), 4408. doi: 10.1038/s41467-020-18317-8.

Shewry, P., and Tatham, A. (1997). Disulphide bonds in wheat gluten proteins. *J. Cereal Sci.* 25(3)**,** 207-227.

Shewry, P.R., Halford, N.G., and Lafiandra, D. (2003). Genetics of Wheat Gluten Proteins. *Advances in Genetics* 49**,** 111-184. doi: 10.1016/S0065-2660(03)01003-4.

Shewry, P.R., Underwood, C., Wan, Y., Lovegrove, A., Bhandari, D., Toole, G., et al. (2009). Storage product synthesis and accumulation in developing grains of wheat. *Journal of Cereal Science* 50(1)**,** 106-112. doi: https://doi.org/10.1016/j.jcs.2009.03.009.

Shiferaw, B., Smale, M., Braun, H.-J., Duveiller, E., Reynolds, M., and Muricho, G. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Secur.* 5(3)**,** 291-317. doi: 10.1007/s12571-013-0263-y.

Singh, H., and MacRitchie, F. (2001). Application of polymer science to properties of gluten. *J. Cereal Sci.* 33(3)**,** 231-243. doi: https://doi.org/10.1006/jcrs.2000.0360.

Singh, N., Donovan, G., Batey, I., and MacRitchie, F. (1990). Use of sonication and size-exclusion high-performance liquid use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of breadmaking quality. *Cereal Chem.* 67**,** 161-170.

Singh, S., Gupta, A.K., and Kaur, N. (2012). Influence of drought and sowing time on protein composition, antinutrients, and mineral contents of wheat. *Sci. World J.* 2012**,** 485751. doi: 10.1100/2012/485751.

Skerritt, J.H., Hac, L., and Bekes, F. (1999). Depolymerization of the glutenin macropolymer during dough mixing: I. Changes in levels, molecular weight distribution, and overall composition. *Cereal Chemistry* 76(3)**,** 395-401.

Sorvaniemi, J., Kinnunen, A., Tsados, A., and Mälkki, Y. (1993). Using partial least squares regression and multiplicative scatter correction for FT-NIR data evaluation of wheat flours. *LWT - Food Sci. Technol.* 26(3)**,** 251-258.

Statkevičiūtė, G., Liatukas, Ž., Cesevičienė, J., Jaškūnė, K., Armonienė, R., Kuktaite, R., et al. (2022). Impact of combined drought and heat stress and nitrogen on winter wheat productivity and end-use quality. *Agronomy* 12(6)**,** 1452.

Sun, G.-Q., Li, L., Li, J., Liu, C., Wu, Y.-P., Gao, S., et al. (2022). Impacts of climate change on vegetation pattern: Mathematical modeling and data analysis. *Phys. Life Rev.* 43**,** 239-270. doi: https://doi.org/10.1016/j.plrev.2022.09.005.

Svec, I., Hrušková, M., Karas, J., and Hofmanova, T. (2012). Solvent Retention Capacity for Different Wheats and Flours Evaluation. *Czech Journal of Food Sciences* 30**,** 429-437. doi: 10.17221/386/2011-CJFS.

Swedish Agency of Agriculture (2023a). *Jordbruksverkets statistikdatabasen*. https://jordbruksverket.se/om-jordbruksverket/jordbruksverkets-officiella-statistik/statistikdatabasen [accessed on 21 March 2023].

Swedish Agency of Agriculture (2023b). *Jordbruksmarkens användning 2022. Slutlig statistic.* https://jordbruksverket.se/om-jordbruksverket/jordbruksverkets-officiella-statistik/jordbruksverkets-statistikrapporter/statistik/2022-10-20-jordbruksmarkens-anvandning-2022.-slutlig-statistik [accessed on 3 March 2023].

Swedish Agency of Agriculture (2023c). *Skörd av spannmål, trindsäd och oljeväxter 2022, preliminär statistik för riket*. https://jordbruksverket.se/om-jordbruksverket/jordbruksverkets-officiella-statistik/jordbruksverkets-statistikrapporter/statistik/2022-11-15-skord-av-spannmal-trindsad-och-oljevaxter-2022-preliminar-statistik-for-riket [accessed on 11 March 2023].

Swedish Meteorological and Hydrological Institute (2023). *Climate indicator – Temperature.* https://www.smhi.se/en/climate/climate-indicators/climate-indicators-temperature-1.91472 [accessed on 21 March 2023].

Tester, R., Morrison, W., Ellis, R., Piggo, J., Batts, G., Wheeler, T., et al. (1995). Effects of elevated growth temperature and carbon dioxide levels on some physiochemical properties of wheat starch. *Journal of Cereal Science - J CEREAL SCI* 22**,** 63-71. doi: 10.1016/S0733-5210(05)80008-6.

Tilley, K.A., Benjamin, R.E., Bagorogoza, K.E., Okot-Kotber, B.M., Prakash, O., and Kwen, H. (2001). Tyrosine cross-links: molecular basis of gluten structure and function. *J. Agric. Food Chem.* 49(5)**,** 2627-2632.

Tosi, P., Gritsch, C.S., He, J., and Shewry, P.R. (2011). Distribution of gluten proteins in bread wheat (Triticum aestivum) grain. *Ann. Bot,* 108(1)**,** 23-35. doi: 10.1093/aob/mcr098.

Tosi, P., Parker, M., Gritsch, C.S., Carzaniga, R., Martin, B., and Shewry, P.R. (2009). Trafficking of storage proteins in developing grain of wheat. *J. Exp. Bot.* 60(3)**,** 979-991. doi: 10.1093/jxb/ern346.

Uhlen, A.K., Hafskjold, R., Kalhovd, A.H., Sahlström, S., Longva, Å., and Magnus, E.M. (1998). Effects of Cultivar and Temperature During Grain Filling on Wheat Protein Content, Composition, and Dough Mixing Properties. *Cereal Chemistry* 75(4)**,** 460-465. doi: https://doi.org/10.1094/CCHEM.1998.75.4.460.

University of Western Australia (2023). *Climate change impact on wheat grain composition and quality.* https://www.uwa.edu.au/news/Article/2022/November/Climate-change-impact-on-wheat-grain-composition-and-quality [accessed on 2 March 2023].

Urban, O., Hlaváčová, M., Klem, K., Novotná, K., Rapantová, B., Smutná, P., et al. (2018). Combined effects of drought and high temperature on photosynthetic characteristics in four winter wheat genotypes. *Field Crops Res.* 223**,** 137-149. doi: 10.1016/j.fcr.2018.02.029.

Vensel, W.H., Tanaka, C.K., and Altenbach, S.B. (2014). Protein composition of wheat gluten polymer fractions determined by quantitative two-dimensional gel electrophoresis and tandem mass spectrometry. *Proteome Sci.* 12(1)**,** 8. doi: 10.1186/1477-5956-12-8.

Walker, C., Garland-Campbell, K., Carter, B., and Kidwell, K. (2008). Using the Solvent Retention Capacity Test When Breeding Wheat for Diverse Production Environments. *Crop Science* 48. doi: 10.2135/cropsci2007.06.0348.

Wan, C., Dang, P., Gao, L., Wang, J., Tao, J., Qin, X., et al. (2022). How does the environment affect wheat yield and protein content response to drought? A meta-analysis. *Front. Plant Sci.* 13**,** 896985. doi: 10.3389/fpls.2022.896985.

Wan, Y., Gritsch, C., Hawkesford, M., and Shewry, P. (2013). Effects of nitrogen nutrition on the synthesis and deposition of the -gliadins of wheat. *Annals of botany* 113. doi: 10.1093/aob/mct291.

Wang, C., and Kovacs, M. (2002). Swelling Index of Glutenin Test. I. Method and Comparison with Sedimentation, Gel-Protein, and Insoluble Glutenin Tests. *Cereal Chemistry* 79. doi: 10.1094/CCHEM.2002.79.2.183.

Webber, H., Ewert, F., Olesen, J.E., Muller, C., Fronzek, S., Ruane, A.C., et al. (2018). Diverging importance of drought stress for maize and winter wheat in Europe. *Nat. Commun.* 9(1)**,** 4249. doi: 10.1038/s41467-018-06525-2.

Wieser, H. (2007). Chemistry of gluten proteins. Food Microbiology 24(2), 115-119. doi: 10.1016/j.fm.2006.07.004.

Wieser, H., Koehler, P., and Scherf, K.A. (2023). Chemistry of wheat gluten proteins: Qualitative composition. Cereal Chemistry 100(1), 23-35. doi: https://doi.org/10.1002/cche.10572.

Woychik, J.H., Boundy, J.A., and Dimler, R.J. (1961). Starch gel electrophoresis of wheat gluten proteins with concentrated urea. Archives of Biochemistry and Biophysics 94(3), 477-482. doi: https://doi.org/10.1016/0003-9861(61)90075-3.

Xiao, D., Bai, H., and Liu, D. (2018). Impact of future climate change on wheat production: A simulated case for china's wheat system. Sustainability 10(4), 1277. doi: 10.3390/su10041277.

Xiao, Z.S., Park, S.H., Chung, O.K., Caley, M.S., and Seib, P.A. (2006). Solvent Retention Capacity Values in Relation to Hard Winter Wheat and Flour Properties and Straight-Dough Breadmaking Quality. Cereal Chemistry 83(5), 465-471. doi: 10.1094/CC-83-0465.

Xie, W., Huang, J., Wang, J., Cui, Q., Robertson, R., and Chen, K. (2020). Climate change impacts on China's agriculture: The responses from market and trade. China Econ. Rev. 62. doi: 10.1016/j.chieco.2018.11.007.

Yang, F., Jørgensen, A.D., Li, H., Søndergaard, I., Finnie, C., Svensson, B., et al. (2011). Implications of high-temperature events and water deficits on protein profiles in wheat (Triticum aestivum L. cv. Vinjett) grain. PROTEOMICS 11(9), 1684-1695. doi: 10.1002/pmic.201000654.

Zahra, N., Hafeez, M.B., Wahid, A., Al Masruri, M.H., Ullah, A., Siddique, K.H.M., et al. (2022). Impact of climate change on wheat grain composition and quality. J. Sci. Food Agric. doi: 10.1002/jsfa.12289.

Zhang, T., He, Y., DePauw, R., Jin, Z., Garvin, D., Yue, X., et al. (2022a). Climate change may outpace current wheat breeding yield improvements in North America. Nat. Commun. 13(1), 5591. doi: 10.1038/s41467-022-33265-1.

Zhang, S., Liu, S., Shen, L., Chen, S., He, L., and Liu, A. (2022b). Application of near-infrared spectroscopy for the nondestructive analysis of wheat flour: A review. Curr. Res. Food Sci. 5, 1305-1312. doi: https://doi.org/10.1016/j.crfs.2022.08.006.

Zhao, C.-X., He, M.-R., Wang, Z.-L., Wang, Y.-F., and Lin, Q. (2009). Effects of different water availability at post-anthesis stage on grain nutrition and

quality in strong-gluten winter wheat. C. R. Biol. 332(8), 759-764. doi: https://doi.org/10.1016/j.crvi.2009.03.003.

Zhao, K., Tao, Y., Liu, M., Yang, D., Zhu, M., Ding, J., et al. (2022). Does temporary heat stress or low temperature stress similarly affect yield, starch, and protein of winter wheat grain during grain filling? J. Cereal Sci. 103. doi: 10.1016/j.jcs.2021.103408.

Zhou, Y., Lao, C., Yang, Y., Zhang, Z., Chen, H., Chen, Y., et al. (2021). Diagnosis of winter-wheat water stress based on UAV-borne multispectral image texture and vegetation indices. Agric. Water Manag. 256. doi: 10.1016/j.agwat.2021.107076.

Popular science summary

Wheat is a staple food for 2.5 billion people in 89 countries around the world. In Sweden, food made from wheat is a part of our daily diet. A day without food from wheat is impossible to imagine. Recent extreme weather events, such as the severe heat and drought in 2018, have affected wheat yield and protein quality negatively in Sweden and worldwide. The future climate will be more extreme and will affect wheat yield and quality severely. To keep a continuing supply of wheat, we need wheat varieties that are able to provide good yields and quality in varying and extreme growing conditions. To develop new wheat varieties, we need to understand how climate change affects wheat yield and quality. We also have a substantial need for rapid and cost-effective wheat yield and quality screening methods to develop new wheat varieties.

We studied the yield, flour, dough mixing and bread-making quality of spring wheat grown in different stress environments, both in the field and in the greenhouse. We used different techniques, such as RGB imaging, to analyze plant biomass and evaluate yield. To analyze protein quality, we used size exclusion high performance liquid chromatography (SE-HPLC), mass spectroscopy (LC-MS/MS), near infrared spectroscopy, swelling index of glutenins (SIG) and solvent retention capacity (SRC). We used a mixograph to study dough mixing quality. Additionally, we baked the bread and measured bread volumes from wheat grown in different environments.

We found a lower yield and biomass in the wheat grown under severe heat and drought. We also found that severe heat and drought increased the gluten strength but reduced the protein concentration in the flour made from wheat grown in field. For the wheat grown in the greenhouse, we found higher gluten strength and protein concentration in wheat grown under the heat and drought conditions. The optimum time for dough mixing was one of the traits that was not affected by the varying climatic conditions. We recommend using dough mixing time, together with gluten strength and

protein concentration, to help with the selection of varities in the breeding projects.
SE-HPLC, SIG and SRC tests used in our study required very small amounts of flour (16 mg to 1 g); in comparison to the industrial flour and dough mixing tests, such as farinograph and extensograph tests, which require a large amount of flour. Thus, SE-HPLC, SIG and SRC could be used as alternative tests to the farinograph and extensograph tests for wheat quality evaluation by both breeding and milling companies. The phenotyping results indicated that RGB cameras and image analysis could be useful tools to evaluate the yield resistance of the wheat genotypes.

In the thesis, the new information we have provided on climate change effects on wheat, as well as the information on the combination of different techniques for measuring yield and quality of wheat, could help breeders to develop new cultivars and millers to evaluate wheat quality at a reduced financial and time cost.

Populärvetenskaplig sammanfattning

Vete är ett av de viktigaste livsmedlen för 2,5 miljarder människor i 89 länder runt om i världen. I Sverige är veteprodukter en del av vår dagliga kost och en dag utan vete är nästintill omöjlig att föreställa sig för de allra flesta. Den senaste tidens extrema väderhändelser, som den svåra värmen och torkan 2018, har påverkat veteskörden och proteinkvaliteten negativt i Sverige och världen över. Det framtida klimatet förväntas bli än mer extremt vilket kommer att påverka både skörden av och kvaliteten på vete allvarligt. För att säkerställa en fortsatt hög tillgång på vete behöver vi vetesorter som kan ge goda skördar med hög kvalitet även under varierande och extrema odlingsförhållanden. För att kunna utveckla nya vetesorter krävs en förståelse för hur klimatförändringarna påverkar dessa egenskaper. Vi har också ett stort behov av snabba och kostnadseffektiva sätt att utvärdera och screena för avkastning och kvalitet.

I denna studie studerade vi avkastning, mjöl-egenskaper, degblandningsförmåga och brödkvalitet hos vårvete som odlats i olika klimat, både på fält och i växthus. Vi använde olika tekniker, såsom RGB bilder, för att analysera växtbiomassan och utvärdera avkastningen. För att analysera proteinkvalitet använde vi kromatografi (size exclusion-high performance liquid chromatography, SE-HPLC), mass spectrometri (LC-MS/MS), infraröd spectroskopi (NIR), ”swelling index of glutenin” (SIG) och ”solvent retention capacity” (SRC). Vi använde en mixograf för att studera degens blandningskvalitet. Dessutom bakade vi bröd på vetet som odlats i olika miljöer och mätte brödets volym.

Vi upptäckte lägre skörd och biomassa i vetet som odlats under extremvärme och torka. Svår värme och torka ökade dessutom glutenhalten men minskade proteinkoncentrationen i mjölet som kom från vetet som odlats på fält. Vetet som odlats i växthus visade däremot både högre glutenhalt och ökad proteinkoncentration i när det utsatts för värme och torka. Den optimala tiden för degblandning var en av de egenskaper som inte påverkades av de

varierande klimatförhållandena. Vi rekommenderar att använda degblandningstid, tillsammans med glutenhalt och proteinkoncentration vid urval av lämpliga vetesorter för förädlingsprojekten.
En tydlig fördel med de metoder som användes i vår studie (SE-HPLC, SIG och SRC tester) är att de endast kräver mycket små mängder mjöl (16 mg-1 g) i jämförelse med de industriella mjöl- och degblandningstesterna, såsom farinograf- och extensograftester, som kräver en betydligt större mängd mjöl. Därför skulle SE-HPLC, SIG och SRC kunna användas som alternativa tester till farinograf- och extensograftesterna för utvärdering av vetekvalitet av både förädlings- och kvarnföretag. Fenotypningsresultaten indikerade att RGB-kameror och bildanalys kan vara användbara verktyg för att utvärdera avkastning hos vete.

I avhandlingen kan den nya informationen vi har tillhandahållit om klimatförändringens effekter på vete och informationen om kombinationen av olika tekniker för att mäta avkastning och kvalitet på vete, hjälpa förädlare att utveckla nya sorter och kvarnmästare att utvärdera vetets kvalitet till en minskad ekonomisk och tidsmässig kostnad.

Acknowledgements

My PhD journey has been exciting, fun, intense and full of challenges, but also a journey of self-doubt. However, I never doubted the support system I have around me which kept me going on in this PhD. A special thanks to this support system, I would not be able to reach this far without your help.

Ramune Kuktaite, I truly admire your confidence, energy and attitude of not compromising with the work and for never giving up. Thank you for your knowledge, experience, uncountable revisions of the papers and for tirelessly thriving to achieve the best in this project.

Pernilla Vallenback, no words are enough to express how grateful I am to have you as my supervisor. You have been kind, caring, truly compassionate, supportive and always stood up for me. I cannot thank you enough for patiently listening to all my struggles and giving me courage and hope when everything felt meaningless.

Aakash Chawade, you have always made time to listen and discuss things with me. Thank you for maintaining harmony in my education, your encouragement to work hard and stay focused and witty ideas to resolve problems.

Marina Kuzmenkova, thank you for unofficially being my fourth supervisor. You are one of the funniest, smartest, direct yet kind and most efficient problem-solver I have ever met. Working hand in hand with you was the best time of my PhD.

Anders Ekholm and Maria Luisa Prieto-Linde, thank you for being available whenever I needed you, for all the support in the lab and for teaching me every bit of HPLC with patience.

Eva Johansson, thank you for sharing your unpublished work with me at the beginning of my PhD, which remained a guidepost throughout my PhD. Your leadership has always been a true inspiration.

Adam Flöhr and Jan-Erik Englund, thank you for the uncountable hours you spend helping me with the data analysis. Without your statistical support, this PhD would not be possible.

Helene Larsson Jönsson, thank you for being a PhD/life savior. You always cared and made time to listen. Taking with you helped me to reflect, make decisions and continue with my education.

Bill Newson, Faraz Muneer and Elaine Ceresino thank you for your support, encouragement and many wonderful discussions.

Malin Olsson, Camilla Stjärnäng and Mirela Beganovic, thank you for helping me with the finance and practical issues, and most importantly, speaking Swedish with me!

Twan America, it was a pleasure to get an opportunity to work with you. Thank you for teaching me LC-MS/MS in detail.

Jörgen Hansson, Rikard Westbom and Linda Cervera, thank you very much for all the tools and technical assistants with the experiments and baking. Baking fresh breads was really fun!

My master supervisor, Yousuf Akhond thank you so much for introducing me to the exciting world of gluten. My co-supervisor, Ashrafi Hossain for inspiring me to do good research and pursue a PhD.

Anna-lovisa Nynäs, Vera Sadovska, Hanna Ohm, Murilo Sandroni, Annika Hinze, Antonio Capezza, Alexander Koc, Awais Zahid, Catja Selga, Emilia Berndtsson, Evelyn Gutierrez, Fernanda Leiva, Joel Markgren, Johanna Osterman, Jonas Skytte af Sätra, Lan Yuzhou, Mahboobeh Yazdani, Mustafa Zakieh, Noelia López-García and Sjur Sandgrind, thank you for all

the coffees, chats, parties, hikes foods and drinks. Knowing you and being friends with you were the best things that happened to me during this PhD.

PhD student council was an integral part of my PhD journey. Thanks to everyone, I came across through the council for making our work fun, interesting and meaningful.

Blenda Agell, Emelie Sundin, Leif Magnusson and Maria Loxbo, I cannot thank you enough for giving me a sense of belonging and for making me feel like home in Sweden.

My friends, Israt Jahan and Fahmidul Haque and beautiful Ayzal Fahmid, thank you for your love, care, support and lots of delicious food. My best friends, Afrin Faria, Afroza Hossain, Farzana Aktar, Momotaj Begum, Salma Aktar, Toufia Jannat, thank you for your friendship, encouragement, support and company through all the ups and downs in life.

Many thanks to my mother Yankey Lama, brothers Dhon Raj and Debendra, sister Anne, brother-in-law Nick and my amazing niece Lyra for your unconditional love and for always being there for me.

I

Check for updates

Received: 6 July 2021 | Revised: 2 January 2022 | Accepted: 1 February 2022

DOI: 10.1002/fes3.376

Food and Energy Security Open Access WILEY

ORIGINAL ARTICLE

Prolonged heat and drought versus cool climate on the Swedish spring wheat breeding lines: Impact on the gluten protein quality and grain microstructure

Sbatie Lama[1] | **Pernilla Vallenback**[2] | **Stephen A. Hall**[3,4] | **Marina Kuzmenkova**[1] | **Ramune Kuktaite**[1]

[1]Department of Plant Breeding, Swedish University of Agricultural Sciences, Lomma, Sweden

[2]Lantmännen Lantbruk, Svalöv, Sweden

[3]Division of Solid Mechanics, Lund University, Lund, Sweden

[4]Lund Institute of advanced Neutron and X-ray Science (LINXS), Lund, Sweden

Correspondence
Sbatie Lama, Department of Plant Breeding, Swedish University of Agricultural Sciences, SE-23422, Lomma, Sweden
Email: sbatie.lama@slu.se

Funding information
SLU Partnerskap Alnarp; SLU Grogrund-Centre for Breeding of Food Crops

Abstract

Fluctuating climate, heat, and drought are expected to considerably impact bread wheat (*Triticum aestivum*) quality in the coming years and, as wheat is an essential food element worldwide, this will have significant implications for future food security and the global economy. This leads to an urgent need for developing wheat varieties with stable yield and gluten quality. In this study, we investigated the effect of heat and drought, compared to a cool climate, on gluten proteins in 294 spring wheat genotypes grown in 2017 and 2018 in Sweden. Gluten protein parameters were studied by size exclusion high-performance liquid chromatography (SE-HPLC) and grain morphology by X-ray tomography. The prolonged heat and drought led to: (i) increased gluten polymerization and the formation of large polymers, as defined by the percentage of unextractable polymers in total polymers (%UPP) and the percentage of large unextractable polymers in total large polymers (%LUPP); and (ii) increase in large monomers, as defined by the percentage of large unextractable monomers in the total large monomers (%LUMP) and the ratio of monomers versus polymers (Mon/Pol) in the flour. The cooler climate also led to an increase in total protein concentration and accumulation of the monomeric proteins and total SDS-extractable proteins (TOTE). No difference in the total amount of SDS-unextractable proteins (TOTU) was found between the studied climates. Due to the heat and drought stress, the grain yield decreased in most of the genotypes, while the grain microstructure varied only to a minor extent. The wheat genotypes identified in the study that provide good yields and stable gluten properties in both prolonged heat–drought and cool environments are strong candidates to contribute to a secure, self-sufficient future wheat supply in the face of an evolving climate in Sweden and in similar climates worldwide.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2022 The Authors. *Food and Energy Security* published by John Wiley & Sons Ltd.

KEYWORDS
bread wheat, breeding for wheat self-sufficiency, gluten quality, high temperature, polymeric and monomeric protein, SE-HPLC

1 | INTRODUCTION

Evolution of climates across the world during recent years has greatly influenced bread wheat (*Triticum aestivum*) production with negative impacts on food security (Hashiguchi et al., 2010; Ray et al., 2019). Several climate parameters, such as rising temperatures and long periods of drought, were found to be among the most severe factors affecting the yield and quality of bread wheat (Magallanes-López et al., 2017; Pennacchi et al., 2018; Xiao et al., 2018; Yu et al., 2014). One such example is the climate in 2018 with high temperatures and severe drought, which caused the major losses in wheat yields, amounting to around 40 million tons (mln.t.) compared to the previous year (FAO), and reduction in wheat bread quality.

The influence of excessive heat and drought on the protein concentration and gluten protein composition in wheat flour is rather sparsely studied (Asseng et al., 2019), although a few observations have been made. For example, Qaseem et al. (2019) observed that the protein concentration of wheat grown under either drought or high heat conditions was reduced by 18% and 15%, respectively, while under the combination of these two conditions (heat and drought) a decrease of 50% in protein concentration was noted.

Climate change is expected to affect wheat production very differently across Europe (Cammalleri et al., 2020). In the northern areas, such as Sweden, higher temperatures during the grain-filling phase may primarily induce positive effects, for example, an increase in protein concentration (Vollmer & Musshoff, 2018). However, Sweden's wheat production in 2018 was nearly 50% lower compared to previous 5 years due to drought and heat (1.6 vs. 2.9 mln.t.; Jordbruksverket, 2019). The loss in yield and quality of wheat resulted in a relatively large import of wheat to Sweden (FAO, 2020). This brings the question of how wheat, gluten protein, and bread-making quality are affected by drought and heat, and, consequently, how to ensure food security in the face of a changing northern climate.

From previous studies, it has been observed that the amount and molecular size distribution of gluten proteins, monomeric gliadins, and polymeric glutenins are strongly influenced by the genotype (G) and growing environment (E), for example, temperature and drought, as well as by their interaction G × E (Guzmán et al., 2016; Hernandez-Espinosa et al., 2018; Johansson et al., 2020; Malik et al., 2013a). Studies on varying climate effects on bread-making and gluten quality of Swedish spring wheat varieties grown in Sweden during 1975–1996 (Johansson et al., 2002; Johansson & Svensson, 1998) have been performed. The study by Johansson et al. (2002) focused on heat and drought conditions, although the climate characteristics were not as excessive as the climate of 2018, for example, an unusually high and prolonged heat plus drought period in Sweden.

Fluctuating temperatures during the wheat grain filling stages are known to specifically impact the gliadins and glutenins in wheat grain (Altenbach et al., 2012; Dupont & Altenbach, 2003; Johansson et al., 2008). For example, the increase in both day and night temperatures by 5-7°C was seen to increase the amount of the large polymeric fraction (%UPP), representing gluten strength, in greenhouse studies (Johansson et al., 2005; Malik et al., 2011). Furthermore, day/night temperatures of 24/22°C, together with drought, increased the formation of gluten protein polymers in several studies (Malik et al., 2013a; Labuschagne et al., 2016; Li et al., 2013). Relatively high growing temperatures during wheat grain filling, for example, 30–35°C, has been seen to increase the formation of large glutenin polymers and %UPP (Balla et al., 2011; Flagella et al., 2010; Zhang et al., 2013). However, a few studies have found that higher than 30°C for ≥3 days, together with drought, decreased both the amount of large glutenin polymers (Balla et al., 2011; Dai et al., 2016; Rakszegi et al., 2019) and wheat dough strength (Li et al., 2013; Randall & Moss, 1990), indicating sensitivity of wheat gluten protein to temperature and drought stresses.

Wheat quality is defined differently by farmers, millers, and bakers. While grain yield and morphology are important for farmers and millers, respectively, gluten strength is primarily important for bakers (Blake et al., 2018; Guzmán et al., 2016). Fluctuation in quality of wheat grain affects export and import of wheat grains, the economy of farmers, millers, and bakers, and the overall supply of wheat-based food products. Because of increased consumption of bread wheat globally and bread wheat being a stable food in many countries, a lack of stability in the supply of wheat has a significant influence on market prices and food security (Battenfield et al., 2016; Enghiad et al., 2017). Therefore, to better manage food security in the near future, a critical question that remains to be answered is how to maintain the quality stability of bread wheat in

a changing and fluctuating climate. Breeding for stable quality wheat and selection of climate stable, good-quality genotypes for a wide range of environments, including heat and drought, is one of the key strategies to tackle climate variation and ensure food security (Bornhofen et al., 2017; FAO, 2009; Kiszonas & Morris, 2018; Lenaerts et al., 2019; Tremmel-Bede et al., 2020). This is of high relevance for Sweden in its aim to increase self-sufficiency in wheat production in the nearest future. Consequently, greater knowledge is needed for a better understanding of the combined effects of heat and drought on wheat gluten protein characteristics and their stability to ensure wheat supplies suitable for bread making in Sweden, as well as elsewhere in the world.

To the best of our knowledge, the present study is the first detailed investigation on how excessive heat and drought, versus a cool climate, impact on wheat gluten protein characteristics and is based on a large collection of 294 spring wheat genotypes grown in the field during 2017 and 2018 in southern Sweden. The aim was to identify the effects of prolonged heat and drought, versus a cool climate, on the gluten protein parameters evaluated by SE-HPLC in the spring wheat breeding lines grown in Sweden. In addition, we also studied heat and drought impact on the structural morphology of wheat grains. Thus, the obtained new knowledge on gluten protein parameters for heat and drought tolerance can sustain wheat breeding in a changing climate, and positively contribute to self-sufficiency in wheat production in Sweden.

2 | MATERIALS AND METHODS

2.1 | Plant material

A collection of 294 Swedish spring wheat genotypes consisting of 9 spring wheat varieties, Diskett, Sonett, Flippen, Happy, Quarna, Rogue, Bumble, Caress, and Levels, plus 285 spring wheat breeding lines were included in this study. The genetic composition of some of the studied wheat genotypes, which consisted of subunits such as Ax1, Ax2*, Dx5 + Dy10, and Dx2 + Dy12, are included in Supporting information (Table S1).

The wheat genotypes were grown by Lantmännen Lantbruk in the field trials in 2017 and 2018 (55°55′N and 13°07′E) in Svalöv, Sweden. The amount of applied nitrogen fertilizer (190 kg/ha) was the same for both years for all the genotypes. The growing period, from the sowing date to harvest date, for the 2017 harvest was 157 days (from 22nd April to 25th September) and for the 2018 harvest was 113 days (from 20th April to 10th August). The lowest temperature, average temperature, highest temperature, and precipitation data for the growing period of the material were extracted from the weather station located in Svalöv Sweden (http://www.ffe.slu.se/lm/LMHome.cfm?LMSUB=1).

2.2 | Sample preparation and protein extraction by SE-HPLC

To compare the gluten protein extractability and polymerization among the different genotypes grown in 2017 and 2018, the samples were analyzed by SE-HPLC according to Ceresino et al. (2020) with some modifications. Dry mature wheat grains from 294 genotypes were ground into flour at 6000 rpm using an Ultra Centrifugal Mill ZM 200 (Retsch) and were freeze-dried (Cool safe Pro, LaboGene). Gluten proteins for SE-HPLC analysis were extracted from freeze-dried flour in two extraction steps, according to the procedure of Gupta et al. (1993) with some modifications following Ceresino et al. (2020). In the first extraction step (first extraction), 16.5 mg of wheat flour was mixed with 1.4 ml extraction buffer (0.05 M NaH_2PO_4 and 0.5% SDS, pH 6.9). The samples were vortexed for 10 seconds with the extraction buffer and centrifuged for 30 min. at 10,000 rpm (Sorvall Legend Micro 17; Thermo Fisher). The supernatants were collected for SE-HPLC analysis. In the second extraction step (second extraction), 1.4 ml extraction buffer was added to the pellet of step 1 and sonicated for 45 s using an ultrasonic disintegrator (Soniprep 150; Sanyo). Samples were centrifuged for 30 min at 96,000 *g* and supernatants were collected for SE-HPLC analysis. The gluten proteins extracted from the first extraction and the second extraction steps were designated SDS-extractable and SDS-unextractable proteins, respectively.

For the SE-HPLC analysis, triplicate samples from each genotype were analyzed and 20 µl of extracted supernatant was injected on a BIOSEP SEC-4000 Phenomenex column and separated for 30 min. Mobile phase solution of 50% acetonitrile with 0.1% trifluroacetic acid (TFA) was used as the eluent. Absorption at 210 nm was used to detect the gluten proteins. The obtained chromatograms for the SDS-extractable and the SDS-unextractable protein fractions were divided into four areas based on retention times. For the SDS-extractable proteins, area 1—indicating large polymeric proteins (LPP), area 2—small polymeric proteins (SPP), area 3—large monomeric proteins (LMP), and area 4—small monomeric proteins (SMP) are shown in Figure 4. Similarly, the SDS-unextractable protein fractions extracted using the same buffer and sonication were designated as following, area 1—LPP sonicated (LPPs), area 2—SPPs, area 3—LMPs, and area 4—SMPs (Figure 4). Retention times for both the SDS-extractable and SDS-unextractable protein for areas 1, 2, 3, and 4 were

8.5–12.0 min, 12.0–14.0 min, 14.0–17.5 min, and 17.5–21.5 min, respectively.

The gluten protein parameters such as TOTE, TOTU, %UPP, %LUPP, %LUMP, and Mon/pol were calculated according to Malik et al. 2013b.

2.3 | Protein concentration

Grain protein concentration (GP%) of 294 genotypes grown in 2017 was determined using near-infrared reflectance (NIR) spectroscopy (Inframatic 9500 NIR Grain Analyser, PerkinElmer, USA), and GP% of 282 genotypes grown in 2018 was determined using near-infrared transmission (NIT) spectroscopy (Infratec 1241 NIT Grain Analyser, Foss analytical, Denmark) at Lantmännen Lantbruk, Svalöv, Sweden. Protein concentration in duplicate is provided in Supporting Information (Table S1). Flour protein concentration (FP%) of 109 genotypes from both 2017 and 2018 was determined by NIT in triplicate is this study.

2.4 | X-ray tomography of wheat grain

The grains of four wheat breeding lines 12, 25, 59, and 156, which varied in %UPP between the studied years, were selected for microstructural study by X-ray tomography. The cross-section of inner structure of the grain was compared between the genotypes and the studied years. The acquisition of 3D volume images was conducted on the whole wheat grains placed in a sample holder (a plastic straw), and the imaging was performed using a Zeiss XRadia XRM520 at the 4D Imaging Lab, Lund University, Sweden. The X-ray source voltage and power used were 60 kV and 5 W, respectively, and the manufacture-supplied Le1 source filter was applied. A total of 1601 radiographic projects were acquired over 360° sample rotation with an exposure time of 1 s for each and using an optical magnification of 4x. Tomographic reconstruction was performed with the Zeiss remonstrator software to produce 3D image volumes with cubic voxels with a width of 4 μm. Images acquired in two batches were merged vertically to cover the full highest of each sample. From the images obtained, 100 images were selected for evaluation of the cross-sectional area in the middle of the grain (excluding the aleurone layer). The images were analyzed using Fiji/ImageJ and the height of the grain (the number of 2D slices for each grain) was counted and converted into μm; 2D slices were taken at a 4 μm interval. Data processing and graphs were prepared using Origin Pro 2017 and Excel 2016.

2.5 | Statistical analysis

The statistical analysis was performed using the software R (https://www.r-project.org/) for evaluating the impact of genotype (G), year (Y), and G x Y on the studied protein parameters LPP, SPP, LMP, SMP, LPPs, SPPs, LMPs, SMPs, TOTE, TOTU, %UPP, %LUPP, %LUMP, and Mon/Pol calculated from SE-HPLC analyses. Two-way analysis of variance (ANOVA) was conducted in order to determine the percentage of variation in the protein parameters from SE-HPLC raised by different factors G, Y, and G x Y. For determination of differences in the protein parameters between years (2017 and 2018), Tukey's post hoc test and a principle component analysis (PCA) were performed. Spearman's rank correlation test was applied on all protein parameters from SE-HPLC and FP% of 294 genotypes grown in 2017 and 2018.

3 | RESULTS AND DISCUSSION

3.1 | Climate characteristics

The wheat genotypes from this study were grown in two very different climatic conditions comparing temperature and precipitation data from 2017 and 2018 in relation to the average temperatures for 2007–2020 period (Figure 1). The average temperature for 2018 season was higher throughout the whole wheat growing period (red part of the graph) when compared to the average temperature for 2007–2020 period. When comparing the average temperature of the 2017 and 2018 seasons, it was found to be 6°C higher (April) and continued up to 11°C higher (August) in 2018 (Figure 1a). The highest (max) and the lowest (min) temperatures observed for 2018 were also higher compared to the average temperatures for 2007–2020 period (Figure 1b,c). The heat waves observed lasted longer compared to the average temperatures, making 2018 season exceptional for Sweden. The greatest fluctuations of

FIGURE 1 Temperatures for 2017 and 2018 during the growing period of the spring wheat genotypes compared with the average temperatures from the 2007–2020 period: (a) average temperature; (b) highest (max) temperature; (c) lowest (min) temperature; red color indicates higher than the 2007–2020 average, and blue color indicates lower than the average temperatures compared to average temperatures of 2007–2020 period; and (d) precipitation (mm) during vegetative and grain filling stages. Data collected from the weather station in Svalöv, Sweden

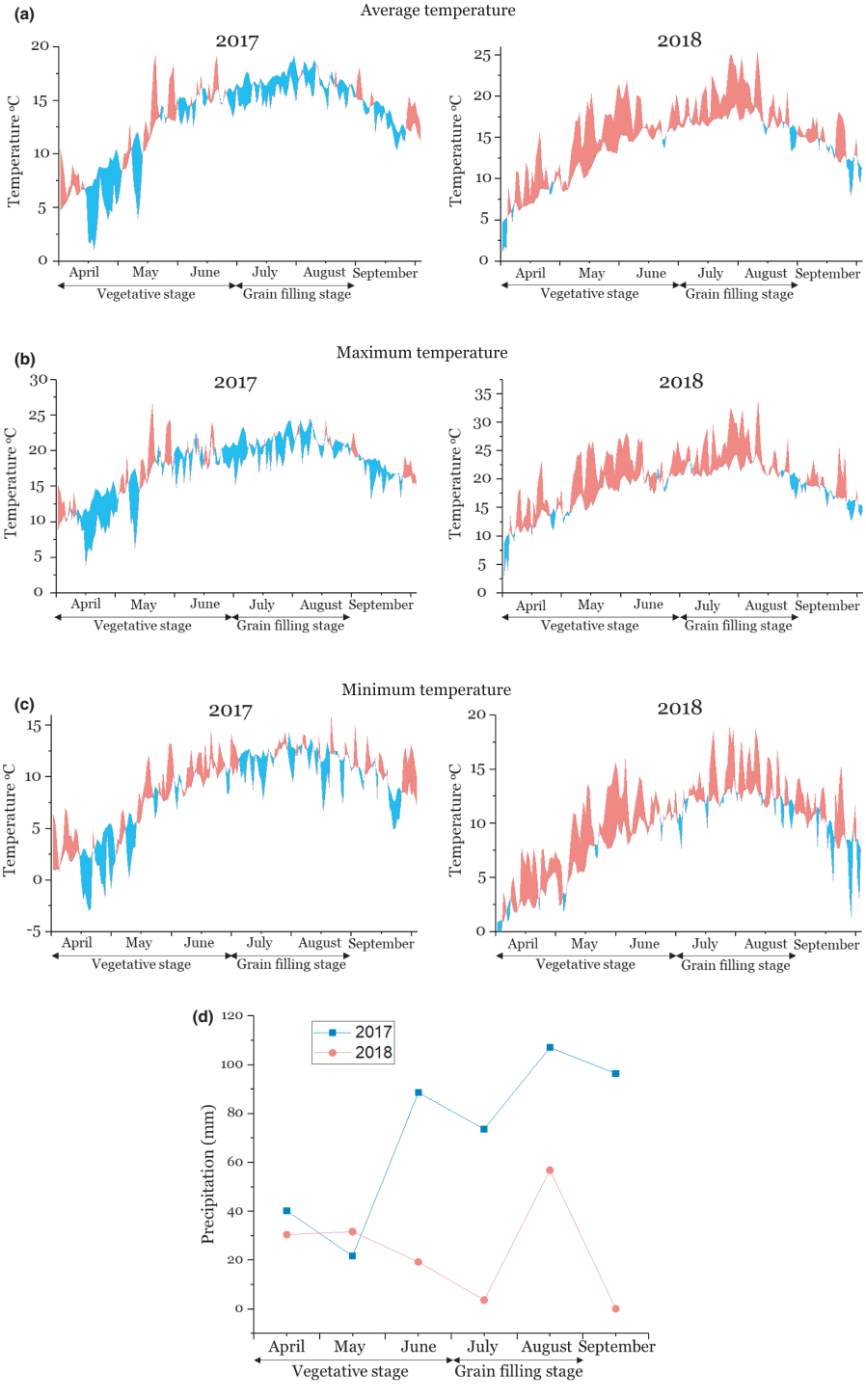

(a)
Average temperature
2017
2018
Temperature °C
April
May
June
July
August
September
Vegetative stage
Grain filling stage
(b)
Maximum temperature
(c)
Minimum temperature
(d)
Precipitation (mm)
2017
2018

TABLE 1 Percentage of variation in the different protein parameters explained by genotype (G), year (Y), and their interaction G × Y

	LPP	SPP	LMP	SMP	LPPs	SPPs	LMPs	SMPs	TOTE	TOTU	%UPP	%LUPP	%LUMP	Mon/Pol
G	46.5	45.8	43.0	31.9	31.3	59.6	58.4	33.6	38.5	64.2	55.1	57.2	43.3	41.8
Y	8.4	20.3	25.8	15.0	35.7	2.7	7.2	24.7	24.9	0.5	16.42	14.1	0.8	4.42
G × Y	41.0	25.4	19.3	46.3	29.8	25.6	23.7	35.0	23.9	20.5	20.1	22.6	28.4	50.8
Residuals	4.1	8.6	11.9	6.7	3.1	12.1	10.7	6.7	12.7	14.7	8.4	6.2	27.6	3.1

Note: Sum of squares was obtained from two-way ANOVA analysis.

high temperature were observed in April, June–August, and were nearly 10°C higher for 2018 compared to 2017, reaching 32–34°C (Figure 1b). The average temperatures in 2018 were also higher compared to the temperatures of the wheat growing seasons in 1994 and 1995 in Sweden (Johansson et al., 2002).

From the precipitation data, roughly four times higher precipitation was experienced for June and seven times higher for July in 2017 compared to 2018 (Figure 1b). Also, in July 2018, precipitation was close to zero. High temperatures and drought in 2018 resulted in a growing period that was 44 days shorter compared to 2017. In this study, we considered the wheat material from 2018 as the prolonged heat and drought representative material, while the material from 2017 as material grown in a cool climate. To conclude, unusually higher temperatures for Sweden and a much longer duration of heat and drought period was observed for the wheat growing period in 2018, compared to the previous climatic conditions, such as the rather hot and dry season in 1994 (Johansson et al., 2002).

3.2 | Variation in the gluten protein parameters and the impact of varying climate

From the studied factors, such as genotype (G) and year (Y), plus their G x Y interaction (here, Y is referred to environment, E), the genotype explained around 60% of the variation in large polymeric proteins (TOTU, %UPP, and %LUPP) and small polymeric proteins (SPPs), and large monomeric proteins (LMPs), while more than 40% of the variation was explained in LPP, SPP, LMP, %LUMP, and Mon/Pol (Table 1; Figure 4). The interaction of genotype and year explained more than 50% of variation in Mon/Pol and 40% of the variation in LPP and SMP (Figure 4). While the varying climate (year) alone contributed to 35% of the variation in LPPs (Table 1; Figure 4).

A principal component analysis was performed to evaluate the climate impact on the gluten protein parameters (Figure 2) and showed that the first principal component (PC1) explained 41.7%, while the second principal component (PC2) explained 27% of the variation (Figure 2). The results clearly indicated that the prolonged heat and drought had a positive effect on the gluten protein parameters related to the large gluten polymers, LPP, LPPs, %LUPP, and %UPP, while the cooler climate contributed positively to TOTE, LMP, SMP, SMPs, and LMPs (Figure 2). Thus, the results clearly indicate that the prolonged heat and drought climate induced the formation of greater amounts of large polymeric gluten proteins. However, the cool growing period in 2017 positively affected the protein concentration (e.g., TOTE) and the amount of monomeric

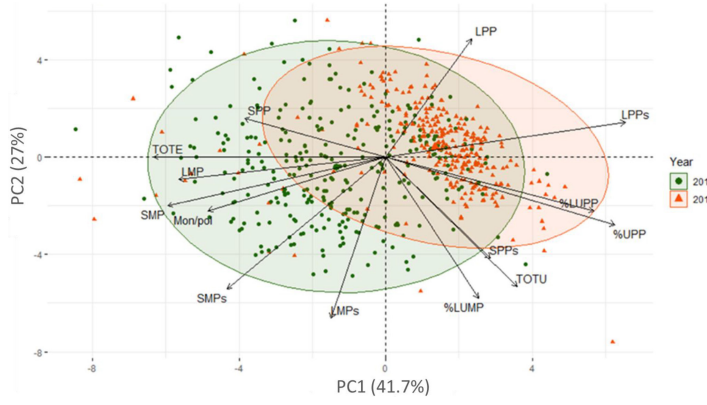


FIGURE 2 Principal component analysis (PCA) of the 14 gluten protein parameters (LPP, SPP, LMP, SMP, LPPs, SPPs, LMPs, SMPs, TOTE, TOTU, %UPP, %LUPP, %LUMP, and Mon/pol) from SE-HPLC of 294 spring wheat genotypes grown in 2017 and 2018

proteins. The impact of the prolonged heat and drought on the increase in the gluten protein polymerization was confirmed by significantly higher mean values for the large gluten polymer parameters (LPPs, %LUPP, and %UPP), as well as the higher values for the large monomeric protein %LUMP and Mon/Pol (Table 2).

The results observed in this study clearly showed a positive effect of the prolonged heat and drought on the gluten strength and the highest importance of a genotype component in regard to response to this stress, while the G × E interaction had a smaller effect. This was in accordance with the previous studies that showed the strong influence of genotype on the gluten polymer (e.g., SDS-unextractable protein), which was positively correlated with strong dough quality and bread-making characteristics in spring and winter wheat (Johansson et al., 2002; Johansson & Svensson, 1998), durum wheat (Li et al., 2013), and wheat/Aegilops addition lines (Rakszegi et al., 2019). This behavior can be explained by the fact that a large fraction of the wheat material in this study contained the combination of alleles Dx5 and Dy10 for the Glu-D1 locus, and Ax1 and Ax2* for the Glu-A1 locus; the first combination (Dx5 + Dy10) having the largest effect on bread quality (Payne et al., 1981), while the second (Ax1 and Ax2*) positively affects bread-making characteristics (Liu et al., 2008). Previous studies indicated that wheat varieties containing Dx2 + Dy12 are more sensitive to the heat stress compared with Dx5 + Dy10 (Blumenthal et al., 1995; Panozzo & Eagles, 2000). A reason behind could be explained by the fact that the subunits Dx5 + Dy10 have an extra cysteine residue, and thus able to form higher number of intermolecular disulfide bonds than the subunit Dx2 + Dy12 (Köhler et al., 1997; Veraverbeke & Delcour, 2002). In addition to disulfide bonds playing an important role in stabilizing the three-dimensional structure of proteins (Zhang et al., 2017), the subunit Dy10 has a longer repetitive domain than Dy12, which results in a higher content of hydrogen bonds (Lafiandra et al., 1999). Both types of bonding seem to lead to greater stability of the wheat varieties containing Dx5 + Dy10 under stress conditions than the wheat varieties containing Dx2 + Dy12. However, the variation in technological performance, for example, bread volume, is known for the genotypes having Dx5 + Dy10, which were grown in different environments (Johansson & Svensson, 1999).

It is also known that the wheat breeding lines containing Dx5 + Dy10 glutenin subunits under the dehydration phase start to accumulate and form very large glutenin polymers quicker compared to the lines containing, for example, Dx2 + Dy12 glutenin subunits (Naeem et al., 2012). Indeed, the heat (temperatures close to 25°C) during the growing period in 2018 started unusually early (already in April) and continued to rise reaching >32°C, while severe drought lasted throughout the whole wheat growing season, making this prolonged heat and drought period unique for the Swedish climate. Around a third of the studied wheat breeding lines (Figure 2; the area close to %UPP, LPPs, and %LUPP) responded positively to such growing conditions and those lines with increased gluten strength might be those with suitable phenotypic characters, for example, longer roots and adaptive shoots (Ahmed et al., 2020; Mathew et al., 2018). Differences in flowering (early flowering) of the wheat genotypes might also explain lower sensitivity to heat and drought (Lin et al., 2019). The grain filling stage in wheat is very sensitive to high temperatures, which speeds up the grain filling and makes a shorter grain filling duration period (Dias & Lidon, 2009; Farooq et al., 2011), as was observed in the wheat material gown in the prolonged heat and drought season in this study.

From previous investigations, high growing temperature (up to 30°C) during grain development increased the amount of large gluten polymers and gluten strength (e.g., TOTU, %UPP, and LUPP%) (Johansson et al., 2002;

TABLE 2 Means of the gluten protein parameters of 294 wheat genotypes grown in 2017 and 2018.

Year	LPP 10^6	SPP 10^6	LMP 10^6	SMP 10^6	LPPs 10^6	SPPs 10^6	LMPs 10^6	SMPs 10^6	TOTE 10^6	TOTU 10^6	%UPP	%LUPP	%LUMP	Mon/Pol
2017	9.7a	20.1a	50.3a	20.8a	9.6b	10.6a	10.7a	7.1a	110.1a	40.9a	44.6b	48.8b	26.7b	1.9b
2018	9.2b	10.7b	40.8b	20.7b	13.1a	10.4b	10.6b	6.3b	100.2b	40.9a	50.0a	56.1a	27.6a	2.4a

Note: Different letters indicating significant difference according to Tukey's post hoc test at $p < 0.05$.

Malik et al., 2011), while temperatures above 30°C during the latter part of the grain development reduced gluten polymers (Blumenthal et al., 1991, 1998; Guzmán et al., 2016; Li et al., 2013; Uhlen et al. 1998). In this study, significantly higher %UPP and LUPP% were found in 2018, while TOTU (a sum of two SDS-unextractable protein types, e.g., polymeric and monomeric) showed no significant differences between the years. Since significantly higher amount of the most protein parameters, except %UPP, %LUPP, and %LUMP, were found in 2017, it can be assumed that the varying climate resulted into similar amounts of the unextractable proteins. However, the protein fractions determined in 2018 contained bigger polymers and monomers compared to 2017. In previous studies, TOTU was positively correlated with the amount of fertilizer and protein concentration (Hailu et al., 2016; Johansson et al., 2008).

In previous studies, early maturing cultivars grown in high temperature had high protein concentration (TOTE) in greenhouse experiments (Malik et al., 2011), as well as in Mediterranean environments (Rharrabti et al., 2003). In this study, the protein concentration (TOTE) was sensitive to heat and drought stresses, which was different from the previous studies. A possible explanation is that a lower growing temperature led to a longer wheat grain maturation period (Johansson et al., 2005) as was observed in this study for 2017 material. Higher temperature is known to reduce nitrogen fertilizer transfer efficiency from the soil to grain during grain filling (Flagella et al., 2010) and grain maturation period (Dupont et al., 2006). A lack of water and prolonged drought during the grain filling in the field in 2018 resulted in a lower nitrogen fertilizer uptake for the genotypes of this study. Therefore, it is of the highest importance to find a suitable genotype optimally coping with nitrogen deficiency and heat–drought resistance. A selection of this genotype for breeding should be based on a good balance of factors leading to sufficient bread-making quality. These factors should include evaluation of genotype and G × E responses, as well as phenotypic and grain development characteristics (e.g., maturation time, time to anthesis, and duration of grain development period).

3.3 | Protein concentration in the varying climate

Flour protein concentration (FP%) of 109 spring wheat genotypes was compared between 2017 and 2018, and a great variation 10.6–16.4% in 2017 and 10.2–15.4% in 2018 was observed (Figure 3). The majority of genotypes (94) showed higher FP% in 2017 comparing to 2018 (Figure 3). The results also showed FP% to be significantly correlated

with the monomeric proteins (LMP, SMP, LMPs, and SMPs), the polymeric proteins (SPPs and TOTU), and Mon/Pol for both years (Table 3). For the cool season, significant correlations were observed between FP% and LPP, TOTE and %LUMP, and, for the heat and drought season, between FP% and SPP.

In this study, an increase in the flour protein concentration in the majority of the studied genotypes found in the cool climate was somewhat unexpected and differed from the previous studies (Johansson et al., 2005; Malik et al., 2011; Rharrabti et al., 2003). Possible explanation might be related to starch development. For example, negative correlations have been observed between the protein content and starch granule size after drought (Balla et al., 2011), suggesting formation of larger starch granules in the genotypes of this study. In fact, the high temperature after flowering is known to reduce the starch content and the starch granule size distribution, for example, B-type granules decrease and A-type granules increase, as well as an increase in starch molecular sizes (Spiertz et al., 2006).

The highest correlations between the gluten protein fractions and protein concentration in this study can be explained to large degree by the large monomeric gluten proteins (LMP and LMPs), for example, gliadins. It seems that the greater amounts and complexity of the gliadins were due to the heat and drought effect. This observation is in agreement with the previous studies that showed an increase in most of the major gliadin types (ω and α/β-gliadins) with high temperature (Daniel & Triboi, 2000). Similarly, the large glutenins were observed either to increase in the amount and molecular complexity or decrease in both due to the varying climate. Nitrogen availability during plant development time, for example, time to anthesis is an important factor influencing the gluten protein concentration (TOTE) in wheat (Malik et al., 2013a). More investigations are needed on the effect of drought on gluten protein concentration and composition to understand how gliadins and glutenins build complex large molecules.

TABLE 3 Spearman rank correlation coefficients between the gluten protein parameters and wheat flour protein concentration (FP%) of 109 spring wheat genotypes grown in 2017 and 2018

2017		2018	
Gluten protein parameters	**FP%**	**Gluten protein parameters**	**FP%**
LPP	−0.25**	LPP	0.03
SPP	0.13	SPP	0.39***
LMP	0.58***	LMP	0.70***
SMP	0.45***	SMP	0.24*
LPPs	−0.16	LPPs	0.12
SPPs	0.46***	SPPs	0.48***
LMPs	0.64***	LMPs	0.60***
SMPs	0.55***	SMPs	0.44***
TOTE	0.45***	TOTE	0.15
TOTU	0.60***	TOTU	0.50***
%UPP	0.10	%UPP	0.04
LUPP%	0.04	LUPP%	0.05
LUMP%	0.35***	LUMP%	0.18
Mon/Pol	0.39***	Mon/Pol	0.26**

Note: ***, **, and * indicate significance at the $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

3.4 | Amount and size distribution of polymeric and monomeric proteins and stability

We have compared the amount and size distribution of polymeric (chromatogram areas 1 and 2) and monomeric proteins (areas 3 and 4); representative SE-HPLC

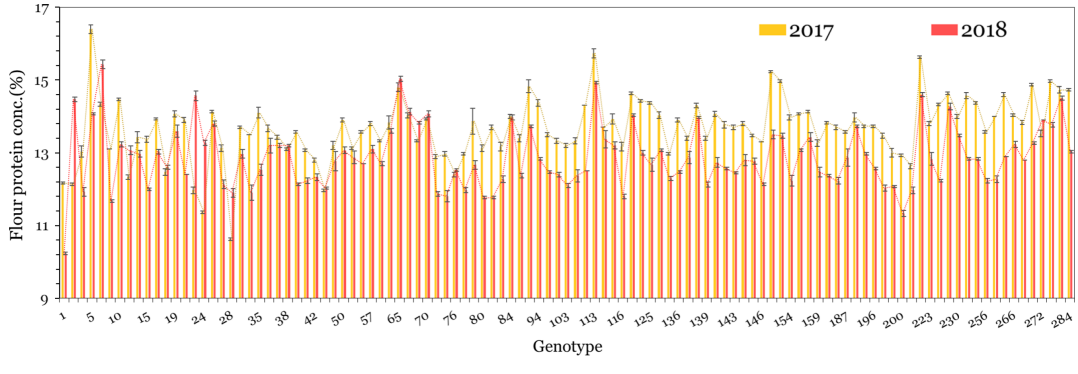


FIGURE 3 Flour protein concentration (%) of 109 spring wheat genotypes grown in 2017 and 2018. Error bars represent standard error

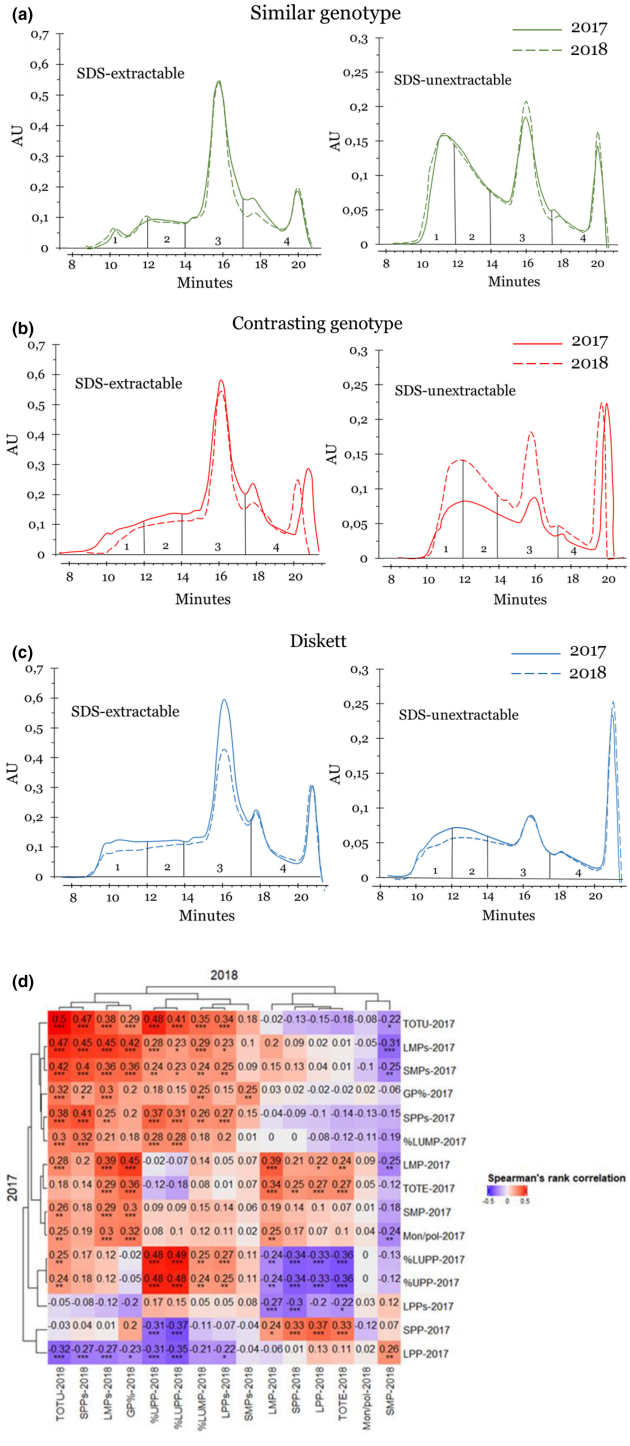


FIGURE 4 Examples of SE-HPLC chromatograms of the SDS-extractable and the SDS-unextractable gluten proteins of the selected wheat genotypes grown in 2017 and 2018; (a) similar protein solubility profile for genotype 187 from both years; (b) contrasting protein solubility profile of genotype 12; and (c) Diskett (a reference variety); 2017—solid line, 2018—dashed line; areas under chromatograms 1–4 represent large polymeric protein (LPP), small polymeric proteins (SPP), large monomeric protein (LMP), and small monomeric protein (SMP), respectively. (d) Spearman correlation matrix and hierarchical clustering of results (dendogram) based on complete linkage method for the 14 studied gluten protein parameters and grain protein concentration (GP%)

chromatograms of three genotypes from the studied climates are shown in Figure 4. A well-established spring wheat variety, Diskett, was used as reference (used as a control by Lantmännen). From the selected chromatograms, examples of similar gluten protein extractability pattern between the studied years (similar genotype 187; Figure 4a) and a varying protein extractability pattern (contrasting genotype 12; Figure 4b) are shown for both SDS-extractable and SDS-unextractable protein fractions in the climates studied (Figure 4). The main variation for the contrasting genotype 12 was observed in the SDS-unextractable protein, areas 1–3 of chromatogram, representing large and small polymeric proteins and large monomeric proteins (Figure 4b). Diskett showed unstable gluten protein pattern due to the variations in climate, for example, the protein solubility varied in both SDS-extractable and SDS-unextractable protein fractions (Figure 4c), indicating sensitivity to heat and drought stress.

A large variation between the genotypes was found in the solubility of both large and small polymeric proteins (chromatogram areas 1 and 2; for both SDS-extractable and SDS-unextractable protein fractions), large monomeric protein (area 3), and %UPP between the years (Figures 4 and 5). The %UPP for the genotypes grown in 2017 varied between 28.5 and 66.9%, while for 2018 material varied 27.6 and 71.1% indicating greater gluten strength for the lines grown in 2018 (Supporting Information, Table S1). From our study, the stability of gluten strength can be evaluated using the %UPP parameter and by comparing the values of each genotype between the studied years (≤5% difference between the years is considered a stable genotype) (genotypes indicated by black arrows; Figure 5). The 18 genotypes that showed stable %UPP, such as the smallest difference (≤5%) between the studied years, indicated their resistance to heat and drought stress, and can be considered as valuable genetic material to be prioritized in a wheat quality breeding program.

3.5 | Correlation among the protein parameters of gluten with the variations in climate

Results of Spearman's correlation coefficients between the studied protein parameters LPP, SPP, LMP, SMP (both SDS-extractable and SDS-unextractable fractions), %UPP, %LUPP, TOTE, %LUMP, Mon/Pol, and grain protein concentration are presented in Figure 4d. Significantly positive correlation coefficients between the compared parameters were 0.5 for TOTU ($p < 0.001$), 0.49 for %LUPP ($p < 0.001$), 0.48 for %UPP ($p < 0.001$), and 0.45 for LMPs ($p < 0.001$) between the different years (Figure 4d). Also, a significantly positive correlation with a coefficient of 0.45 between GP% from 2018 and LMP from 2017 ($p < 0.001$) was observed.

The protein parameter values in the correlation matrix for different years were displayed in a dendrogram and for the 2017 year indicated three clear clusters describing the largest gluten polymers (LPPs, %UPP, and %LUPP), the smaller monomeric proteins (Mon/Pol, SMP, TOTE, and LMP) were followed by the largest monomeric proteins (%LUMP, SPPs, GP%, SMPs, and LMPs) (Figure 4d). For 2018, different clusters were observed, the first cluster showing a mix of different parameters (TOTU, SPPs, LMPs, and GP%), the second cluster including large polymers and monomers (%UPP, %LUPP, %LUMP, and LPPs), and the third one smaller and medium large proteins (LMP, SPP, LPP, TOTE, Mon/Pol, and SMP; Figure 4d). It is interesting to point out that in 2017, %LUMP was related to SPPs and monomeric protein fractions, while in 2018 only to the large polymeric fractions, suggesting the large monomeric protein similarity to large polymers in terms of molecular sizes.

From the studied parameters, TOTU, %UPP, %LUPP, and LMPs were those parameters showing the highest correlations between the studied years. The results of

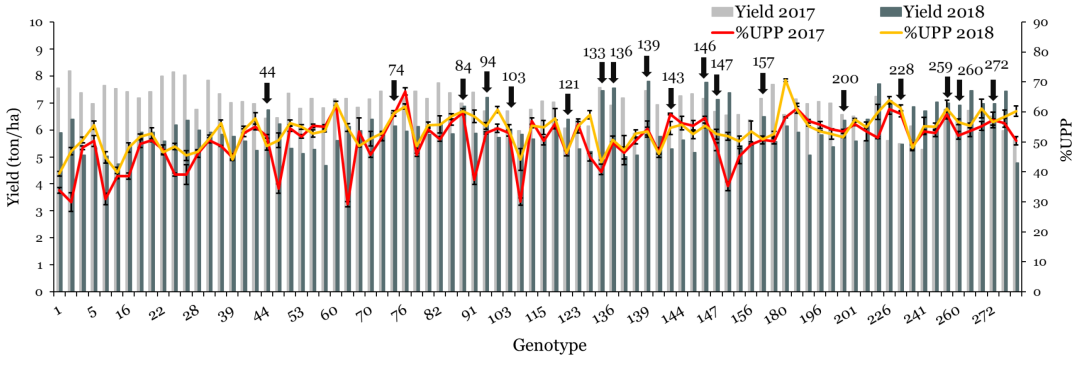


FIGURE 5 Wheat grain yields (ton/ha) and %UPP of 84 genotypes grown in 2017 and 2018. Error bars represent standard error

the Tukey's test (Table 2) and Spearman's correlation test (Figure 4d) indicate TOTU being least effected by the varying climate; this potential can be further explored in evaluating gluten quality stability.

3.6 | Grain yield

The effect of prolonged heat and drought on grain yield measured on 84 genotypes is shown in Figure 5. High temperature and drought decreased the yield in most of the studied genotypes. However, 21% of genotypes (18 of 84) showed similar yields for the studied years (resistant genotypes are indicated by arrows; Figure 5). The yields in 2017 ranged from 5.3 to 8.2 ton/ha, while in 2018 4.7 to 7.8 ton/ha (Figure 5). The wheat breeding lines that showed stability in yield during the studied years were genotypes 44, 74, 84, 94, 103, 121, 133, 136, 139, 143, 146, 147, 157, 200, 228, 259, 260, and 272. These 18 genotypes also showed stable %UPP between the years (Figure 5). In the similar study by Fleitas et al., 2020, the superior-yielding genotypes under heat stress delivered more than 5 ton/ha with attractive thousand kernel values, while the lowest yields in this study during a heat and drought season were similar (from 4.7 ton/ha) as in the study referred. In addition, combined heat and drought stress is known to induce higher yield losses than single heat or drought stress (Qaseem et al., 2019; Zhang et al., 2013). Combined heat and drought stress applied after anthesis reduced chlorophyll content (Qaseem et al., 2019) and caused a higher yield reduction compared with stress during anthesis and pre-anthesis (Zhang et al., 2013). However, in this study, a small increase (0.2–1.5 ton/ha) in yield during the heat and drought season was observed in 20 genotypes (44, 94, 121, 136, 139, 146, 147, 150, 220, 223, 226, 230, 241, 242, 259, 260, 267, 268, 272, 275) suggests that photosynthesis and chlorophyll production in those plants were not disturbed and the plants used some mechanisms to cope with the stress. More investigation is needed in order to understand the mechanisms behind this stress coping response. The results obtained in this study clearly indicate that the genotypes that delivered both high yields and satisfactory gluten strength (%UPP) are very promising genetic material to consider in breeding of climate-resistant bread wheat with attractive yields and quality.

3.7 | Wheat grain morphology by X-ray tomography

Decrease in grain number and size caused by heat stress is a rather well-known assumption (Akter & Rafiqul Islam, 2017). In this study, we examined the impact of the prolonged heat and drought and cool climate on the components of wheat grain such as, protein and starch, grain shape, and cross-sectional area of the grain by X-ray tomography (Ceresino et al., 2020, 2021). Unfortunately, we were not able to differentiate gluten protein and starch components in the grain due the lack of contrast between these components. We compared the cross-sectional structure of the grain from the three genotypes that were similar in %UPP and one contrasting genotype in %UPP between the years (Figure 6). A large variation in the grain microstructure in 2D images and grain characteristics (protein concentration and %UPP stability) was observed between the studied genotypes, although no clear differences due to the prolonged heat and drought was noted (Figure 6). The main observation of changes in the microstructure was related to the heat- and drought-exposed grains, which were either shrunken or asymmetric in the 2D cross-section of the grains (genotypes 12, 59, and 156) or contained dry fractures seen in the endosperm (genotype 25 from 2018; Figure 6a). The only significant cross-sectional differences in the area observed between the years were for the genotypes 12 and 25 (Figure 6b). Possible explanations for the differences observed are related to the fact that the genotype 12 was sensitive to the heat and drought stress for both %UPP and protein concentration (showed higher in 2017) and most likely accumulated more starch compared to 2018 grain (Figure 6b). In contrast, the genotype 25 was more tolerant to the heat and drought stress in regard to protein concentration. In general, the 3D images of reconstructed grain (Figure 6c; Supporting information, Videos S1–S4) indicated that the most uniform outer layer of the grain was observed in the genotypes grown in 2017. In general, the response of the grain microstructure to prolonged heat and drought was more related to genotypical variations in response. For the genotypes grown in 2017, with a longer grain maturation period, this could be related to higher accumulation of the starch component in the grain (Johansson et al., 2005; Koga et al., 2015). More investigations are needed to better explore and understand the morphological and structural responses of the prolonged heat and drought stress on wheat grain with stable protein characteristics (e.g., %UPP and TOTE).

4 | CONCLUSIONS

The 2018 season in Sweden was unique for growing spring wheat in the prolonged heat and drought period. To our knowledge, the results presented in this study are the first reported from this period. The excessive and prolonged heat and drought substantially affected

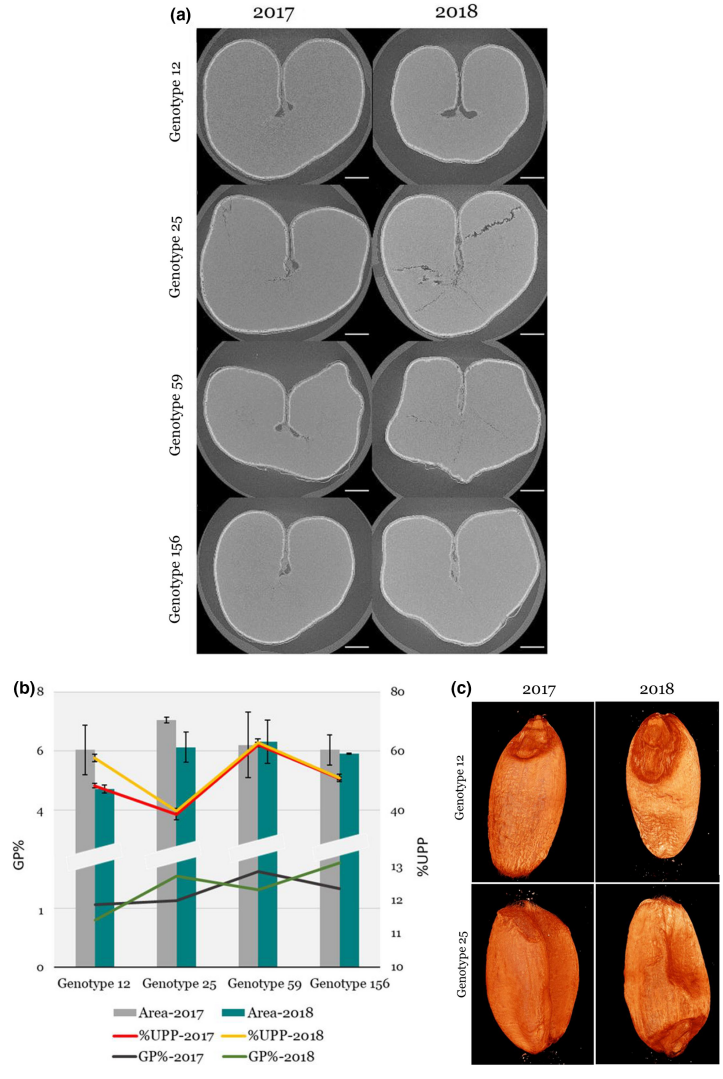


FIGURE 6 Microstructure of wheat grains from the genotypes differing in %UPP grown in 2017 and 2018 studied by X-ray tomography; (a) 2D images of the cross-section of wheat grain from the genotypes 12, 25, 59, and 156; scale bar is 500 μm; (b) grain endosperm area (mm^2), %UPP, and grain protein concentration (GP%) of four genotypes 12, 25, 59, and 156 grown in 2017 and 2018; and (c) 2D images of the genotypes 12 and 25. For the endosperm area and %UPP, standard deviations are included

the wheat grain development time, which was 44 days shorter compared to the grain development in the cool climate.

The prolonged heat and drought increased gluten protein polymerization and induced formation of large gluten polymers (LPPs, %UPP, and %LUPP) and large monomeric proteins (%LUMP and Mon/Pol). The cool climate increased the amount of monomeric gluten proteins (LMP and SMP) and the protein concentration (TOTE) in the grain and flour. Unexpectedly, the protein concentration was sensitive to heat and drought stress, most likely due to the fact that nitrogen was not accessible to the plant due to excessive heat and drought period.

The prolonged heat and drought were also seen to have positively impacted the gluten strength, and the genotype played the most important role in this response to the stress, while G x E had a smaller effect. Furthermore, it resulted in the formation of large monomeric proteins that were found to be similar to polymeric gluten proteins due to their molecular sizes and possibly formed greater amounts of intermolecular disulfide bridges.

TOTU was the gluten protein parameter least effected by the varying climate. For the gluten strength stability evaluation, gluten protein parameter %UPP can be used, while the use of TOTU for gluten stability evaluation should be further explored.

Eighteen genotypes 44, 74, 84, 94, 103, 121, 133, 136, 139, 143, 146, 147, 157, 200, 228, 259, 260, and 272 showed both stable yield and stable %UPP between the years, and are attractive breeding materials for climate-resistant bread wheat with increased food security.

The grain morphology and microstructure of the grain varied to a minor extent due to the prolonged heat and drought. X-ray tomography might be a valuable tool to be further explored if contrast between the grain components could be improved.

The new knowledge obtained on gluten protein parameters related to environmental effects is important in searching for new genotypes with tolerance for fluctuating climatic conditions and can help breeders in improving the performance of Swedish spring wheat genotypes and self-sufficiency in bread wheat for Sweden.

ACKNOWLEDGEMENTS

This study was financially supported by the Swedish governmental initiative SLU Grogrund-Centre for Breeding of Food Crops and SLU Partnerskap Alnarp. We acknowledge Maria Luisa Prieto-Linde for her technical support in the laboratory, and William R. Newson for English language editing.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

ORCID

Sbatie Lama https://orcid.org/0000-0003-0093-8915

REFERENCES

Ahmed, K., Shabbir, G., Ahmed, M., & Shah, K. N. (2020). Phenotyping for drought resistance in bread wheat using physiological and biochemical traits. *Science of the Total Environment*, *729*, 139082. https://doi.org/10.1016/j.scitotenv.2020.139082

Akter, N., & Rafiqul Islam, M. (2017). Heat stress effects and management in wheat. A review. *Agronomy for Sustainable Development*, *37*(5), 1–17. https://doi.org/10.1007/s13593-017-0443-9

Altenbach, S. B. (2012). New insights into the effects of high temperature, drought and post-anthesis fertilizer on wheat grain development. *Journal of Cereal Science*, *56*(1), 39–50. https://doi.org/10.1016/j.jcs.2011.12.012

Asseng, S., Martre, P., Maiorano, A., Rötter, R. P., O'Leary, G. J., Fitzgerald, G. J., Girousse, C., Motzo, R., Giunta, F., Babar, M. A., Reynolds, M. P., Kheir, A. M. S., Thorburn, P. J., Waha, K., Ruane, A. C., Aggarwal, P. K., Ahmed, M., Balkovič, J., Basso, B., … & Ewert, F. (2019). Climate change impact and adaptation for wheat protein. *Glob Chang Biology*, *25*(1), 155–173. https://doi.org/10.1111/gcb.14481

Balla, K., Rakszegi, M., Li, Z., Bekes, F., Bencze, S., & Veisz, O. (2011). Quality of winter wheat in relation to heat and drought shock after anthesis. *Czech Journal of Food Sciences*, *29*, 117–128. https://doi.org/10.17221/227/2010-CJFS

Battenfield, S. D., Guzman, C., Gaynor, R. C., Singh, R. P., Pena, R. J., Dreisigacker, S., Fritz, A. K., & Poland, J. A. (2016). Genomic selection for processing and end-use quality traits in the CIMMYT spring bread wheat breeding program. *The Plant Genome*, *9*(2), 1–12. https://doi.org/10.3835/plantgenome2016.01.0005

Blake, N. K., Varella, A. C., Bicego, B., Martin, J. M., Cook, J. P., Heo, H.-Y., Acharya, R., Sherman, J. D., Nash, D., & Talbert, L. E. (2018). Maturity traits related to climate adaptation affect quality characteristics in hard red spring wheat. *Crop Science*, *58*(5), 1954–1963. https://doi.org/10.2135/cropsci2018.04.0228

Blumenthal, C., Gras, P. W., Bekes, F., Barlow, E. W. R., & Wrigley, C. W. (1995). Possible role for the Glu-D1 locus with respect to tolerance to dough-quality change after heat stress. *Cereal Chemistry*, *72*(1), 135–136.

Blumenthal, C. S., Batey, I. L., Bekes, F., Wrigley, C. W., & Barlow, E. W. R. (1991). Seasonal changes in wheat-grain quality associated with high temperatures during grain filling. *Australian Journal of Agricultural Research*, *42*(1), 21–30. https://doi.org/10.1071/AR9910021

Blumenthal, C., Stone, P. J., Gras, P. W., Bekes, F., Clarke, B., Barlow, E. W. R., Appels, R., & Wrigley, C. W. (1998). Heat-shock protein 70 and dough-quality changes resulting from heat stress during grain filling in wheat. *Cereal Chemistry*, *75*, 43–50. https://doi.org/10.1094/CCHEM.1998.75.1.43

Bornhofen, E., Benin, G., Storck, L., Marchioro, V. S., Meneguzzi, C., Milioli, A. S., & Trevizan, D. M. (2017). Environmental effect on genetic gains and its impact on bread-making quality traits in Brazilian spring wheat. *Chilean Journal of Agricultural Research*, *77*(1), 27–34. https://doi.org/10.4067/S0718-58392017000100003

Cammalleri, C., Naumann, G., Mentaschi, L., Formetta, G., Forzieri, G., Gosling, S., Bisselink, B., De Roo, A., & Feyen, L. (2020). *Global warming and drought impacts in the EU, EUR 29956 EN*. Publications Office of the European Union. ISBN 978-92-76-12947-9. https://doi.org/10.2760/597045

Ceresino, E. B., Johansson, E., Sato, H., Plivelic, T., Hall, S., Bez, J., & Kuktaite, R. (2021). Lupin Protein isolate structure diversity in frozen-cast foams: effects of transglutaminases and edible fats. *Molecules*, *26*(6), 1717. https://doi.org/10.3390/molecules26061717

Ceresino, E. B., Johansson, E., Sato, H. H., Plivelic, T. S., Hall, S. A., & Kuktaite, R. (2020). Morphological and structural heterogeneity of solid gliadin food foams modified with transglutaminase and food grade dispersants. *Food Hydrocolloids*, *108*, 105995. https://doi.org/10.1016/j.foodhyd.2020.105995

Dai, Z. M., Xu, T. S., Li, X. G., Zhang, H., Li, Y., & Zhang, X. L. (2016). Effect of different water supply on accumulation of high molecular weight glutenin subunits and glutenin macropolymers in near-isogenic wheat lines. *Plant, Soil and Environment*, *62*, 53–59. https://doi.org/10.17221/728/2015-PSE

Daniel, C., & Triboi, E. (2000). Effects of temperature and nitrogen nutrition on the grain composition of winter wheat: Effects on gliadin content and composition. *Journal of Cereal Science*, *32*(1), 45–56. https://doi.org/10.1006/jcrs.2000.0313

Dias, A. S., & Lidon, F. C. (2009). Evaluation of grain filling rate and duration in bread and durum wheat, under heat stress after

anthesis. *Journal of Agronomy and Crop Science*, *195*(2), 137–147. https://doi.org/10.1111/j.1439-037X.2008.00347.x

Dupont, F. M., & Altenbach, S. (2003). Molecular and biochemical impacts of environmental factors on wheat grain development and protein yield. *Journal of Cereal Science*, *38*(2), 133–146. https://doi.org/10.1016/S0733-5210(03)00030-4

Dupont, F. M., Hurkman, W. J., Vensel, W. H., Tanaka, C., Kothari, K. M., Chung, O. K., & Altenbach, S. B. (2006). Protein accumulation and composition in wheat grains: Effects of mineral nutrients and high temperature. *European Journal of Agronomy*, *25*(2), 96–107. https://doi.org/10.1016/j.eja.2006.04.003

Enghiad, A., Ufer, D., Countryman, A., & Thilmany, D. (2017). An overview of global wheat market fundamentals in an era of climate concerns. *International Journal of Agronomy*, *2017*(19), 1–15. https://doi.org/10.1155/2017/3931897

FAO (2009). *Tackling climate change through plant breeding and better use of plant genetic resources*. Retrieved from: http://www.fao.org/3/a-at911e.pdf

FAO (Food and Agriculture Organization of the United Nations). FAOSTAT: FAO Statistical Databases. (Accessed June 1, 2020).

Farooq, M., Bramley, H., Palta, J. A., & Siddique, K. H. M. (2011). Heat stress in wheat during reproductive and grain-filling phases. *Critical Reviews in Plant Sciences*, *30*(6), 491–507. https://doi.org/10.1080/07352689.2011.615687

Flagella, Z., Giuliani, M., Giuzio, L., Volpi, C., & Masci, S. (2010). Influence of water deficit on durum wheat storage protein composition and technological quality. *European Journal of Agronomy*, *33*(3), 197–207. https://doi.org/10.1016/j.eja.2010.05.006

Gupta, R. B., Khan, K., & Macritchie, F. (1993). Biochemical basis of flour properties in bread wheats. I. effects of variation in the quantity and size distribution of polymeric protein. *Journal of Cereal Science*, *18*(1), 23–41. https://doi.org/10.1006/jcrs.1993.1031

Guzmán, C., Autrique, J. E., Mondal, S., Singh, R. P., Govindan, V., Morales-Dorantes, A., Posadas-Romano, G., Crossa, J., Ammar, K., & Peña, R. J. (2016). Response to drought and heat stress on wheat quality, with special emphasis on bread-making quality, in durum wheat. *Field Crops Research*, *186*, 157–165. https://doi.org/10.1016/j.fcr.2015.12.002

Hailu, F., Labuschagne, M., van Biljon, A., Hovmalm, H., & Johansson, E. (2016). Quality assessment with HPLC in released varieties of tetraploid (Triticum durum Desf.) wheat from Ethiopia and Spain. *Cereal Research Communications*, *44*(4), 617–627. https://doi.org/10.1556/0806.44.2016.040

Hashiguchi, A., Ahsan, N., & Komatsu, S. (2010). Proteomics application of crops in the context of climatic changes. *Food Research International*, *43*(7), 1803–1813. https://doi.org/10.1016/j.foodres.2009.07.033

Hernandez-Espinosa, N., Mondal, S., Autrique, E., Gonzalez-Santoyo, H., Crossa, J., Huerta-Espino, J., Shing, R. P., & Guzmán, C. (2018). Milling, processing and end-use quality traits of CIMMYT spring bread wheat germplasm under drought and heat stress. *Field Crops Research*, *215*, 104–112. https://doi.org/10.1016/j.fcr.2017.10.003

Johansson, E., Branlard, G., Cuniberti, M., Flagella, Z., Hüsken, A., Nurit, E., Peña, R. J., Sissons, M., & Vazquez, D. (2020). Genotypic and environmental effects on wheat technological and nutritional quality. In G. Igrejas, T. Ikeda, & C. Guzmán (Eds.), *Wheat quality for improving processing and human health*. (pp. 171–204). Springer: Cham. https://doi.org/10.1007/978-3-030-34163-3_8

Johansson, E., Malik, A. H., Hussain, A., Rasheed, F., Newson, W. R., Plivelic, T., Hedenqvist, M. S., Gällstedt, M., & Kuktaite, R. (2013). Wheat gluten polymer structures: The impact of genotype, environment, and processing on their functionality in various applications. *Cereal Chemistry*, *90*(4), 367–376. https://doi.org/10.1094/CCHEM-08-12-0105-FI

Johansson, E., Nilsson, H., Mazhar, H., Skerritt, J., MacRitchie, F., & Svensson, G. (2002). Seasonal effects on storage proteins and gluten strength in four Swedish wheat cultivars. *Journal of the Science of Food and Agriculture*, *82*, 1305–1311. https://doi.org/10.1002/jsfa.1185

Johansson, E., Prieto-Linde, M. L., & Gissén, C. (2008). Influences of weather, cultivar and fertiliser rate on grain protein polymer accumulation in field-grown winter wheat, and relations to grain water content and falling number. *Journal of the Science of Food and Agriculture*, *88*(11), 2011–2018. https://doi.org/10.1002/jsfa.3312

Johansson, E., Kuktaite, R., Andersson, A., & Prieto-Linde, M. L. (2005). Protein polymer build-up during wheat grain development: influences of temperature and nitrogen timing, (Eds.), *Journal of the Science of Food and Agriculture 85*(3), 473–479. https://doi.org/10.1002/jsfa.2006

Johansson, E., & Svensson, G. (1998). Variation in bread-making quality: Effects of weather parameters on protein concentration and quality in some Swedish wheat cultivars grown during the period 1975–1996. *Journal of the Science of Food and Agriculture*, *78*(1), 109–118. https://doi.org/10.1002/(SICI)1097-0010(199809)78:1<109:AID-JSFA92>3.0.CO;2-0

Johansson, E., & Svensson, G. (1999). Influences of yearly weather variation and fertilizer rate on bread-making quality in Swedish grown wheats containing HMW glutenin subunits 2+12 or 5+10 cultivated during the period 1990–96. *The Journal of Agricultural Science*, *132*(1), 13–22. https://doi.org/10.1017/S0021859698006029

Jordbruksverket (2019). *Utrikeshandel inom livsmedelsområdet*. Retrieved from: https://djur.jordbruksverket.se/amnesomraden/handelmarknad/allmantomhandelsochjordbrukspolitik/statistikutrikeshandel.4.7a34cbce172e8d52fe81ffeb.html

Kiszonas, A. M., & Morris, C. F. (2018). Wheat breeding for quality: A historical review. *Cereal Chemistry*, *95*(1), 17–34. https://doi.org/10.1094/CCHEM-05-17-0103-FI

Koga, S., Böcker, U., Moldestad, A., Tosi, P., Shewry, P. R., Mosleth, E. F., & Uhlen, A. K. (2015). Influence of temperature on the composition and polymerization of gluten proteins during grain filling in spring wheat (Triticum aestivum L.). *Journal of Cereal Science*, *65*, 1–8. https://doi.org/10.1016/j.jcs.2015.05.012

Köhler, P., Keck-Gassenmeier, B., Wieser, H., & Kasarda, D. D. (1997). Molecular modeling of the n-terminal regions of high molecular weight glutenin subunits 7 and 5 in relation to intramolecular disulfide bond formation. *Cereal Chemistry*, *74*(2), 154–158. https://doi.org/10.1094/CCHEM.1997.74.2.154

Labuschagne, M. T., Moloi, J., & van Biljon, A. (2016). Abiotic stress induced changes in protein quality and quantity of two bread wheat cultivars. *Journal of Cereal Science*, *69*, 259–263. https://doi.org/10.1016/j.jcs.2016.03.018

Lafiandra, D., Turchetta, T., Ovidio, R., Anderson, O. D., Facchiano, A. M., & Colonna, G. (1999). Conformational polymorphism of

high Mr glutenin subunits detected by transverse urea gradient gel electrophoresis. *Journal of Cereal Science*, *30*(2), 97–104. https://doi.org/10.1006/jcrs.1999.0260

Lenaerts, B., Collard, B. C. Y., & Demont, M. (2019). Review: Improving global food security through accelerated plant breeding. *Plant Science*, *287*, 110207. https://doi.org/10.1016/j.plantsci.2019.110207

Li, Y.-F., Wu, Y., Hernandez-Espinosa, N., & Pena, R.J. (2013). Heat and drought stress on durum wheat: Responses of genotypes, yield, and quality parameters. *Journal of Cereal Science*, *57*(3), 398–404. https://doi.org/10.1016/j.jcs.2013.01.005

Lin, Y., Yi, X., Tang, S., Chen, W., Wu, F., Yang, X., Jiang, X., Shi, H., Ma, J., Chen, G., Chen, G., Zheng, Y. W., & Liu, Y. (2019). Dissection of phenotypic and genetic variation of drought-related traits in diverse Chinese wheat landraces. *The Plant Genome*, *12*(3), 190025. https://doi.org/10.3835/plantgenome2019.03.0025

Liu, S., Chao, S., & Anderson, J. A. (2008). New DNA markers for high molecular weight glutenin subunits in wheat. *Theoretical and Applied Genetics*, *118*(1), 177–183. https://doi.org/10.1007/s00122-008-0886-0

Magallanes-López, A. M., Ammar, K., Morales-Dorantes, A., González-Santoyo, H., Crossa, J., & Guzmán, C. (2017). Grain quality traits of commercial durum wheat varieties and their relationships with drought stress and glutenins composition. *Journal of Cereal Science*, *75*, 1–9. https://doi.org/10.1016/j.jcs.2017.03.005

Malik, A. H., Prieto-Linde, M. L., Kuktaite, R., Andersson, A, & Johansson, E. (2011). Individual and interactive effects of cultivar maturation time, nitrogen regime and temperature level on accumulation of wheat grain proteins. *Journal of the Science of Food and Agriculture*, *91*(12), 2192–2200. https://doi.org/10.1002/jsfa.4439

Malik, A. H., Kuktaite, R., & Johansson, E. (2013a). Combined effect of genetic and environmental factors on the accumulation of proteins in the wheat grain and their relationship to bread-making quality. *Journal of Cereal Science*, *57*(2), 170–174. https://doi.org/10.1016/j.jcs.2012.09.017

Malik, A. H., Holm, L., Kuktaite, R., & Andersson, A. (2013b). Individual and combined effects of pre- and post-anthesis temperature on protein composition of two malting barley cultivars. *Journal of Cereal Science*, *58*(2), 341–347. https://www.sciencedirect.com/science/article/pii/S0733521013001227

Malik, A. H., Prieto-Linde, M. L., Kuktaite, R., Andersson, A., & Johansson, E. (2011). Individual and interactive effects of cultivar maturation time, nitrogen regime and temperature level on accumulation of wheat grain proteins. *Journal of the Science of Food and Agriculture*, *91*(12), 2192–2200. https://doi.org/10.1002/jsfa.4439

Mathew, I., Shimelis, H., Mwadzingeni, L., Zengeni, R., Mutema, M., & Chaplot, V. (2018). Variance components and heritability of traits related to root: shoot biomass allocation and drought tolerance in wheat. *Euphytica*, *214*, 1–12. https://doi.org/10.1007/s10681-018-2302-4

Naeem, H. A., Paulon, D., Irmak, S., & MacRitchie, F. (2012). Developmental and environmental effects on the assembly of glutenin polymers and the impact on grain quality of wheat. *Journal of Cereal Science*, *56*(1), 51–57. https://doi.org/10.1016/j.jcs.2011.10.014

Panozzo, J. F., & Eagles, H. A. (2000). Cultivar and environmental effects on quality characters in wheat. II. Protein. *Australian Journal of Agricultural Research*, *51*(5), 629–636. https://doi.org/10.1071/AR99137

Payne, P. I., Corfield, K. G., Holt, L. M., & Blackman, J. A. (1981). Correlations between the inheritance of certain high-molecular weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. *Journal of the Science of Food and Agriculture*, *32*(1), 51–60. https://doi.org/10.1002/jsfa.2740320109

Pennacchi, J. P., Carmo-Silva, E., Andralojc, P. J., Lawson, T., Allen, A. M., Raines, C. A., & Parry, M. A. J. (2018). Stability of wheat grain yields over three field seasons in the UK. *Food and Energy Security*, *8*(2), e00147. https://doi.org/10.1002/fes3.147

Qaseem, M. F., Qureshi, R., & Shaheen, H. (2019). Effects of pre-anthesis drought, heat and their combination on the growth, yield and physiology of diverse wheat (Triticum aestivum L.) genotypes varying in sensitivity to heat and drought stress. *Scientific Reports*, *9*(1), 6955. https://doi.org/10.1038/s41598-019-43477-z

Rakszegi, M., Darkó, É., Lovegrove, A., Molnár, I., Láng, L., Bedő, Z., Molnár-Láng, M., & Shewry, P. (2019). Drought stress affects the protein and dietary fiber content of wholemeal wheat flour in wheat/Aegilops addition lines. *PLoS One*, *14*(2), e0211892. https://doi.org/10.1371/journal.pone.0211892

Randall, P., & Moss, H. (1990). Some effects of temperature regime during grain filling on wheat quality. *Australian Journal of Agricultural Research*, *41*(4), 603. https://doi.org/10.1071/AR9900603

Ray, D. K., West, P. C., Clark, M., Gerber, J. S., Prishchepov, A. V., & Chatterjee, S. (2019). Climate change has likely already affected global food production. *PLoS One*, *14*(5), e0217148. https://doi.org/10.1371/journal.pone.0217148

Rharrabti, Y., Villegas, D., Royo, C., Martos-Núñez, V., & García del Moral, L. F. (2003). Durum wheat quality in Mediterranean environments: II. Influence of climatic variables and relationships between quality parameters. *Field Crops Research*, *80*(2), 133–140. https://doi.org/10.1016/S0378-4290(02)00177-6

Spiertz, J. H. J., Hamer, R. J., Xu, H., Primo-Martin, C., Don, C., & van der Putten, P. E. L. (2006). Heat stress in wheat (Triticum aestivum L.): Effects on grain growth and quality traits. *European Journal of Agronomy*, *25*(2), 89–95. https://doi.org/10.1016/j.eja.2006.04.012

Tremmel-Bede, K., Szentmiklóssy, M., Tömösközi, S., Török, K., Lovegrove, A., Shewry, P. R., Láng, L., Bedő, Z., Vida, G., & Rakszegi, M. (2020). Stability analysis of wheat lines with increased level of arabinoxylan. *PLoS One*, *15*(5), e0232892. https://doi.org/10.1371/journal.pone.0232892

Uhlen, A. K., Hafskjold, R., Kalhovd, A. H., Sahlström, S., Longva, Å., & Magnus, E. M. (1998). Effects of cultivar and temperature during grain filling on wheat protein content, composition, and dough mixing properties. *Cereal Chemistry*, *75*(4), 460–465. https://doi.org/10.1094/CCHEM.1998.75.4.460

Veraverbeke, W. S., & Delcour, J. A. (2002). Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. *Critical Reviews in Food Science and Nutrition*, *42*(3), 179–208. https://doi.org/10.1080/10408690290825510

Vollmer, E., & Musshoff, O. (2018). Average protein content and its variability in winter wheat: A forecast model based on weather

parameters. *Earth Interactions*, *22*(19), 1–24. https://doi.org/10.1175/EI-D-18-0011.1

Xiao, D., Bai, H., & Liu, D. (2018). Impact of future climate change on wheat production: A simulated case for China's wheat system. *Sustainability*, *10*(4), 1277. https://doi.org/10.3390/su10041277

Yu, Q., Li, L., Luo, Q., Eamus, D., Xu, S., Chen, C., Wang, E., Liu, J., & Nielsen, D. C. (2014). Year patterns of climate impact on wheat yields. *International Journal of Climatology*, *34*(2), 518–528. https://doi.org/10.1002/joc.3704

Zhang, X., Cai, J., Wollenweber, B., Liu, F., Dai, T., Cao, W., & Jiang, D. (2013). Multiple heat and drought events affect grain yield and accumulations of high molecular weight glutenin subunits and glutenin macropolymers in wheat. *Journal of Cereal Science*, *57*(1), 134–140. https://doi.org/10.1016/j.jcs.2012.10.010

Zhang, Y., Pan, J., Huang, X., Guo, D., Lou, H., Hou, Z., Su, M., Liang, R., Xie, C., You, M., & Li, B. (2017). Differential effects of a post-anthesis heat stress on wheat (Triticum aestivum L.) grain proteome determined by iTRAQ. *Scientific Reports*, *7*, 3468. https://doi.org/10.1038/s41598-017-03860-0

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Lama, S., Vallenback, P., Hall, S. A., Kuzmenkova, M., & Kuktaite, R. (2022). Prolonged heat and drought versus cool climate on the Swedish spring wheat breeding lines: Impact on the gluten protein quality and grain microstructure. *Food and Energy Security*, *11*, e376. https://doi.org/10.1002/fes3.376

II

plants

MDPI

Article

Striving for Stability in the Dough Mixing Quality of Spring Wheat under the Influence of Prolonged Heat and Drought

Sbatie Lama [1,*], Marina Kuzmenkova [1], Pernilla Vallenback [2] and Ramune Kuktaite [1]

[1] Department of Plant Breeding, Swedish University of Agricultural Sciences (Alnarp), SE-234 22 Lomma, Sweden
[2] Lantmännen Lantbruk, SE-26831 Svalöv, Sweden
* Correspondence: sbatie.lama@slu.se

Abstract: The effects of prolonged heat and drought stress and cool growing conditions on dough mixing quality traits of spring wheat (*Triticum aestivum* L.) were studied in fifty-six genotypes grown in 2017 and 2018 in southern Sweden. The mixing parameters evaluated by mixograph and the gluten protein characteristics studied by size exclusion high-performance liquid chromatography (SE-HPLC) in dough were compared between the two growing seasons which were very different in length, temperature and precipitation. The genotypes varying in gluten strength between the growing seasons ($\leq$5%, $\leq$12%, and $\leq$17%) from three groups (stable (S), moderately stable (MS), and of varying stability (VS)) were studied. The results indicate that most of the mixing parameters were more strongly impacted by the interaction between the group, genotype, and year than by their individual contribution. The excessive prolonged heat and drought did not impact the buildup and mixing time expressed as peak time and time 1–2. The gluten polymeric proteins (unextractable, %UPP; total unextractable, TOTU) and large unextractable monomeric proteins (%LUMP) were closely associated with buildup and water absorption in dough. Major significant differences were found in the dough mixing parameters between the years within each group. In Groups S and MS, the majority of genotypes showed the smallest variation in the dough mixing parameters responsible for the gluten strength and dough development between the years. The mixing parameters such as time 1–2, buildup, and peak time (which were not affected by prolonged heat and drought stress) together with the selected gluten protein parameters (%UPP, TOTU, and %LUMP) are essential components to be used in future screening of dough mixing quality in wheat in severe growing environments.

Keywords: mixing quality; wheat plant; gluten polymers and monomers; dough mixing time

check for updates

Citation: Lama, S.; Kuzmenkova, M.; Vallenback, P.; Kuktaite, R. Striving for Stability in the Dough Mixing Quality of Spring Wheat under the Influence of Prolonged Heat and Drought. *Plants* **2022**, *11*, 2662. https://doi.org/10.3390/plants11192662

Academic Editor: Cristina Crosatti

Received: 14 September 2022
Accepted: 5 October 2022
Published: 10 October 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Bread-making characteristics of wheat (*Triticum aestivum* L.) are largely determined by the quantity and quality of storage proteins, and the baking ability is strongly influenced by the genotype and environmental factors [1,2]. Gluten protein is the main determinant of the bread-making quality of wheat, and its content in flour is strongly influenced by abiotic stress factors such as heat and drought. An increase in gluten protein content under drought stress was observed in wheat grain [3]. The stress magnitude was found to vary between the different periods of drought (early, late, prolonged, etc.) [3–5], suggesting different consequences for the processing quality of bread wheat flour [6].

Dough mixing is a vital step in bread making; it includes blending of the wheat flour with water and developing a three dimensional network of gluten in which the starch granules are embedded [7]. During dough mixing, the rheological properties such as elasticity, viscosity, and extensibility change and these properties are important in predicting the quality of the final product [8], especially under varying growing conditions. The rheological properties of dough are governed by the specific types of gluten protein, such as polymeric glutenins and monomeric gliadins. From these, the unextractable polymeric protein (%UPP)

fraction, which can be solubilized in sodium dodecyl sulfate (SDS)-phosphate buffer with the use of sonication, is the most important for gluten quality [7,9–11]. Thus, %UPP is known to be directly correlated to the gluten strength in dough and bread volume [10,12,13].

The mixograph is a widely used instrument which mixes wheat flour and water into a dough to assess the processing quality of the wheat flour [14–17]. Specifically, the mixograph was developed to evaluate mixing characteristics of strong high-protein flour [18,19]. Different versions of the mixograph are available, such as mixographs with 2, 5, 10, and 35 g of flour [18], where the 10 g mixograph is more commonly used [12,14,19]. From the mixograph parameters, the peak time and buildup (the difference between the maximum stress in the dough during deformation and the stress in the dough at the point in time when all the liquid has been absorbed) were found to be positively correlated with the SDS-unextractable polymeric proteins (i.e., gluten strength) [12,20,21] and bread volume (correlation of more than 80%) [19]. However, there have been only few studies conducted using a mixograph to evaluate the processing quality of wheat flour under varying environmental conditions, and none which involve extreme heat or drought stress.

An extreme stress environment can be defined as a growing environment in which heat or drought stress (or a combination of these) occurring around plant flowering, which is known to cause severe losses in yield and quality. Few studies have indicated that heat and drought stresses induce the formation of large gluten polymers (i.e., %UPP) and overall increase the gluten strength of wheat flour [22,23]. This trend was also observed in our latest study involving wheat flour from genotypes grown during excessively long period of heat and drought [5]. For wheat flour dough, it was reported that a relatively high temperature (e.g., 27 °C) caused a longer dough development time and higher dough stability, resulting in greater loaf volume compared to cooler temperatures (e.g., 18 °C) [24,25]. In particular, heat stress was found to increase both the extensibility and strength of wheat dough [26,27]. Drought was found to increase the optimum dough mixing time [28]. Still, it is unknown how severe heat and drought stresses impact wheat dough characteristics and whether any of these characteristics could be used in the screening of wheat material for climate stability. So far, no studies have focused on the combination of extreme heat and drought stress and its impact on the rheological properties and gluten protein characteristics of the dough of Swedish bread wheat; thus, a major knowledge gap still exists.

The main goal of this study was to investigate the effect of the combination of extreme heat and drought stress—observed in 2018 in a field in Sweden—on the mixing properties and composition of gluten proteins in wheat dough, studied by mixograph and size exclusion liquid chromatography (SE-HPLC). The novel approach of this study is exposing of wheat genotypes to an extreme growing environment: a temperature rise of 6–11 °C above the average temperature and a very low level of precipitation (below 30 mm), and evaluating how the mixing parameters vary in three groups of spring wheats with diverse gluten strengths. The results of this paper highlight how the specific dough mixing parameters, if tuned with the gluten protein characteristics, might be the key criteria to achieve more uniform bread baking performance under varying growing conditions in the future.

2. Results

2.1. Effect of Year and Groups on the Dough Mixing Characteristics

Analysis of variance (ANOVA) results show that the interactions of group × genotype, group × year, and group × year × genotype significantly ($p < 0.001$) influenced all the mixing characteristics from the water absorption and dough development phases and IHTP, except the peak width and breakdown (Table 1). The most highly significant ($p < 0.001$) impact from the group × genotype interaction was on IHTP, followed by the group × year × genotype impact.

Table 1. Analysis of variance (ANOVA) showing the effect of group, year, and wheat genotype and their interaction on the mixing characteristics of the 56 spring wheat genotypes grown in cool (2017) and heat–drought (2018) years. Data are presented as mean values calculated from three replicates for each parameter.

	Group	Year	Group × Genotype	Group × Year	Group × Year × Genotype	Residuals
Df	2	1	53	2	53	224
Water absorption						
Initial slope	0.37 *	4.41 ***	39.92 ***	2.23 ***	18.31 ***	9.75
Initial width	0.05 **	0.58 ***	4.84 ***	0.23 ***	1.79 ***	1.01
Initial build-up	0.45 *	3.52 ***	49.22 ***	1.93 ***	14.24 ***	16.02
Time 1–2	6.30 ***	0.08	53.71 ***	1.32 ***	8.99 ***	4.85
Initial build width	0.07	0.37 ***	19.51 ***	0.45 ***	3.14 ***	4.65
Dough development						
Buildup	0.69	0.05	27.88 ***	1.56 **	13.70 **	31.57
Peak time	25.30 ***	0.32	215.09 ***	5.23 ***	35.94 ***	19.37
Peak height	1.07 *	5.77 ***	70.57 ***	2.78 ***	26.64 ***	28.09
Peak width	1.43 ***	0.35 ***	24.50 ***	0.05	4.24 ***	5
Build width	1.10 ***	0.60 ***	10.31 ***	0.75 ***	6.00 ***	9.63
Dough breakdown						
Breakdown	0.45	0.07	7.33	0.05	5.05	260.240
IHTP	500.4 ***	13.5 *	4830.6 ***	164.8 ***	1017.8 ***	597.0
Water absorption	0.09	0.32***	-	0.12	-	2.41

Note: IHTP—Integrated height to the peak. ***, **, and * indicate significance at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively. The mixograph parameters (initial slope, initial width, initial buildup, build width, buildup, peak height, peak width, width build, breakdown, and IHTP) are measured as torque (N·m); mixing time (time 1–2 and peak time) are measured in minutes; water absorption is measured ml/10 g flour.

The strongly significant impact of year ($p < 0.001$ and $p < 0.05$) was clear on almost all of the mixing parameters, except time 1–2, buildup, and peak time (Table 1), indicating the stability of these parameters in the studied years. The year impact on IHTP was much weaker ($p < 0.05$) compared to the impact of the group ($p < 0.001$) (Table 1). The group showed a clearly significant impact ($p < 0.001$) on time 1–2 and the main dough development phase parameters (peak time, peak width, and build width) (Table 1).

From the Tukey test, the major significant differences between the C and HD years were found for the initial slope and initial width among S and VS groups, and time 1–2 for M group (Table 2). In Group S, the strongest impacts of the different years were for peak height, width built, IHTP, and water absorption (Table 2). The HD year had a stronger impact only on width build in this group (compared with group S-C). Meanwhile, in group MS, the HD year showed a greater impact on time 1–2 and peak time compared to the C year. No impact of HD year was noted in Group VS, indicating that the C year caused higher values for initial slope and initial width (Table 2).

2.2. Variation in Dough Mixing Characteristics among the Groups

The subtracted values between the C and HD years were compared between the groups to show data intervals and to refer this to lower variability (eventual stability). The mixing parameters responsible for dough development and water absorption (peak time, initial width, initial build width, and water absorption) are shown in Figure 1. The studied groups showed similar data distribution between the years, however, some minor differences were observed for the selected mixing parameters. Smaller data distribution intervals between the years were observed for the peak time and initial build width for Groups S and MS compared to Group VS (Figure 1A,C), indicating lower data variation

between the years. The different years did not impact the initial width among the groups, although a greater data variation interval was found for Group VS compared to the other groups (Figure 1B). Water absorption is an important dough mixing parameter largely dependent on gluten protein properties and amounts, and the data distribution was found to be similar between Groups S and VS (Figure 1B,D). This indicates that genotypes with varying mixing stability are present across all groups in this study. In conclusion, Groups S and MS are the main groups that offer genotypes with less variation in some of the mixing properties, while Group VS includes genotypic material with mixing properties that vary more broadly.

Table 2. Tukey's post hoc test ($p < 0.05$) of dough mixing characteristics of groups (S—stable, MS—moderately stable, and VS—varying stability) of 56 spring wheat genotypes grown in cool (C, 2017) and heat–drought (HD, 2018) years. Data are presented as means ± standard errors, calculated from three replicates for each parameter.

	Group S-C	Group S-HD	Group MS-C	Group MS-HD	Group VS-C	Group VS-HD
Water absorption						
Initial slope	4.53 ± 0.07 ab	4.23 ± 0.07 c	4.33 ± 0.10 abc	4.40 ± 0.10 abc	4.64 ± 0.11 a	4.27 ± 0.11 cb
Initial width	1.23 ± 0.02 ab	1.12 ± 0.02 cd	1.14 ± 0.04 abcd	1.15 ± 0.04 abcd	1.24 ± 0.04 ac	1.12 ± 0.04 bd
Initial buildup	3.47 ± 0.07 a	3.13 ± 0.08 b	3.41 ± 0.12 ab	3.30 ± 0.12 ab	3.23 ± 0.13 ab	3.26 ± 0.13 ab
Time 1–2	1.65 ± 0.08 ab	1.57 ± 0.08 b	1.85 ± 0.12 b	2.05 ± 0.12 a	1.67 ± 0.13 ab	1.81 ± 0.13 ab
Initial build width	1.97 ± 0.05 a	1.84 ± 0.05 b	1.92 ± 0.07 ab	1.89 ± 0.07 ab	1.84 ± 0.07 ab	1.90 ± 0.07 ab
Dough development						
Buildup	1.46 ± 0.06 a	1.50 ± 0.07 a	1.57 ± 0.10 a	1.37 ± 0.09 a	1.48 ± 0.10 a	1.69 ± 0.10 a
Peak time	3.80 ± 0.16 ab	3.65 ± 0.15 b	4.20 ± 0.23 b	4.60 ± 0.25 a	3.85 ± 0.24 ab	4.12 ± 0.24 ab
Peak height	5.50 ± 0.09 a	5.10 ± 0.10 b	5.54 ± 0.14 ab	5.31 ± 0.15 ab	5.38 ± 0.15 ab	5.44 ± 0.16 ab
Peak width	3.37 ± 0.05 a	3.30 ± 0.05 a	3.22 ± 0.08 a	3.13 ± 0.08 a	3.32 ± 0.08 a	3.30 ± 0.08 a
Width build	0.17 ± 0.04 b	0.33 ± 0.04 a	0.15 ± 0.06 ab	0.09 ± 0.06 b	0.24 ± 0.06 ab	0.28 ± 0.06 ab
Breakdown phase						
Breakdown	0.70 ± 0.09 a	0.65 ± 0.09 a	0.59 ± 0.15 a	0.59 ± 0.15 a	0.69 ± 0.15 a	0.69 ± 0.15 a
IHTP	13.8 ± 0.72 a	12.1 ± 0.72 b	15.2 ± 1.12 ab	16.7 ± 1.12 a	13.6 ± 1.16 ab	14.4 ± 1.16 ab
Water absorption	6.60 ± 0.03 a	6.49 ± 0.03 bc	6.63 ± 0.04 ab	6.45 ± 0.04 c	6.62 ± 0.05 abc	6.60 ± 0.04 abc

Note: Different letters (a, b, c and d) indicate significant differences among the groups and years for each parameter. The mixograph parameters (initial slope, initial width, initial buildup, build width, buildup, peak height, peak width, width build, breakdown, and IHTP) are measured as torque (N·m); mixing time (time 1–2 and peak time) is measured in minutes; water absorption is measured in ml/10 g flour.

2.3. Relationships between the Mixograph Parameters and the Gluten Proteins

PCA analysis was performed to investigate relationships between the mixing characteristics of dough and the gluten protein characteristics of flour (Figure 2). PCA results showed that PC1 and PC2 explained 27.9% and 17.1% variability, respectively (Figure 2). The gluten protein parameters describing large polymeric proteins (%UPP, TOTU, %LUPP) and large monomers (%LUMP) were most closely associated only with buildup and water absorption, parameters that are strongly related to protein content of the flour (Figure 2). The mixing parameters such as end height, time 1–2, peak time, and IHTP were those more closely associated with the large polymeric and monomeric proteins.

The protein content indicating parameters such as, total SDS-extractable proteins (TOTE) and ratio of monomers to polymers (Mon/pol) were positioned in the opposite direction of %UPP, %LUPP, total SDS-unextractable proteins (TOTU) and %LUMP, showing a negative correlation between the parameters compared. Initial slope and initial width were the most closely positioned mixing parameters to TOTE and Mon/pol, followed by break down, peak width, and end width (Figure 2). Total amount of monomeric proteins (TMP) was the most closely related to the mixing parameters responsible for dough development, such as area within, initial build width, initial buildup, and peak height.

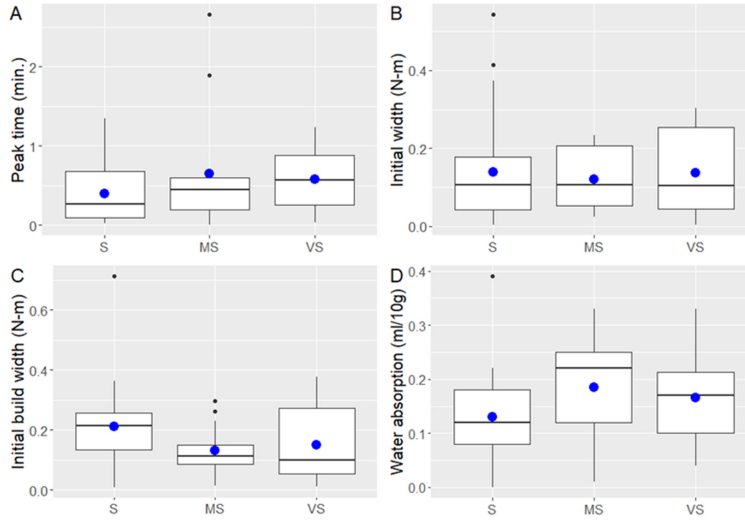


Figure 1. Differences (subtracted between the years) in the dough mixing parameters of 56 spring wheat genotypes grown in the cool (2017) and heat–drought (2018) years: peak time (**A**), initial width (**B**), initial build width (**C**), and water absorption (**D**). Peak time is expressed as minutes (min.), initial width and initial build width are expressed as mixing torque in N·m, water absorption is expressed in ml/10 g of flour. Blue dots represent mean values.

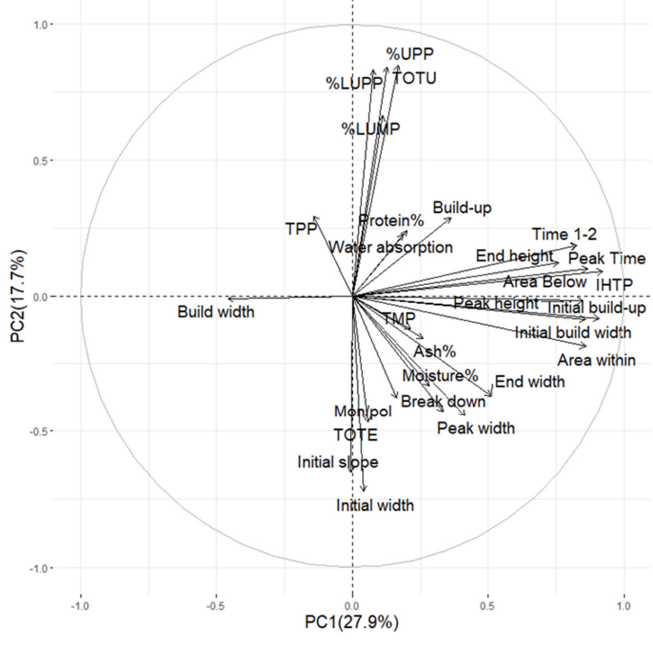


Figure 2. Principal component analysis (PCA) plot of mixograph parameters in dough and protein composition parameters in flour of 56 spring wheat genotypes grown in cool (2017) and heat–drought (2018) years evaluated by SE-HPLC; protein (%), ash (%), and flour moisture (%) were determined in flour by NIT (taken from Lama et al., 2022).

From Spearman's correlation analysis, the highest significant correlations were found between the water absorption and TMP (0.79), TOTE (0.59), TOTU (0.59), and Mon/pol (0.49) (Figure 3). The mixing parameters, buildup, end height, and peak height showed significant correlations of 0.39, 0.43, and 0.42 with protein content, respectively. The highest negative significant correlations were observed between the initial width and %LUMP (−0.46) and %UPP (−0.43) (Figure 3).

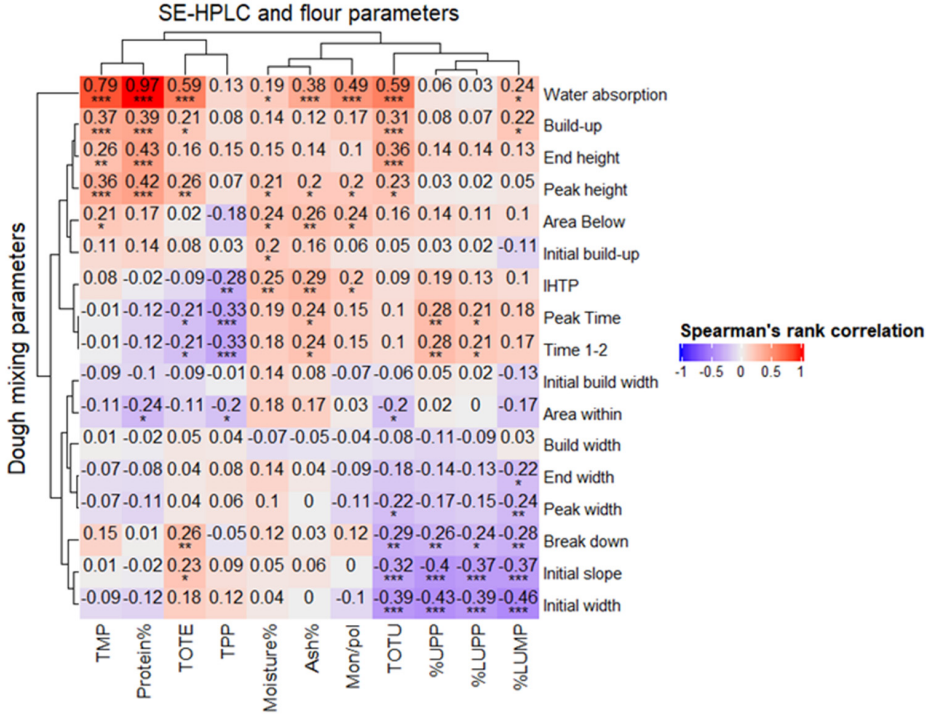


Figure 3. Spearman's rank correlation matrix and hierarchical clustering of results (dendogram) based on complete linkage method for the gluten protein parameters obtained by SE-HPLC; flour protein (%), moisture (%), and ash (%) determined by NIT (taken from Lama et al. 2022) and 17 dough mixing parameters of 56 spring wheat genotypes grown in cool (2017) and heat–drought (2018) years. ***, **, and * indicate significance at $p < 0.001$, $p < 0.01$, and $p < 0.05$.

2.4. Variation of Monomeric and Polymerics Proteins in Dough under Diverse Growing Conditions

The diverse growing conditions of the studied years impacted the monomeric and polymeric gluten proteins in dough differently, as shown by SE-HPLC (Figure 4). Heat and drought stress resulted in lower values in both TMP and TPP in most of the samples from all groups. The least variation in TMP between the wheat genotypes and between the years accounted for around 50% of the studied genotypes in Group S (Figure 4A). A great number of genotypes from Group S showed similar TPP values between the years (indicating robustness to prolonged heat and drought) as compared to Groups MS and VS (Figure 4B). Several genotypes from Group VS showed rather similar TMP and TPP values in both studied years, showing certain robustness potential to the contrasting growing conditions of these genotypes.

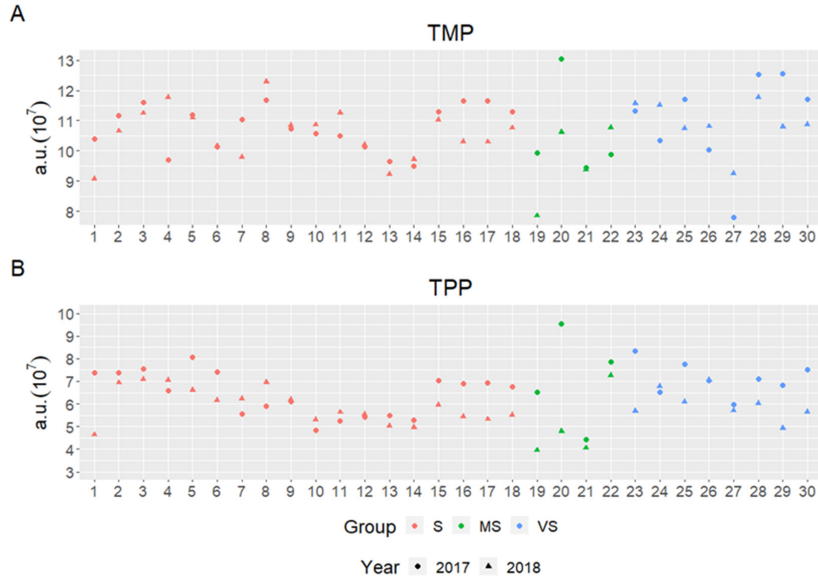


Figure 4. Total amount of monomeric proteins (TMP) (**A**) and total amount of polymeric proteins (TPP) (**B**) in dough of wheat samples shown in Groups S, MS, and VS of 30 spring wheat genotypes grown in cool (2017) and heat–drought (2018) years.

The amount of polymeric proteins (%UPP) in dough was compared between the diverse groups and years, and higher values were observed for the majority of wheat genotypes from the HD year as compared to the C year in all the groups (Figure 5). The highest %UPP in dough was around 15% for several wheat genotypes from Groups S and MS. The wheat genotypes that were least impacted by the different years (under 3% difference) were 1, 3, 4, 5, 7, 13, 14, and 18 from Group S; all the genotypes from Group MS; and two genotypes (24 and 26) from Group VS (Figure 5). From these, the genotypes 3, 13, and 14 were those that also showed similar TMP and TPP values compared with other genotypes in this study (Figure 4).

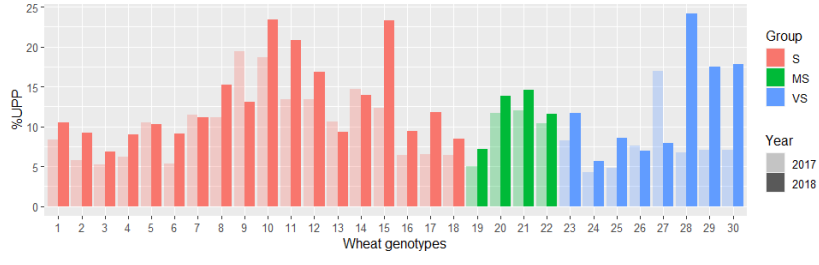


Figure 5. Unextractable polymeric protein (%UPP) in wheat dough samples in Groups S, MS, and VS of 30 spring wheat genotypes grown in cool (2017) and heat–drought (2018) years.

3. Discussion

3.1. Environment-Induced Changes in Dough Mixing Quality

The impact of heat and drought stress on wheat dough quality has been vaguely investigated, with the few studies performed so far indicating roughly equal impacts from the wheat genotype and the growing environment [28,29]. In the present study, we had unique excessive growing conditions for the Nordic climate, such as prolonged drought

and heat in 2018, which positively affected the gluten strength in flour for a number of genotypes [5], and a similar trend was observed in dough for some of the mixing parameters of the individual genotypes. In this study, interestingly, we observed that the excessive heat and drought (i.e., the year) did not impact the two mixing parameters responsible for dough strength and development (time 1–2 and peak time). This observation was different to the previously reported trends such as heat stress induced dough weakening [30,31]. A possible explanation is that differential changes occurring in the size and amount of polymeric proteins are negatively compensated by the lower protein content in the excessive growing environment [5].

It is important to point out that, in this study, the interaction between the wheat genotype, year, and group made a larger impact on the dough mixing parameters than the group and year individually, confirming that the environmental events are an important source of variation. This was also previously found for the dough mixing time, which was found to be largely influenced by the interaction between the wheat genotypes and environment [32]. In fact, in our study, the strongest impact from genotype × environment was observed on IHTP, which can be used as a dough strength and development indicator, and in several studies this parameter has been shown to have a good correlation with bread volume. Previous studies have also shown that buildup and peak time are closely linked to the gluten strength [12,21,33], while water absorption is highly correlated with the protein content [18], a trend that was also shown in our study. The peak time and buildup were among those mostly related to the polymeric and large monomeric gluten proteins (i.e., %UPP, TOTU, %LUPP, and %LUMP). Furthermore, time 1–2, peak time, peak width, and IHTP were parameters strongly correlated with the group and genotype. Another aspect strengthening the impact of the interactions group × genotype and group × year involves a clear impact of the genotype in this study. Our results align well with the concept that dough optimum mixing time and torque are parameters controlled by a strong genetic factor [29]. This also explains why the year in this study did not impact time 1–2 or peak time. Since dough mixing time is an indicator of the wheat flour strength [34] and peak time is known to be controlled by the gluten protein composition [35,36], it seems that mixing time and time 1–2 are parameters that might have some potential in wheat genotype prediction in excessive growing environments. However, there remains a key point to understand: where is the important switch putting genetics over environment and vice versa? Some of the water absorption phase parameters, such as initial width and initial slope, were closely related to protein content (as TOTE) in this study. Indeed, polymeric glutenins are known to correlate positively with peak time, while monomeric gliadins show negative impact and weakening of dough [37,38], overall, the protein content decreased in the excessive heat and drought year. Sufficient hydration of wheat flour particles and proteins therein facilitates optimum gluten development during mixing [39]. From the difference in water absorption between the years, which decreased considerably between the groups during the prolonged heat–drought year and varied mostly within the groups in the different years (Table 2), it can be assumed that gluten protein qualitative and quantitative components differed greatly between the years. The higher protein content of flour and weaker gluten in the cool year and an opposite trend for heat and drought stress suggest that water absorption and mixing time might not be optimal for such flours and should be further explored. Extreme mixing (i.e., overmixing) to evaluate hydration of the flour and individual tuning of water amount according to gluten strength should be further studied and not overlooked. Similar findings were also observed in wheat flours used in noodle dough by Liu et al. [40], where flours with different protein contents and gluten strengths behaved differently during mixing.

3.2. Growing Conditions-Induced Variation between the Groups

In our latest study [5] and a few other studies [28,29], we pointed out that either heat or combined heat and drought stress favor gluten strength. When comparing the differences between the years and the groups in this study, we observed this favoring trend for a great

number of mixing parameters in the studied groups (initial slope, initial width, initial buildup, initial build width, peak height, width build, IHTP, and water absorption in Group S; time 1–2, peak time, and water absorption in Group MS; and initial slope and initial width in Group VS (Table 2). In general, we did not see clear differences in the mixing characteristics between the groups for most of the mixing parameters in this study, except the peak time and water absorption (Figure 1), most likely due to the fact that differentiation was mainly based on the polymeric proteins that were used in PCA differentiation and grouping. A reason for this choice was based on Swedish baking industries' needs for high-gluten strength flour, which is further used as fortifying "material" in blends with weaker wheat flours. The produced wheat flour blends are further processed into different types of bread and other wheat products, which is a common practice in Sweden and other countries in Europe.

The clear impact of heat and drought stress on most of the mixing parameters observed for Group S, which differed for Groups MS and VS, could be explained by higher variation in %UPP in the majority of wheat genotypes and the larger size of Group S. The sensitivity of certain wheat genotypes from Group S to heat and drought stress (such as nr 7) led to a decrease in %UPP in the flour from 66.91% to 61.86%, which could also be one of explanations for observed differences in the dough. Smaller variation in peak time and initial buildup in Group MS indicates similar dough development and water absorption patterns, and these parameters can be suggested for further screening tests.

Heat and drought reduced the glutenin content (and moisture of flour) in this study, indicating a similar tendency observed in other studies [41,42]. However, this trend is rather uncommon when compared with a significant rise in grain protein due to heat, which was reported in several other studies [30,43,44].

3.3. Relation between the Mixing Parameters and Protein Composition

Mixograph parameters are important in predicting bread baking performance, and have been shown to around 90% correlate to bread volume. The bread baking process is a time- and resource-consuming process, and if well correlated with protein composition, can be a very useful tool in breeding. Among mixing parameters, the greatest positive correlations (0.79 and 0.59) were observed between the water absorption and the monomeric gluten proteins (i.e., TMP and TOTE) and polymeric proteins (i.e., TOTU) (Figure 3), which suggests a close relationship between the studied parameters; as such, they might potentially be further used in flour and dough prediction studies. Negative correlations (-0.43 and -0.46) between the initial width and initial slope and gluten strength (i.e., %UPP) as well as %LUMP shown in this study can be explained by the decrease in protein content. More thorough correlation studies taking into account repetitive measurements of the relationships between the mixing and protein composition parameters should be performed. Besides the initial experiments we performed in this study, water absorption, TMP, TOTE, and TOTU may be valuable parameters for investigating genotype selection under diverse growing conditions.

In this study, we observed differences in the gluten protein composition of dough between the years for the total polymeric protein fraction (TPP) (Figure 5), which confirms different genotypes' sensitivity to the excessive growing environment originating from genetic makeups [26]. It is important to point out that much lower %UPP values were observed in dough in comparison to those in flour for all the groups [5]. This indicates that mixing action might have been insufficient to contribute to the optimum development of a protein network, where a matrix of glutenins and gliadins develops. The monomeric and polymeric types of gluten from the heat and drought year were larger in size and complexity than those from the cool year, as was observed in flour by Lama et al. [5]. Meanwhile, in dough, these differences were expected to be more pronounced, and were possibly the main reason for very different strengths of gluten networks under the studied growing conditions. Different mixograph dough development curves in terms of shape were observed between the years for more than half of the studied wheat material. Width of

the mixograph curve can commonly be related to dough extensibility and mixing tolerance, whereas height of the curve represents dough strength and consistency [45,46]. In fact, in this study, initial slope and initial width were negatively correlated with %UPP, %LUPP, and %LUMP, indicating a negative relation with dough extensibility. A negative correlation between the gluten polymeric proteins (LMW-GSs) and dough extensibility during severe heat and drought stress was also found by Phakela et al. [47].

In breeding, screening of wheat genotypes according to mixing properties and gluten protein properties (e.g., %UPP and other characteristics of protein) is very important, and this study reveals a number of parameters that might be important in varying growing environments, including excessive heat and drought. However, it should not be forgotten that not only flour and dough, but also bread properties should be considered and evaluated in further techno-functional studies of wheat plant materials. To conclude, in this study, the key parameters to consider in further investigations and screening are time 1–2, peak time and water absorption. These parameters are known for their ability to retain gas in the dough during proofing and baking, and thus are related to the bread volume [48]. Other parameters such as buildup, initial slope, and initial width might also be important in selecting wheat genotypes for less-variable mixing quality. The mixing parameters' screening and tuning should be performed in relation to qualitative and quantitative gluten protein characteristics such as TOTE, TOTU, %UPP, %LUPP, and to some extent Mon/pol in both flour and dough.

4. Materials and Methods

4.1. Plant Growing Environment

This study is an extension of our previous study on 294 spring wheat genotypes grown in 2017 and 2018 [5]. In this study, 56 spring wheat genotypes were used in this study to investigate dough mixing characteristics. The growing seasons in 2017 and 2018 were designated as cool (C) and prolonged heat–drought (HD), respectively. The extreme prolonged HD season was designated due to the higher temperature (6–11 °C higher than the average) and its unusual length lasting from May until grain harvesting in August 2018 (Figure 6).

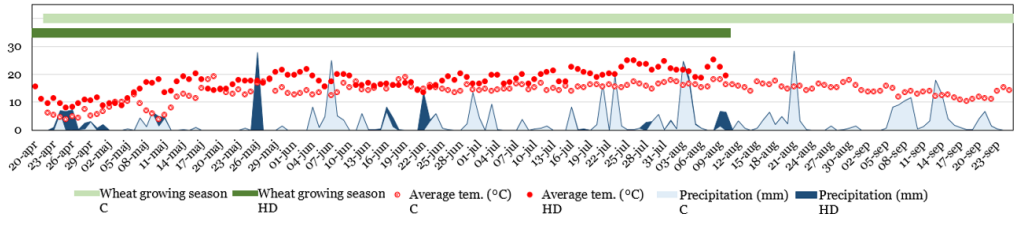


Figure 6. Growing conditions of 56 wheat genotypes grown in two varying environments used in this study; average temperature (°C) and precipitation (mm) during the wheat growing seasons (C—cool, 2017; HT—heat–drought, 2018) expressed as number of days.

The genotypes were grown in a lattice design with two replicates in the C year and a modified augmented design 2 (MAD2) with one replicate in the HD year. Out of two replicates produced, plants from one replicate were used for the C year in order to compare with the HD year. Specific details regarding the growing conditions of the plants in the C and HD years are included in Lama et al. (2022).

4.2. Flour Materials

The flour of 56 genotypes used in this study were divided into three groups according the variance of the gluten protein parameters %UPP, TOTE, TOTU, %LUPP, and Mon/pol measured by SE-HPLC [12,13] in C and HD environments (data obtained in our previous study by Lama et al. 2022) in PCA analysis. Three PCA distance intervals were designated

as follows: (1) stable (S) group (31 genotypes; interval 0.17–1.42; %UPP $\leq$ 5%), (2) moderately stable (MS) group (13 genotypes; interval 1.45–2.57; %UPP $\leq$ 12%), and (3) varying stability (VS) group (12 genotypes; interval 2.58–9.10; %UPP $\leq$ 17%).

4.3. Dough Mixing Using Mixograph

The whole wheat flour (10 g) of 56 genotypes from the two years was mixed with water into a dough using a mixograph (Bohlin Reologi AB, Lund, Sweden), and mixing was performed at 26 °C [49]. Each flour sample was mixed for 10 min in order to determine the optimum mixing time. Two replicates of dough mixed to the optimum time were used in this study. The dough samples after mixing were stored immediately at −80 °C and freeze-dried (Cool safe Pro, LaboGene, Denmark) afterwards for 48 h. Freeze-dried dough samples were ground into a fine powder using a grinder (Yellow line, A10, IKA-Werke, Staufen, Germany) and used for further analysis.

Seventeen dough parameters were obtained from mixograph curve for the wheat genotype Mirakel grown in the C year (Figure 7). The parameters are (1) initial slope (A1/T1), (2) initial width (A1−B1), (3) initial buildup (A2−A1), (4) time 1–2 (T2−T1), (5) initial build width (A2−B2)−(A1−B1), (6) buildup (A3−A2), (7) peak time (T3), (8) peak width (A3−B3), (9) peak height ((A3+B3)/2), (10) width build (A3−B3)−(A2−B2), (11) break down (A3−A4), (12) end width (A5−B5), (13) end height ((A5+B5)/2), (14) area below (A1−A5), (15) area within (area between A1−A5 and B1−B5), (16) IHTP (integrated height to the peak), and (17) water absorption (obtained according to Wikström and Bohlin [14]). The process of mixing dough was divided into three phases, designated as water absorption (parameters 1–5), dough development (parameters 6–10), and break down of dough (parameter 11) [14,50]. Overall, 13 mixing parameters were used for evaluating the effect of year and groups, and 17 mixing parameters were used for Spearman's rank correlations between the protein parameters studied by SE-HPLC and flour protein content determined by NIT; gluten protein and flour parameters borrowed from Lama et al. (2022) were used in this study for Spearman's rank correlations.

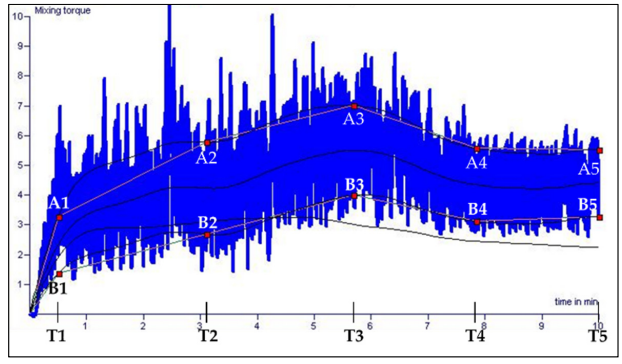


Figure 7. Mixograph curve for wheat genotype Mirakel grown in the cool year (2017).

4.4. Size Exclusion High-Performance Liquid Chromatography of Doughs (SE-HPLC)

Thirty genotypes were selected for gluten protein analysis of dough by SE-HPLC. The selected wheat materials comprised 18 genotypes from Group S, 4 genotypes from Group MS, and 8 genotypes from Group VS.

Freeze-dried dough samples were blended with the buffer and two step extractions were performed to investigate the gluten protein polymerization (extractability) in dough according to Lama et al. [5] and Kuktaite et al. [12,51], with some modifications. The modifications were as follows: after first extraction (1Ex, referred to as SDS-extractable protein) and second extraction (2Ex, referred to as SDS-unextractable protein), the supernatants were collected in SE-HPLC vials and heated at 80 °C for 2 min (to inactivate proteases) in a

water bath according to Islas-Rubio et al. [52]. Immediately after heating, the vials were cooled down in ice cold water for 1 min, followed by SE-HPLC analysis.

We injected 20 µLof extracted proteins from 1Ex and 2Ex into an SE-HPLC column (BIOSEP SEC-4000 Phenomenex column), which were separated for 30 min in a solution of 50% acetonitrile with 0.1% trifluoroacetic acid (TFA). The extracted chromatograms at 210 nm UV wavelength were divided into four areas according to the retention times of different gluten proteins. The gluten protein parameters of TOTE, TOTU, %UPP, %LUPP, percentage of large unextractable monomer into total large monomer (%LUMP), and mon/pol were calculated according to Lama et al. [5]. Total polymeric proteins (TPP) and total monomeric proteins (TMP) were calculated as LPP+SPP+LPPs+SPPs and LMP+SMP+LMPs+SMPs, respectively.

4.5. Statistical Analysis

The statistical analysis was performed using the software R (https://www.r-project.org/). Three way ANOVA was conducted for evaluating the impact of the group (S, M, and VS) and year (C and HT), and the interactions of group × genotype and genotype × year × group on the mixing parameters (for this analysis, "wheat genotypes" were nested within the "group"; since each wheat genotype belongs to exactly one level of group, group and wheat genotype effects were not differentiated). For water absorption (ml/10 g flour) the values did not differ between the replicates and therefore average value was used for two way ANOVA analysis. Tukey's post hoc test, PCA, and Spearman's rank correlation were performed to study the variation of dough mixing parameters in the C and HT years.

5. Conclusions

Striving for stability of wheat quality characteristics in varying and excessive growing environmental conditions is essential in wheat breeding programs. From this point of view, there is a continuous interest to define wheat quality parameters that are most reproducible and are more influenced by genotype than by growing conditions. In this context, from our study, there was a significant impact of the year on most of the dough mixing parameters, except time 1–2, buildup, and peak time. These parameters were strongly impacted by the genetic background and might be very useful in the screening of wheat material from contrasting environments. Therefore, in screening procedures of wheat breeding activities, the interaction of genotype × environment should be thoroughly explored.

The varying growing conditions were the main factor causing differences in the dough mixing parameters within the studied groups (S, MS, and VS) and minor differences between the groups. In Groups S and MS, the majority of wheat genotypes showed less variation in dough mixing characteristics such as peak time, initial width, initial build width, and water absorption, which could be related with the gluten strength and dough development. Group VS included genotypic material which broadly varied in the mixing characteristics.

The gluten protein parameters for the large polymeric proteins (%UPP, TOTU, %LUPP) and the large monomers (%LUMP) in the flour showed a close association with the buildup and water absorption in dough, indicating their potential to be used as screening parameters for wheat dough stability. However, further studies are needed to better fine tune those gluten protein parameters for diverse excessive growing environments and different dough mixing conditions (e.g., overmixing and optimal hydration).

To sum up, screening of wheat genotypes according to the dough mixing characteristics and the gluten protein parameters (e.g., %UPP and others) is very important, and this study reveals a number of parameters that might be important to focus on in contrasting growing environments. However, screening of wheat quality properties in flour, dough, and bread should be evaluated in further techno-functional studies of wheat plant materials.

Author Contributions: Conceptualization, R.K. and S.L.; methodology, R.K., M.K. and S.L.; software, S.L.; validation, S.L.; formal analysis, S.L.; investigation, S.L.; resources, P.V. and R.K.; data curation, S.L. and R.K.; writing—original draft preparation, S.L.; writing—review and editing, S.L., R.K. and P.V.; visualization, S.L.; supervision, R.K.; project administration, R.K.; funding acquisition, R.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by SLU Grogrund center for plant breeding of food crops, Trees and Crops for the Future (TC4F), and Partnerskap Alnarp.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are openly available from the first author and can be accessed upon reasonable request.

Acknowledgments: We thank Maria Luisa Prieto-Linde and Anders Ekholm for providing technical and laboratory assistance.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rekowski, A.; Wimmer, M.A.; Tahmasebi, S.; Dier, M.; Kalmbach, S.; Hitzmann, B.; Zörb, C. Drought stress during anthesis alters grain protein composition and improves bread quality in field-grown Iranian and German wheat genotypes. *Appl. Sci.* **2021**, *11*, 9782. [CrossRef]
2. Souza, E.J.; Martin, J.M.; Guttieri, M.J.; O'Brien, K.M.; Habernicht, D.K.; Lanning, S.P.; McLean, R.; Carlson, G.R.; Talbert, L.E. Influence of genotype, environment, and nitrogen management on spring wheat quality. *Crop Sci.* **2004**, *44*, 425–432. [CrossRef]
3. Rakszegi, M.; Darkó, É.; Lovegrove, A.; Molnár, I.; Láng, L.; Bedő, Z.; Molnár-Láng, M.; Shewry, P. Drought stress affects the protein and dietary fiber content of wholemeal wheat flour in wheat/Aegilops addition lines. *PLoS ONE* **2019**, *14*, e0211892. [CrossRef]
4. Lan, Y.; Chawade, A.; Kuktaite, R.; Johansson, E. Climate change impact on wheat performance—Effects on vigour, plant traits and yield from early and late drought stress in diverse lines. *Int. J. Mol. Sci.* **2022**, *23*, 3333. [CrossRef]
5. Lama, S.; Vallenback, P.; Hall, S.A.; Kuzmenkova, M.; Kuktaite, R. Prolonged heat and drought versus cool climate on the Swedish spring wheat breeding lines: Impact on the gluten protein quality and grain microstructure. *Food Energy Secur.* **2022**, *11*, e376. [CrossRef]
6. Jiang, D.; Yue, H.; Wollenweber, B.; Tan, W.; Mu, H.; Bo, Y.; Dai, T.; Jing, Q.; Cao, W. Effects of post-anthesis drought and waterlogging on accumulation of high-molecular-weight glutenin subunits and glutenin macropolymers content in wheat grain. *J. Agron. Crop Sci.* **2009**, *195*, 89–97. [CrossRef]
7. Gao, X.; Tong, J.; Guo, L.; Yu, L.; Li, S.; Yang, B.; Wang, L.; Liu, Y.; Li, F.; Guo, J.; et al. Influence of gluten and starch granules interactions on dough mixing properties in wheat (*Triticum aestivum* L.). *Food Hydrocoll.* **2020**, *106*, 105885. [CrossRef]
8. Cao, X.; Tong, J.; Ding, M.; Wang, K.; Wang, L.; Cheng, D.; Li, H.; Liu, A.; Liu, J.; Zhao, Z.; et al. Physicochemical properties of starch in relation to rheological properties of wheat dough (*Triticum aestivum* L.). *Food Chem.* **2019**, *297*, 125000. [CrossRef]
9. Gupta, R.B.; Khan, K.; Macritchie, F. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. *J. Cereal Sci.* **1993**, *18*, 23–41. [CrossRef]
10. Johansson, E.; Nilsson, H.; Mazhar, H.; Skerritt, J.; MacRitchie, F.; Svensson, G. Seasonal effects on storage proteins and gluten strength in four Swedish wheat cultivars. *J. Sci. Food Agric.* **2002**, *82*, 1305–1311. [CrossRef]
11. Ceresino, E.B.; Johansson, E.; Sato, H.H.; Plivelic, T.S.; Hall, S.A.; Kuktaite, R. Morphological and structural heterogeneity of solid gliadin food foams modified with transglutaminase and food grade dispersants. *Food Hydrocoll.* **2020**, *108*, 105995. [CrossRef]
12. Kuktaite, R.; Larsson, H.; Johansson, E. Variation in protein composition of wheat flour and its relationship to dough mixing behaviour. *J. Cereal Sci.* **2004**, *40*, 31–39. [CrossRef]
13. Malik, A.H.; Prieto-Linde, M.L.; Kuktaite, R.; Andersson, A.; Johansson, E. Individual and interactive effects of cultivar maturation time, nitrogen regime and temperature level on accumulation of wheat grain proteins. *J. Sci. Food Agric.* **2011**, *91*, 2192–2200. [CrossRef] [PubMed]
14. Wikstrom, K.; Bohlin, L. Multivariate analysis as a tool to predict bread volume from mixogram parameters. *Cereal Chem.* **1996**, *73*, 686–690.
15. Bordes, J.; Branlard, G.; Oury, F.X.; Charmet, G.; Balfourier, F. Agronomic characteristics, grain quality and flour rheology of 372 bread wheats in a worldwide core collection. *J. Cereal Sci.* **2008**, *48*, 569–579. [CrossRef]
16. Caffe-Treml, M.; Glover, K.D.; Krishnan, P.G.; Hareland, G.A. Variability and relationships among mixolab, mixograph, and baking parameters based on multienvironment spring wheat trials. *Cereal Chem.* **2010**, *87*, 574–580. [CrossRef]
17. Tozatti, P.; Güldiken, B.; Fleitas, M.C.; Chibbar, R.N.; Hucl, P.; Nickerson, M.T. The interrelationships between wheat quality, composition, and dough rheology for a range of Western Canadian wheat cultivars. *Cereal Chem.* **2020**, *97*, 1010–1025. [CrossRef]

18. Ohm, J.B.; Chung, O.K. Gluten, pasting, and mixograph parameters of hard winter wheat flours in relation to breadmaking. *Cereal Chem.* **1999**, *76*, 606–613. [CrossRef]
19. Bohlin, L. Method for Determining the Quality of Cereals. U.S. Patent No. 6,548,091 B2, 2003.
20. Ohm, J.B.; Dykes, L.; Graybosch, R.A. Variation of protein molecular weight distribution parameters and their correlations with gluten and mixing characteristics for winter waxy wheat. *Cereal Chem.* **2019**, *96*, 302–312. [CrossRef]
21. Kuktaite, R. Protein Quality in Wheat: Changes in Protein Polymer Composition during Grain Development and Dough Processing. PhD Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden, 2004.
22. Flagella, Z.; Giuliani, M.; Giuzio, L.; Volpi, C.; Masci, S. Influence of water deficit on durum wheat storage protein composition and technological quality. *Eur. J. Agron.* **2010**, *33*, 197–207. [CrossRef]
23. Zhang, X.; Cai, J.; Wollenweber, B.; Liu, F.; Dai, T.; Cao, W.; Jiang, D. Multiple heat and drought events affect grain yield and accumulations of high molecular weight glutenin subunits and glutenin macropolymers in wheat. *J. Cereal Sci.* **2013**, *57*, 134–140. [CrossRef]
24. Blumenthal, C.S.; Batey, I.L.; Bekes, F.; Wrigley, C.W.; Barlow, E.W.R. Seasonal changes in wheat-grain quality associated with high temperatures during grain filling. *Aust. J. Agric. Res.* **1991**, *42*, 21–30. [CrossRef]
25. Maphosa, L.; Langridge, P.; Taylor, H.; Emebiri, L.C.; Mather, D.E. Genetic control of grain protein, dough rheology traits and loaf traits in a bread wheat population grown in three environments. *J. Cereal Sci.* **2015**, *64*, 147–152. [CrossRef]
26. Fleitas, M.C.; Mondal, S.; Gerard, G.S.; Hernández-Espinosa, N.; Singh, R.P.; Crossa, J.; Guzmán, C. Identification of CIMMYT spring bread wheat germplasm maintaining superior grain yield and quality under heat-stress. *J. Cereal Sci.* **2020**, *93*, 102981. [CrossRef]
27. Sakr, N.; Rhazi, L.; Aussenac, T. Bread wheat quality under limiting environmental conditions: II—Rheological properties of Lebanese wheat genotypes. *J. Saudi Soc. Agric. Sci.* **2021**, *20*, 235–242. [CrossRef]
28. Li, Y.; Wu, Y.; Hernandez-Espinosa, N.; Peña, R.J. The influence of drought and heat stress on the expression of end-use quality parameters of common wheat. *J. Cereal Sci.* **2013**, *57*, 73–78. [CrossRef]
29. Guzmán, C.; Autrique, J.E.; Mondal, S.; Singh, R.P.; Govindan, V.; Morales-Dorantes, A.; Posadas-Romano, G.; Crossa, J.; Ammar, K.; Peña, R.J. Response to drought and heat stress on wheat quality, with special emphasis on bread-making quality, in durum wheat. *Field Crops Res.* **2016**, *186*, 157–165. [CrossRef]
30. Wrigley, C.; Blumenthal, C.; Gras, P.; Barlow, E. Temperature variation during grain filling and changes in wheat-grain quality. *Funct. Plant Biol.* **1994**, *21*, 875–885. [CrossRef]
31. Tahir, I.S.A. Genotypic and temperature effects on wheat grain yield and quality in a hot irrigated environment. *Plant breeding* **2006**, *125*, 323–330. [CrossRef]
32. Yong, Z.; Zhonghu, H.; Ye, G.; Aimin, Z.; Van Ginkel, M. Effect of environment and genotype on bread-making quality of spring-sown spring wheat cultivars in China. *Euphytica* **2004**, *139*, 75–83. [CrossRef]
33. Khatkar, B.S.; Bell, A.E.; Schofield, J.D. A comparative study of the inter-relationships between mixograph parameters and bread-making qualities of wheat flours and glutens. *J. Sci. Food Agric.* **1996**, *72*, 71–85. [CrossRef]
34. Boyacioglu, M.; D'appolonia, B. Characterization and utilization of durum wheat for breadmaking. I. Comparison of chemical, rheological, and baking properties between bread wheat flours and durum wheat flours. *Cereal Chem.* **1994**, *71*, 21–27.
35. Labuschagne, M.T.; Moloi, M.J. The influence of abiotic stress conditions on dough mixing characteristics of two hard red spring wheat cultivars. *J. Cereal Sci.* **2015**, *63*, 134–139. [CrossRef]
36. Hussain, A.; Larsson, H.; Kuktaite, R.; Prieto-Linde, M.L.; Johansson, E. Towards the understanding of bread-making quality in organically grown wheat: Dough mixing behaviour, protein polymerisation and structural properties. *J. Cereal Sci.* **2012**, *56*, 659–666. [CrossRef]
37. MacRitchie, F. Evaluation of contributions from wheat protein fractions to dough mixing and breadmaking. *J. Cereal Sci.* **1987**, *6*, 259–268. [CrossRef]
38. Khatkar, B.S.; Fido, R.J.; Tatham, A.S.; Schofield, J.D. Functional properties of wheat gliadins. I. Effects on mixing characteristics and bread making quality. *J. Cereal Sci.* **2002**, *35*, 299–306. [CrossRef]
39. Hou, G.G.; Otsubo, S.; Okusu, H.; Shen, L. Noodle Processing Technology. In *Asian Noodles*; John Wiley & Sons: Hoboken, NJ, USA, 2010; pp. 99–140.
40. Liu, R.; Xing, Y.; Zhang, Y.; Zhang, B.; Jiang, X.; Wei, Y. Effect of mixing time on the structural characteristics of noodle dough under vacuum. *Food Chem.* **2015**, *188*, 328–336. [CrossRef] [PubMed]
41. Balla, K.; Rakszegi, M.; Li, Z.; Bekes, F.; Bencze, S.; Veisz, O. Quality of winter wheat in relation to heat and drought shock after anthesis. *Czech J. Food Sci.* **2011**, *29*, 117–128. [CrossRef]
42. Blumenthal, C.; Bekes, F.; Gras, P.; Barlow, E.; Wrigley, C. Identification of wheat genotypes tolerant to the effects of heat stress on grain quality. *Cereal Chem.* **1995**, *72*, 539–544.
43. Daniel, C.; Triboi, E. Effects of temperature and nitrogen nutrition on the grain composition of winter wheat: Effects on gliadin content and composition. *J. Cereal Sci.* **2000**, *32*, 45–56. [CrossRef]
44. Labuschagne, M.; Elago, O.; Koen, E. The influence of temperature extremes on some quality and starch characteristics in bread, biscuit and durum wheat. *J. Cereal Sci.* **2009**, *49*, 184–189. [CrossRef]
45. Martinant, J.P.; Nicolas, Y.; Bouguennec, A.; Popineau, Y.; Saulnier, L.; Branlard, G. Relationships between mixograph parameters and indices of wheat grain quality. *J. Cereal Sci.* **1998**, *27*, 179–189. [CrossRef]

46. Neacşu, A.; Stanciu, G.; Săulescu, N. Most suitable mixing parameters for use in breeding breadwheat for processing quality. *Cereal Res. Commun.* **2009**, *37*, 83–92. [CrossRef]
47. Phakela, K.; van Biljon, A.; Wentzel, B.; Guzman, C.; Labuschagne, M. Gluten protein response to heat and drought stress in durum wheat as measured by reverse phase-high performance liquid chromatography. *J. Cereal Sci.* **2021**, *100*, 103267. [CrossRef]
48. Jacobs, P.J.; Bogaerts, S.; Hemdane, S.; Delcour, J.A.; Courtin, C.M. Impact of wheat bran hydration properties as affected by toasting and degree of milling on optimal dough development in bread making. *J. Agric. Food Chem.* **2016**, *64*, 3636–3644. [CrossRef]
49. Ceresino, E.B.; Kuktaite, R.; Sato, H.H.; Hedenqvist, M.S.; Johansson, E. Impact of gluten separation process and transglutaminase source on gluten based dough properties. *Food Hydrocoll.* **2019**, *87*, 661–669. [CrossRef]
50. Sedláček, T.; Horčička, P. Prediction of wheat baking quality using reomixer analysis of whole-grain meal. *Cereal Res. Commun.* **2014**, *42*, 274–281. [CrossRef]
51. Kuktaite, R.; Repo-Carrasco-Valencia, R.; de Mendoza, C.C.; Plivelic, T.S.; Hall, S.; Johansson, E. Innovatively processed quinoa (Chenopodium quinoa W illd.) food: Chemistry, structure and end-use characteristics. *J. Sci. Food Agric.* **2021**, *102*, 5065–5076. [CrossRef]
52. Islas-Rubio, A.R.; Singh, H.; Chittrakorn, S.; MacRitchie, F. Stability of wheat proteins in solution. *J. Cereal Sci.* **2006**, *43*, 169–174. [CrossRef]

Acta Universitatis agriculturae Sueciae
Doctoral Thesis No. 2023:26

This thesis is designed to broaden our understanding of how different climatic conditions affect the quality of the gluten proteins in Swedish wheat genotypes and imported wheat varieties. Combinations of different innovative methods to examine the quality and yield of wheat such as, SE-HPLC, SIG, SRC and RGB imaging were tested in this thesis, which could be of potential interest to both the breeding and bread-baking industries.

Sbatie Lama received her graduate education at the Department of Biosystem and Technology, SLU, Alnarp. She received her MSc in Biochemistry and BSc in Agriculture from Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

Acta Universitatis agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

ISSN 1652-6880
ISBN (print version) 978-91-8046-104-7
ISBN (electronic version) 978-91-8046-105-4