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

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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.

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

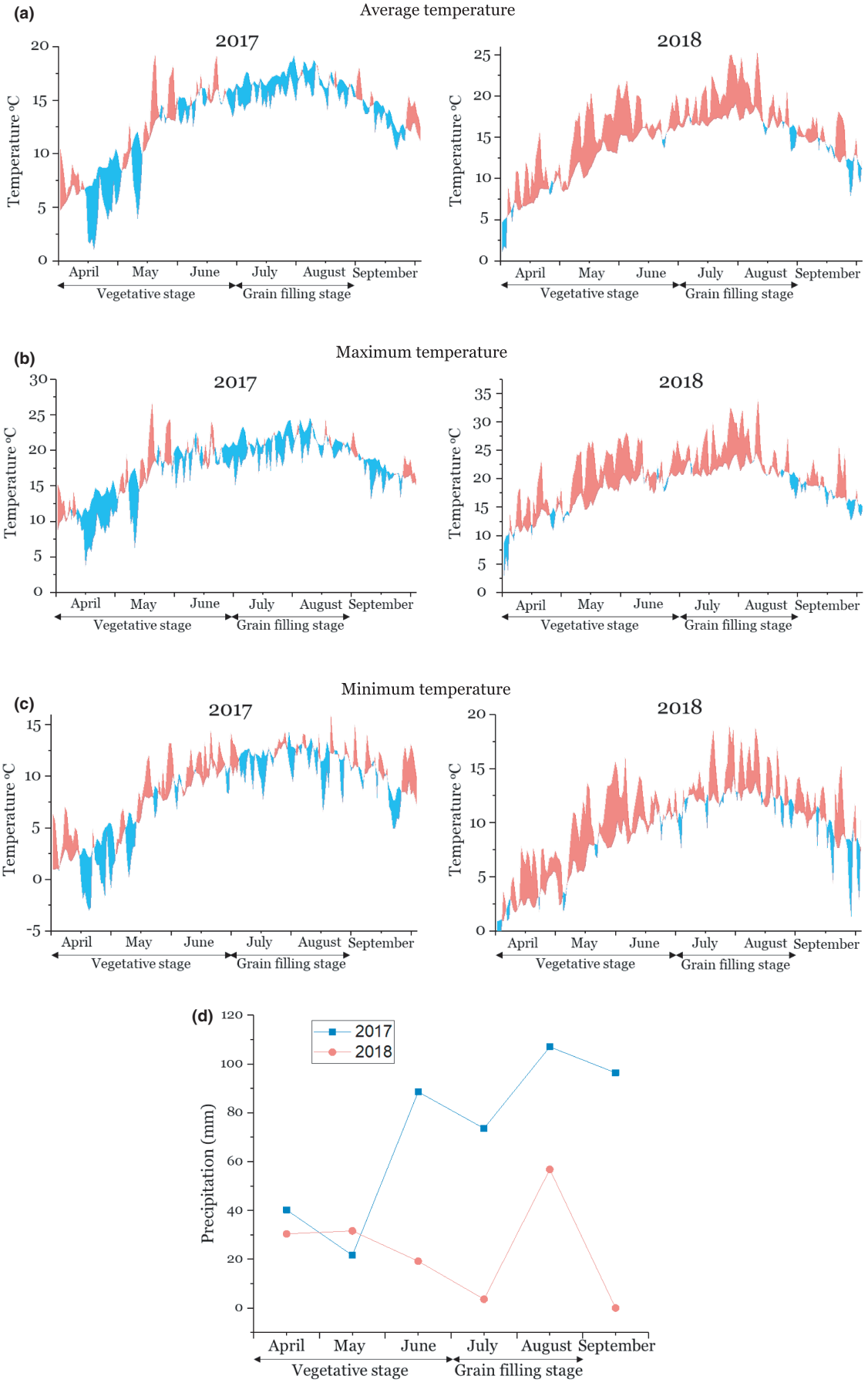
The statistical analysis was performed using the software R (https://www.r-project.org/) for evaluating the impact of genotype (G), year (Y), and G x Y on the studied protein parameters LPP, SPP, LMP, SMP, LPPs, SPPs, LMPs, SMPs, TOTE, TOTU, %UPP, %LUPP, %LUMP, and Mon/Pol calculated from SE-HPLC analyses. 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
2017
2018
Temperature °C
April
May
June
July
August
September
Vegetative stage
Grain filling stage
(c)
Minimum temperature
2017
2018
Temperature °C
April
May
June
July
August
September
Vegetative stage
Grain filling stage
(d)
2017
2018
Precipitation (mm)
April
May
June
July
August
September
Vegetative stage
Grain filling stage

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

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.

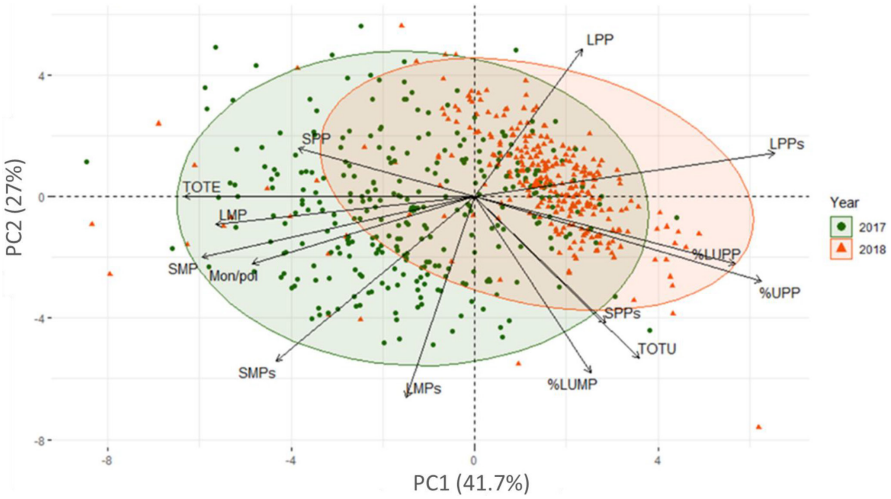


FIGURE 2 Principal component analysis (PCA) of the 14 gluten protein parameters (LPP, SPP, LMP, SMP, LPPs, SPPs, LMPs, SMPs, TOTE, TOTU, %UPP, %LUPP, %LUMP, and Mon/pol) from SE-HPLC of 294 spring wheat genotypes grown in 2017 and 2018

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

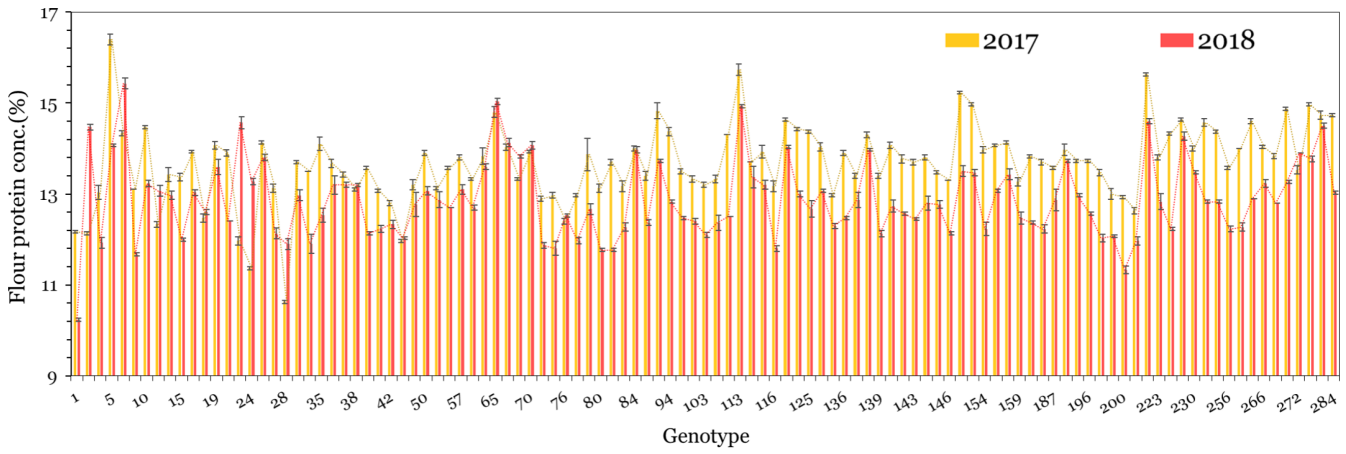


FIGURE 3 Flour protein concentration (%) of 109 spring wheat genotypes grown in 2017 and 2018. Error bars represent standard error

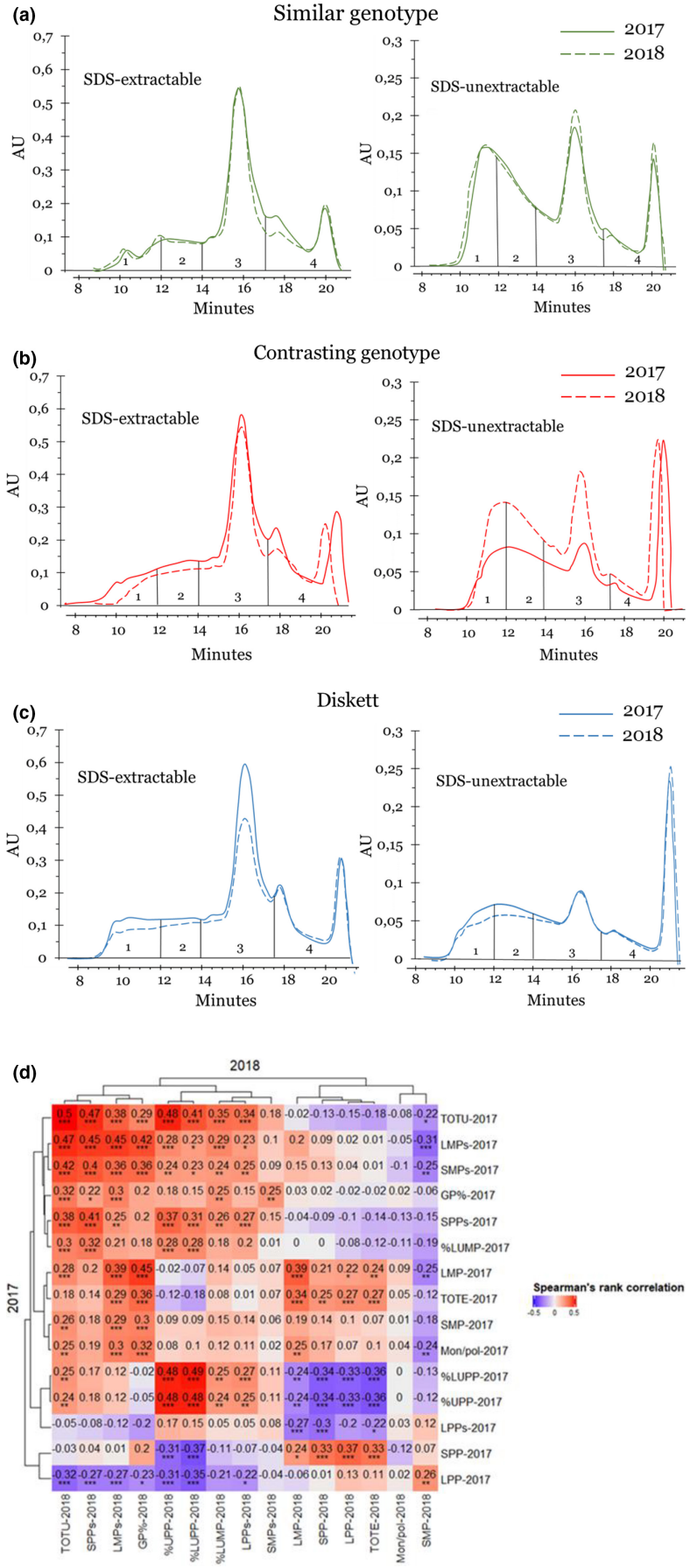


FIGURE 4 Examples of SE-HPLC chromatograms of the SDS-extractable and the SDS-unextractable gluten proteins of the selected wheat genotypes grown in 2017 and 2018; (a) similar protein solubility profile for genotype 187 from both years; (b) contrasting protein solubility profile of genotype 12; and (c) 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

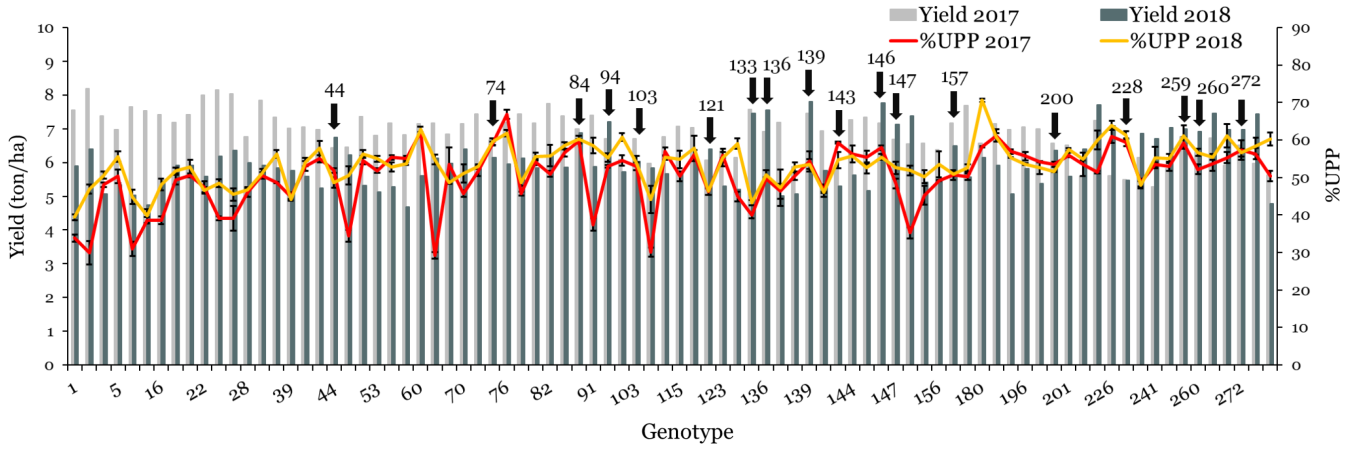


FIGURE 5 Wheat grain yields (ton/ha) and %UPP of 84 genotypes grown in 2017 and 2018. Error bars represent standard error

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

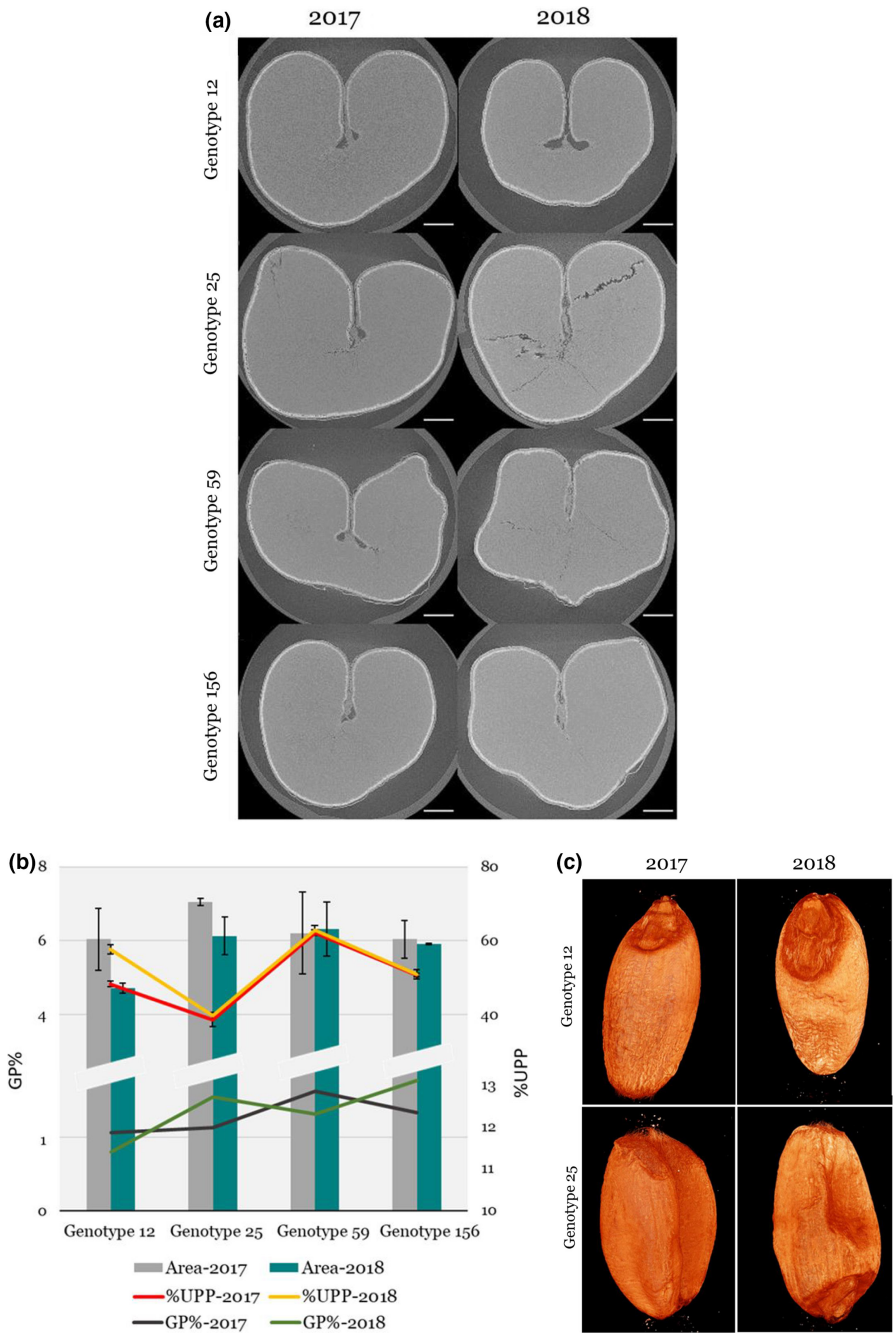


FIGURE 6 Microstructure of wheat grains from the genotypes differing in %UPP grown in 2017 and 2018 studied by X-ray tomography; (a) 2D images of the cross-section of wheat grain from the genotypes 12, 25, 59, and 156; scale bar is 500 μm; (b) grain endosperm area (mm^2), %UPP, and grain protein concentration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

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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