

UNVEILING PROTEIN CHANGES IN THE CONTEXT OF CLIMATE CHANGE:
REVELATIONS FROM A HISTORICAL WHEAT SEED COLLECTIONIRIS ALOISI¹, GIOVANNI MARIA POGGI^{1,2}, SIMONA CORNETI¹, STEFANO DEL DUCA¹, FRANCESCA VENTURA²¹DEPARTMENT OF BIOLOGICAL, GEOLOGICAL AND ENVIRONMENTAL SCIENCES, UNIVERSITY OF BOLOGNA, ITALY²DEPARTMENT OF AGRICULTURAL AND FOOD SCIENCES, UNIVERSITY OF BOLOGNA, ITALY

INTRODUCTION

In Southern Europe, the impacts of rising temperatures, heat waves, and decreased annual precipitation, have been apparent even prior to the widespread recognition of the term 'climate change' in the 1980s. These environmental changes have not only intensified in frequency but also in intensity, profoundly influencing the development and phenology of plants¹. Within the agricultural context, major crops have already experienced alarming declines in productivity, and this trend is projected to exacerbate in the future². The consequences of climate change extend to crop quality as well, particularly in the case of cereals. Phenological alterations, resulting from shifts in climate patterns, can profoundly impact the composition of storage proteins³.

The objective of this study was to investigate the presence of traces and indications of climate change in previous decades, with a specific focus on bread wheat in Southern Europe. To achieve this, an analysis was conducted utilizing historical phenological and meteorological data. Additionally, the protein content and composition of grain samples collected over the past 50 years were examined. Through these investigations, the study aimed to uncover potential correlations between climate change and observed changes in phenology and protein composition, shedding light on the long-term effects of climate change on bread wheat in Southern Europe.

METHODS

1

The Bolognese Plain, located in Emilia-Romagna, was selected as study site. Here the effects of climate change have become evident as reported in the "Climate change mitigation and adaptation strategy for the Emilia-Romagna Region" summary document⁶.

2

Phenological data for the past period (1951/52–1965/66) were obtained from long-lasting surveys. Phenological bulletins were used for the present period (2005/06–2019/20). The days after sowing (DAS) and the cumulative growing degree days (CGDDs) necessary to reach BBCH stages 31 (stem elongation), 40–49 (booting), 50–59 (heading), 61 (anthesis), and 89 (full ripening) were calculated.

3

Weather data (daily maximum, minimum, and mean temperature) were provided by the University of Bologna agrometeorological stations. These mechanically measured weather data were used, representing a continuous and uniform historical series from 1952 to today, measured near the plots where the phenological data were collected.

4

Historical San Pastore grains were provided by Laras Laboratory (University of Bologna). Seed companies provided modern grains. Proteins were extracted⁵ and analyzed by liquid chromatography–high-resolution mass spectrometry. Peak areas were calculated using PeakView to compare protein composition among samples.

5

Statistical analysis was performed in R statistical environment (R version 4.0.3)⁴. Specifically, R packages used were Kendall, strucchange, stats, lawstat, pgirmess, ggplot2, and ggpubr.

RESULTS

From 1952 to 2020, a significant increasing trend emerged for annual mean air temperature (T_{mean}) from the Mann-Kendall test ($t = 0.538$, p -value < 0.05), with a breakpoint in 1989.

Present period showed an evident aridity period, during summer, due to a huge decrease in water supply (Figure 1A) and due to the increase in mean temperatures in the two 15-year periods. Changes in T_{max} were recorded mostly during the months from January to April, as well as July and August ($>2^{\circ}\text{C}$ on average). January and February, as well as July and August, also showed the highest increases in T_{min} ($>2^{\circ}\text{C}$ on average) (Figure 1B).

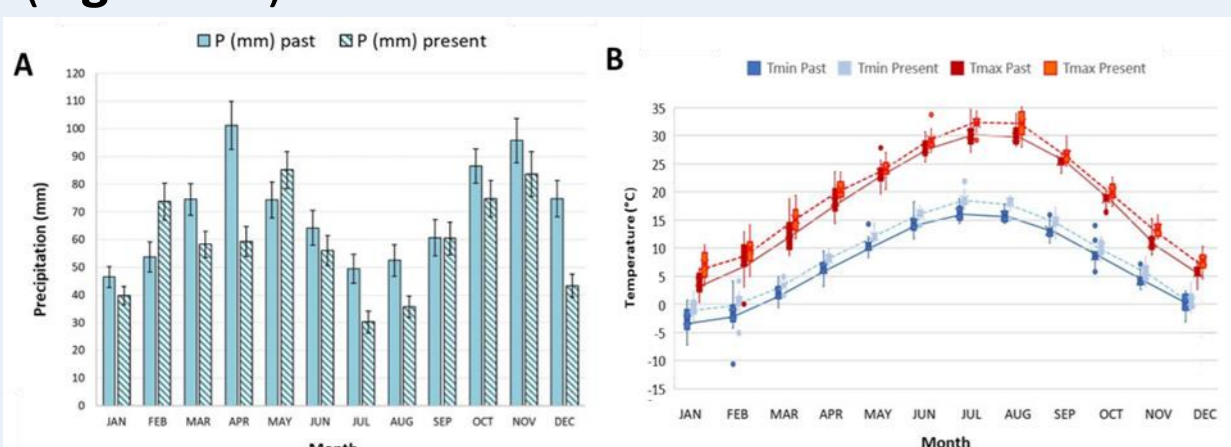


Figure 1. Average monthly precipitation (A) and mean monthly T_{max} and T_{min} (B) for past and present period. Boxplots for monthly temperatures data are presented: the box goes from first to the third quartile. The line through the box represents the median. The whiskers go from each quartile respectively to the minimum and maximum value. Individual points outside the ends of the whiskers are outliers.

To verify whether wheat life cycle has significantly shortened compared to the past, DAS between the average values of four past period varieties, *i.e.*, San Giorgio, San Pastore, Mara, and Fortunato, and the values of the present period variety Mieta in 15 growing seasons were compared.

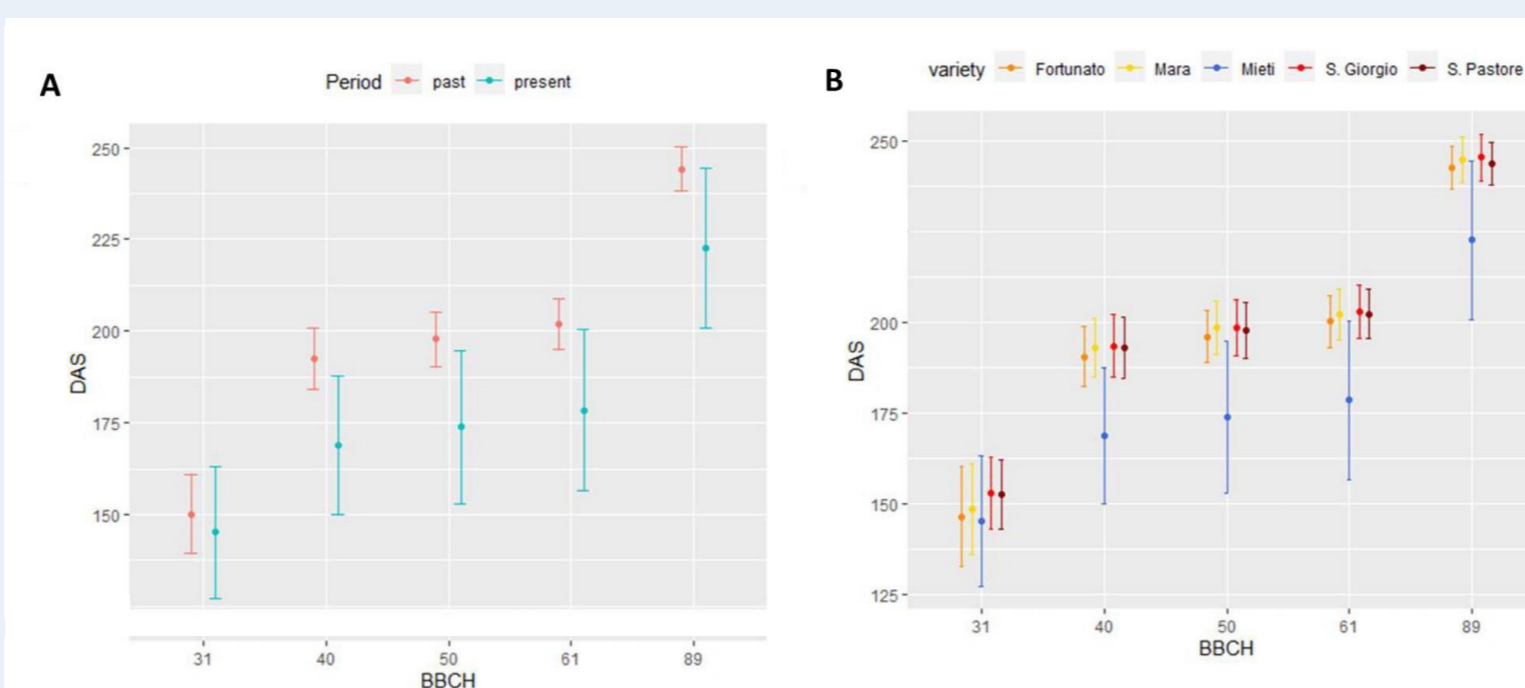


Figure 2. DAS necessary to reach 31, 40–49, 50–59, 61, and 89 BBCH stages in past period (mean of 4 varieties) and present period (Mieta) (A). Data for each variety are also presented (B). Dots represent the mean of 15 agronomic seasons, and upper and lower bars represent one standard deviation.

A significant shortening of the chronological time necessary to reach the BBCH stages 40–49, 50–59, 61, and 89, was observed for the present period (p -value < 0.05 , Wilcoxon rank-sum test). The average life cycle length in the past period was 244 ± 6 days, compared to the average of 223 ± 22 days in the present period (Figure 2).

Seed samples were homogenous in total protein content (average $13.1 \pm 1.2\%$) and gliadins generally accounted for between 66 and 75% in the analysed samples, whereas glutenins between 27 and 37%.

Protein fraction distribution was further investigated for correlation with the number of CGDDs accumulated in the period 1 May–15 June, during which wheat grain filling occurs in the region, for each year in analysis.

Strong and significant (p -value < 0.05) Pearson's r correlation resulted between CGDDs and high vs low molecular weight glutenins ($r = 0.83$), high molecular weight glutenins (%) ($r = 0.9$), and gliadins (%) ($r = -0.82$) (Figure 3).

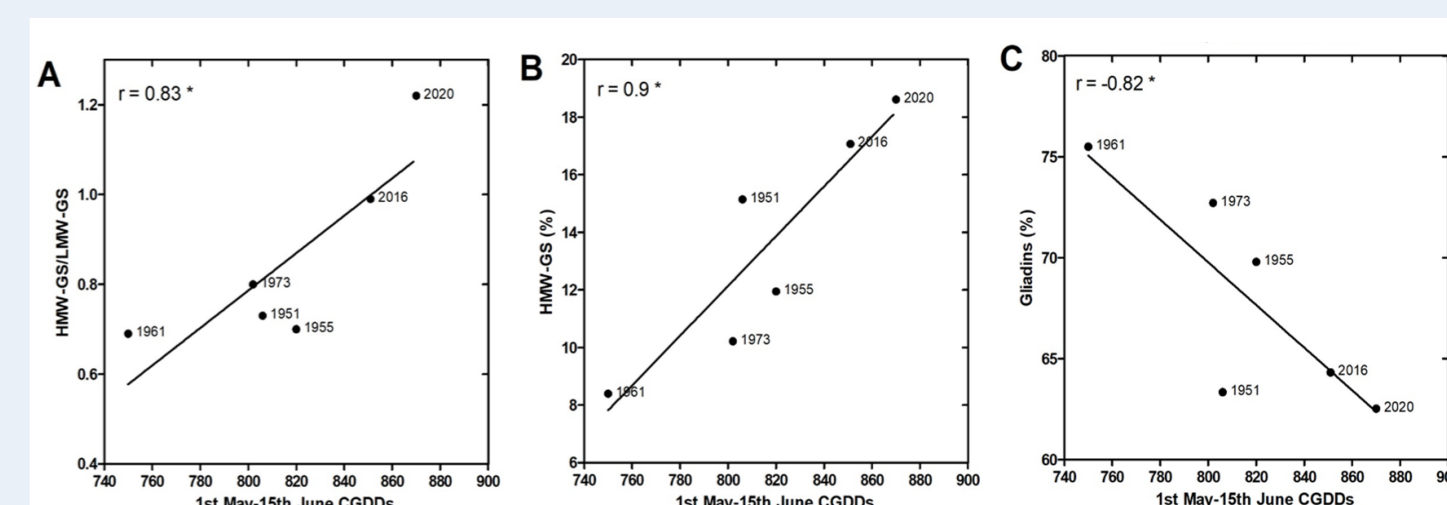


Figure 3. 1 May–15 June CGDDs Pearson's r correlation with high molecular weight glutenin subunits (HMW-GSs) and low molecular weight glutenin subunits (LMW-GSs) ratio (A), HMW-GS (%) (B), and total gliadins (%) (C). For 1955 and 2020, average year values are presented. *Means significant at $p = 0.05$ level.

DISCUSSION AND CONCLUSION

Although it is well known that climate change can cause a shift in crops' phenological development, studies that exactly quantify this shift are few, due to the lack of long lasting phenological surveys, conducted in the same site and on the same varieties along an appreciable time span.

In this scenario, this study precisely assesses the shortening of bread wheat phenological development, in a key area for its cultivation, such as Southern Europe. The acceleration of wheat life cycle can have negative consequences on grain yield, due to the shortening of phenological phases, such as vegetative and grain-filling period.

Moreover, we report for the first time on how gluten composition mirrors phenological alterations in the Mediterranean climate by analysing a historical seed collection of the same variety, cropped in the same study area, highlighting how insights of climate change are secretly conserved within seed proteins.

The information included in this study might represent a tool for modelling, for both predictive purposes and decision supporting systems for farmers, as well as a guide for future breeding choices for varietal innovation.

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