Post-anthesis thermal stress induces differential accumulation of bioactive compounds in field-grown barley

Mariona Martínez-Subirà, Maria-Paz Romero, Marian Moralejo, Alba Macià, Eva Puig, Roxana Savin and Ignacio Romagosa*

Abstract

BACKGROUND: Barley (Hordeum vulgare L.) is a healthy grain because of its high content of dietary fibre and phenolic compounds. It faces periods of high temperature during grain filling, frequently reducing grain weight. Heat stress may also affect some of the bioactive compounds present in the grain. To produce quality grains that provide nutritional and health benefits, it is important to understand the effect of environmental stresses on the quantity and quality of bioactive compounds.

RESULTS: We have studied the effect of post-anthesis thermal stress on barley bioactive compounds and antioxidant capacity under Mediterranean field conditions during two consecutive growing seasons in four barley genotypes. Thermal stress affected grain weight and size and changed the relative composition of bioactive compounds. The relationship between heat stress and grain β-glucans and arabinoxylans content was indirect, as the resulting increases in concentrations were due to the lower grain weight under stress. Conversely, heat stress had a significant direct impact on some phenolic compounds, increasing their concentrations differentially across genotypes, which contributed to an improvement in antioxidant capacity of up to 30%.

CONCLUSION: Post-anthesis thermal stress had a significant effect on β-glucans, arabinoxylans, phenolic compound concentrations and antioxidant capacity of barley grains. Final grain quality could, at least partially, be controlled in order to increase the bioactive concentrations in the barley grain, by cultivation in growing areas prone to heat stress. Late sowings or late flowering genotypes could also be considered, should a premium be implemented to compensate for lower yields.

Supporting information may be found in the online version of this article.

Keywords: barley grain; thermal stress; dietary fibre; phenolic compounds; antioxidant capacity

INTRODUCTION

Barley (Hordeum vulgare L.) is the fourth most abundant cereal in the world, being well adapted against extreme environmental conditions.1 Most barley is used for animal feed, about 6% for brewing malt and less than 2% for food. Consumption is highest in Morocco, with 20% of barley grain used in a variety of traditional dishes. Barley flour is increasingly used in some industrialized countries in new bread and pasta formulations, and whole grains, flours, differential pearling fractions and bioactive extracts are being evaluated to develop new food products, from non-alcoholic power drinks to meat-analogue burgers. Barley is a good source of bioactive compounds, components with potential health-promoting effects, such as β-glucans, arabinoxylans, phenolic compounds (PC), vitamin E (tocols), sterols and folates.2 β-Glucans and arabinoxylans are the major non-starch polysaccharides present in cell walls of the barley grain. β-Glucans are polymers of β-D-glucose with glycosidic linkages (1,4) and (1,3). They are related to several positive health effects, such as maintaining normal blood cholesterol levels, reduction of blood glucose after meals,3,4 and improving the responsiveness of the immune system against infectious diseases, inflammation and some types of cancer.5 Arabinoxylans consist of (1,4)-β-linked xylopyranosyl residues, being the second most abundant barley cell wall polysaccharide. Arabinoxylans have been associated with reduction of postprandial glycaemic responses6 and other health-promoting properties, such as the nutritional benefits of soluble and insoluble fibre and antioxidant properties due to the presence of phenolic acids attached to its structure.7 Barley is also a good source of PC, secondary metabolites characterized by having at least one phenol unit, that can be free or bound to the fibre.

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They possess antioxidant capacity and have been associated with the reduction of cardiovascular disease, inflammation and a diversity of cancers.8

Agronomic and environmental conditions during the barley growing cycle strongly influence grain yield and grain composition9,10 and, thus, in order to produce grains with a certain composition to provide health and nutritional benefits, it is imperative to better understand the effect of environmental stresses on the quantity and quality of bioactive compounds. Deleterious effects of high temperature on barley yield and quality are well documented in the literature. For instance, it is well known that higher temperatures during grain filling reduce grain weight (GW) in barley in experiments performed under both controlled11,12 and field conditions,13,14 with a decrease in GW ranging from 5% to 30% depending on the cultivar, time of exposure and duration of the stress.15 Furthermore, it is commonly accepted that accumulation of starch is more sensitive to high temperature than accumulation of nitrogen,16,17 as most of the experiments in barley when heat stress was applied during grain filling period showed increases in grain nitrogen proportion when GW was reduced as a consequence of heat stress.12-14 However, investigations on the impact of heat stress on grain bioactive compounds content are very limited. There are contradictory reports on the effect of high temperatures on the β-glucan content in barley: some studies reported an increase,18 while in others barley β-glucan levels were reduced12 or not affected.13 There have been very few studies on the variability of arabinoxylans content affected by environmental factors9,10 and none, which we know of, describing the effect of high-temperature stress on barley. Environmental conditions may also have a significant impact on total phenolic content and antioxidant capacity in barley.21 Narwal et al. showed that the free phenol content was more influenced by the genotype, while the bound phenols were more influenced by the environment.22 The few studies that have examined variation in phenolic content due to the environmental conditions on barley have either focused on different locations or year of growth,10,21,22 rather than focusing on a specific environmental effect such as heat stress. In addition, the effects of heat stress depend on the time, duration and intensity of exposure of the genotypes to heat,23 and this determines its impact on the final bioactive compound content. Therefore, it is relevant to quantify the thermal stress effects on bioactive compounds under field conditions. Furthermore, in areas such as the Mediterranean basin, where high temperature stress is normally associated with the end of the growing season,24 thermal stress is expected to be more frequent in the future.25 Thus the purpose of the current study was to investigate the effect of high temperatures from the mid-grain filling period to physiological maturity on GW, grain size, β-glucans, arabinoxylans, PC and their antioxidant capacity in four distinct barley genotypes under field conditions during two consecutive seasons.

MATERIALS AND METHODS

Plant materials and treatments

Four barley genotypes were used in this study, differing in presence/absence of husks, number of rows, type of starch, grain quality and colour (Table S1): Annapurna – two-rowed variety with hull-less (naked) grain, waxy endosperm and high β-glucan content; Hindukusch – Afghan two-rowed landrace with purple and partially hull-less grain, non-waxy endosperm and medium β-glucan content; Hispanic – two-rowed variety with hulled grain and non-waxy endosperm; Tamalpais – six-rowed variety with hull-less grain, non-waxy endosperm and high β-glucan content.

Heat stress was induced as described by Elia et al.26 Two temperature conditions were induced: a control and a high-temperature treatment, starting 15 days after heading (decimal code,17 DC55) and continuing up to physiological maturity (DC 90). The heat treatment was carried out by enclosing half of the plots with transparent polyethylene film (125 µm) mounted on wooden structures 1.5 m in height above soil level,26 but leaving the bottom 30 cm of the four sides of each structure open and punctures made in the top of the plastic to facilitate free gas exchange and reduce humidity. Stress increased maximum temperatures up to 8 °C (Supporting Information, Table S2), while the plastic cover reduced solar radiation by up to 15%. The two growing seasons differed significantly (Table S2 and Fig. S1). Spring 2017 was warmer (average 15 °C vs. 13 °C), drier (100 L m⁻² vs. 175 L m⁻² accumulated precipitation) and with higher solar radiation (+10 vs. −10% long-term average); 2018 was warmer immediately after sowing.28,29 Temperatures were continuously registered from the start of the treatments during the two seasons (Table S2). Average daily temperatures for the stressed and control treatments were 21.5 and 19.3 °C and 20.6 and 18.0 °C in 2017 and 2018, respectively. Average and maximum difference in thermal amplitudes under stress vs. control were 7.3 and 9.2 °C in 2017 and 8.6 and 10.2 °C in 2018. Temperatures under stress reached 45.1 and 44.4 °C in 2017 and 2018, respectively. These extremely high temperatures are not that unusual at the end of grain filling under warm Mediterranean conditions.

Experimental design

Fully irrigated and well-fertilized field experiments were conducted in Semillas Batlle, located in Bell-lloc d’Urgell (41°37’N, 0°47’E), Lleida, Spain, under irrigation and well-fertilized conditions. The sowing dates were 21 December 2016 and 20 December 2017, at rates of 350 seeds m⁻². The main plot size was 4 x 1.8 m², from which two subplots of the same size were generated to apply the control treatment and the artificially induced continuous heat stress during grain filling.

Measurements and analyses

Grain weight and grain size

The barley grain was harvested at maturity, 45 days after anthesis. The spikes were threshed and cleaned with an LT-15 thresher (FOSS, Denmark) and milled using a Cyclotec 1093 mill equipped with a 0.5 mm screen to produce wholemeal and a 2.5 mm sieve to produce middlings. Grain plumpness was estimated by the percentage weight of grains retained over a 2.5 mm sieve.

Milling

The barley seeds were milled using a Cyclotec 1093™ (FOSS, Denmark, Spain) mill equipped with a 0.5 mm screen to produce whole meal flour, which was immediately kept at −20 °C in the dark until analysis.

Quantitative determination of β-glucans and arabinoxylans

The total amounts of mixed-linkage β-glucans and arabinoxylans in wholemeal flours were determined using the β-glucan assay
(K-BGLU) and d-xylose assay (K-XYLOSE) kits from Megazyme (Wicklow, Ireland).

PC extraction and ultra-performance liquid chromatographic–tandem mass spectrometric analysis
Free and bound PC were extracted according to Martínez et al.20 subjected to a micro-elution solid-phase extraction (μSPE) (Waters, Milford, MA, USA)31 and analysed by liquid chromatography (for more details see Martínez et al.20). The phenolics were quantified by commercial reference to a 0.02–25 ng calibration curve of commercially available standard compounds and the results were expressed as micrograms per gram of dry sample. The limits of detection (LOD) ranged from 0.007 to 0.09 ng and limits of quantification (LOQ) from 0.02 to 0.30 ng. Total PC were calculated by adding all PC.

Determination of antioxidant capacity
The antioxidant capacity of the total PC in the barley grain was determined by the oxygen radical absorbance capacity (ORAC) assay according to Huang et al.32 The determination of ORAC was carried out using a FLUOstar OPTIMA fluorescence reader (BMG Labtech) in a 96-well polystyrene microplate controlled by OPTIMA 2.10R2 software, working at 485 nm for excitation and 520 nm for emission. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as control, which increased radiation use efficiency. Therefore, the reduction in incoming radiation was offset by an increase in radiation use efficiency.

Grain weight and grain size
GW for the controls over the two growing seasons ranged from 44 to 52 mg across the four genotypes (Supporting Information, Table S2). As expected, both environmental and genetic effects significantly influenced GW (Table 1 and Fig. 1(A)). Heat stress was the most important source of the differences, with control grains weighing on average 10% more than the stressed ones (Fig. 1(A)). The reduction of GW under heat stress from 15 days after heading to maturity was in agreement with previous studies on barley, which suggested that high temperature causes inactivation of sucrose synthase, leading to a reduction in the synthesis of starch that reduces grain growth.9,11,13,14 The difference in the average weight was reflected in grain size as grain plumpness was

### Table 1

<table>
<thead>
<tr>
<th>Source</th>
<th>Grain weight (mg)</th>
<th>Grain plumpness (%)</th>
<th>β-Glucans (mg g⁻¹)</th>
<th>Arabinoxylans (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-ratio</td>
<td>P-value</td>
<td>F-ratio</td>
<td>P-value</td>
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<td>0.0995</td>
<td>21.95</td>
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<td>Y * G * E</td>
<td>2.21</td>
<td>0.1647</td>
<td>11.73</td>
<td>0.0001</td>
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</tbody>
</table>

Bold font indicates significance at P < 0.05.

RESULTS AND DISCUSSION
The four genotypes studied differed widely in an array of bioactive compounds, potentially susceptible to heat stress from mid-grain filling to physiological maturity. However, we recognize that it may not be a representative set of food barley diversity.

Imposing high temperatures under field conditions
Experiments under controlled environments such as growth chambers are useful in understanding responses of plants to specific environmental factors, but they can differ considerably from field conditions and cannot be simply extrapolated to interpret variations in actual yield and quality observed in the field.33 In this study, high temperature was adequately and consistently imposed in the field with polyethylene film chambers (Supporting Information, Table S2). However, reduced incident radiation (up to 15% at noon on very sunny days) was also registered. This reduction in incoming radiation did not significantly modify the source–sink balance for grain filling, as shown by Elia et al.26 The polyethylene film changed the partitioning of incoming radiation between direct and diffuse, favouring the latter,34 which increased radiation use efficiency.35 Therefore, the reduction in incoming radiation was offset by an increase in radiation use efficiency.
much lower under heat stress (Table 1, Fig. 1(B) and Supporting Information, Fig. S2), as also reported by Passarella et al. Genotypic differences were also significant, with Annapurna and Hispanic, both two-rowed commercial varieties, producing heavier and plumper grains than Hindukusch, a two-rowed landrace, and Tamalpais, a six-rowed cultivar.

**Dietary fibre**

Genotype was the most important factor explaining the β-glucan content. This ranged from 80 ± 2 mg g⁻¹ in Tamalpais to 50 ± 2 mg g⁻¹ in Hispanic over 2 years (Fig. 1(C) and Supporting Information, Table S1). Although it has reported that waxy genotypes have higher β-glucans content than non-waxy types, the non-waxy genotype Tamalpais did not differ from the waxy genotype Annapurna. The grain β-glucan content was not significantly altered by the continuous stress treatment (Table 1 and Fig. 1(C)). However, β-glucans were affected by annual variability, as the genotype × year interaction was statistically significant (Table 1). β-Glucan levels were lower in 2017 (warm with higher solar radiation) than in 2018, especially for Annapurna and Tamalpais. There are contradictory reports on the effect of high temperatures on the β-glucan levels in barley grain. Most of these studies were performed under controlled conditions, which are not easy to extrapolate to field conditions, and some of them study heat stress by comparing different sites or sowing dates, which have confounding effects. In our study, we did not detect any effect associated with the artificially imposed thermal stress but the year effect (annual variability) was highly significant, not interacting with any other term (Table 1).

Arabinoxylans also varied among genotypes. Annapurna and Hindukusch had the highest average arabinoxylan content (55 ± 3 mg g⁻¹), followed by Tamalpais and Hispanic (Fig. 1(D) and Supporting Information, Table S1). Although it has been suggested that six-rowed cultivars generally contain slightly higher levels of arabinoxylans than two-rowed genotypes, in our study the highest arabinoxylan contents were observed in the two-rowed genotypes: Annapurna (waxy) and Hindukusch (non-waxy); presence of the waxy gene was not associated with a higher content of arabinoxylans as found by Izydorczyk and Dexter. Arabinoxylan content could also be influenced by the environment. Arabinoxylan content in wheat increased under high temperature stress. Our results showed that the arabinoxylan concentration in barley grain was apparently affected by thermal-induced stress; however, covariance analysis showed that any difference in arabinoxylans detected disappeared once GW was introduced as covariable in the model (Table 1). The apparently higher arabinoxylan concentration under stress could be explained by a concentration effect of the same amount of this pentosan in lighter grains, and not an apparent direct response to the induced heat stress. Although heat stress produced low flour yields due to thinner grains, the grains had dietary fibre concentrations equal to or greater than under non-stressed conditions and thus enhanced healthy properties.

**Antioxidant capacity**

Antioxidant capacity was significantly influenced by genotype and environment, both in the artificially induced stress and in year-to-year variation, either as main effects or at the level of some of their interactions (Table 2 and Fig. 2(A)). The highest antioxidant capacity was found in Hindukusch (140 ± 7 μmol Trolox g⁻¹) and the lowest in Hispanic (91 ± 20 μmol Trolox g⁻¹), in accordance with their total PC content (Fig. 2(A,B) and Supporting Information, Table S1). These results were in line with those previously reported by Suriano et al., who found that grain of coloured barley genotypes had the highest antioxidant capacity and correlated significantly with their anthocyanin levels, as discussed below. Genotypes grown under heat stress had higher antioxidant capacity except for Hindukusch, which decreased from...
Table 2. Fixed-effect F-tests for the REML analyses of variance (ANOVA) and covariance (ANCOVA) for antioxidant capacity (AC) and total phenolic compound (PC) concentrations in the grain, using GW as a covariable, of four barley genotypes grown under control and induced heat stress, 'Environment', for two consecutive years in Lleida, Spain.

<table>
<thead>
<tr>
<th>Source</th>
<th>ANOVA</th>
<th>ANCOVA</th>
<th>ANOVA</th>
<th>ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-ratio</td>
<td>P-value</td>
<td>F-ratio</td>
<td>P-value</td>
</tr>
<tr>
<td>GW</td>
<td>6.49</td>
<td>0.0349</td>
<td>19.01</td>
<td>0.0024</td>
</tr>
<tr>
<td>Year (Y)</td>
<td>35.40</td>
<td>0.0271</td>
<td>11.38</td>
<td>0.0064</td>
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<td>6.98</td>
<td>0.0223</td>
<td>15.95</td>
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<td>Environment (E)</td>
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<td>0.0000</td>
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<td>0.0011</td>
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<tr>
<td>Y * E</td>
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<td>0.3167</td>
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<td>G * E</td>
<td>13.73</td>
<td>0.0016</td>
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<td>0.0080</td>
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<tr>
<td>Y * G * E</td>
<td>0.30</td>
<td>0.8267</td>
<td>6.96</td>
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</table>

Bold font indicates significance at $P < 0.05$.

Figure 2. ANCOVA least squares means for (A) antioxidant capacity (AC) and (B) total phenolic compounds (PC) in grain of four barley genotypes (A, Annapurna; Hk, Hindukusch; Hp, Hispanic; T, Tamalpais) grown under control (light gray) and heat stress (dark gray) conditions for two consecutive years in Lleida, Spain. Standard error is shown by error bars. For statistical significance, see Table 2.

143 ± 4 to 126 ± 4 μmol Trolox g⁻¹. This reduction could be associated with a decrease in some PC, particularly anthocyanins due to the polyethylene film as further discussed under ‘Anthocyanins’, below. The highest increase in antioxidant capacity due to stress was observed in Tamalpais (from 110 ± 2 to 148 ± 4 μmol Trolox g⁻¹; i.e., 34%), and the lowest in Hispanic (91 ± 9 to 107 ± 9 μmol Trolox g⁻¹; i.e., 18%).

Total PC

A total of 61 PC were identified in the four barley genotypes (Supporting Information, Table S3). The 37 quantitatively most relevant – seven phenolic acids, nine flavan-3-ols and 21 anthocyanins – were selected to investigate the effect of high temperature. Phenolic acids detected in the free and bound fractions were ferulic and p-coumaric acids and their derivatives,
representing an average of 72% of the total PC. The predominant flavan-3-ols were catechin and two dimers: procyanidin B3 and prodelpiholinid B4 (average 77% of free fraction). The anthocyanin content was extremely high in the purple Hindukusch genotype, which was characterized by a high concentration of cyanidin-dimaloyl glucoside and cyanidin glucoside (81% of the total anthocyanins).

Genotype was the most important factor in determining differences in total PC concentrations (Table 2 and Fig. 2(B)). Hindukusch and Tamalpais had the highest average content: 1649 ± 450 and 1496 ± 54 μg g⁻¹, respectively (Supporting Information, Table S1). This is in agreement to what has been reported, that purple39 and six-rowed genotypes39 had higher content of PC. Total PC was also affected by thermal-induced stress, increasing content in all genotypes. However, significance decreased once the GW covariable was introduced in the ANCOVA model. Year-to-year variability affected PC concentration more than other measuring factors. Weather conditions for all PC analysed; for a few of them the response to heat stress was genotypic dependent. The highest levels of free and bound ferulic acids were observed in the purple genotype 9 ± 1 and 1246 ± 37 μg g⁻¹, respectively (Fig. 3(A,B)). Conversely, Hispanic and Annapurna had the lowest, with 4 ± 1, 5 ± 1 μg g⁻¹ and 786 ± 64, 607 ± 65 μg g⁻¹, respectively. Hispanic (hulled genotype) had the highest coumaric acid concentration (233 ± 17 μg g⁻¹), while Annapurna had the lowest (56 ± 17 μg g⁻¹) (Fig. 3(C)). Our results agree with those of Holtekjølen et al., who observed higher content of coumaric acid in hulled barleys.39 Free and bound phenolic acids were indirectly associated with GW (Table 3), suggesting that lower concentration in heavier grains (non-stressed) could be attributed to dilution effects. Phenolic acids differed across the genotypes, with lesser influence associated with stress. Conversely, bound coumaric acids differentially increased among genotypes (13–47%) under heat-induced stress. Previous research suggested that high temperature stress could influence the metabolic pathway of PC by increasing phenylalanine ammonia lyase activity, which catalyses the conversion of phenylalanine to trans-cinnamic acid, increasing the levels of some PC.41

Phenolic acids

There was not a common response in all four genotypes studied for all PC analysed; for a few of them the response to heat stress was genotypic dependent. The highest levels of free and bound ferulic acids were observed in the purple genotype 9 ± 1 and 1246 ± 37 μg g⁻¹, respectively (Fig. 3(A,B)). Conversely, Hispanic and Annapurna had the lowest, with 4 ± 1, 5 ± 1 μg g⁻¹ and 786 ± 64, 607 ± 65 μg g⁻¹, respectively. Hispanic (hulled genotype) had the highest coumaric acid concentration (233 ± 17 μg g⁻¹), while Annapurna had the lowest (56 ± 17 μg g⁻¹) (Fig. 3(C)). Our results agree with those of Holtekjølen et al., who observed higher content of coumaric acid in hulled barleys.39 Free and bound phenolic acids were indirectly associated with GW (Table 3), suggesting that lower concentration in heavier grains (non-stressed) could be attributed to dilution effects. Phenolic acids differed across the genotypes, with lesser influence associated with stress. Conversely, bound coumaric acids differentially increased among genotypes (13–47%) under heat-induced stress. Previous research suggested that high temperature stress could influence the metabolic pathway of PC by increasing phenylalanine ammonia lyase activity, which catalyses the conversion of phenylalanine to trans-cinnamic acid, increasing the levels of some PC.41

Flavan-3-ols

The total flavan-3-ols were strongly affected by genotype and year x genotype interaction (Table 3). The highest flavan-3-ol content was observed in Tamalpais (523 ± 24 μg g⁻¹), while the lowest was in Hindukusch (247 ± 18 μg g⁻¹) (Fig. 3(D)). The flavan-3-ol concentration varied between years. Tamalpais and Hindukusch had higher flavan-3-ol content in 2017, marked by higher maximum temperatures and higher solar radiation during the grain-filling period. In a previous study, we also found higher procyanidin C2 content in barley samples grown in a warm environment than in a cool climate.30 Therefore, warm climate could have a significant impact on the flavan-3-ol profile in barley. Differential flavan-3-ol content of the genotypes was observed as a response to environmental changes; it increased under heat stress in Annapurna (12%), Hispanic (23%) and Tamalpais (7%). To the best of our knowledge, the mechanism of flavan-3-ol

Figure 3. ANCOVA least squares means for the main phenolic compounds concentrations: (A) free ferulic acids; (B) bound ferulic acids; (C) bound coumaric acids; (D) flavan-3-ols; and (E) anthocyanins in grain of four barley genotypes (A, Annapurna; Hk, Hindukusch; Hp, Hispanic; T, Tamalpais) grown under control (light gray) and heat stress (dark gray) conditions for two consecutive years in Lleida, Spain. Standard error is shown by error bars. For statistical significance, see Table 3.
synthesis upregulation in response to abiotic stress, such as temperature and solar radiation, has not been fully elucidated in cereals. However, several studies have shown the influence of environmental conditions on flavan-3-ol content in other crops. Yao et al. showed in tea that the catechin contents were higher during warm months, while the catechin and proanthocyanidin contents were not greatly affected by partial exclusion of solar radiation in tea or by UV-B radiation in apples. Although similar results had not been reported in cereals, variations in flavan-3-ol content could be more closely related to high-temperature stress than to changes in solar radiation.

Anthocyanins

Anthocyanins act as specific light protectors that absorb visible and UV radiation in vacuoles and prevent UV rays from penetrating into the tissue. High anthocyanin content enhances absorption and tolerance to UV radiation as well as increasing its antioxidant capacity. Therefore, blocking UV radiation with a conventional polyethylene film may affect the accumulation of these compounds in the barley grain and, therefore, may reduce the antioxidant capacity. Differential genotypic responses associated with pigmentation of the barley grain were observed for the anthocyanin content (Table 3). The highest total anthocyanin content was observed in Hindukusch (50 ± 4 μg g⁻¹), an old landrace collected from a high-altitude area, where protection from excess UV radiation is important. Concentrations for the other three yellow grain genotypes were extremely low: less than 0.8 ± 4.7 μg g⁻¹ (Fig. 3E). Anthocyanin concentrations in Hindukusch under stress conditions decreased 61% on average over the 2 years (Fig. 3E). Previous studies have suggested that UV radiation has a significant effect on anthocyanin accumulation. Blocking or decreasing UV radiation has been observed to reduce anthocyanin content in strawberries and apples, while higher UV radiation levels increased the anthocyanin accumulation in purple wheat. Bustos et al. also observed a lower anthocyanin content in wheat grains from the shading of the spikes, proposing an effect of light on the genes controlling anthocyanin biosynthesis. These results reflect the influence of solar radiation on the accumulation of anthocyanins, suggesting that their decrease in Hindukusch was due to reduction of the incident radiation caused by the polyethylene film.

CONCLUSIONS

Heat stress during the mid-grain filling period not only reduced final GW (on average by more than 10%) and size but also changed the relative composition of its bioactive compounds. In the case of β-glucans and arabinoxylans, the relationship between heat stress and their content was indirect because the resulting increases in concentrations were due to the lower GW under stress. However, heat stress had indirect and direct significant impacts on some PC, increasing their concentrations differentially across genotypes (up to 20%). Grain under heat stress had more PC, which contribute to a higher antioxidant capacity of up to 30%, depending on the genotype. The lower incidence of solar radiation due to the use of conventional UV blocking polyethylene film reduced the anthocyanin accumulation in the purple grain genotype. Despite the influence of genotypic variations on the final grain quality, these findings highlight the importance of assessing the impact of heat stress periods on barley bioactive compounds, especially PC, to develop a better understanding of its subsequent impact on functional properties of these compounds for human health. Future research would be necessary to determine whether the structure of some of these bioactive compounds is affected by heat stress as it can influence the final quality of the barley-based product.

These findings support growing food barley in high-temperature stress-prone areas, as some bioactive compound and antioxidant capacity will increase, regardless of the smaller size grains. Furthermore, if a market develops for food barley, late sowings or late flowering genotypes could also be recommended for any barley-growing area, should a potential premium be implemented to compensate for the expected lower grain yield.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

Table 3. Fixed-effect F-tests for the REML analyses of covariance (ANCOVA) for main phenolic compounds concentration in the grain, using GW as a covariable, of four barley genotypes grown under control and induced heat stress, ‘Environment’, for two consecutive years in Lleida, Spain

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<thead>
<tr>
<th>Source</th>
<th>Free ferulics</th>
<th>Bound ferulics</th>
<th>Bound coumarics</th>
<th>Flavan-3-ols (μg g⁻¹)</th>
<th>Anthocyanins (μg g⁻¹)</th>
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Bold font indicates significance at P < 0.05.
SUPPORTING INFORMATION
Supporting information may be found in the online version of this article.

REFERENCES