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

Mariona Martínez-Subirà, © María-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 concentration 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 fibber; 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.3 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 responses5 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.6 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.

* Correspondence to: I Romagosa, University of Lleida – AGROTECNIO CERCA Center, Av. Rovira Roure 191, 25198 Lleida, Spain. E-mail: ignacio.romagosa@udl.cat

University of Lleida – AGROTECNIO-CERCA Center, Lleida, Spain

© 2021 The Authors. Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry. 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.
They possess antioxidant capacity and have been associated with the reduction of cardiovascular disease, inflammation and a diversity of cancers. 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 composition 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 controlled1,12 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. 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 factors19,20 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 years 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. 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.25 Two temperature conditions were induced: a control and a high-temperature treatment, starting 15 days after heading (decimal code, 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,25 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 × 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 (Filtra, Barcelona, Spain) to determine the percentage of grains retained through nested slotted sieves of 2.2, 2.5 and 2.8 mm. Grain plumpness was estimated by the percentage weight of grains retained over a 2.5 mm sieve.

**Milling**

The barred seeds were milled using a Cyclotec 1093™ (FOSS, Barcelona, Spain) mill equipped with a 0.5 mm screen to produce whole meal flour, which was immediately kept at −20 °C until analysis. Grain weight was determined with a Marvin System according to the standard MSZ 6367/4-86 (1986) method. The grains were sieved using an electromagnetical siever shaker (Filtra, Barcelona, Spain) to determine the percentage of grains retained through nested slotted sieves of 2.2, 2.5 and 2.8 mm. Grain plumpness was estimated by the percentage weight of grains retained over a 2.5 mm sieve.

**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 method. The total amounts of mixed-linkage β-glucans and arabinoxylans in wholemeal flours were determined using the β-glucan assay method.
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, with one ORAC unit being equal to the antioxidant protection given by 1 µmol Trolox. The antioxidant capacity of the extracts was calculated as micromoles of Trolox per gram of dry sample.

Statistical analysis

Chemical determinations were carried out in triplicate and means used for the subsequent statistical analyses. A split-split-plot-like model with two full replicates was used, with year as main plots, genotypes in subplots and environment (heat stress and control) as sub-sub plots. Two complementary statistical analyses were carried out. First, a direct standard analysis of variance (ANOVA) of grain weight (GW) and grain plumpness (% grains >2.5 mm) and of α-glucans and arabinoxylans concentrations in the grain, using GW as a covariable, of four barley genotypes grown under field conditions and cannot be simply extrapolated to interpret variations in actual yield and quality observed in the field. 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,[^24] which increased radiation use efficiency.[^35] 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 S1). 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
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 $\beta$-glucan content. This ranged from $80 \pm 2 \text{ mg g}^{-1}$ in Tamalpais to $50 \pm 2 \text{ mg g}^{-1}$ in Hispanic over 2 years (Fig. 1(C) and Supporting Information, Table S1). Although it has reported that waxy genotypes have higher $\beta$-glucans content than non-waxy types, the non-waxy genotype Tamalpais did not differ from the waxy genotype Annapurna. The grain $\beta$-glucan content was not significantly altered by the continuous stress treatment (Table 1 and Fig. 1(C)). However, $\beta$-glucans were affected by annual variability, as the genotype × year interaction was statistically significant (Table 1). $\beta$-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 $\beta$-glucan levels in barley grain. Genotypes grown under heat stress had higher antioxidant capacity except for Hindukusch, which decreased from 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...
This reduction could be associated with a decrease in some PC, particularly anthocyanins due to use 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%).

| Source       | ANOVA F-ratio | ANOVA P-value | ANCOVA F-ratio | ANCOVA P-value | ANOVA F-ratio | ANOVA P-value | ANCOVA F-ratio | ANCOVA P-value |
|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| GW           | 6.49          | 0.0349        |               |               | 19.01         | 0.0024        |               |               |
| Year (Y)     | 35.40         | 0.0271        | 11.38         | 0.0064        | 5.03          | 0.1848        | 3.70          | 0.0863        |
| Genotype (G) | 91.50         | 0.0000        | 67.32         | 0.0000        | 17.82         | 0.0028        | 4.59          | 0.0517        |
| G * Y        | 6.98          | 0.0223        | 15.95         | 0.0019        | 6.21          | 0.0218        | 11.53         | 0.0060        |
| Environment (E) | 73.73       | 0.0000        | 1.20          | 0.3019        | 18.68         | 0.0076        | 7.87          | 0.0227        |
| Y * E        | 1.14          | 0.3167        | 0.14          | 0.7133        | 0.16          | 0.7090        | 9.08          | 0.0137        |
| G * E        | 13.73         | 0.0016        | 12.07         | 0.0011        | 2.20          | 0.2063        | 4.11          | 0.0618        |
| Y * G * E    | 0.30          | 0.8267        | 6.96          | 0.0080        | 0.37          | 0.7795        | 3.58          | 0.0773        |

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 use 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 prodelphinidin 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-dimalonyl 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 purple and six-rowed genotypes 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.

Flavan-3-ols were strongly affected by genotype and year × genotype interaction (Table 3). The highest level of 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. 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...
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 grapes. 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 UV-blocking 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. 3(E)). Anthocyanin concentration in Hindukusch under stress conditions decreased 61% on average over the 2 years (Fig. 3(E)). 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 weat 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 decreases in concentrations were due to the lower GW under stress. However, heat stress had direct and indirect 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**

This work was funded by project AGL 2015–69435-C3-1 from the Spanish Ministry of Economy and Competitiveness. Mariona Martinez-Subirà was supported by a pre-doctoral fellowship (BES-2016-078654/AGL 2015–69435-C3-1).

**CONFLICTS OF INTEREST**

The authors declare no conflicts of interest.
SUPPORTING INFORMATION

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

REFERENCES

1 FAOSTAT (2019). Available: http://www.fao.org/faostat/es/ [6 November 2020].

2 Balik BK and Ullrich SE, barley for food: characteristics, improvement, and renewed interest. J Cereal Sci 48:233–242 (2008).

3 FDA. Food Labeling: Health Claims; Soluble Dietary Fiber from Certain Foods and Coronary Heart Disease. [Docket No. FDA-2008-P0090]. Available: https://pubmed.ncbi.nlm.nih.gov/12361061/ [5 June 2020].

4 EFSA. Scientific opinion on the substantiation of health claims related to β-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycemic responses (ID 821, 824), and ‘digestive function’ (ID 850) pursuant to article 13(1) of regulation (EC) no 1924/2004. EFSA J 9:2207 (2011). Available: http://www.efsa.europa.eu/en/efsajournal/pub/2207 [5 June 2020].

5 Bashir KM and Choi JS, Clinical and physiological perspectives of β-glucans: the past, present, and future. Int J Mol Med 18:1906 (2007).

6 EFSA. Scientific opinion on the substantiation of health claims related to arabinoxylans produced from wheat endosperm and reduction of post-prandial glycemic responses (ID 830) pursuant to Article 13(1) of Regulation (EC) no 1924/2004. Available: https://www.efsa.europa.eu/en/efsajournal/pub/2205 [5 June 2020].

7 Izudorczyk MS and Dexter JE, barley, β-glucans and arabinoxylans: molecular structure, physicochemical properties, and uses in food products – a review. Food Res Int 41:850–868 (2008).

8 Pandey KB and Risvi SI, Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2:270–278 (2009).

9 Rani S, Chaudhary A and Rani K, Management strategies for abiotic stresses in barley. Wheat Barley Res 10:151–165 (2018).

10 Zhou B, Jin Z, Schwarz P and Li Y, Impact of genotype, environment and matting quality on the antioxidant activity and phenolic content in US malting barley. Fermentation 6:48 (2020).

11 Savin R and Nicolas ME, Effects of short periods of drought and high temperature on grain growth and starch accumulation of two malting barley cultivars. Aust J Plant Physiol 23:201–210 (1996).

12 Wallwork MAB, Logue SJ, MacLeod LC and Jenner CF, Effect of high temperature during grain filling on starch synthesis in the developing barley grain. Aust J Plant Physiol 25:173–181 (1998).

13 Savin R, Stone PJ and Nicolas ME, Responses of grain growth and matting quality of barley to short periods of high temperature in field studies using portable chambers. Aust J Agric Res 47:465–477 (1996).

14 Passarella VS, Savin R and Slaf er GA, Grain weight and matting quality in barley as affected by brief periods of increased spike temperature under field conditions. Aust J Agric Res 53:1–9 (2002).

15 Savin R and Molina-Canjo JL, Changes in malting quality and its determinants in response to abiotic stresses, in barley Science: Recent Advances from Molecular Biology to Agronomy of Yield and Quality, ed. by Slaf er GA, Molina-Canjo JL, Savin R, Araus JL and Romagosa I. Food Product Press, New York, NY, pp. 523–550 (2002).

16 Buñol S and Jenner C, Differential responses to high temperatures of starch and nitrogen accumulation in the grain of four cultivars of wheat. Funct Plant Biol 12:363–375 (1985).

17 Jenner CF, Ugalde DT and Aspinal D, The physiology of starch and protein deposition in the endosperm of wheat. Aust J Plant Physiol 18:211–226 (1991).

18 Swanson JS, Ellis RP, Perez-Vendrell A, Voltas J and Molina-Canjo JL, Patterns of barley grain development in Spain and Scotland and their implications for malting quality. Cereal Chem 74:456–461 (1997).

19 Henry RJ, Genetic and environmental variation in the pentosan and β-glucan contents of barley, and their relation to malting quality. J Cereal Sci 4:269–277 (1986).

20 Zhang X, Wu F and Zhang G, Genotypic and environmental variations of arabinoxylan content and endoxylanase activity in barley grains. J Integr Agric 12:1489–1494 (2013).

21 Rao S, Santhakumar AB, Chinkwo KA and Blanchard CL, Investigation of phenolic compounds with antioxidant activity in barley and oats affected by variation in growing location. Cereal Chem 97:772–782 (2020).

22 Narval S, Kumar D and Verma RPS, Effect of genotype, environment and malting on the antioxidant activity and phenolic content of Indian barley. J Food Biochem 40:91–99 (2016).

23 Iqbal M, Raja N, Yasmeen F, Hussain M, Ejaz M and Shah MA, Impacts of heat stress on wheat: a critical review. Adv Crop Sci Technol 5:251 (2017).

24 Bavei V, Vaeei B, Abdipour M, Kamali MRJ and Roustaii M, Screening of tolerant spring barleys for terminal heat stress: different importance of yield components in barleys with different row type. Int J Plant Breed Genet 5:175–193 (2011).

25 Li J, Lin X, Chen A, Peterson T, Ma K, Bertzky M et al., Global priority conservation areas in the face of 21st century climate change. PLoS One 8:e54839 (2013).

26 Elia M, Slaf er GA and Savin R, Yield and grain weight responses to post-anthesis increases in maximum temperature under field grown wheat as modified by nitrogen supply. Field Crops Res 221:228–237 (2018).

27 Zadoks JC, Chang TT and Konzak CF, A decimal code for the growth stages of cereals. Weed Res 14:415–421 (1974).

28 Servei Meteorologic de Catalunya (2017). Available: https://static-met.eo.cat/wordpresdf/wp-content/uploads/2018/11/22160838/Bullet%20%20Primavera_2017_v4.pdf [8 July 2020].

29 Servei Meteorologic de Catalunya (2018). Available: https://static-met.eo.cat/wordpresdf/wp-content/uploads/2018/11/22161004/Bullet%20%20Primavera_2018_v2.pdf [8 July 2020].

30 Martínez M, Mottilva MJ, López D I HMC, Romero MP, Vaculova K and Ludwig IA, Phytochemical composition and β-glucan content of barley genotypes from two different geographic origins for human health food production. Food Chem 245:61–70 (2018).

31 Serra A, Rubió L, Macia A, Valls RM, Catalan Ú, de la Torre R et al., Application of dried spot cards as a rapid sample treatment method for determining hydroxytyrosol metabolites in human urine samples: comparison with microelution solid-phase extraction. Anal Bioanal Chem 405:9179–9192 (2013).

32 Huang D, Ou B, Hampsch-Woodill M, Flanagan JA and Prior RL, High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. J Agric Food Chem 50:4437–4444 (2002).

33 Passioura JB, Translational research in agriculture: can we do it better? Crop Past Sci 71:517–528 (2020).

34 Soar CJ, Collins MJ and Sadras VO, Irrigated shiraz vines (Vitis vinifera) up regulate gas exchange and maintain berry growth in response to short spells of high maximum temperature in the field. Funct Plant Biol 36:801–814 (2009).

35 Sinclair TR, Shiraïwa T and Hammer GL, Variation in crop radiation-use efficiency with increased diffuse radiation. Crop Sci 32:1281–1284 (1992).

36 Rakszegi M, Lovegrove A, Balla K, Láng L, Bedo Z, Veisz O et al., Effect of heat and drought stress on the structure and composition of arabinoxylans and β-glucan in wheat grain. Carbohydr Polym 102:557–565 (2014).

37 Duriano S, Savino M, Codianni P, Iannuccini A, Cateronolo G, Russo M et al., Anthocyanin profile and antioxidant capacity in coloured barley. Int J Food Sci Technol 54:2478–2486 (2019).

38 Kim M-J, Hyun JN, Kim J-A, Park J-C, Kim M-Y, Kim J-G et al., Relationship between phenolic compounds, anthocyanins content and antioxidant activity in colored barley germplasm. J Agric Food Chem 55:4802–4809 (2007).

39 Holtekljøen AK, Knitz C and Knutens SH, Flavonol and bound phenolic acid contents in different barley varieties. J Agric Food Chem 54:2253–2260 (2006).

40 Di Silvestro R, Di Loreto A, Bosi S, Bregola V, Marotti I, Benedettelli S et al., Environment and genotype effects on antioxidant properties of organically grown wheat varieties: a 3-year study. J Sci Food Agric 97:641–649 (2017).

41 Shamloo M, Babawale EA, Furtado A, Henry RJ, Eck PK, Peter JH et al., Effects of genotype and temperature on accumulation of plant secondary metabolites in Canadian and Australian wheat grown under controlled environments. Sci Rep 7:9133 (2017).

42 Yao L, Caffin N, D’arcy B, Jiang Y, Shi J, Singanusong R et al., Seasonal variations of phenolic compounds in Australia-grown tea (Camellia sinensis). J Agric Food Chem 53:6477–6483 (2005).
43 Song RD, Kelman DKL, Johns KL and Wright AD, Correlation between leaf age, shade levels, and characteristic beneficial natural constituents of tea (Camellia sinensis) grown in Hawaii. *Food Chem* **133**:707–714 (2012).

44 Martínez-Lüscher J, Lee Chen CC, Brillante L and Kurtural SK, Partial solar radiation exclusion with color shade nets reduces the degradation of organic acids and flavonoids of grape berry (Vitis vinifera L.). *J Agric Food Chem* **65**:10693–10702 (2017).

45 Lancaster JE, Reay PF, Norris J and Butler RC, Induction of flavonoids and phenolic acids in apple by UV-B and temperature. *J Hortic Sci Biotechnol* **75**:142–148 (2000).

46 Sharma A, Shahzad B, Rehman A, Bhardwaj R, Landi M and Zheng B, Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules* **24**:2452 (2019).

47 Tsormpatsidis E, Henbest RGC, Davis FJ, Battey NH, Hadley P and Wagstaffe A, UV irradiance as a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce ‘Revolution’ grown under polyethylene films. *Environ Exp Bot* **63**:232–239 (2008).

48 Josuttis M, Dietrich H, Treutter D, Will F, Linnemannstönös L and Krüger E, Solar UVB response of bioactives in strawberry (Fragaria × ananassa Duch. L.): a comparison of protected and open-field cultivation. *J Agric Food Chem* **58**:12692–12702 (2010).

49 Henry-Kirk RA, Plunkett B, Hall M, McGhie T, Allan AC, Wargent JJ et al., Solar UV light regulates flavonoid metabolism in apple (Malus × domestica). *Plant Cell Environ* **41**:675–688 (2018).

50 Wang F, Xu Z, Fan X, Zhou Q, Cao J, Ji G et al., Transcriptome analysis reveals complex molecular mechanisms underlying UV tolerance of wheat (Triticum aestivum L.). *J Agric Food Chem* **58**:12692–12702 (2019).

51 Bustos DV, Riegel R and Calderini DF, Anthocyanin content of grains in purple wheat is affected by grain position, assimilate availability and agronomic management. *J Cereal Sci* **55**:257–264 (2012).