Current Research in Nutrition and Food Science

Evaluation of Diverse Barley Cultivars and Landraces for Contents of Four Multifunctional Biomolecules with Nutraceutical Potential

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Abstract

Barley is long-identified as a functional food due to its content of micronutrients, β-glucans and vitamins. However, there is scant literature on a number of other nutritionally important biomolecules in the barley grain. This study determined the contents of four biomolecules, each with multiple known human and/or other animal health benefits, in the grains of 27 commercial barley cultivars and 7 landraces of barley from diverse countries of origin. These included the antioxidants, comprised of various vitamin E isomers and polyphenols, the osmoprotectant glycine betaine (GB) that protects cellular cytoplasm from osmotic shock, and the 'plant stress hormone' abscisic acid (ABA) which is endogenously expressed in humans and has multiple roles in physiology. All grains exhibited the presence of all biomolecules, suggesting they could potentially make some contribution to the health benefits of barley. The total vitamin E content varied between 19.20 - 54.56 μg/g DW, with α-tocotrienol being the major component (33.9 - 60.7%). The phenolics made up 3.21 - 9.73 mg gallic acid equivalent (GAE)/g DW, exceeding the amounts in the two major cereals, rice and wheat. GB ranged between 0.41-1.40 mg/g DW. The total vitamin E contents and GB typically exceeded those in corn. ABA ranged as 8.50 - 235.46 ng/g dry weight (DW), with the highest inter-variety variability. The data confirm barley to be an excellent source of these nutraceuticals, generally better than other major cereals. Our results thus offer more detailed insights into the potential of barley as a functional food and suggests the need to investigate in depth the health effects of this grain as well as the contribution of genetic and environmental factors.

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Introduction

Barley (*Hordeum vulgare* L.) is a member of the family Poacea. In terms of production and area of harvest, it is the fourth major cereal crop in the world (http://faostat.fao.org). Barley is used either for malting as a precursor in the production of beer or as a feed-stock for animals.[1] However, over the last decade, there is an increasing interest in barley due to significant amounts of soluble dietary fibre such as β-glucans, arabinoxylans and pectins in its grains.[1,2] The main component of barley grain is starch (70-80%), followed by proteins (10-15%) and non-starch polysaccharides including β-glucans, arabinoxylans and pectins (3-8%)2. Other components include lipids (3%), minerals including iron and zinc,[3] vitamins (vitamin B complex, vitamin E, vitamin K, vitamin A) (~0.07 mg/kg)[4] and phenolic compounds (0.3-0.6% gallic acid equivalent; GAE).[5,6] Whole grain barley products increase satiety, assist in obesity management and reduce plasma cholesterol, collectively reducing susceptibility to heart diseases and type-2 diabetes.[1] The polyphenolics, vitamin E isomers and arabinoxylans also impart antioxidant properties on barley foods.[1,2] Hence barley foods have 'nutraceutical' (nutritional and pharmaceutical) value and are considered 'functional foods'[7], defined as 'foods and food components that provide a health benefit beyond basic nutrition'.[8]

Interestingly, some of the biomolecules in the barley grain have dual significance, as potential nutraceuticals in the grain, and in abiotic stress responses of plants (reviewed by Derakhshani *et al.,*[9]) These include the polyphenolics, which possess strong antioxidant activities and the ability to scavenge free radicals, break radical chain reactions and chelate metals.[10] Extracts of juvenile barley (barley grass or seedlings or green barley) also contain numerous vitamins, minerals and polyphenolic compounds and exhibit strong antioxidant activity and are a potential source of medicinal value. The extracts exhibit intervarietal differences in the polyphenolic compounds as well as other bioactive compounds.[11,12] Further, variations are observed in the polyphenolics profile and antioxidant activity of the extracts under abiotic stress conditions.[12] Foods rich in polyphenolics can increase the antioxidant capacity of plasma, or influence the absorption of pro-oxidative food components such as iron, thus having the potential to reduce the risk of degenerative diseases associated with oxidative stress.[10] Polyphenolics could also prevent the initiation and progression of certain cancers by regulating the genes involved in transformation of normal to cancer cells, tumour growth, angiogenesis and metastasis.[13]

Vitamin E components (tocochromanols or tocols) are lipid-phase chain-breaking antioxidants that scavenge free radicals and protect biological molecules and tissues against oxidative damage.[14] A lack of α-tocopherol has been shown to negatively affect colorectal, prostate[15] and lung cancers.[16] Vitamin E components reduce the oxidation of low-density lipoprotein cholesterol (LDL-c), a factor involved in cardiovascular diseases, by decreasing the systemic oxidative stress.[17] However, there are some ambiguities in literature regarding the benefits of different vitamin E isomers. There are eight isomers of vitamin E, i.e., α-, β-, γ- and δ-tocopherol (T), and α-, β-, γ- and δ-tocotrienol (T3). Yoshida *et al.,*[14] reported that the corresponding tocopherols and tocotrienols have similar antioxidant activities against radicals and lipid peroxidation in solution and liposomal membranes. However, α-T3 was reported to be a more effective antioxidant than α-T, and tocotrienols were found to display unique bioactivities such as being anti-cholesterolemic.[18] Interestingly, vitamin E amounts quantified in barley grains were higher than in other cereal grains.[19]

Glycine betaine (GB) has the key role of an osmolyte, protecting plant cells from dehydration stresses by adjusting the osmotic pressure of the cytoplasm, due to its high solubility and non-cytotoxicity in large amounts.[20] In mammals, GB has two crucial functions, i.e., an osmolyte that regulates cell volume, and a protector of cells, proteins and enzymes against adverse conditions such as water deficit, high salinity or extreme temperatures.[21] It also facilitates the transport of waste products against their concentration gradient into urine,[22] ameliorating the negative impacts of alcohol on liver.[23] It also acts as a donor in the methylation of homocysteine in the methionine cycle in human liver and kidneys.[23] Betaine-deficient diets may result in hypomethylation of DNA and disruption of DNA repair, contributing to diseases including cancers.[24]

Abscisic acid (ABA) is a key plant hormone well known for triggering plant responses to diverse abiotic stresses including salinity and drought.[25] Interestingly, the presence of ABA has been confirmed in mammalian brain,[26] and it is produced endogenously by many other human cells and tissues.[27] ABA maintains its stress-response related functions in mammals.[28] It promotes cellular defence mechanisms against inflammation, stimulates the secretion of insulin by pancreatic β cells,[27] and acts as an endogenous immune regulator, significantly stimulating the immune, pancreatic and vascular cells under various inflammatory disease conditions.[29] Moreover, it is shown to reduce tumour growth and cell proliferation rate and induce apoptosis in four human cancer cell lines.[30]

Some of the above molecules also have synergies that promote health effects. For example, vitamin E components have synergistic effects with phenolics to reduce the susceptibility of LDL-c to oxidation, which is a risk factor in cardiovascular diseases.[17] However, studies on genetic diversity in barley related to these four dual-functional biomolecules, i.e., ABA, GB, phenolics and vitamin E, are scant. It is essential to assess the whole grains of different genotypes of barley for the individual and combinatorial nutraceutical potential. Among the genetic resources worldwide, landraces from the centre of origin of barley, i.e., the 'fertile crescent' in the middle east[31] are expected to be genetically diverse and a rich resource for breeding programs. Hence this study aimed to assess the composition of these four biomolecules in the grains of 34 barley genotypes, comprised of 27 cultivars and 7 landraces, as a measure of their nutraceutical potential. The outcomes are expected to assist in varietal selection and breeding programs of barley for enhanced nutritional value as well as environmental stress tolerance potential.

Materials and Methods
Barley Lines
Seeds of 27 barley cultivars were provided by Dr Joseph Panazzo (Agriculture Victoria Research, Horsham, Victoria). Seeds of 7 landraces (one each from Syria, Russia, Turkey, Azerbaijan, Iran, Kyrgyzstan and Ukraine) from countries around the Fertile Crescent[31] were obtained from Australian Winter Cereals Collection (AWCC) (Tamworth, New South Wales; now held at Horsham, Victoria). Altogether 32 grain samples were analysed.

Determination of Total Phenolic Content (Tpc)
The phenolics extracts were prepared according to the process used by Rababah *et al.,*[32], for fenugreek seeds, with modifications (mainly, removal of the defatting step and extraction with methanol instead of ethanol). In brief, 0.5 g of seeds were frozen in liquid nitrogen and ground to a fine powder then mixed with 10 mL of 80% methanol and stirred for 1 h at 60°C for extraction. The suspension was cooled to room temperature, then filtered and TPC determined by the spectrophotometric method.[33] First, 0.5 mL of the methanolic extract of seeds or the gallic acid (GA) standards was mixed with 2.5 mL of 10% Folin-Ciocalteu's reagent in water, and after 5 min incubation, 2.5 mL of 7.5% $NaHCO_3$ was added. The samples were incubated at 45°C for 45 min and their absorbance at 765 nm was recorded using a microplate reader (BioRad, Australia). Concentrations of the standards used 0-120 µg/mL of GA, and the TPC was expressed as GA Equivalent (mg GAE/g seeds).

Determination of Vitamin E Components
To extract the vitamin E compounds (tocochromanols or tocols) from barley grains, avoiding degradation of the isomers, a hot saponification extraction method[34] was applied. Grains (0.5 g) were added to a solution of 1 mL 100% ethanol, 0.4 mL water and 20 mg ascorbic acid. After the addition of 100 µL potassium hydroxide (10.7 M), the tubes were transferred to a boiling water bath and saponified for 25 min. The tubes were cooled on ice for 10 min, and 0.5 mL of 50% (v/v) ethanol added. To extract tocols and other unsaponifiable lipids, three portions (each 2 mL) of n-hexane:ethyl acetate (8:2 v/v) were used. After shaking for 10 min, two phases formed, and the upper organic layer was transferred into a new test tube. This process was repeated three times and the combined extracted organic phase then dried under nitrogen. The residue was dissolved in 1 mL methanol and filtered through a 0.45 µM filter for HPLC analysis. Tocochromanols were analysed according to the parameters provided by Phenomenex (Australia) HPLC application (App ID: 22953). The chromatographic system was equipped with a Kinetex F5 2.6µM 100 Å, LC column (150 x 4.6 mm), a gradient mobile phase

(0-17 min 85% methanol, 17-23 min 100% methanol, 23-26 min 85% methanol) with a flow rate of 1.2 mL per min, oven temperature of 42˚C and UV-Vis detector set at 290 nm. α-tocopherol (Sigma Aldrich, Australia) and α-, β-, λ and δ-tocotrienols (Cayman, Germany) were dissolved in methanol to make standards in the concentration ranges of 5-200 µg/ mL. In the HPLC analyses, the standards appeared at retention times of 5.5-5.6 min for δ-T3, 6.3-6.5 min for β-T3, 6.6-6.8 min for γ-T3, 7.5-7.6 min for α-T3 and 10.3-10.5 for α-T.

Determination of Glycine Betaine (GB)
Plant extracts were prepared according to Joseph *et al.,*[35] 50 mg of seeds were mixed with 150 µL of the solvent (methanol: chloroform: water 60:25:15). An equal volume of sterile Milli-Q water was added and the homogenate shaken gently for 20 min. After centrifugation, the clear methanol-water supernatant phase was separated and freeze-dried. The concentrated sample was dissolved in 0.5 mL of 50% ethanol and filtered through a 0.45 µM membrane filter. A Shimadzu high-performance liquid chromatography (HPLC) system equipped with a UV-VIS detector and a Prevail™ Carbohydrate ES (250 × 4.6 mm) column were used for analysis. The typical analytical conditions were temperature of 30°C, mobile phase (acetonitrile: Milli-Q water 75:25) flow rate of 1 mL/min, injection volume of 10 µL, maximum pressure (P_{max}) around 2200 psi, UV detection at wavelength of 190 nm and a total run time of 10 min. Peak areas were calculated using Shimadzu CLASS VP chromatography analysis software. The identification of GB in seed samples was based on retention time of the GB standard (Betaine anhydrous, $\geq$98.0%, CAS Number 107-43-7, Sigma-Aldrich), found to be 3.9-4.3 min. The concentration range of the standard used for quantitation purposes was 25 to 500 µg/mL. The amount of GB in unknown sample was determined by reporting the peak areas on the standard curve, and the amounts expressed as mg of GB per g dry weight (DW) of seeds.[35]

Determination of Abscisic Acid (ABA)
2 mL of 80% methanol was added to the crushed seeds (0.5 g) and incubated in dark at 4˚C for 24 h. This was followed by addition of 2 mL of 100% methanol and holding at 4˚C for another 24 h. 2 mL of 10 mg/L butylated hydroxytoluene was

the added and the mixture incubated for another 6 h at 4˚C. The extracts were dried under nitrogen flow and the residue dissolved in methanol and tris-buffered saline.[36] Quantitative immunoassay analysis of free ABA was performed by an Enzyme-Linked Immunosorbent Assay (ELISA) kit (PDK 09347/0096, Sigma-Aldrich) using a competitive binding reaction between a constant amount of ABA-tracer labelled with alkaline phosphatase, a limited amount of anti-ABA monoclonal antibody and the unknown sample. Any ABA in the sample competes with the ABA-tracer for antibody binding sites. The assay was performed in a microtitre plate coated with anti-ABA antibody. Concentrations of the ABA standard (strips provided in the kit) were in the range of 0-20 picomoles/mL. After dispensing 100 µL of the standard (or sample extracts) to each well, 100 µL of ABA-tracer labelled with alkaline phosphatase diluted with TBS buffer (1:400) were added, and the wells incubated at 4˚C for 3 h, then emptied. 200 µL of substrate were added to each well, the plates incubated in a humid box at 37˚C for 1 h, and absorbance at 490 nm noted as above.

Statistical Analysis
The Coefficient of Variation (% CV) was applied to determine variability of the above biomolecules, calculated as ratio of the standard deviation to the mean, using Excel.

Results and Discussion
Total Phenolic Content
Table 1 summarises the total phenolic content (TPC) of the grains of diverse barley lines. The TPC in the cultivars ranged between 3.21 mg GAE/g DW (Dhow) and 4.80 mg GAE/g DW (Sloop), while in the landraces, it was higher and ranged between 5.38 (403130, from Kyrgyzstan) to 9.73 mg GAE/g DW (411822, from Turkey).

The coefficient of variation (% CV) is a parameter that can be used to compare the relative levels of variability between crop plant traits.[37] In the current study, % CV was used to estimate the dispersion of nutraceutical values across the barley lines and to compare their relative dependance to genotypic variation. Hence it was calculated for the polyphenolics, and was 34.93% (Table 1), being the second large dispersion among the lines compared to other nutraceuticals studied

The range of TPC is in agreement with the Kruma *et al.,* 6 study of various hulled and hull-less barley varieties, wherein the TPC was reported to be in the range of 3.51- 4.60 mg GAE/g DW, and it was suggested that the key determining factor was the variety (genotype). Our results on the cultivars also agree with those of Griffiths and Welch[5] who reported TPC in barley grains as 4.3- 5.3 mg/g DW in 16 UK cultivars and 3.7-5.4 mg/g DW in 85 genotypes of diverse origins. However, in our study, the TPC of landraces was higher than that in those 85 genotypes. While it is known that TPC in leaves is affected by environmental factors12, it is unknown whether the TPC of grains is also affected by environmental factors during plant growth and/or grain storage. The TPC in defatted barley seeds from 6 cultivars[38] was 13.58 to 22.93 mg of ferulic acid equivalent/g DW, and it was concluded that a complex of compounds including phenolics collectively determined the final antioxidant capacity of barley grains. It is noteworthy that the TPC found in our study appears to be higher than that reported for rice (1.3 mg/g DW) and wheat (2.3 mg/g DW).[39] indicating barley to be a better functional food grain. Also, the grains of landraces in our study exhibited more phenolics content compared to cultivars. However, it will be necessary to carry out studies on grains grown simultaneously under the same environmental conditions for more thorough and statistically valid comparisons and assessments of genetic versus environmental factors.

Table 1: TPC, ABA, GB and vitamin E content in grains of barley cultivars and landraces

Cultivars		Total phenolics (mg GAE/g)	Total Vitamin E (µg/g DW)	GB (mg/g DW)	ABA (ng/g DW)
1	Arapiles	3.34±0.01	35.46±2.40	0.87±0.00	42.59±1.93
2	Barque	3.81±0.05	26.89±1.45	0.69±0.00	47.74±4.56
3	Baudin	3.45±0.02	29.37±1.32	0.68±0.00	36.30±3.19
4	Capstan	4.17±0.01	43.87±2.63	0.72±0.00	24.29±2.72
5	Clipper	3.92±0.04	19.20±1.04	0.81±0.01	66.45±5.27
6	Dhow	3.21±0.06	42.32±3.02	0.86±0.00	21.80±1.63
7	Fitzgerald	4.29±0.08	28.47±1.54	0.92±0.00	227.33±17.01
8	Franklin	3.27±0.04	43.29±2.10	1.40±0.01	235.46±15.54
9	Galleon	3.98±0.18	27.39±1.21	0.47±0.00	19.74±2.03
10	Hamelin	3.71±0.02	33.95±1.52	1.19±0.01	43.85±2.63
11	Harrington	3.58±0.03	32.19±1.76	0.57±0.00	195.51±14.43
12	Hindmarsh	4.60±0.04	29.80±1.72	0.83±0.00	26.31±2.02
13	Kaputar	3.65±0.00	37.67±2.01	0.57±0.00	21.86±2.18
14	Keel	3.40±0.03	24.12±0.95	0.41±0.00	61.57±4.80
15	Lofty Nijo	3.69±0.02	45.66±2.91	0.62±0.00	25.70±1.08
16	Mackay	3.70±0.03	28.06±1.50	0.58±0.00	135.65±10.33
17	Maritime	3.96±0.03	35.19±1.73	0.57±0.00	100.29±8.13
18	Mundah	3.82±0.16	37.92±2.02	0.69±0.00	49.20±3.66
19	Onslow	3.73±0.06	22.19±1.22	0.80±0.00	39.83±2.85
20	Sloop	4.80±0.16	31.52±1.75	0.89±0.01	58.80±4.93
21	Sloop SA	3.79±0.02	54.56±3.65	0.70±0.00	54.50±3.63
22	Sloop VIC	3.25±0.04	24.12±1.10	0.47±0.00	8.50±1.04
23	Stirling	3.28±0.04	21.79±0.83	0.99±0.01	89.54±5.76
24	Tantangara	3.86±0.03	26.5±1.16	0.50±0.00	67.96±4.10
25	Tilga	3.80±0.04	24.94±1.03	0.63±0.00	46.84±3.65
26	Wyalong	3.89±0.11	38.38±2.18	0.49±0.00	49.03±3.10
27	Yagan	3.52±0.16	19.48±0.93	0.72±0.00	15.92±2.37

Landraces (AWCC accession #, origin)		Total phenolics (mg GAE/g)	Total Vitamin E (µg/g DW)	GB (mg/g DW)	ABA (ng/g DW)
28	400125 (Syria)	6.54±0.08	21.40±0.87	0.69±0.00	57.22±4.82
29	400254 (Russia)	7.07±0.15	19.33±0.90	0.62±0.01	51.17±4.35
30	411822 (Turkey)	9.73±0.04	29.71±1.08	0.42±0.00	44.83±4.50
31	403130 (Azerbaijan)	6.15±0.01	28.04±1.21	1.16±0.01	9.20±0.62
32	407328 (Iran)	5.38±0.03	21.86±1.14	0.64±0.01	63.72±2.63
33	411813 (Kyrgyzstan)	8.44±0.13	22.36±1.32	0.60±0.00	36.20±3.61
34	408603 (Ukraine)	6.46±0.11	29.96±1.20	1.03±0.01	28.00±0.97
Coefficient of Variation (%)		**34.93**	**28.16**	**31.42**	**91.07**

Vitamin E Isomers

In this study, all 4 isomers of tocotrienols (α-, γ-, β- and δ-T3), as well as α-tocopherol (α-T) were analysed as the main vitamin E components (tocols) in barley grains, as other forms of tocopherols are reported to be negligible. Except for α-T, no other tocopherols have been detected in barley grains,[40] and the γ-T, β-T and δ-T together constitute less than 6%, 5% or 4% of the average total vitamin E content in studies on barley grains by Panfili *et al.,*[19] Cavallero *et al.,*[41] and Do *et al.,*[42], respectively. The results confirmed α-T3 to be the major tocol, constituting 33.9-60.7% of total vitamin E. The α-T3 content ranged from 8.09 to 28.20 µg/g DW in cultivars with the lowest and highest contents being for Yagan and Sloop SA (Table 2). In landraces, this range was between 8.11 (landrace 400254) and 15.46 µg/g DW (landrace 408603).

Table 2: Vitamin E isomer contents in grains of barley cultivars and landrace

	Cultivars	α-T (µg/g DW)	α-T3 (µg/g DW)	β-T3 (µg/g DW)	γ-T3 (µg/g DW)	δ-T3 (µg/g DW)
1	Arapiles	9.78±0.41	14.51±0.61	4.36±0.16	5.39±0.08	1.42±0.05
2	Barque	6.89±0.36	11.75±0.44	3.50±0.06	4.75±0.08	-
3	Baudin	7.42±0.25	12.82±0.69	3.50±0.05	4.76±0.07	0.87±0.03
4	Capstan	10.31±0.37	23.89±1.30	3.76±0.08	5.91±0.10	-
5	Clipper	4.21±0.11	8.18±0.38	3.18±0.05	3.63±0.06	-
6	Dhow	7.28±0.32	24.30±1.03	3.91±0.08	5.63±0.25	1.20±0.03
7	Fitzgerald	4.87±0.18	16.07±1.01	3.47±0.03	4.06±0.09	-
8	Franklin	6.79±0.22	26.29±1.11	3.82±0.07	5.54±0.18	0.85±0.03
9	Galleon	6.31±0.09	12.19±0.57	3.80±0.05	5.09±0.23	-
10	Hamelin	7.03±0.21	15.80±0.85	4.14±0.09	5.97±0.15	1.01±0.05
11	Harrington	7.17±0.18	14.33±0.65	3.94±0.07	5.77±0.18	0.98±0.03
12	Hindmarsh	7.30±0.13	12.26±0.50	3.99±0.05	5.30±0.19	0.95±0.04
13	Kaputar	7.71±0.24	18.73±1.03	4.32±0.07	6.91±0.25	-
14	Keel	6.45±0.17	9.84±0.42	3.29±0.05	4.54±0.09	-
15	Lofty Nijo	7.50±0.40	25.13±1.09	4.74±0.09	7.39±0.40	0.90±0.06
16	Mackay	7.89±0.42	11.26±0.48	3.37±0.03	4.55±0.14	0.99±0.05
17	Maritime	5.71±0.21	19.04±0.81	4.32±0.07	5.03±0.24	1.09±0.05
18	Mundah	8.54±0.50	18.49±0.75	4.11±0.08	5.90±0.31	0.88±0.03
19	Onslow	6.56±0.36	8.19±0.31	3.31±0.07	4.13±0.08	-
20	Sloop	8.17±0.14	13.38±0.76	3.91±0.09	4.82±0.13	1.24±0.05
21	Sloop SA	11.49±0.45	28.20±1.67	6.51±0.13	7.01±0.42	1.35±0.05

		α-T	α-T3	β-T3	γ-T3	δ-T3
22	Sloop VIC	6.65±0.38	8.25±0.42	3.68±0.07	4.50±0.10	1.04±0.06
23	Stirling	5.70±0.11	8.18±0.52	3.37±0.08	4.54±0.11	-
24	Tantangara	6.58±0.16	12.47±0.45	3.26±0.04	4.19±0.13	-
25	Tilga	7.29±0.20	8.45±0.23	3.35±0.05	4.82±0.12	1.03±0.05
26	Wyalong	8.27±0.35	19.16±0.60	4.01±0.12	5.58±0.17	1.36±0.07
27	Yagan	4.10±0.14	8.09±0.33	3.26±0.05	4.03±0.20	-
Landraces (AWCC accession #, origin)		**α-T (µg/g DW)**	**α-T3 (µg/g DW)**	**β-T3 (µg/g DW)**	**γ-T3 (µg/g DW)**	**δ-T3 (µg/g DW)**
28	400125 (Syria)	4.44±0.15	9.57±0.36	3.39±0.03	4.00±0.14	-
29	400254 (Russia)	4.01±0.10	8.11±0.35	3.29±0.02	3.92±0.09	-
30	411822 (Turkey)	7.18±0.37	14.81±0.80	3.04±0.03	3.49±0.12	1.19±0.01
S31	403130 (Azerbaijan)	4.94±0.16	14.28±0.65	3.87±0.07	4.61±0.15	-
32	407328 (Iran)	4.56±0.18	9.52±0.56	3.41±0.05	4.37±0.16	-
33	411813 (Kyrgyzstan)	5.60±0.19	8.38±0.40	4.18±0.06	4.20±0.12	-
34	408603 (Ukraine)	5.59±0.10	15.46±0.82	3.54±0.05	4.54±0.16	0.83±0.01
Coefficient of Variation (%)		**25.64**	**40.6**	**16.64**	**19.14**	**17.50**

The content of β-T3 was 3.18-6.51 µg/g DW in cultivars and 3.04-4.18 µg/g DW in landraces. γ-T3 was 3.63-7.39 µg/g DW in cultivars, and 3.49-4.61 µg/g DW in landraces. δ-T3 exhibited the lowest amounts, being 0.85-1.42 µg/g DW in cultivars, 0.83-1.19 µg/g DW in landraces, and undetectable in several lines. The range of α-T in the cultivars was between 4.10 (Yagan) and 11.49 µg/g DW (Sloop SA), and that for landraces was 4.01 (400254) to 7.18 µg/g DW (411822) (Table 2).

The sum of all tocol isomers was used to determine the total vitamin E content, which averaged between 19.20 and 54.56 µg/g DW in cultivars (Table 1), with Clipper and Sloop SA having the lowest and highest contents, respectively. The landraces showed a total vitamin E range of 19.33 to 29.96 µg/g DW, with the minimum and maximum amounts noted in landraces 400254 and 408603 (from Russia and Ukraine, respectively). The % CV of total vitamin E was the lowest among the other three nutraceuticals (Table 1) representing that its content in barley seeds has the least dependence to genotypic variation. Further, the % CV was calculated for each tocol isomer (Table 2), the lowest and highest variations being noted for β-T3 (16.64%) and α-T3 (40.60%), respectively.

The levels of α-T3 (8.09-28.20 µg/g DW) found were broadly comparable to those reported in 30 different barley lines (23.8-43.0 µg/g)[43] and one barley line (17.1 µg/g).[40] The isoforms α-T (4.01-11.49 µg/g DW), γ-T3 (3.49-7.39 µg/g DW), β-T3 (3.04-6.51 µg/g DW) and δ-T3 (0.83-1.42 µg/g DW) were sequentially the next abundant forms, also in agreement with other studies on grains of different barley genotypes.[42,43,44] The total content of vitamin E (19.20-54.56 µg/g DW) is in good agreement with that in the whole grain of one variety (23.14 µg/g)[40] and five varieties (23.63-32.68 µg/g),[44] but less than that reported in another study on 25 barley genotypes (20.3-102.4 µg/g).[42] In relation to other major cereals, the total vitamin E content in our study appears to be comparable to that in wheat (35.02 µg/g DW),[40] higher than corn (4.76 µg/g DW),[45] and comparable to oat (19.00-30.32 µg/g DW)[43] or better (15.11 µg/g DW).[40] These results suggest a strong potential of barley as a functional cereal grain, as also observed in the TPC studies.

Glycine Betaine (GB)
The GB contents of barley cultivars ranged between 0.41 mg/g DW in Keel, and 1.40 mg/g DW in Franklin with a % CV of 31.42% (Table 1), while the range for landraces was 0.42 mg/g in 400254 (Russia) to 1.16 mg/g in 403130 (Azerbaijan). The content of GB noted is similar to that noted in the wholemeal in 10 barley lines (0.71–1.36 mg/g DW)[46] and a grain sample (460 mg/g DW).[47] The GB content in many barley samples in our study is higher than that in

oat (0.43 mg/g DW) but lower than that reported in rye (2.27 mg/g DW) and bread wheat (1.59-1.62 mg/g DW).[46]

Abscisic Acid (ABA)

The amount of grain ABA in the cultivars was 8.50- 235.46 ng/g DW (Table 1). The ABA variations were the most significant among the four biomolecules analysed, with the largest % CV (91.07%) (Table 1). The minimum and maximum grain ABA contents were recorded in Sloop VIC (8.50 ng/g DW) and Franklin (235.46 ng/g DW). Among the landraces, landrace 403130 (from Azerbaijan) (9.20 ng/g) and 407328 (from Iran) (63.72 ng/g) exhibited the lowest and highest levels, respectively (Table 1).

ABA is well-known as a plant stress hormone with key roles in the regulation of plant responses to environmental stresses, induction of seed dormancy and biosynthesis of seed storage proteins and lipids.[25] It is also a bioactive molecule produced endogenously in many human cells with proven roles in stress-related functions in mammals,[28] and as an endogenous immune regulator,[29] potentially making it an important nutraceutical. The ABA concentrations determined here exhibited a wide range, from 7.37 to 235.46 ng/g, the upper limit being similar to the 262 ng/g reported in another study on barley grains.[48] The % CV for ABA content (91.07%) represents the highest dispersion and dependence on genotypic diversity among the biomolecules studied. The biosynthetic pathway of ABA is complex and has numerous steps,[48] suggesting involvement of many genetic factors. ABA levels are reported to increase during seed maturation and in response to different abiotic stresses.[25] In barley, certain genes with key roles in ABA biosynthesis and catabolism are regulated in a growth dependent manner in the grains but are also affected by environmental conditions prevailing during grain development.[48] Thus the ABA contents of barley grains may be controlled by environmental as well as genetic factors, and both need investigations.

In summary, our results show that the grains of all lines exhibited the presence of all four biomolecules, often in proportions higher than those reported in other major cereals such as wheat, supporting that these molecules would be expected to contribute the functional food properties of barley. Further work using cell culture, physiological and gene expression studies will help test this hypothesis. Some inter-varietal differences were also noted in the relative proportions of the biomolecules studied. The results also indicate that the landraces may be suitable for utilisation in breeding approaches, for developing barley as a functional food. Detailed analysis of grains of barley lines grown in identical or diverse environmental conditions (such as growth temperatures, drought, soil salinity) will also provide data on their genetic versus environmentally determined variability.

Conclusions

The study confirms barley to be a rich source of the four biomolecules with nutraceutical potential, particularly phenolics and total vitamin E. Landraces also exhibit these biomolecules, confirming the potential of such lines as a rich genetic resource. Interestingly, the compounds assessed here have significant roles in abiotic stress tolerance. Hence developing a greater understanding of expression and distribution of these molecules in diverse germplasm and environments during plant development and grain filling will enable selection and breeding of genotypes suitable for cultivation under challenging conditions, in addition to improved nutritional value. Study of the genetic variability in key genes involved in the biosynthetic pathways of these compounds and their expression patterns will aid such developments.

Conflict of Interest

The authors declare that they have no conflict of interest.

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