Genetic Resources and Crop Evolution

Morpho-physiological and qualitative variation of domesticated einkorn (Triticum monococcum L. ssp. monococcum).

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Corresponding Author:	Andrea Brandolini Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA) Sant'Angelo Lodigiano, ITALY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA)
Corresponding Author's Secondary Institution:	
First Author:	Andrea Volante
First Author Secondary Information:	
Order of Authors:	Andrea Volante
	Volkan Arif Yilmaz
	Alyssa Hidalgo
	Andrea Brandolini
Order of Authors Secondary Information:	
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Morpho-physiological and qualitative variation of domesticated einkorn (*Triticum monococcum* L. ssp. *monococcum*).

Andrea Volante[1], Volkan Arif Yilmaz[2] Alyssa Hidalgo[3], Andrea Brandolini[4*]

[1] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di Ricerca Cerealicoltura e Colture Industriali (CREA-CI), Strada Provinciale 11 per Torino km 2.5, 13100 Vercelli, Italy. E-mail: andrea.volante@crea.gov.it

[2] Food Engineering Department, Ondokuz Mayıs University, Kurupelit, 55139 Samsun, Turkey. E-mail: volkan.yilmaz@omu.edu.tr

[3]Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, via Celoria 2, 20133 Milan, Italy. E-mail: alyssa.hidalgovidal@unimi.it

[4] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di Ricerca Zootecnia e Acquacoltura (CREA-ZA), Via R. Forlani 3, Sant'Angelo Lodigiano (LO), Italy. Telephone: + 39 0371 211261; e-mail: andrea.brandolini@crea.gov.it; ORCID: 0000-0002-4552-4081

* Corresponding author.

Abstract

A pool of 158 *Triticum monococcum* L. ssp. *monococcum* accessions, originating from different traditional cropping areas and representative of a broader germplasm collection, was characterised for 20 morpho-physiological and qualitative descriptors. The accessions were cultivated for four years in two different Po plain (Italy) locations. The traits analysed were growth habit, awn length, glume colour and hairiness, rachis brittleness, heading date, plant height, spike length, n° spikelets/spike, spikelet density, n° kernels/spikelet, kernel length, width, thickness and volume, thousand kernels weight, protein and carotenoid content, sodium dodecyl sulphate sedimentation volume and specific sedimentation volume.

A broad variation for all the traits studied was detected and promising accessions for breeding purposes were identified. Several characteristics showed a clear region-specific pattern: the samples tracing their origin to warmer climates were earlier-maturing, taller, had shorter spikes, fewer spikelet/spike, bigger kernels and lower protein content than those from cooler regions. A Principal Components Analysis highlighted the existence of two clusters composed mainly of Maghreb/Iberia and of Prealpine genotypes, whose peculiar characteristics are most likely a consequence of adaptation, by natural selection or by human practices and ingenuity, to their growing environments.

Keywords. Carotenoids; einkorn; germplasm; kernel; plant; spike

Introduction

Einkorn (*Triticum monococcum* L. ssp. *monococcum*; briefly, *T. monococcum*) is a diploid (2n=2x=14) wheat, close relative of durum and bread wheats. Einkorn was domesticated from its wild progenitor (*T. monococcum* L. ssp. *boeoticum*; briefly *T. boeoticum*) about 10,000 years before present (Salamini et al, 2002) in the Karacadağ mountains area (southeast Turkey), where present-day wild population are genetically very similar to the cultivated form (Heun et al, 1997; Heun et al, 2008). This part of the world has been dubbed "the cradle of agriculture"(Lev-Yadun et al, 2000) because here thrived and still grow the Near East wild ancestors of seven founder crops i.e. einkorn, emmer, barley, chickpea, lentil, pea and bitter vetch.

Einkorn rapidly spread outside its original area, and by 5,000 before present was cropped all over Europe (Nesbitt and Samuel, 1996) contributing, together with emmer and barley, to the establishment of agriculture. For several thousand years it was a staple food, as indicated by archaeological remains and by the analysis of Ötzi, a Copper Age man found in the Alps (Oeggl, 2000). Einkorn cultivation and use diminished after the Copper Age (Nesbitt and Samuel, 1996), possibly because of lower yields and more labour-intensive post-harvest management in comparison to recently-introduced free-threshing polyploid wheats. Afterwards, *T. monococcum* survived in isolated pockets of marginal areas. By the end of the twentieth century, scattered einkorn populations were still cropped in Turkey, the Balkans, France, Italy, Switzerland, Germany, Spain and Morocco (Perrino et al, 1996). Furthermore, samples collected in the course of the last century were stored and are currently preserved in germplasm banks.

An upwards shift in the fortunes of einkorn has been fostered by recent trends toward low-impact and sustainable agriculture, as well as an increased awareness about the nutritional aspects of food. In fact, einkorn is able to grow in harsh environments and poor soils, where other wheat species cannot survive (Dinu et al 2018); additionally, einkorn has a higher content of protein, monosaturated lipids, tocols, carotenoids (especially lutein) and selected minerals than other wheat species (Hidalgo and Brandolini, 2019).

CREA maintains at the Sant'Angelo Lodigiano (Italy) premises a broad collection of diploid wheats that includes about 670 *T. monococcum* and 880 *T. boeoticum*. This germplasm collection represents a unique stock of genetic variation and a valuable source of useful genes for many agronomic and quality traits, very handy for modern breeding programmes to cope with rapidly changing environmental conditions. Aims of this research were to evaluate the morpho-physiological and qualitative variation available in this pool of domesticated einkorn, and to ascertain the existence of origin-related patterns of the characteristics.

Material and methods

Materials

One hundred fifty eight einkorn landraces, representative of the whole domesticated einkorn collection maintained at CREA-ZA Sant'Angelo Lodigiano, were selected mainly based on their provenance. The complete accessions list, with country of origin and collection site (when available) is presented in Online Resource 1, along with the average values (four years and two locations) of the traits evaluated.

Methods

About 50 kernels of each landrace were manually planted in single rows 1.2 m long and 0.40 cm apart in Sant'Angelo Lodigiano (SAL, silty sand soil, organic matter 27.8 g/kg, N content 1.5 g/kg, pH 7.1) and Lodi (LO; sandy silt soil, organic matter 16.6 g/kg, N content 1.27 g/kg, pH 6.2) during four growing seasons, i.e. 2011-2012, 2012-2013, 2013-2014 and 2014-2015. Standard cultural practices were employed, including nitrogen fertilisation (40 kg/ha) at tillering and chemical weed control (Ariane II: Fluroxipir + Clopiralid + MCPA). The spikes were manually harvested at full maturity and stored under refrigeration (5 °C). Whole meal flour was prepared from 10 g dehulled kernels using a Cyclotec 1093 laboratory mill (FOSS Tecator, Denmark) and stored at -20 °C until analysis.

The landraces were characterised for16 morpho-agronomic descriptors: growth habit (1=prostrate to 3=erect), awn length (1=no awns to 5=very long awns), glume colour (1=white-cream, 2=brown, 3=black), glume hairiness (1=no hair to 3=very hairy), rachis brittleness (1= very brittle to 5 non-brittle), heading date (number of days, starting from 1 May, to have about 50% fully visible spikes), plant height (excluding awns), spike length (excluding awns), n° spikelets/spike, spike density (n° spikelets/cm spike), n° kernels/spikelet, kernel length, kernel width, kernel thickness, kernel volume ($\frac{2}{3} * \pi * d1 * \frac{d2}{2} * \frac{d3}{2}$, where d_1, d_2 and d_3 are the length, width and thickness of the kernel) and thousand kernels weight (computed from two 100-kernel samples). The data were collected from five plants, ten spikes or 20 kernels for each landrace.

Additionally, four quality traits were assessed: protein content (N × 5.7, dry matter basis) was determined according to method 46-10.01 (AACC International), using a NIR System Model 6500 (FOSS NIRSystems, Laurel, MD, USA), sodium dodecyl sulphate sedimentation volume (SDS) was recorded as described by Preston et al (1982), specific sedimentation volume (SSV) was computed as ratio between SDS and protein content, and carotenoid content was assessed following method 14-60.01 (AACC International) using a DU-62 spectrophotometer (Beckman Coulter Inc., Brea, CA, USA). The flour quality descriptors were measured from at least two technical replicates.

Statistical analysis

After normality checking, an analysis of variance (ANOVA) was performed on the quantitative traits considering landrace and year as main factors; the two locations were used as repetitions. An exploratory Principal Components Analysis (PCA) was also carried out. Afterwards, a multivariate ANOVA was performed only on the 148 landraces of known provenience, considering geographic area of origin and year as main factors; when the ANOVA detected significant differences ($p \leq 0.05$) least significant differences (LSD) were computed. ANOVAs, means, standard errors (s.e.) and LSD were computed using the software STATGRAPHICS© Centurion XVI (StatPoint Technologies Inc., Warrenton, VA, USA) while PCA was performed with the software Past3 (Hammer et al, 2001).

Results and discussion

Genotypes variation

The frequency distributions for the five qualitative descriptors are presented in Table 1 while the overall mean, maximum and minimum values recorded for the quantitative descriptor are reported in Table 2. The qualitative characteristics analysed presented a broad variation. The growth habit of juvenile plants ranged from semi-erect to erect; the awns, always present, were short in two accessions (ID69 and ID127) belonging to the *sinskajae* group, but in general were well developed and quite long. The glume colour was mainly beige or light brown, but black spikes were sometimes observed; the glumes were generally glabrous, albeit about one quarter (22.8%) of the landraces had hair, sometimes abundant. The rachis of bread wheat does not fracture even in overripe spikes and often remains intact during threshing, while wild einkorn rachis is very fragile and breaks into single spikelets even before maturity; einkorn rachis is semi-tough, because it is still whole at maturity but breaks during threshing, resulting in kernels still enclosed by the glumes (Brandolini and Heun, 2019). Nevertheless, our results demonstrate that some variation for this trait is present in the cultivated genepool.

The quantitative descriptors showed normal data distribution; the only exception was the SDS sedimentation volume, whose results were log-transformed before analysis. The ANOVA (Online Resource 2A) highlighted significant effects due to the cropping year as well as to the landrace; their interaction, even when significant, was always of minor relevance. All the quantitative characteristics showed a broad variation, as also observed by Empilli et al (2000). The average heading date was around the end of May, but early-maturing landraces (ID2, ID3 and ID347), flowering before mid-May, were observed; the heading period spanned about one month. Equally impressive was the variation for plant height: the shortest landraces (ID366 and ID237) were 50-70 cm, while the tallest

(IDS1516) reached 127 cm; overall, einkorn plants were quite tall, averaging 109.0 cm. The mean spike length was 7.19 cm and ranged from 5.43 (ID69 and ID127) to 8.51 cm (ID1341), while the number of spikelets per spike hovered around 28.43 (range: 23.24-34.84). Kernel dimensions also fluctuated broadly: the length ranged between 6.61 and 8.24 mm, the width between 2.41 and 3.31 mm and the thickness between 1.37 and 2.22 mm, leading to volumes from 14.60 to 30.20 mm^3, and thousand kernel weights from light (16.05 g) and similar to wild diploid wheats to fairly heavy (35.66 g) and comparable to bread wheat (Brandolini et al, 2008). The high protein content (17.11 g/100 g) confirmed the results reported for einkorn by other authors (e.g. Brandolini et al, 2008; Corbellini et al, 1999); some landraces (ID147, ID568 and ID 1341) reached concentrations above 20 g/100 g. Similarly, the mean carotenoids content was 8.18 mg/kg, but 13 samples with > 10 mg/kg carotenoids were found; these results are in line with several reports (Abdel-Aal et al, 2002; Brandolini et al, 2008; Hidalgo et al, 2006) which describe einkorn as a high-lutein wheat. The breadmaking quality of the accessions was low, even considering that whole meal flour was tested; nevertheless, some landraces with SDS values over 40 mL (ID193, ID365, ID514, ID358, IDS1652) and SSV values above 3.0 (IDS652 and ID358) were spotted, confirming the existence of einkorn with good breadmaking quality (Brandolini et al, 2008; Corbellini et al, 1999).

Geographic origin

The ANOVA performed on the 148 landraces of known origin (Online Resource 2B) stressed again the importance of the cropping year; nevertheless, highly significant differences were recorded among groups of accessions coming from different areas. The interactions between the two factors were not significant or of minor relevance.

Figure 1 depicts the mean scores of the landraces, divided by area of origin, for the five qualitative characteristics. In general, the landraces from cold areas had a more prostrate growth habit than those from warm climates and the awn length was shorter among Mediterranean samples (except those from Maghreb and Italy). The glumes colour was darkest for the French samples, but there was no clear regional cline; on the other hand, the accessions from cold areas had the hairiest glumes, while those from warm areas were consistently glabrous. Rachis fragility was limited, particularly among the einkorns originating from the Mediterranean area (except for Italy).

Figure 2 presents the country-wise mean results relative to plant and spike quantitative traits. The heading date was earlier in the samples tracing their origin to warmer climates (Maghreb, Iberia, Near East) and later in those coming from cold and/or mountain areas (e.g. Switzerland, Austria, France and Germany). Additionally, the samples from the above-mentioned cold areas were generally shorter, had longer spikes and higher number of spikelet/spike than those from the other regions. The landraces from the Mediterranean area, instead, in general had shorter spikes with a lower number of kernels. Spike density, maximum in the Austria, Caucasus, Bulgaria and Switzerland accessions and minimum in the Italy, Maghreb, Germany, Hungary and France ones, did not display any particular gradient. The number of kernels/spikelet was maximum in the Near East samples, followed by Maghreb and Iberia, intermediate in the Central Europe group and minimum in the French, Italian, Turkish and Caucasian landraces.

The kernel characteristics (Figure 3) showed a clear cline. Kernel length, width, thickness and their derived variables (kernel volume and thousand kernels weight) were highest in the samples coming from the Maghreb, Italy, Iberia, Turkey and former Yugoslavia, and lowest in those from Germany, Switzerland and Austria. The peculiar behavior of the Near East landraces was due to their long and narrow kernels, similar in size and shape to wild einkorn. Figure 4 depicts the average protein and carotenoid contents, as well as the sedimentation volumes, of the landraces grouped by origin. The protein concentration was highest in the cold/mountain accessions, as well as in those from the Near East, probably because their small kernels had a higher proportion of external layers, including the protein-rich aleurone (Hidalgo and Brandolini, 2008). Carotenoids content was low in samples from the Maghreb, Iberia and Italy, and high in those from Albania, Romania, Switzerland, Greece and former Yugoslavia; Hidalgo et al. (2006) and Brandolini et al. (2008) observed similar results in their screenings of smaller einkorn pools, with the exception of the Western Europe samples. Finally, the landraces with the best sedimentation volumes (hence, potentially with good bread-making attitude) hailed from Albania and former Yugoslavia, while those with the worst were from Iberia, Maghreb and Near East; however, a broad intra-location variability was observed, as hinted by the large standard error bars.

An exploratory Principal Components Analysis, carried out on the mean values (four years, two locations) of the 158 einkorn accessions, showed that the first three eigenvalues summarized 56.2% of total variation; the plot of Principal Component 1 (33.2% of total variation) and Principal Component 2 (13.0%) evidenced the presence of two clusters (Figure 5) hailing largely from Maghreb and Iberia (bottom-left quadrant) and from the Prealpine region (Switzerland, Austria and Germany; bottom-right quadrant); this result was corroborated by the outcome of the PCA performed on the country-by-country data (Online Resource 3).

In conclusion, this work allowed to get a deeper insight of the phenotypic variability across an einkorn panel representative of different cultivation sites in the Mediterranean and Northern Europe area. A high range of variation was observed for the parameters measured, and this was somehow correlated with the different provenance. The results are in general agreement with the findings of Brandolini et al (2016), who studied einkorn diffusion in Europe by fingerprinting 136 einkorn landraces with DArT-seq markers and identified two major groups, one from the Prealpine region (Switzerland, Austria, Germany and France) and one from the Maghreb/Iberian region. We therefore suggest that late heading, increased number of spikelets per spike, low thousand-kernel weight and glume hairiness of the “Prealpine” lines are most likely a consequence of adaptation by selection, either natural or human-mediated, to longer growing season, more abundant rainfall and more intense frost. Conversely, early-heading, low number of spikelets per spike, high thousand-kernel weight and glabrous glumes of the Mediterranean and the Maghreb/Iberian accessions are probably a consequence of shorter growing season, milder climate and dry summer spells.

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Conflict of interest. The authors declare that they have no conflict of interest.

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Captions to Figures

Figure 1. Growth habit, awn length, glume colour, glume hairiness and rachis fragility of the 158 *Triticum monococcum* analysed, according to country of origin. The bars represent the standard error.

Figure 2. Heading date, plant height, spike length, number of spikelets/spike, spike density and number of kernels/spikelet of the 158 *Triticum monococcum* analysed, according to country of origin. The bars represent the standard error.

Figure 3. Kernel length, width, thickness and volume, and thousand kernels weight of the 158 *Triticum monococcum* analysed, according to country of origin. The bars represent the standard error.

Figure 4. Protein and carotenoid content, sodium sedimentation value (SDS) and specific sedimentation value (SSV) of the 158 *Triticum monococcum* analysed, according to country of origin. The bars represent the standard error.

Figure 5. Score plot of the Principal Component Analysis carried out on the mean values of the 20 traits for each of the 158 *Triticum monococcum* analysed.

Symbols: + Maghreb, x Iberia, * Italy, o Turkey, □ Greece, ◊ East Europe, ⬡ Near East, ● Caucasus, ▲ Germany, ▼ France, ■ Austria, ▌ Hungary, ▬ Romania, △ Former Yugoslavia, ▽ Albania, ⬭ Bulgaria.

Captions to Online Resources

Online Resource 1. Code, geographic area, country, location of origin and traits results of the 158 Triticum *monococcum* ssp. *monococcum* accessions tested. The values are mean over four years and two locations.

Online Resource 2. ANOVAs (mean square and significance) of the quantitative traits analysed on the 158 *Triticum monococcum* landraces studied (2A) and of the quantitative traits analysed on the 148 einkorn landraces of known origin (2B).

Online Resource 3. Score plot of the Principal Component Analysis carried out for the country of origin mean values of the 20 traits scored on the 158 *Triticum monococcum* analysed.

Figure 1

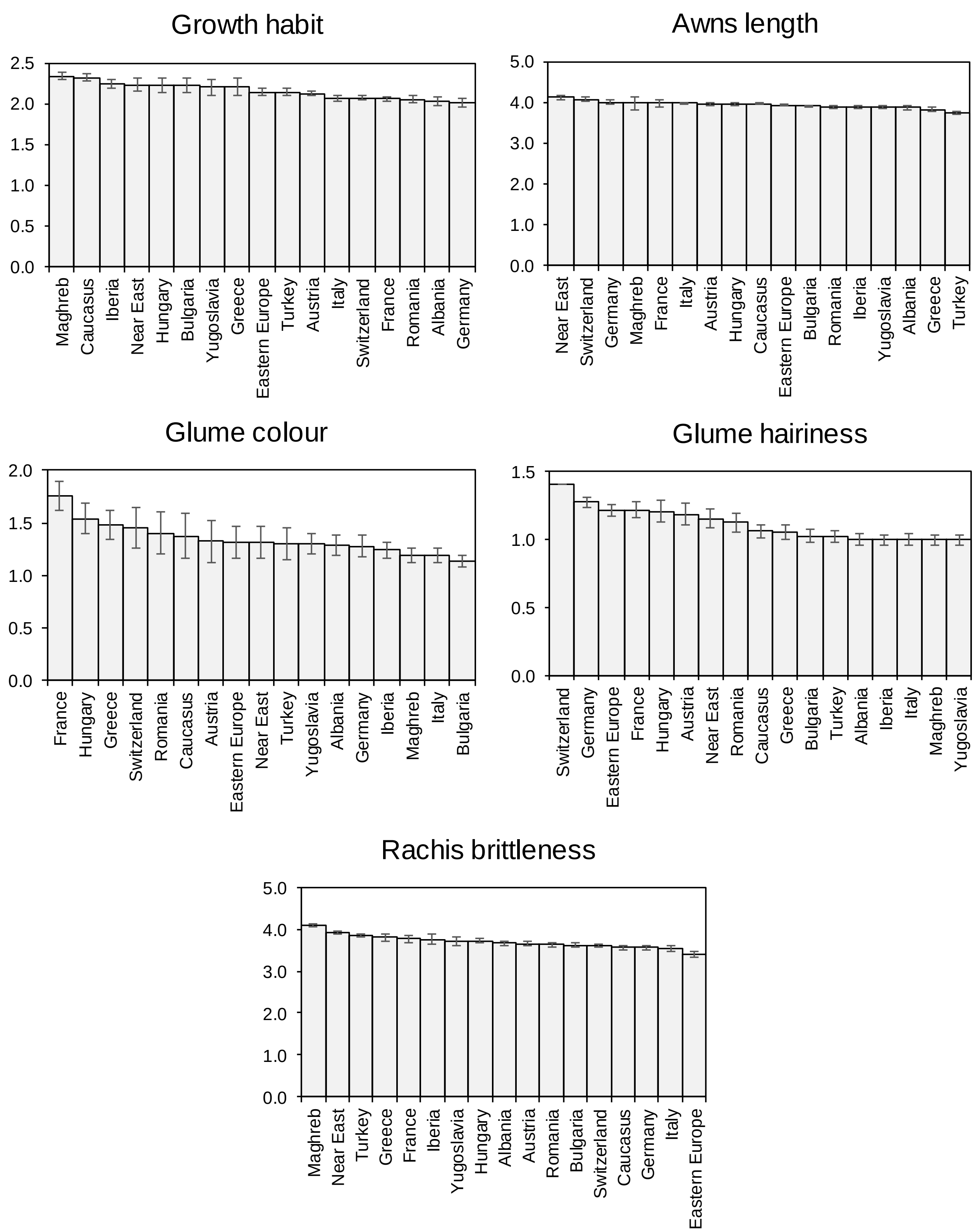
Growth habit
2.5
2.0
1.5
1.0
0.5
0.0
Maghreb
Caucasus
Iberia
Near East
Hungary
Bulgaria
Yugoslavia
Greece
Eastern Europe
Turkey
Austria
Italy
Switzerland
France
Romania
Albania
Germany
Awns length
5.0
4.0
3.0
2.0
1.0
0.0
Near East
Switzerland
Germany
Maghreb
France
Italy
Austria
Hungary
Caucasus
Eastern Europe
Bulgaria
Romania
Iberia
Yugoslavia
Albania
Greece
Turkey
Glume colour
2.0
1.5
1.0
0.5
0.0
France
Hungary
Greece
Switzerland
Romania
Caucasus
Austria
Eastern Europe
Near East
Turkey
Yugoslavia
Albania
Germany
Iberia
Maghreb
Italy
Bulgaria
Glume hairiness
1.5
1.0
0.5
0.0
Switzerland
Germany
Eastern Europe
France
Hungary
Austria
Near East
Romania
Caucasus
Greece
Bulgaria
Turkey
Albania
Iberia
Italy
Maghreb
Yugoslavia
Rachis brittleness
5.0
4.0
3.0
2.0
1.0
0.0
Maghreb
Near East
Turkey
Greece
France
Iberia
Yugoslavia
Hungary
Albania
Austria
Romania
Bulgaria
Switzerland
Caucasus
Germany
Italy
Eastern Europe

Figure 2

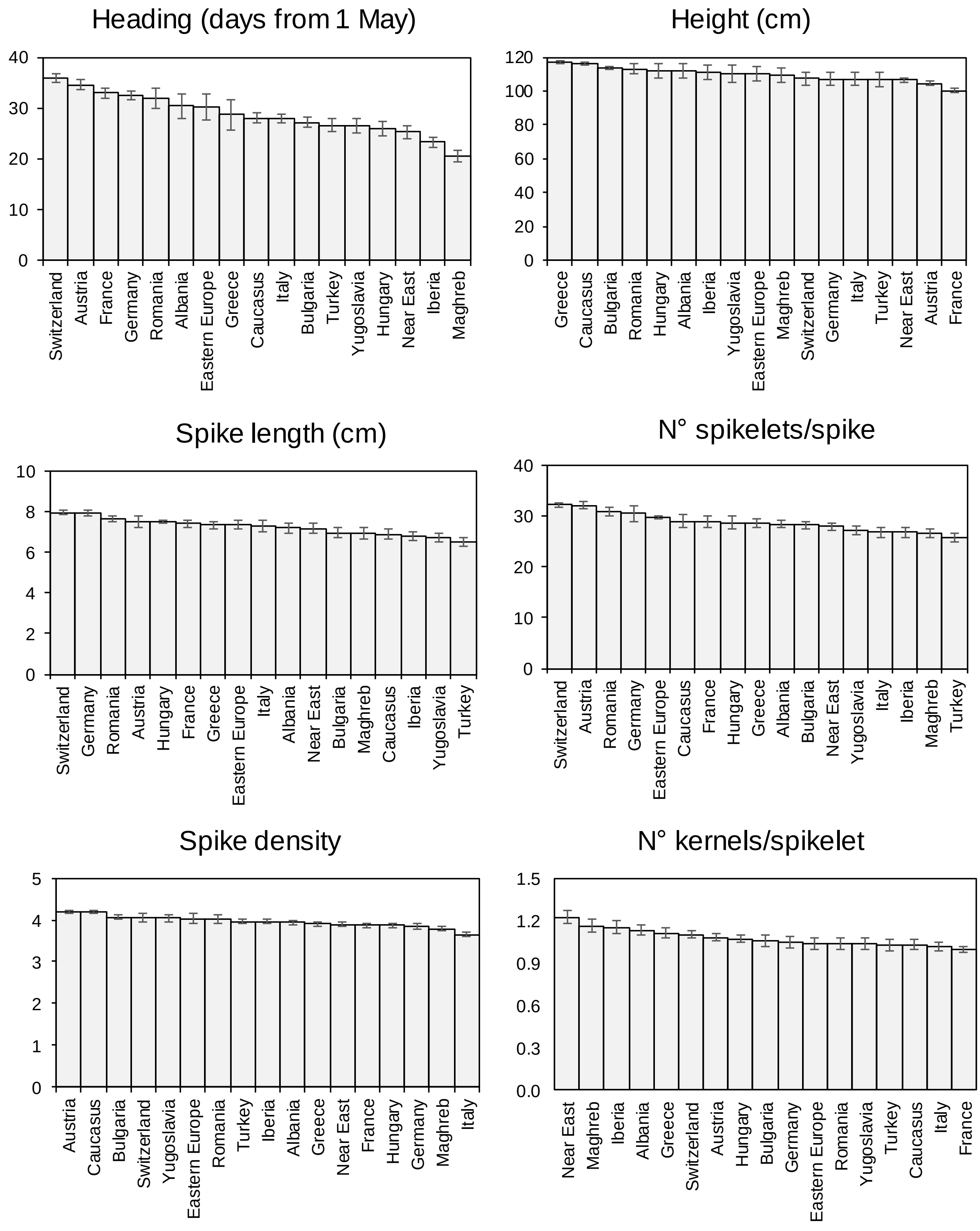
Heading (days from 1 May)
0
10
20
30
40
Switzerland
Austria
France
Germany
Romania
Albania
Eastern Europe
Greece
Caucasus
Italy
Bulgaria
Turkey
Yugoslavia
Hungary
Near East
Iberia
Maghreb
Height (cm)
0
20
40
60
80
100
120
Greece
Caucasus
Bulgaria
Romania
Hungary
Albania
Iberia
Yugoslavia
Eastern Europe
Maghreb
Switzerland
Germany
Italy
Turkey
Near East
Austria
France
Spike length (cm)
0
2
4
6
8
10
Switzerland
Germany
Romania
Austria
Hungary
France
Greece
Eastern Europe
Italy
Albania
Near East
Bulgaria
Maghreb
Caucasus
Iberia
Yugoslavia
Turkey
N° spikelets/spike
0
10
20
30
40
Switzerland
Austria
Romania
Germany
Eastern Europe
Caucasus
France
Hungary
Greece
Albania
Bulgaria
Near East
Yugoslavia
Italy
Iberia
Maghreb
Turkey
Spike density
0
1
2
3
4
5
Austria
Caucasus
Bulgaria
Switzerland
Yugoslavia
Eastern Europe
Romania
Turkey
Iberia
Albania
Greece
Near East
France
Hungary
Germany
Maghreb
Italy
N° kernels/spikelet
0.0
0.3
0.6
0.9
1.2
1.5
Near East
Maghreb
Iberia
Albania
Greece
Switzerland
Austria
Hungary
Bulgaria
Germany
Eastern Europe
Romania
Yugoslavia
Turkey
Caucasus
Italy
France

Figure 3

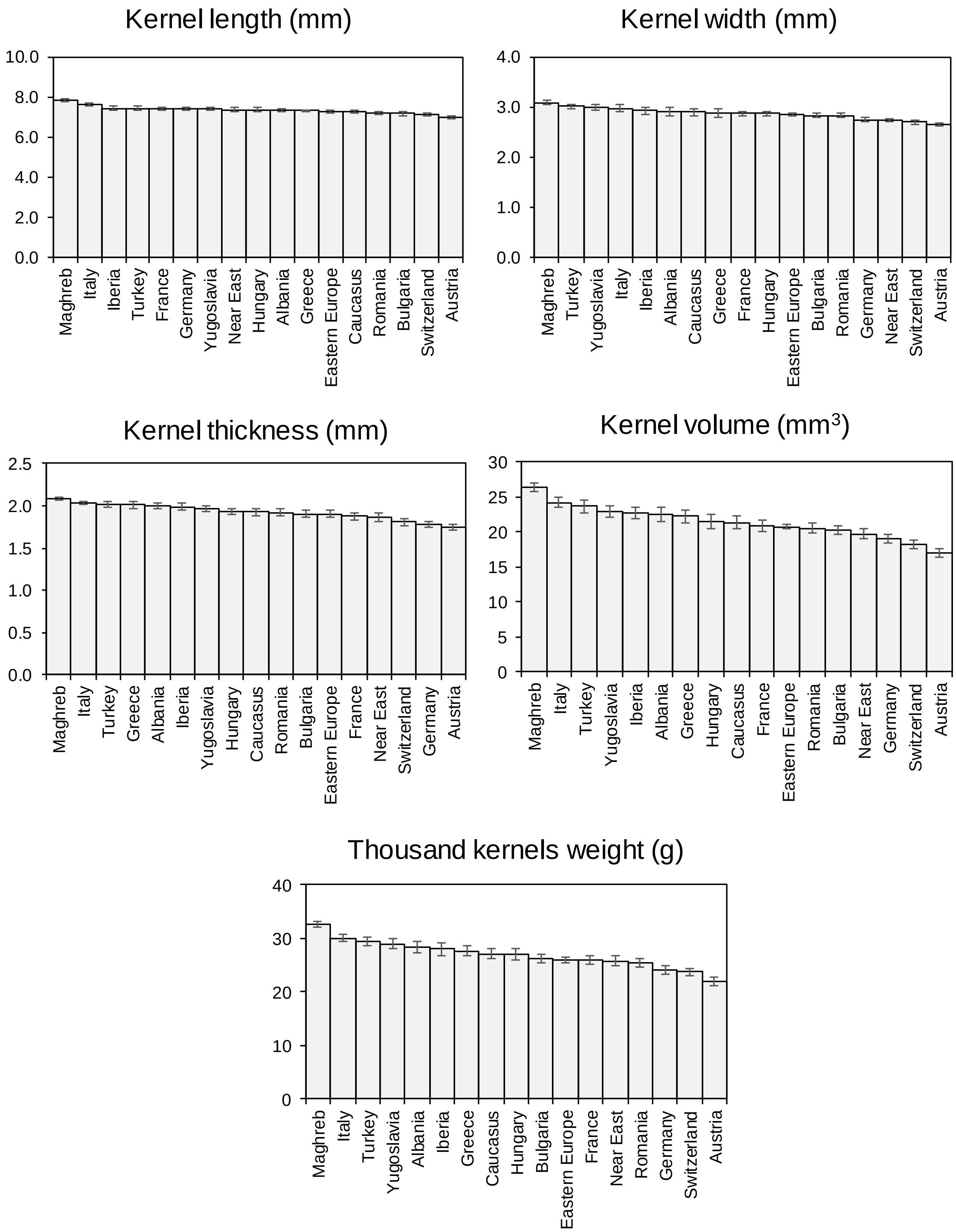
Kernel length (mm)
10.0
8.0
6.0
4.0
2.0
0.0
Maghreb
Italy
Iberia
Turkey
France
Germany
Yugoslavia
Near East
Hungary
Albania
Greece
Eastern Europe
Caucasus
Romania
Bulgaria
Switzerland
Austria
Kernel width (mm)
4.0
3.0
2.0
1.0
0.0
Maghreb
Turkey
Yugoslavia
Italy
Iberia
Albania
Caucasus
Greece
France
Hungary
Eastern Europe
Bulgaria
Romania
Germany
Near East
Switzerland
Austria
Kernel thickness (mm)
2.5
2.0
1.5
1.0
0.5
0.0
Maghreb
Italy
Turkey
Greece
Albania
Iberia
Yugoslavia
Hungary
Caucasus
Romania
Bulgaria
Eastern Europe
France
Near East
Switzerland
Germany
Austria
Kernel volume (mm3)
30
25
20
15
10
5
0
Maghreb
Italy
Turkey
Yugoslavia
Iberia
Albania
Greece
Hungary
Caucasus
France
Eastern Europe
Romania
Bulgaria
Near East
Germany
Switzerland
Austria
Thousand kernels weight (g)
40
30
20
10
0
Maghreb
Italy
Turkey
Yugoslavia
Albania
Iberia
Greece
Caucasus
Hungary
Bulgaria
Eastern Europe
France
Near East
Romania
Germany
Switzerland
Austria

Figure 4

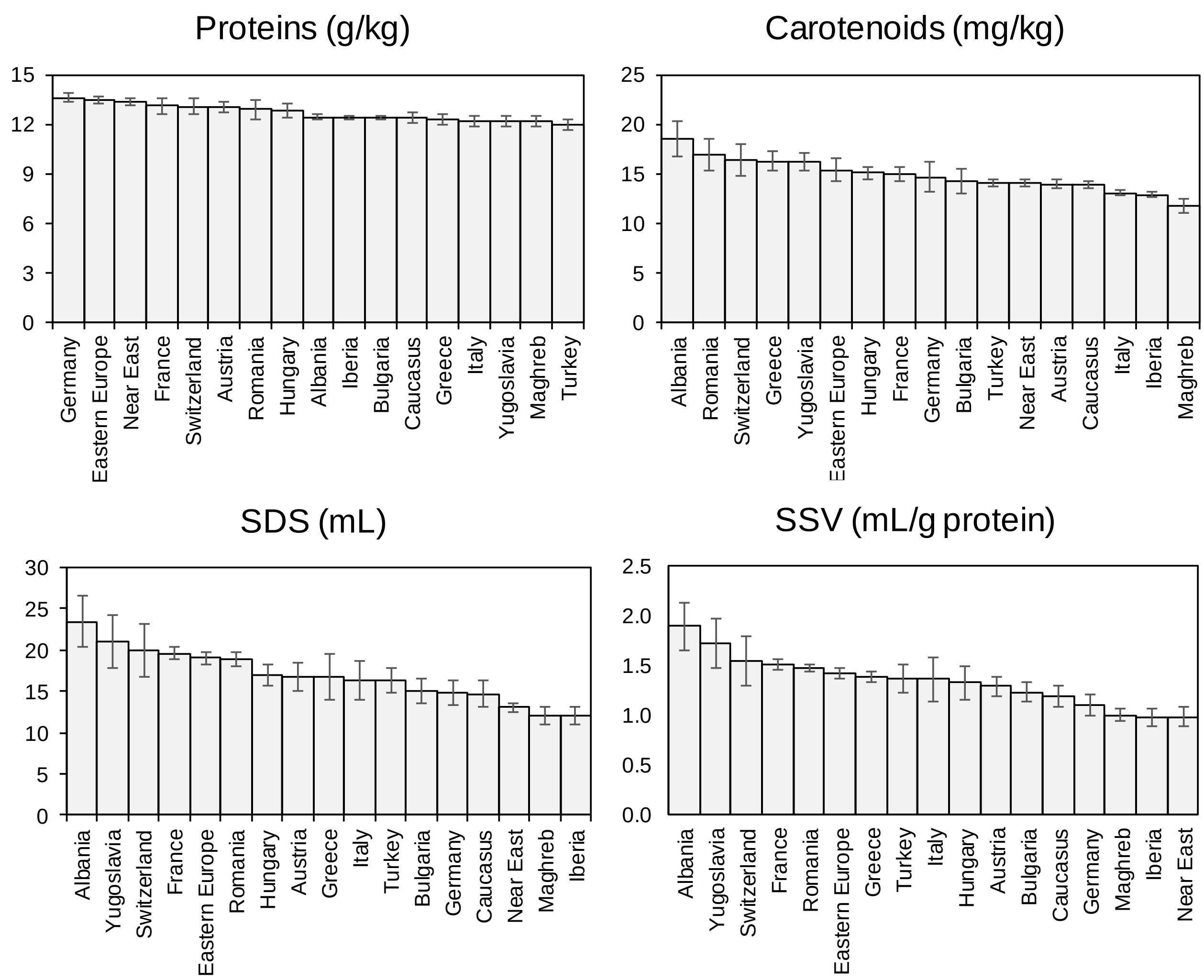
Proteins (g/kg)
15
12
9
6
3
0
Germany
Eastern Europe
Near East
France
Switzerland
Austria
Romania
Hungary
Albania
Iberia
Bulgaria
Caucasus
Greece
Italy
Yugoslavia
Maghreb
Turkey
Carotenoids (mg/kg)
25
20
15
10
5
0
Albania
Romania
Switzerland
Greece
Yugoslavia
Eastern Europe
Hungary
France
Germany
Bulgaria
Turkey
Near East
Austria
Caucasus
Italy
Iberia
Maghreb
SDS (mL)
30
25
20
15
10
5
0
Albania
Yugoslavia
Switzerland
France
Eastern Europe
Romania
Hungary
Austria
Greece
Italy
Turkey
Bulgaria
Germany
Caucasus
Near East
Maghreb
Iberia
SSV (mL/g protein)
2.5
2.0
1.5
1.0
0.5
0.0
Albania
Yugoslavia
Switzerland
France
Romania
Eastern Europe
Greece
Turkey
Italy
Hungary
Austria
Bulgaria
Caucasus
Germany
Maghreb
Iberia
Near East

Figure 5 Click here to access/download;Figure;Figura 5 AB.docx

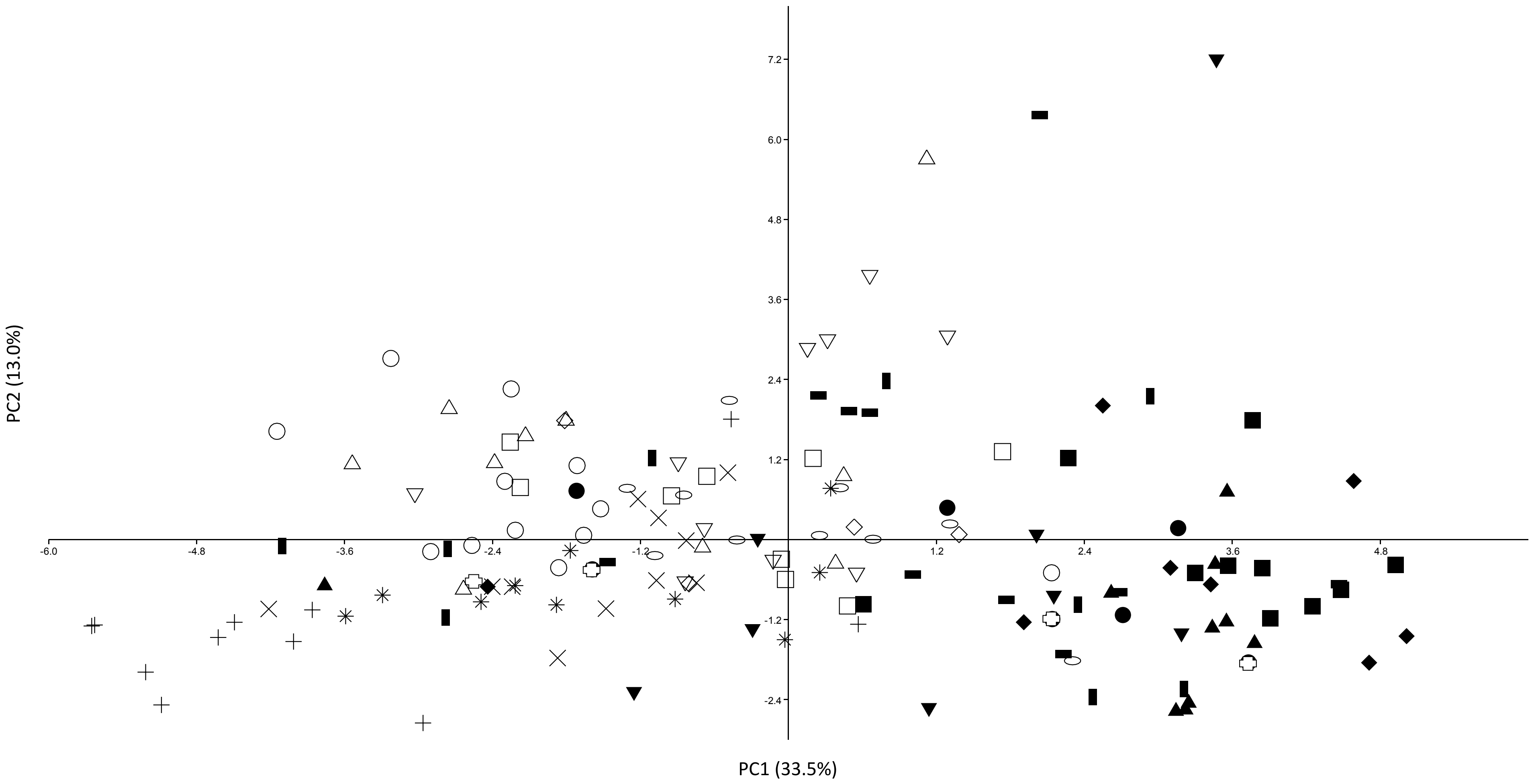

Table 1. Frequency distribution (%) for the discontinuously variable descriptors measured on 158 einkorn accessions. The data are the average of four growing seasons (2011-12, 2012-13, 2013-14 and 2014-15) and two locations (SAL and LO).

Descriptor		Class				
		1	2	3	4	5
Growth habit	1= prostrate, 2=semi-erect, 3= erect		28.1	71.9		
Awns length	1=none, 3=short, 5=long			2.9	85.5	13.1
Glume colour	1=beige, 2=brown, 3=black	28.5	63.3	8.2		
Glume hairiness	1=no hair, 2= sparse hair, 3= thick hair	71.2	21.5	1.3		
Rachis brittleness	1= high, 3= medium, 5= low				91.9	8.1

Table 2. Mean, minimum and maximum values for 15 continuous variables measured on 158 einkorn accessions. The data are the average of four growing seasons (2011-12, 2012-13, 2013-14 and 2014-15) and two locations (SAL and LO).

		Mean	Minimum	Maximum
Heading (from 1 May)	days	28.13	10.25	38.50
Plant height	cm	109.02	47.66	126.88
Spike length	cm	7.19	5.43	8.51
N° spikelets/spike		28.43	23.24	34.84
Spike density		3.95	3.38	4.92
N° kernels/spikelet		1.07	0.85	1.44
Kernel length	mm	7.38	6.61	8.24
Kernel width	mm	2.88	2.41	3.31
Kernel thickness	mm	1.93	1.37	2.22
Kernel volume	mm^3	21.65	14.60	30.20
Thousand kernels weight	g	27.19	16.05	35.66
Proteins content	g/100 g DM	17.11	14.36	20.70
Carotenoids content	mg/kg DM	8.18	4.92	11.46
SDS sedimentation volume	mL	19.04	11.63	56.61
Specific sedimentation volume	mL/g protein	1.12	0.64	3.28

Online Resource 1

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