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Re-Introduction of Ancient Wheat Cultivars into Organic Agriculture—Emmer and Einkorn Cultivation Experiences under Marginal Conditions

Szilvia Bencze [1,*], Marianna Makádi [2], Tibor J. Aranyos [2], Mihály Földi [1], Péter Hertelendy [1], Péter Mikó [3], Sara Bosi [4], Lorenzo Negri [4] and Dóra Drexler [1]

1 ÖMKi, Research Institute of Organic Agriculture, 1033 Budapest, Hungary; mihaly.foldi@biokutatas.hu (M.F.); herpeter@vipmail.hu (P.H.); dora.drexler@biokutatas.hu (D.D.)
2 IAREF Research Institute of Nyíregyháza, University of Debrecen, 4400 Nyíregyháza, Hungary; makadim@agr.unideb.hu (M.M.); aranyostibor@agr.unideb.hu (T.J.A.)
3 Agricultural Institute, Centre for Agricultural Research, 2462 Martonvásár, Hungary; miko.peter@agrar.mta.hu
4 Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna, 40127 Bologna, Italy; sara.bosi@unibo.it (S.B.); lorenzo.negri4@unibo.it (L.N.)
* Correspondence: szilvia.bencze@biokutatas.hu

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Abstract: Modern agriculture depends on the production of very few crop species, which provide lower nutritive value for consumers. The present work summarizes the results of a three-year experiment on hulled wheat varieties as potential candidates for food system diversification. The organic field cultivation tests with 10 emmer and five einkorn landraces and varieties were conducted on ~10m^2 plots on sandy soil, and from 2017, under on-farm conditions in eastern Hungary. Most accessions adapted well to the marginal conditions, with some landraces even yielding higher than registered varieties—over 3 t per ha on average over three years. Compared to emmer, einkorn had higher maximum grain yields, but its yield performance varied more than that of emmer. Grain protein and the total phenolic content were high in both species. Compared to emmer, einkorn seeds exhibited a 3.8 times higher content of bound flavonoids and had 3.4 times higher antioxidant activity. Four einkorn accessions were resistant to leaf spot, as well as yellow and leaf rusts. *Fusarium* infected both species similarly. Our findings indicate that not only registered varieties of ancient wheat species but also their landraces can provide sustainable alternatives both for organic farmers and also for the diversification of agriculture.

Keywords: emmer; einkorn; landraces; organic; disease resistance; yield stability; grain quality; diversifood

1. Introduction

Ancient people recognized and consumed thousands of wild and locally produced plants. Gathering tribes in Australia still gather and use over 400 different plant species belonging to more than 250 genera. However, in modern societies, only 150 species are actively cultivated worldwide, and just 30 of these provide, directly or indirectly, 95% of the calories and protein for humans [1]. This enormous decrease in crop diversity resulted in vulnerable farming practices depending on only two or three crops produced on farms and also in a great loss in genetic and nutritive values due to intensified growing systems. In fact, grain yield and year of cultivar release are negatively correlated with microelement content [2,3]. The dilution involves microelements, macroelements, bioactive components, and vitamins [3–7]. The progression of chronic, non-communicable diseases is associated with the modern human diet and is the consequence of a complex interplay between genetic and environmental factors, in which diet is a key component [8]. With sustainability and soil and environment protection in focus, organic agriculture might outperform conventional farming in terms of quality [9]. Even so, species diversity could be increased further in organic practice.

In Hungary, the wider spread of organic farming has only started recently. On a considerable proportion of the organic lands, cereal cultivation tackles lower mechanization levels and poor, often marginal conditions in some areas, such as the sand ridge area of the confluence of the Danube and the Tisza and northern Hungary, where decreasing soil water levels, poor and dry soils, acidic pH, and salinization can cause severe problems for farming [10]. Changing climatic conditions further increase the occurrence of combined water and nutrient deficiencies. Ancient wheat species, which have historically been cultivated under low-input conditions, could provide a more sustainable alternative that could help increase both field biodiversity and climate adaptation, especially in the case of the heterogeneous landraces. Considering the higher marketing price of these niche products, ancient wheats could also be profitable for organic farmers.

Emmer and einkorn are also considered to be more resistant to diseases than modern wheats. Certain accessions have been identified as resistance sources against fungal diseases, including powdery mildew, stem, yellow and leaf rusts, tan spot, *Septoria* blotch, bunts, and *Fusarium* [11–13]. Even though there is a high level of variability regarding different accessions, such traits can be important assets in organic production where the use of pesticides is prohibited.

Emmer and einkorn are much less well recognized than spelt, although they are all considered healthy cereals and are recommended for people that suffer from allergies, colitis, high blood cholesterol, and diabetes [12,14–16]. Their advantages can partly be attributed to their low glycaemic index value, high satiety value, and higher total dietary fiber content (this latter feature is not consistent over all studies), in association with reduced starch digestion rate [12]. However, not all the mechanisms behind the beneficial effects are properly understood, as quantitative measurements cannot be strictly associated with functional performance or qualitative potential [16].

One of the components of most concern is protein content, which may vary with the growing season, cultivation, fertilizer use, and variety. However, hulled wheats are reported to have consistently higher protein concentrations compared to modern wheats cultivated under the same conditions [15,17,18]. These high values were, however, suggested to be a consequence of low grain yields. This theory seems to be supported by the fact that the protein yield (per area) was lower for emmer than for wheats [19]. Even so, emmer and einkorn are reported to have better amino acid profiles (e.g., high lysin content) accompanied by high protein digestibility [15,20,21]. They are also rich in carotenoids (lutein), antioxidants, and certain vitamins (the vitamin E level is especially high in einkorn) and have a low fat-content [15]. Both emmer and einkorn may contain more bioactive compounds, including total phenolics and phenolic acids, than bread wheat [22–24].

With respect to the most important micro-elements for humans, selenium is reported to be found in considerable amounts in ancient wheats [22,25], while emmer was found to contain higher levels of Li, Mg, P, Se, and Zn than durum wheat or bread wheat [22,26]. This has special importance from a nutritional point of view, as a 7–50% decrease was demonstrated in modern spring wheats compared to historical varieties [3] in the levels of various microelements (Cu, Fe, Mg, Mn, P, Se, and Zn).

Considering the various health benefits of emmer and einkorn consumption, the rising market demand for ancient wheats, and the urgent need to diversify our cropping systems in light of climate change, the aim of the present work was to investigate whether their re-introduction under marginal conditions would be a feasible alternative for organic farmers in rural areas. In order to increase crop diversity and combat varying climatic and extensive growth conditions in a sustainable way, emmer and einkorn landraces (with possible heterogeneity) were chosen to be tested and compared with commercial varieties with respect to (a) their grain yield, (b) yield stability (as a measure of adaptation capacity), and (c) grain quality. In sustainable agriculture, crops should be cultivated without the use of synthetic pesticides. Therefore, another goal of the experiments was to determine (d) how effective was the disease resistance of the landrace populations.

2. Materials and Methods

2.1. Plant Material and Experimental Designs

For the investigations, 10 winter emmer (*Triticum turgidum* subsp. *dicoccum* Schrank) and five winter einkorn (*Triticum monococcum* L. subsp. *monococcum*) landraces were kindly provided by Pro Specie Rara (Basel, Switzerland) and Plant Diversity Centre (NöDiK, Tápiószele, Hungary). Registered varieties were kindly offered by the Agricultural Institute, Centre for Agricultural Research (Martonvásár, Hungary) and the Louis Bolk Institute (Driebergen-Rijsenburg, the Netherlands), as shown in Table 1. The investigations were carried out under low-input marginal conditions at the organic trial site of the Research Institute of Nyíregyháza (eastern Hungary) in sandy soil, which is characterized by acidic pH over most of the area, low organic carbon content, no stable structure, and low water holding capacity. The experiment was sown on 10.7 m^2 (1.2 × 8.9 m wide) plots, first in 2015, with one to four replications (with respect to the availability of seeds) in an incomplete block design. From 2016, it was sown in a complete block design, in four replicates. The trial site received 30 tonnes of manure per hectare each year. Manual weed control was applied whenever necessary. In the first crop year (2015–2016), the sowing rate was 12 grams per m^2 for Mv Alkor; 15 grams per m^2 for Nödik einkorn, Tifi, and GT 2139; 17.5 grams per m^2 for Mv Menket; and 20.25 grams per m^2 for all emmer accessions. The seeds were sown after ploughing, discing, and harrowing, with a Wintersteiger Plotseed TC sowing machine (soil pH(H_2O) = 7.29, organic matter content (m/m%) = 1.18, AL-P_2O_5 (mg per kg) = 240, AL-K_2O (mg per kg) = 375, nKCl-soluble Mg (mg per kg) = 66.7), after the pre-crop lacy phacelia (*Phacelia tanacetifolia* L. cv. Mira)). The following treatments were applied: In May 2018, Funguran-OH 50 WP 2 kg per ha and Biokal 01 4 l per ha, in June 17: Funguran-OH 50 WP 2 kg per ha and Biosol 0.8%. Sowing date: 26 October 2015, emmer harvest: 12 July 2016, einkorn harvest: 21 July 2016. The mean medium temperature in the growing season was 10.4 °C, the total amount of precipitation was: 562 mm. In the second crop year (2016–2017), the accessions were the same excluding GT 2140 (which froze out in the previous year), with the sowing rate 16 grams per m^2. Conditions were the same as before except when indicated otherwise (soil pH(KCl) = 5.35, organic matter content (m/m%) = 0.9, AL-P_2O_5 (mg per kg) = 86.7, AL-K_2O (mg per kg) = 215, nKCl-soluble Mg (mg per kg) = 40.6), after the pre-crop lacy phacelia (*Phacelia tanacetifolia* L. cv. Mira)). Treatments applied: in May 30: Biokal 01 4 l per ha, on June 6: Funguran-OH 50 WP 2 kg per ha and Biokal 01 4 l per ha. Sowing date: 3 November 2016, harvest date: 20 July 2017. The mean medium temperature in the growing season was: 8.7 °C, the total amount of precipitation was: 404 mm. In crop year three (2017–2018), conditions were the same except when indicated otherwise (soil pH(KCl) = 5.49, organic matter content (m/m%) = 1.25, AL-P_2O_5 (mg per kg) = 314, AL-K_2O (mg per kg) = 256, nKCl-soluble Mg (mg per kg) = 44.2), after vetch (*Vicia sativa* L. cv. Emma) and oat (*Avena sativa* L. cv. Lota)). Treatments applied: on June 20: Funguran-OH 50 WP 2kg/ha. Sowing date: 28 September 2017, harvest date: 11–12 July 2018. The mean medium temperature in the growing season was: 11.0 °C, the total amount of precipitation was: 592 mm. Further details of weather data at the experimental sites are provided in Supplementary Materials.

The morphological, phenological, and agronomical parameters were determined as described below: Plant density after emergence (number of single plants determined on two quadrates of 50 × 50 cm for each plot), autumn and spring plant density (same method). Phenological development was monitored on several occasions, according to the BBCH Zadoks growth scale [27] on 10 random plants from each plot. Diseases were assessed during May–July and reported as the percentage of plants (or spikes in the case of *Fusarium* head blight; FHB) infected (i.e., frequency) and the percentage coverage of the surface of foliage or spikes infected (severity). Plant height was measured in each plot when the final height had been reached. Lodging was assessed a week before harvest, determining the percentage of plants lodged and the angle of lodging from the vertical. Plots were harvested using a plot combine harvester Zürn 130 SE, and yields were reported as hulled grain yield. Winter survival (percentage) was calculated from the autumn and spring plant density data (autumn = 100%). Yield parameters: spike number per m^2 was determined on two quadrates of 50 × 50 cm in each plot, thousand (hulled) grain weight (TGW) was calculated from 5 × 200 seed weight per plot, and test weight (hectolitre weight) was determined according to the ISO 7971-1:2009 standard method.

On-farm multi-variety tests were started in autumn 2017 in one location, Füzesgyarmat (eastern Hungary), on 455m^2 plots for each of the 11 accessions (excluding GT 196, GT 1402, and Mv Menket). No fertilization was applied within five years. The pre-crop was white mustard (*Sinapis alba* L.). The seeds were sown after discing and harrowing with an Agro-Masz SR300 sowing machine, and no treatment or weed control was applied. Due to severe lodging and considerable yield loss, the grain yield could not be determined on this site, so only the protein content was measured on the collected grain samples (details are given below).

Table 1. Winter emmer and einkorn cultivars tested in the small-plot experiment, Nyíregyháza.

	Name/Code of Accession	Common Name/Origin	Number of Replicates 2015	2016–
Winter emmer	Mv Hegyes	registered variety—HU	4	4
	NÖDIK emmer (RCAT 004664)	Emmer roter (German landrace)	4	4
	GT 143	Schwarzwerdender—CH	3	4
	GT 196	Zweikorn—CH	1	4
	GT 381	Schwarzer Samtemmer—CH	1	4
	GT 831	Blauemmer—CH	3	4
	GT 1399	Grauer—CH	2	4
	GT 1400	Schwarzbehaarter—CH	3	4
	GT 1402	Weisser behaarter—CH	1	4
	GT 2140	"Züblin" WS—CH	3	0
Winter einkorn	Mv Alkor	registered variety—HU	4	4
	Mv Menket	registered variety—HU	4	4
	NÖDIK einkorn (RCAT 074129)	Morocco (COLL. SCHIEMANN)	4	4
	Tifi	registered variety—NL	4	4
	GT 2139	unknown—CH	1	4

Legend: CH = from the collection of the gene bank Pro Specie Rara in Switzerland.

2.2. Grain Quality Determination

Grain samples collected from the small-plots were transferred to the University of Bologna for quality analyses, which were carried out using the protocols described below. Dehulled whole grain samples were ground to whole-flour using a domestic stone mill (100% flour extraction, Billy 200, Hawos Mulini, Bad Hamburg, Germany). All determinations were replicated twice, and the results were expressed on a dry matter (DM) basis assuming 14% water content according to Shewry and Hey [28]. Folin-Ciocalteu reagent, gallic acid, and catechin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents were analytical grade unless otherwise stated.

2.2.1. Macronutrient Content

In the small-plot experiment, the protein content of the whole-flour was determined using an Infratec® 1229 Whole Grain Analyzer (Foss Tecator AB, Global calibration No. WH000003). In the on-farm experiment, the wholemeal flour of the dehulled grain samples collected were measured using the Kjeldahl method (based on the Hungarian standard MSZ 6830/4, applying a factor of 5.7).

Lipid analysis was carried out according to standardized methods [29]. Briefly, 500 mg of whole-flour was treated with 10 ml chloroform–methanol (2:1, v/v) under continuous shaking for 20 minutes and then centrifuged (10,000 rpm, 20 min). The supernatant was collected, and the extraction was repeated once. Finally, supernatants were pooled, evaporated to dryness, and the residue was weighed to quantify the lipid content.

2.2.2. Dietary Fiber Components

Total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) content was determined following the enzymatic/gravimetric method described by Prosky et al. [30] using a Megazyme assay kit (Megazyme International Ireland Ltd.). The procedure was based on sequential enzymatic digestion steps using heat stable α-amylase, protease and amyloglucosidase, allowing the determination of IDF, SDF, and TDF amounts.

2.2.3. Phenolic Compounds

Free phenolic compounds (FPC) and bound phenolic compounds (BPC) were extracted as previously described by Dinelli et al. [31]. Wholegrain flours were extracted with cold 80% ethanol (4 °C) in order to dissolve the free soluble compounds, followed by acid and alkaline hydrolyses to release the bound forms. Extracts were analysed for polyphenol quantification following the colorimetric procedure based on the Folin–Ciocalteu reagent, as described by Singleton et al. [32]. Furthermore, extracts containing free and bound phenolic compounds were analysed for flavonoid content following the spectrophotometric method previously described [31]. The absorbance values were converted using gallic acid and catechin as standards for polyphenols and flavonoids, respectively.

2.2.4. Antioxidant Activity Assays

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay was carried out according to the procedure described by Brand-Williams et al. [33]. The final concentration of the methanolic DPPH solution was 0.225 mmol L^{-1}. Each sample was tested in duplicate, and the absorbance was recorded at 517 nm, along with the control and blank test tubes. The Trolox calibration curve (0–998.84 μmol L^{-1}) was plotted as a function of the percentage of DPPH radical scavenging activity, and the final results were expressed as micromoles of Trolox equivalents (TE) per gram of the whole-wheat flour (μmol TE g^{-1}).

2.2.5. Statistical Analyses

Due to the variation in the number of repetitions in various years, two-way ANOVA without repetitions was applied on the annual mean yield data (the two factors being the variety and the year) to compare the three-year performances of different accessions (using Microsoft Excel 2010). The least significant differences ($LSD_{5\%}$) were calculated using the equation $LSD_{5\%} = t_{P5\%} \sqrt{(2 \text{ Error MQ } r_a^{-1})}$, where r_a is the number of versions of factor "*A*". The Wilcoxon signed rank test was used to make comparisons between years on the species level in both species (related samples in pairs), while for comparisons between emmer and einkorn, all data on various parameters was evaluated using the Mann–Whitney U test (IBM SPSS Statistics 25.0). Correlation analyses in emmer and einkorn were made between various parameters using the statistical package of Microsoft Excel 2010.

3. Results

3.1. Yield Potential and Stability

Under the marginal conditions of the experiments, the calculated mean grain yield values for the harvesting years 2016, 2017, and 2018 were 3.16, 1.76, and 3.58 t per ha for emmer and 4.33, 1.34, and 2.82 t per ha for einkorn, respectively. The differences between the years were significant ($p < 0.05$). For both species, the poorest yield values were recorded in 2017, when the sowing of early November was accompanied by low temperatures from November until January (which hindered emergence and early development), and damaging sandstorms resulted in extreme variations between single plots. Maximum yields were obtained in different years for emmer and einkorn (Figure 1). The first year of the experiment was the most favorable for einkorn, which may be partly attributable to the lower winter-kill in this species (34% compared to 46.6% in emmer—disregarding the facultative emmer landrace GT 2140, which froze out totally). For emmer, 2018 was the best yielding year.

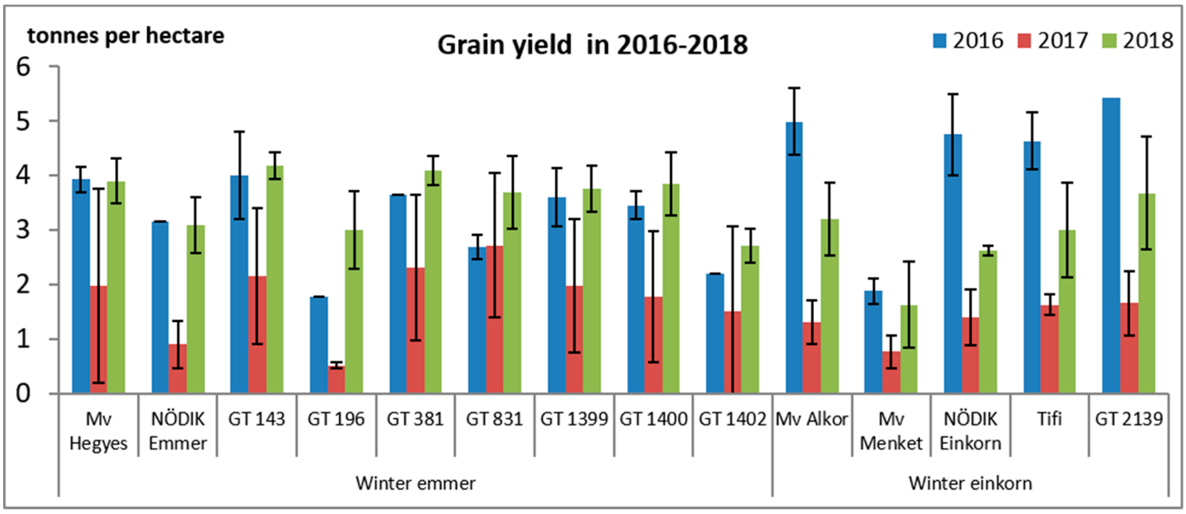

Figure 1. Grain yield of winter emmer and einkorn accessions in 2016–2018, Nyíregyháza.

Comparing the accessions based on their yields on average over three years, some landraces even tended to rank higher than the registered varieties (Figure 1), with the top three cultivars being GT 143, GT 381 and Mv Hegyes (with 3.44, 3.35, 3.26 t per ha) for emmer and GT 2139, Mv Alkor, and Tifi (3.58, 3.16, and 3.08 t per ha) for einkorn. There was, however, only a statistically significant difference between the group of the lower yielding varieties—consisting of Nödik emmer, GT 1402, GT 196 in emmer, and Mv Menket in einkorn—and the remaining, higher yielding cultivars. Mv Menket, a registered semi-dwarf einkorn variety, which was bred for short straw (to resist lodging) and intensive growing conditions (not only for organic cultivation), could not tolerate these marginal conditions.

Although some einkorn accessions were able to yield nearly five t per ha in 2016, their yield was more unstable in less advantageous years than that of emmer. Annual yield fluctuations were large, and even higher for einkorn (within accessions, standard deviations (SD) were 0.58–1.88 t per ha in einkorn, compared to 0.57–1.28 t per ha in emmer, with average values 1.50 and 0.99, respectively). On average over years and accessions, however, emmer and einkorn both produced 2.83 t per ha in grain yields.

3.2. Factors Affecting Grain Yield

In emmer, there were significant correlations between the grain yields of various years; the Pearson correlation coefficient, r was 0.843 and 0.726 for 2016–2018 and 2017–2018, respectively. For 2016–2017, the correlation coefficient was only 0.555 because one accession, GT 831, was an outlier in 2017, in the lowest yielding year (Figure 2). This might be because this landrace produced the most stable grain yields (2.67–3.68 t per ha), irrespective of the year. From all the landraces, GT 831 developed the longest, branching, netty root system by spring (data not shown here), while its shoot development was more moderate. These characteristics might have contributed to its stable performance. Excluding this landrace from the analysis, the correlation coefficient became highly significant, $r = 0.807$. Correlation coefficients for einkorn between yields of 2016–2017, 2016–2018, and 2017–2018 were 0.909, 0.941, and 0.888, respectively. These results in both emmer and einkorn suggest a strong genetic determination of actual grain yield, which might have resulted in the more or less conservative ranking order of accessions under the present experimental conditions.

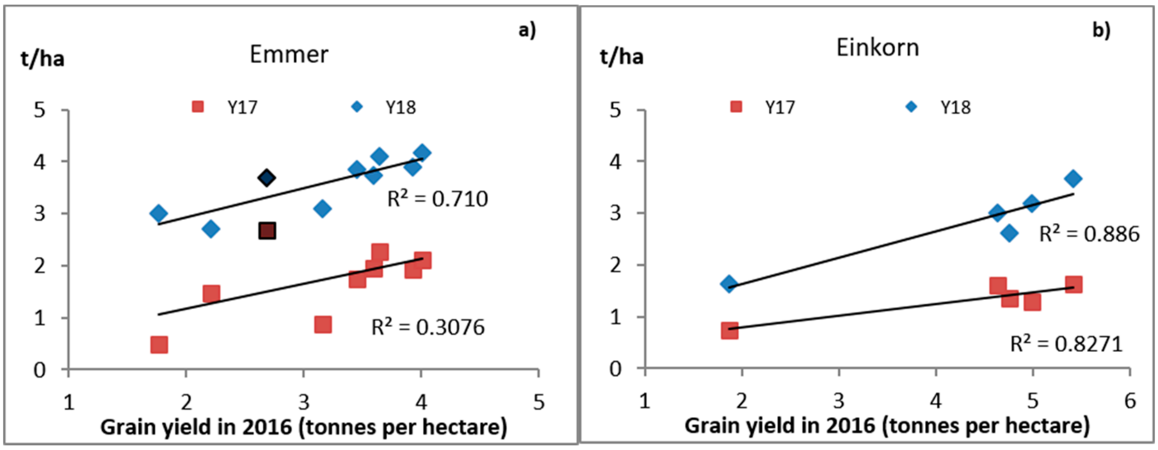

Figure 2. Relationship between grain yield data of 2016 (displayed on axis *x*) and the grain yields in 2017 and 2018 (axis *y*). (**a**) Emmer, (**b**) einkorn. Results of the outlier GT 831 are marked with darker, framed symbols in the graph to the left. Abbreviations: Y17 and Y18: yields in 2017 and 2018, respectively.

Besides the genetic background, different environmental factors had the highest impact on grain yield in different years. In 2016, it was the rate of winter-kill ($r = -0.977$ and -0.611 in einkorn and emmer with $p = 0.01$ and 0.1, respectively). The germination rate was the key factor for emmer in 2017 (exhibiting the unfavourable weather conditions described above), with a correlation coefficient $r = 0.850$ as germination frequency was 10% in Nödik emmer, GT 196, and 1402 and 30–50% in the rest of emmer varieties. In contrast, einkorn accessions had 70% germination rate in the same year. In this year, the germination rate correlated strongly with the spike number per square meter, which significantly affected emmer grain yield. In the case of emmer in 2018, where plant density was the highest, lodging was more severe in accessions with higher yields, with $r = 0.755$ and 0.803 relating the percentage and degree of lodging. Thousand (hulled) grain weight did not influence grain yields in either emmer or einkorn in any year, except for einkorn in 2016, when TGW was negatively correlated with the spring plant number and positively with the rate of dying out due to freezing, which suggested that varieties on plots with low plant density (resulting from a higher rate of winter-kill) compensated in the grain yield with a larger grain size. Both the plant height and test weight were conservative traits in einkorn, exhibiting significant correlations between years.

The rate of phenological development was only associated with grain yield in emmer in 2018, the most favorable year for this species; the Zadoks value in early May exhibited a significant negative correlation with the grain yield indicating that later heading/ripening accessions produced significantly higher yields. Due to the limited number of accessions, this relationship could not be proven properly for einkorn as the correlation coefficient was only significant at the probability level $p < 0.1$, although a similar tendency was confirmed. In both emmer and einkorn, Zadoks values on many sampling dates of each year were significantly correlated, while data in different years also corresponded in many cases, suggesting a consistency in phenological development in different years. The latest cultivars were GT 2139 and Tifi in einkorn, while the earliest ones were Nödik einkorn and the emmer landraces Nödik emmer and GT 196.

3.3. Grain Quality Attributes

The analyses carried out on the grains produced in the last year of the experiment (2018) revealed significant differences in quality traits between the two species. Although the average grain yield was 28% (0.8 kg) higher in emmer than in einkorn ($p = 0.042$) in this year, einkorn seeds contained a relatively much higher proportion of bioactive compounds, including 277% higher bound, 63% higher total flavonoids, and 62% more lipids, and also had 243% higher antioxidant activity (DPPH) than the seeds of emmer ($p = 0.001$, Figure 3). There were, however, no significant differences in the free, bound and total phenolic and the free flavonoid contents between the two species. Total phenolic content was on average 2490 and 2519 µg/g in einkorn and emmer, while the lipid fraction was 4.61% and 2.84%, respectively. Total dietary fiber data are only available for einkorn accessions, in which its content ranged between 21–25%, with an average of 23%.

Figure 3. Grain quality components (± SD) and the protein yields of the emmer and einkorn accessions cultivated in Nyíregyháza in 2018 (**a**) Bound phenolic content (BPC), free phenolic content (FPC) and total (BPC + FPC) phenolic content; (**b**) bound flavonoid content (BFC), free flavonoid content (FFC) and total (BFC + FFC) flavonoid content; (**c**) antioxidant activity; (**d**) lipid content; (**e**) grain protein content; (**f**) protein yield. (Standard deviation (SD) values are given for the total component parameter when free and bound values are indicated).

Grain protein content varied between 14.8% and 20.2% in einkorn and 15.3% and 19.7% in emmer (Figure 3). When the protein data obtained in the two trials (Nyíregyháza and Füzesgyarmat) were compared for consistency (Figure 4a), emmer grains were found to contain significantly less protein under the on-farm conditions (14.7% in average, varying between 13.4% and 18.3%, $p = 0.028$). The average protein content of einkorn did not differ at the two sites ($p = 0.144$, Wilcoxon signed rank test), which indicates that einkorn had more stable protein values even under the lower nutrient supply levels of the on-farm conditions. Compared to the highly variable data in Nyíregyháza, the variation in the protein content of einkorn was very low in Füzesgyarmat (SD = 2.26 and 0.28, in the small-plot and on-farm tests, respectively). In emmer, only two varieties exhibited a variation, while the rest of accessions did not differ significantly in Füzesgyarmat. The Nödik emmer had outstandingly high protein content on both sites (18.3% and 19.7%), while the registered variety, Mv Hegyes, had stably high values around 16.3% under both conditions.

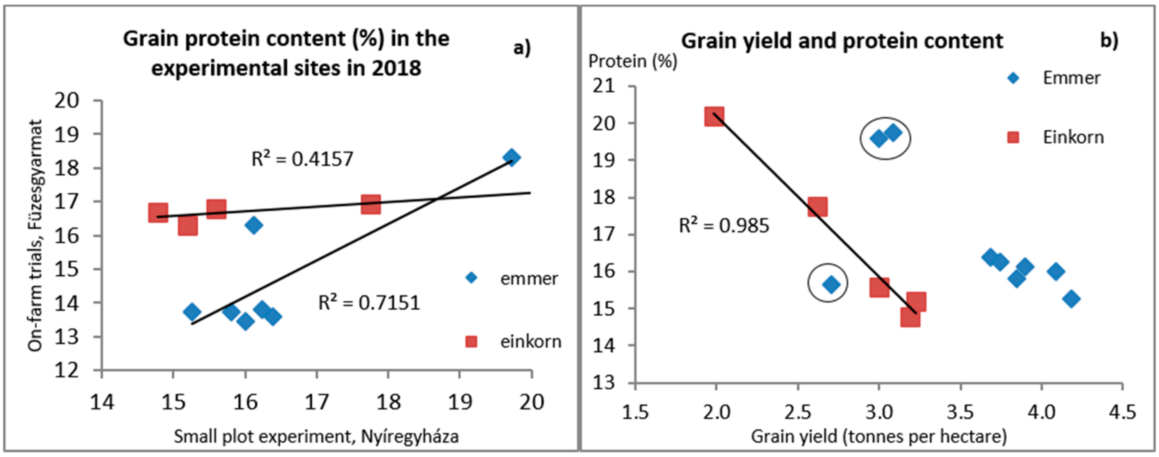

Figure 4. Relationship between the protein contents of grains produced in the two experiments, in Nyíregyháza and Füzesgyarmat (**a**), and between the grain yield and protein content of emmer and einkorn cultivated in Nyíregyháza 2018 (**b**). Outliers are indicated with circles.

Although the average grain protein content was similar in both species (16.7%), the protein yields were significantly lower in einkorn and in the emmer accession GT 1402 than in the rest of emmer varieties ($p = 0.001$). The range was 344–422 and 364–548 kg per ha, with mean values 394 and 512 kg per ha for einkorn and emmer, respectively. Grain protein content was negatively related to the grain yield in the case of einkorn (Figure 4b). For emmer, however, there were some discrepancies. The landraces Nödik emmer and GT 196 had both fairly high yields (around 3 t per ha) and very high protein contents, while GT 1402 had a lower yield without higher protein content in compensation.

3.4. Disease Resistance of Emmer and Einkorn

The first two years of the experiments were favorable for fungal diseases as several pathogens were detected. In 2016, *Drechslera graminea* was identified as the main cause of leaf spot diseases in emmer. GT 196, Nödik emmer, and GT1402 were infected more severely (in this order), with 100/100/90% incidences, while the rest of emmer varieties were not affected considerably (Table 2). All the emmer cultivars proved to be susceptible to yellow rust (*Puccinia striiformis* West.) to some extent, with 20–70% of the plants being covered in pustules. In 2017, the less severe yellow rust infection could only affect four accessions, however, leaf rust (*Puccinia triticina* Eriks.) had an epidemic occurrence in this year, resulting in 47–76% infection frequency of all the emmer accessions. Leaf spot symptoms were caused by *Septoria tritici* in 2017. Nödik emmer was affected the most severely, while GT 196 was infected moderately by this disease. The last year, 2018, did not favor any fungal diseases. The pathogens present only caused slight, insignificant symptoms. In all three years, einkorn accessions were found to be resistant to all leaf fungal diseases, and only one landrace, GT 2139 was reported to have been severely infected with leaf rust in 2016.

Table 2. Disease occurrence in emmer and einkorn accessions between 2016–2018 (Nyíregyháza, Hungary).

	Nyíregyháza, 2016					Nyíregyháza, 2017								Nyíregyháza, 2018							
Name of Accession	*Drechs. gram.* (%)	YR (%)	LR (%)	FHB/10 spikes		*S.tritici* (%)		YR (%)		LR (%)		FHB (%)		*S.tritici* (%)		YR (%)		LR (%)		FHB (%)	
	freq/sev	freq/sev		freq.	sev.	freq.	sev.	freq.	sev.	freq.	sev.	freq.	sev.	freq.	sev.	freq.	sev.	freq.	sev.	freq.	sev.
Emmer																					
Mv Hegyes	2/2	35/100	0	2.5	2.5	0	0	17.5	3.8	72.5	10.0	31.3	8.5	1.25	1.25	22.5	3.5	2.5	1.25	2.5	0.75
NÖDIK emmer	100/65	60/100	0	17.1	24.4	91.3	26.3	11.3	2.0	63.8	6.8	22.5	7.5	5	3.75	7.5	2.5	1.25	1.25	0	0
GT-143	0	35/100	0	3.3	3.3	0	0	0.5	0.3	70.0	13.8	21.3	6.3	0	0	11.25	2.25	0	0	0	0
GT-196	100/80	50/100	0	20	20	40.0	8.3	1.7	0.7	46.7	5.0	16.7	6.7	3.75	2.5	8.75	2	1.25	1.25	5	1.25
GT-381	5/5	30/100	0	30	70	0.3	0.3	2.5	1.3	67.5	13.8	21.7	7.0	0	0	2.5	1.5	5	2.5	0	0
GT-831	10/15	45/100	0	3.3	23.3	0	0	8.8	1.5	62.5	10.0	22.5	6.5	1.25	1.25	13.75	5.5	0	0	0	0
GT-1399	8/10	40/100	0	0	0	0	0	1.3	0.8	76.3	14.8	12.0	6.0	3.75	1.25	10	2.5	7.5	3.75	0	0
GT-1400	15/15	70/100	0	13.3	12.3	0	0	13.8	4.8	50.0	13.3	27.5	7.5	1.25	1.25	17.5	5	6.25	2	0	0
GT-1402	90/10	20/100	0	10	30	0	0	0	0	60.0	13.0	21.3	7.0	1.25	1.25	16.25	4.5	6.25	5	3.75	0.75
Einkorn																					
Mv Alkor	0	0	5/15	40	25.5	0	0	0	0	0	0	0.25	0.75	10	3.75	0	0	0	0	0	0
Mv Menket	0	0	0	100	100	0	0	0	0	0	0	0.75	1.25	20	10	0	0	2.5	11.25	0	0
NÖDIK einkorn	0	0	5/15	22.5	22.0	5	1.25	0	0	0	0	0	0	7.5	3.25	0	0	0	0	0	0
Tifi	0	0	10/5	25.0	32.5	0	0	0	0	0	0	0	0	5	3.75	0	0	0	0	2.5	1.75
GT-2139	0	0	80/100	20	15	0	0	0	0	0	0	1.5	3.25	3.75	2.5	0	0	0	0	2.5	1.25

Abbreviations: Drechs. gram. = *Drechslera graminea*, YR = yellow (stripe) rust LR = leaf (brown) rust, FHB = *Fusarium* head blight, S. tritici = *Septoria tritici*, freq = frequency (% of plants/spikes infected), sev. = severity (disease coverage on the plant/spike infected)).

Although 2018 was not the year of *Fusarium* species either, *Fusarium* head blight was present in the first two years. Based on the visual microscopic evaluation of 10 randomly collected spikes (from each plot), only the emmer landrace GT 1399 was found to be free from this pathogen in 2016 (Table 2). The least infected cultivars were Mv Hegyes, GT 143, and GT 831, while all of the remaining emmers were infected moderately. From the einkorns, Mv Menket was severely affected with 100/100% incidence and severity, and Mv Alkor was infected to medium level. The two landraces and Tifi, however, were infected to a moderate degree. The field survey in 2017 revealed that the incidence of *Fusarium* infection was 12–31% for all the emmers with 6-8.5% infection rates (GT 1399 still had the lowest infection level). In this year, einkorn infection was restricted to a minimum. This difference between FHB infection in the two years might be due to the timing of weather events relative to anthesis of each accession but also due to changes in *Fusarium* species or pathotypes.

4. Discussion

4.1. Grain Yield and Yield Stability

Despite the low input conditions of the experiments, both species had fairly high average grain yields (in most accessions it was around 3 t per ha). Some landraces produced as high yields as 4 t per ha and 5 t per ha in favorable years in emmer and einkorn, respectively. Owing to the special layout of the small-plot experiment (with some minor edge-effect around the plots), the obtained data might be a slight over-estimation of the actual yield. However, the grain yields and variation obtained in the present work are in line with reports from other European countries (e.g., Czech Republic, Germany or Italy) where 1.2–3.3 t per ha and 0.84–4.5 t per ha yields were recorded under conventional farming practices for emmer and einkorn, respectively, depending on the year, location, nitrogen supply, and sowing rate [34–36]. Most cultivars adapted well to the conditions of the experiment while there were some which did not, either because of insufficient winter hardiness or due to high-input farming requirements (e.g., Mv Menket). The grain yields of the best landraces were not statistically different from those of the registered varieties. In fact, the emmer landrace GT 143 and the einkorn landrace GT 2139 even tended to yield more in all three years than the registered varieties (Tifi and the standard varieties Mv Hegyes and Mv Alkor). Emmer exhibited greater yield stability than einkorn (one emmer landrace, GT 831 proved to yield especially stably—independently of the extremities of the crop year—possibly due to its special root architecture). From the two species, however, einkorn had higher yield maximum, which matched other data [34,36], but was in contrast with some Italian findings where emmer was better adapted to the location, yielding 3.54 t per ha compared to 1.42 t per ha of einkorn [37].

Our results indicated that genetic determination affected the ranking of the varieties the most. Abiotic stress factors, such as frost, cold temperatures during early development, and sandstorms, also had negative impacts on the yield in some years or cultivars. A compensation in the seed size in response to decreased plant density and highest rate of lodging in accessions with higher yields were also confirmed. This was in accordance with other findings on the relationship between lodging and yield in emmer [35].

4.2. Grain Quality

In spite of the low-input conditions in Nyíregyháza, the grain protein content of both emmer and einkorn was high, ranging between 15% and 20%, which matched previous reports [18,21,35,38] and was in the upper region of the range 8.7–22%, reported for emmer [15]. Lower values were only obtained for emmer in the on-farm experiment (13–14%). Compared to emmer, einkorn was found to be more stable. It had higher protein values even under poor nutrient conditions. At the cultivar level, there were some emmer accessions with either stable, moderately high (Mv Hegyes), or high values but at the expense of lower grain yields (Nödik emmer).

The protein yields were lower in einkorn, while the mean values obtained for emmer were higher than those reported elsewhere and were comparable with the protein yields of durum wheats, which produced mean protein yields of 550 kg per ha [19]. However, these results of 2018 might not be in correspondence—if there had been such a measurement—with those of 2016, the year in which einkorn produced outstandingly high (5 t per ha) yields. This is a point worthy of note and requires further investigation.

Grain protein content is considered to be in a negative correlation with grain yield [15]. This was supported by the present work in the case of einkorn, and to some extent also for emmer. Other components of the grain such as lipids and bioactive compounds were highly variable through different accessions but had no relation to their grain yields. However, einkorn seeds contained much higher amounts of flavonoids and lipids, and exhibited higher antioxidant activity than emmer, while the concentration of phenolic compounds did not differ in the two species. Although a correlation between the content of total polyphenols and the antioxidant activity had been reported previously [39], the present findings did not confirm this. Opposite results were obtained in another study [40], where the total flavonoid content was 1.4 times higher in emmer than in einkorn, and emmers had a 1.9-fold higher total phenolic content than einkorn samples. Total phenolic values determined in the present work were, however, relatively high compared to literature data, being in the upper range of those reported for modern bread wheats, einkorn and emmer [28]. Similarly, the total dietary fiber content in the einkorn accessions was even higher than in the modern wheats reported by Shewry and Hey [28]. Lipid content in emmer was in line with the literary mean data of 2.8% [15], while the higher values determined in the einkorn accessions fitted other data published for einkorn [16,41].

The results obtained here on grain quality are promising but, as they represent only one year, they need to be confirmed by further investigation—namely, how the crop year and the characteristics of the growing site affect these parameters.

4.3. Disease Resistance of Emmer and Einkorn

Based on the three years of pathological data, it can be stated that einkorn was more resistant to leaf fungal infections (including leaf spot diseases and yellow and leaf rusts) than emmer. This was in line with other findings on certain diseases including powdery mildew and leaf rust [42]. The susceptibility of the two species to *Fusarium* (FHB) was similar, and matched other reports by other authors [13]. The most resistant cultivars against all diseases were GT 1399 in emmer and Nödik einkorn and Tifi in einkorn. All five einkorn accessions were resistant to *Drechslera graminea*, *Septoria tritici*, and yellow rust.

Our findings indicate that there are differences in susceptibility both between the two species and between cultivars. The most resistant emmer accessions identified here could be the subject of further studies as they could be used as new disease resistance sources for durum breeding.

5. Conclusions

In conclusion, our findings indicate that most accessions adapted successfully to the low-input conditions of the experiments, and the performance of most landraces in either quantity or quality were not statistically different from the registered varieties. The yields of the best landraces even tended to rank higher in all three years than those of the commercial varieties. Although einkorn had higher yield maximum values, yield stability was higher in emmer as yield fluctuations due to the crop year were greater in einkorn. The average grain quality was also satisfactorily high, and both the protein and the bioactive components were present in high amounts in the grains of both species. Einkorn seeds were even richer in flavonoids and lipids and had higher antioxidant activity than emmer grains. There were differences between accessions, although the impact and interaction of the genotype and the environment on these traits should also be determined in the future. Investigations on quality are also planned to include processing requirements and protein digestibility issues.

Some accessions had outstandingly high disease resistance, and einkorn was resistant to most diseases. Even if emmer and einkorn are less susceptible than modern wheats (durum and bread wheat), *Fusarium* head blight can still be a problem, which should be addressed in future research. Possible mycotoxin contamination will be studied in the future.

Summing up, in the present work we demonstrated that einkorn, which could be considered as the most ancient wheat, still has invaluable characteristics, despite the several thousand years of cultivation. These unique features include exceptional disease resistance and very high bioactive component content, which even exceeds that of the emmer accessions. We proved that both emmer and einkorn can be grown successfully under low-input organic conditions and still keep their high grain quality. The cultivation of both species could lead to a more diverse and sustainable agriculture.

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References

1. Füleky, G. Cultivated plants, primarily as food sources. In *Knowledge for Sustainable Development*; UNESCO: Paris, France, 2004; pp. 1035–1057.
2. Garvin, D.F.; Welch, R.M.; Finley, J.W. Historical shifts in the seed mineral micronutrient concentration of US hard red winter wheat germplasm. *J. Sci. Food Agric.* **2006**, *86*, 2213–2220. [CrossRef]
3. Murphy, K.M.; Reeves, P.G.; Jones, S.S. Relationship between yield and mineral nutrient concentrations in historical and modern spring wheat cultivars. *Euphytica* **2008**, *163*, 381–390. [CrossRef]
4. Mayer, A.M. Historical changes in the mineral content of fruits and vegetables. *Br. Food J.* **1997**, *99*, 207–211. [CrossRef]
5. White, P.J.; Broadley, M.R. Historical variation in the mineral composition of edible horticultural products. *J. Hortic. Sci. Biotech.* **2005**, *80*, 660–667. [CrossRef]
6. Davis, D.R. Declining fruit and vegetable nutrient composition: What is the evidence? *J. Am. Soc. Hortic. Sci.* **2009**, *44*, 15–19. [CrossRef]
7. Marles, R.J. Mineral nutrient composition of vegetables, fruits and grains: The context of reports of apparent historical declines. *J. Food Compos. Anal.* **2017**, *56*, 93–103. [CrossRef]
8. Dwivedi, S.L.; Bueren, E.T.; Ceccarelli, S.; Grando, S.; Upadhyaya, H.D.; Ortiz, R. Diversifying Food Systems in the Pursuit of Sustainable Food Production and Healthy Diets. *Trends Plant Sci.* **2017**, *22*, 842–856. [CrossRef]
9. Mie, A.; Andersen, H.R.; Gunnarsson, S.; Kahl, J.; Kesse-Guyot, E.; Rembiałkowska, E.; Quaglio, G.; Grandjean, P. Human health implications of organic food and organic agriculture: A comprehensive review. *Environ. Health* **2017**, *16*, 111. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5658984/ (accessed on 19 February 2020). [CrossRef]
10. European Soil Data Centre (ESDAC) Database. Available online: https://esdac.jrc.ec.europa.eu/content/soil-map-hungary-clay-mineral-association-soils-main-substance-regime-type-soils-main (accessed on 19 February 2020).
11. Yao, G.; Zhang, J.; Yang, L.; Xu, H.; Jiang, Y.; Xiong, L.; Zhang, C.; Zhang, Z.; Ma, Z.; Sorrells, M.E. Genetic mapping of two powdery mildew resistance genes in einkorn (*Triticum monococcum* L.) accessions. *Theor. Appl. Genet.* **2007**, *114*, 351–358. [CrossRef]

12. Zaharieva, M.; Ayana, N.G.; Al Hakimi, A.; Misra, S.C.; Monneveux, P. Cultivated emmer wheat (*Triticum dicoccon* Schrank), an old crop with promising future: A review. *Genet. Resour. Crop. Evol.* **2010**, *57*, 937–962. [CrossRef]
13. Góral, T.; Ochodzki, P. *Fusarium* head blight resistance and mycotoxin profiles of four *Triticum* species genotypes. *Phytopathol. Mediterr.* **2017**, *56*, 175–186.
14. Buvaneshwari, G.; Yenagi, N.B.; Hanchinal, R.R.; Naik, R.K. Glycaemic responses to dicoccum products in the dietary management of diabetes. *Ind. J. Nutr. Dietet.* **2003**, *40*, 363–368.
15. Čurná, V.; Lacko-Bartošová, M. Chemical composition and nutritional value of emmer wheat (*Triticum dicoccon* Schrank): A Review. *J. Cent. Eur. Agric.* **2017**, *18*, 117–134.
16. Dinu, M.; Whittaker, A.; Pagliai, G.; Benedettelli, S.; Sofi, F. Ancient wheat species and human health: Biochemical and clinical implications. *J. Nutr. Biochem.* **2018**, *52*, 1–9. [CrossRef]
17. Loje, H.; Moller, B.; Laustsen, A.M.; Hansen, A. Chemical composition, functional properties and sensory profiling of einkorn (*Triticum monococcum* L.). *J. Cereal. Sci.* **2003**, *37*, 231–240. [CrossRef]
18. Nakov, G.; Stamatovska, V.; Vasileva, N.; Damyanova, S.; Necinova, L. Nutritional properties of einkorn wheat (*Triticum monococcum* L.)—Review. Reports Awarded with "Best Paper" Crystal Prize. In *55th Science Conference of Ruse University, Razgrad, Bulgaria, 4 November 2016*; "Angel Kanchev" University of Ruse: Ruse, Bulgaria, 2016; pp. 381–384.
19. Piergiovanni, A.R.; Laghetti, G.; Perrino, P. Characteristics of meal from hulled wheats (*Triticum dicoccon* Schrank and *T. spelta* L.): An evaluation of selected accessions. *Cereal. Chem.* **1996**, *73*, 732–735.
20. Arzani, A. Emmer (*Triticum tugidum* spp. *Dicoccum*) flour and breads. In *Flour and Breads and Their Fortification in Health and Disease Prevention*; Preedy, V.R., Watson, R.R., Patel, V.B., Eds.; Academic Press: Cambridge, MA, USA; Elsevier: Amsterdam, The Netherlands, 2011; pp. 69–78. ISBN 978-0-12-380886-8.
21. Akar, T.; Cengiz, M.F.; Tekin, M. A comparative study of protein and free amino acid contents in some important ancient wheat lines. *Qual. Assur. Saf. Crop.* **2019**, *11*, 191–200. [CrossRef]
22. Lachman, J.; Miholová, D.; Pivec, V.; Jírů, K.; Janovská, D. Content of phenolic antioxidants and selenium in grain of einkorn (*Triticum monococcum*), emmer (*Triticum dicoccum*) and spring wheat (*Triticum aestivum*) varieties. *Plant Soil Environ.* **2011**, *57*, 235–243. [CrossRef]
23. Şahin, Y.; Yıldırım, A.; Yücesan, B.; Zencirci, N.; Erbayram, Ş.; Gürel, E. Phytochemical content and antioxidant activity of einkorn (*Triticum monococcum* ssp. *monococcum*), bread (*Triticum aestivum* L.), and durum (*Triticum durum* Desf.) wheat. *Progr. Nutr.* **2018**, *19*, 450–459. Available online: https://mattioli1885journals.com/index.php/progressinnutrition/article/view/5847 (accessed on 10 January 2020).
24. Zrcková, M.; Capouchová, I.; Paznocht, L.; Eliášová, M.; Dvořák, P.; Konvalina, P.; Janovská, D.; Orsák, M.; Bečková, L. Variation of the total content of polyphenols and phenolic acids in einkorn, emmer, spelt and common wheat grain as a function of genotype, wheat species and crop year. *Plant Soil Environ.* **2019**, *65*, 260–266. [CrossRef]
25. Zhao, F.J.; Su, Y.H.; Dunham, S.J.; Rakszegi, M.; Bedő, Z.; McGrath, S.P.; Shewry, P.R. Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. *J. Cereal. Sci.* **2009**, *49*, 290–295. [CrossRef]
26. Piergiovanni, A.R.; Rizzi, R.; Pannacciulli, E.; Della Gatta, C. Mineral composition in hulled wheat grains: A comparison between emmer (*Triticum dicoccon* Schrank) and spelt (*T. spelta* L.) accessions. *Int. J. Food Sci. Nutr.* **1997**, *48*, 381–386. [CrossRef]
27. Zadoks, J.C.; Chang, T.T.; Konzak, C.F. A decimal code for the growth stages of cereals. *Weed Res.* **1974**, *14*, 415–421. [CrossRef]
28. Shewry, R.P.; Hey, S. Do "ancient" wheat species differ from modern bread wheat in their contents of bioactive components? *J. Cereal. Sci.* **2015**, *65*, 236–243. [CrossRef]
29. AOAC. *Approved Methods of Association of Official Analytical Chemists*, 5th ed.; Association of Official Analytical Chemists, Inc.: Arlington, VA, USA, 1990.
30. Prosky, L.; Asp, N.G.; Scheweizer, T.F.; de Vries, J.W.; Furda, I. Determination of insoluble, soluble, and total dietary fiber in foods and food products: Interlaboratory study. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 1017–1023. [CrossRef] [PubMed]

31. Dinelli, G.; Segura-Carretero, A.; Di Silvestro, R.; Marotti, I.; Fu, S.; Benedettelli, S.; Ghiselli, L.; Fernandez-Gutierrez, A. Profiles of phenolic compounds in modern and old common wheat varieties determined by liquid chromatography coupled with time-of-flight mass spectrometry. *J. Chromatogr. A* **2011**, *1218*, 7670–7681. [CrossRef] [PubMed]
32. Singleton, V.L.; Orthofer, R.; Lamuela–Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.
33. Brand-Williams, W.; Cuvelier, H.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
34. Castagna, R.; Borghi, B.; Di Fonzo, N.; Heun, M.; Salamini, F. Yield and related traits of einkorn (*T. monococcum ssp. monococcum*) in different environments. *Eur. J. Agron.* **1995**, *4*, 371–378. [CrossRef]
35. Konvalina, P.; Capouchová, I.; Stehno, Z.; Moudrý, J. Differences in yield parameters of emmer in comparison with old and new varieties of bread wheat. *Afr. J. Agric. Res.* **2012**, *7*, 986–992.
36. Marino, S.; Tognetti, R.; Alvino, A. Crop yield and grain quality of emmer populations grown in central Italy, as affected by nitrogen fertilization. *Eur. J. Agron.* **2009**, *31*, 233–240. [CrossRef]
37. Troccoli, A.; Codianni, P. Appropriate seeding rate for einkorn, emmer, and spelt grown under rainfed condition in southern Italy. *Eur. J. Agron.* **2005**, *22*, 293–300. [CrossRef]
38. Dhanavath, S.; Rao, U.J. Nutritional and nutraceutical properties of *Triticum dicoccum* wheat and its health benefits: An overview. *J. Food Sci.* **2017**, *82*, 2243–2250. [CrossRef] [PubMed]
39. Lachman, J.; Orsák, M.; Pivec, V.; Jírů, K. Antioxidant activity of grain of einkorn (*Triticum monococcum* L.), emmer (*Triticum dicoccum* Schuebl [Schrank]) and spring wheat (*Triticum aestivum* L.) varieties. *Plant Soil Environ.* **2012**, *58*, 15–21. [CrossRef]
40. Serpen, A.; Gökmen, V.; Karagöz, A.; Scanlon, M.G. Phytochemical quantification and total antioxidant capacities of emmer (*Triticum dicoccon* Schrank) and einkorn (*Triticum monococcum* L.) wheat landraces. *J. Agric. Food Chem.* **2008**, *56*, 7285–7292. [CrossRef]
41. Hidalgo, A.; Brandolini, A.; Ratti, S. Influence of genetic and environmental factors on selected nutritional traits of *Triticum monococcum*. *J. Agric. Food Chem.* **2009**, *57*, 6342–6348. [CrossRef]
42. Konvalina, P.; Capouchová, I.; Stehno, Z.; Moudrý, J. Agronomic characteristics of the spring forms of the wheat landraces (einkorn, emmer, spelt, intermediate bread wheat) grown in organic farming. *J. Agrobiol.* **2010**, *27*, 9–17. [CrossRef]