agronomy

MDPI

Article

A Comparative Study of Organic and Conventional Management on the Rhizosphere Microbiome, Growth and Grain Quality Traits of Tritordeum

Giovanna Visioli [1,*], Marta Lauro [1], Teofilo Vamerali [2], Cristian Dal Cortivo [2], Anna Panozzo [2], Silvia Folloni [3], Cristina Piazza [4] and Roberto Ranieri [3]

1 Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy; marta.lauro@unipr.it
2 Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padua, Viale dell'Università 16, Legnaro, 35020 Padua, Italy; teofilo.vamerali@unipd.it (T.V.); cristian.dalcortivo@unipd.it (C.D.C.); anna.panozzo@unipd.it (A.P.)
3 Open Fields s.r.l., Strada Consortile 2, Collecchio, 43044 Parma, Italy; s.folloni@openfields.it (S.F.); r.ranieri@openfields.it (R.R.)
4 Stuard Demonstration Farm, Strada Madonna dell'Aiuto 7/a, 43126 Parma, Italy; c.piazza@stuard.it
* Correspondence: giovanna.visioli@unipr.it

Received: 30 September 2020; Accepted: 3 November 2020; Published: 5 November 2020

Abstract: Tritordeum is a novel hexaploid cereal derived from the cross between a wild Chilean barley species (*Hordeum chilense* Roem. et Schultz) and durum wheat (*Triticum turgidum* ssp. *durum* Desf.) that is potentially of great interest for human nutrition. In this study, a commercial and an experimental Tritordeum cultivar were analyzed in comparison with a reference durum wheat under conventional and organic management. We demonstrate that Tritordeum is better adapted to organic farming through an increase in the below-ground rhizosphere community of the Bacteroidetes phylum, which includes many bacteria species known to exert beneficial effects on plants, particularly for root growth. Despite a considerably lower grain yield, Tritordeum had better quality traits than durum wheat, particularly under organic farming *vs.* conventional management, with respect to total protein contents, high molecular weight glutenin subunits, antioxidant free phenols and nutrients (i.e., calcium, potassium, sulphur, iron, and zinc), depending on the cultivar. We conclude that Tritordeum is a promising cereal in light of its quality traits and adaptability to sustainable crop management practices, such as organic farming, although further improvement in yield potential should be pursued by breeding and by optimising the cultivation method.

Keywords: resilient cereals; grain quality; rhizosphere; root growth; sustainable management

1. Introduction

The Triticeae tribe contains more than 350 species, including some important staple crops such as bread wheat (*Triticum aestivum* ssp. *aestivum* L.), durum wheat (*Triticum turgidum* ssp. *durum* Desf.), barley (*Hordeum vulgare* ssp. *vulgare*), and rye (*Secale cereale* L.). Wheat is the third most important cereal in the world in terms of total production; it is widely grown in Europe with 61 million hectares in 2018 [1], and provides 19% of the calories and 20% of protein requirements for human nutrition in Western and Northern Asia. Wheat grains are central dietary components worldwide due to their high nutritional content and organoleptic qualities, and the unique biomechanical properties of the derived dough, from which a wide range of leavened products are produced. Historically, wheat bread has been a staple of European-type diets, and in recent decades wheat products have increasingly replaced other staple carbohydrates in Asia and Africa [2].

The ongoing worldwide decrease in the genetic variability of the main cereal crops (e.g., wheat, corn, and rice) is one of the reasons for the increased vulnerability of agriculture to climate change [3] and related problems associated with emerging diseases and difficulties in ensuring food security [4].

Increasing the genetic diversity of wheat-like cereals by growing crops of minor or novel species can improve the stability, robustness and sustainability of farming systems, contribute to sound pest and disease management, increase product diversity and income opportunities, help to maximize the effective use of resources and the environment, reduce dependency on external inputs and improve human nutrition [5]. Since the beginning of the last century, plant breeding programmes have focused their efforts on developing interspecific cereal hybrids with better agronomic performances, environmental adaptability, and technological/nutritional qualities [6]. This has been the case with triticale (×*Triticosecale* Wittmark), a cross between *T. turgidum*/*T. aestivum* and *Secale cereale*, and could also be the case with Tritordeum making it a potentially interesting candidate for use in sustainable farming systems.

Tritordeum (×*Tritordeum* Asch. & Graebn.) is a novel fertile hexaploid cereal species derived from the cross between a wild barley species of Chilean origin (*Hordeum chilense* Roem. & Schult.) as the maternal parent and the cultivated tetraploid durum wheat (*Triticum turgidum* ssp. *durum* Desf.) [7,8]. Due to its favorable agronomical (i.e., biomass, number of spikelets per spike, seed size, adaptation to drought, and salinity) and qualitative traits (i.e., protein content, high seed carotenoid content, and prolamins) it was later used in a breeding programme to produce new varieties [9]. The first two commercial lines have been registered as "Aucan" (in 2010) and "Bulel" (in 2015). Tritordeum is now available in several European and non-European countries [10], and it is becoming widely cultivated in Spain, Italy, and Portugal. Villegas et al. [11] confirmed the growing performance of Tritordeum to be quite close to that of wheat and triticale in marginal and drought (rain-fed) environments, and although its grain yield is lower, its grain quality is better. Its lutein content is approximately 5.2 times higher than that of durum wheat, with some intraspecific variability and, interestingly, a higher degree of esterification [12], potentially increasing the stability of this carotenoid and its bioavailability for absorption and transport [13]. Tritordeum has been acknowledged as an excellent raw material for the production of healthier foods compared with barley and wheat, due to its phytochemical composition (i.e., lutein and β-glucans) and bioactive antioxidant compounds, such as carotenoids and phenolic acids [14], suggesting the high potential of this species to become a functional staple food [15]. However, the significant gap in yield that currently exists between Tritordeum and other widely-cultivated small grain cereals seems to be the main limitation to its wider distribution in fertile agricultural lands.

The present study, taking its cue from the information reported above, compares two Tritordeum cultivars (cvs.) with a reference durum wheat cultivar (cv.) under both conventional and organic management systems in order to evaluate: (i) root development and the associated rhizosphere microbiome as indicators of adaptability to organic farming; (ii) grain yield and gluten protein composition in relation to nutritional and technological characteristics, and (iii) mineral and polyphenol contents.

2. Materials and Methods

2.1. Field Trial Set-up and Agronomic Parameters

The durum wheat (*Triticum turgidum* ssp. *durum*) cv. Iride (Syngenta Italy, Milan, Italy) and two cvs. of Tritordeum (×*Tritordeum martini*), i.e., one commercial (cv. Bulel) and one experimental (cv. HTC-444) (Agrasys S.L., Barcelona, Spain) were grown in the field at the Stuard Experimental Farm in Parma (Italy, GPS 44.79 N 10.27 E) during the 2017–2018 growing season. The experiment was carried out in a randomized block design with 10.5-m^2 plots (n = 3) arranged in two closed fields, one under a conventional farming system (~3 hectares) and the other under an organic farming

system (~2.7 hectares). The two systems differed in that only the conventional farm management used nitrogen fertilization, provided as ammonium nitrate in a total amount of 120 kg N ha^{-1}, and chemical treatments against *Septoria* and *Fusarium* fungal pathogens.

The soil was silty-clay, previously cultivated with tomato (conventional) and spinach (organic). Sowing took place on 21 November 2017 at a density of 400 seeds m^{-2}. At end of the heading stage (BBCH 59) [16] the final plant height (from the soil surface to the ear tip) and the lodging rate were recorded. At the grain over-ripe stage (BBCH 92), on 25 June 2017, yield was measured by collecting the grains from the central area of each plot with a mini combine harvester. Thousand kernel weight (TKW) was determined on three sets of each sample using an electronic balance. Test weight (TW) was determined with a GAC-2000 grain analysis meter (Dickey-John Corp., Auburn, IL, USA).

2.2. Rhizosphere Soil Sampling and Root Growth Analyses

Destructive sampling of the root systems of the Tritordeum and durum wheat cvs. down to a depth of 0.3 m was carried out at the 3 unfolded leaves stage (BBCH 13; December 2017) using the monolith method over a soil surface area of 0.2 × 0.2 m. Three biological replicates per cvs. and species under each management system were collected. The samples, comprising the soil block and the entire root systems, were immediately processed in the laboratory to isolate the rhizosphere soil. Roots were gently extracted from the soil blocks and after removing most of the soil by shaking, the remaining rhizosphere soil adhering to the roots was carefully collected with a small sterile brush. Rhizosphere soil samples from the three plants of each replicate were pooled to obtain a 2-g sample, which was placed in a sterile Falcon tube and stored at −80 °C until DNA extraction and analysis. Washed roots from the same monoliths were stored in a 15% v/v ethanol solution at 4 °C until digitalization as 1-bit 400-DPI TIFF format images using a flatbed scanner. The images were processed with the WinRhizo software (Regent Instruments Inc., Ville de Québec, QC, Canada), with a minimum area of 40 pixels adopted for thresholding background noise. The root parameters length, surface area, diameter, and number of tips and forks were recorded [17].

2.3. DNA Extraction from Rhizosphere Soil, Rhizobiome Identification by 16S rRNA Gene Sequencing, and Data Analysis

DNA was extracted from the rhizosphere soil of three biological replicates of Iride, Bulel, and HTC-444 cvs. from conventional and organic fields, and from non-vegetated soils (bulk soil, BS). Specifically, 0.5 g of each sample was extracted with the FastDNA® Spin Kit for Soil (MP Biomedicals Inc., Santa Ana, CA, USA) according to the manufacturer's protocols, visualized by electrophoresis on 0.8% (*w*/*v*) agarose gel to test for DNA integrity, and quantified by Nanodrop ND1000. The bacterial community profiles of the samples were generated by NGS technologies at the Genprobio Srl Laboratory (Parma, Italy; www.genprobio.com). Partial 16S rRNA gene sequences were obtained from the extracted DNA by polymerase chain reaction (PCR) using the primer pair Probio_Uni and Probio_Rev and targeting the V3 region of the bacterial 16S rRNA gene (Probio_Uni CCTACGGGRSGCAGCAG and Probio_Rev *ATTACCGCGGCTGCT)* [18]. The PCR conditions were 5 min at 95 °C, 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 90 s at 72 °C, followed by 10 min at 72 °C. Amplification was carried out using a Veriti Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The integrity of the PCR amplicons was analyzed by electrophoresis on an Experion workstation (Bio-Rad Laboratories Inc., Hercules, CA, USA). After amplification and amplicon checks, 16S rDNA was sequenced with a MiSeq (Illumina Inc., San Diego, CA, USA) and analyzed at the DNA sequencing facility of GenProbio Srl, according to a previously-reported protocol [19].

The 16S rDNA reads were sent to GenBank to obtain their accession numbers. They are available as bioproject PRJNA590242.

2.4. Determination of Micro- and Macronutrients in the Grain

Nitrogen concentrations in grain flour were determined according to the Kjeldahl method. Grain mineral contents, namely calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), phosphorus (P), sulphur (S), and zinc (Zn), were measured by inductively coupled plasma–optical emission spectroscopy (ICP-OES) (SPECTRO CirOS Vision EOP, SPECTRO Analytical Instruments GmbH & Co. KG, Kleve, Germany). Oven-dried grain samples of about 0.4 g were mixed with 7 mL HNO_3 (65% *v/v*) and 1 mL H_2O_2 (30% *v/v*), then microwave acid-digested (Mileston ETHOS 900, Bergamo, Italy) following the EPA method 3052 [20]. The samples were then diluted to 25 mL with distilled water, filtered (0.45-µm CA), and analyzed by ICP-OES. Measurement accuracy was ensured with certified reference materials (ERM-CD281 and BRC-402; JRC-IRMM, Geel, Belgium).

2.5. Determination of Free Phenolic Acids

Free phenolic acids were extracted from mature grains according to the modified procedure by [21]. Specifically, extraction was carried out from 0.1-g samples mixed with 5 mL of 80% *v/v* chilled acetonitrile in 10 mL tubes. The mixture was shaken for 5 min at 70 rpm at room temperature, and centrifuged at 10,000× *g* for 10 min. The supernatant was filtered at 0.2 µm (Acrodisc syringe filters with GHP membranes) and kept in clean tubes at −20 °C until processing. Free phenolic acids were analyzed by high-performance liquid chromatography (HPLC; Shimadzu Corporation, Kyoto, Japan) using an Ultra Tech sphere C18 column (1.5 µm particle size, 33 mm × 4.6 mm i.d.; CIL Cluzeau, Sainte-Foy-La-Grande, France) at 36 °C, while the phenols were identified with a Shimadzu SPD-M20A Photodiode Array Detector (282 nm wavelength). The flow rate and time settings of the solvents (trifluoroacetic acid and pure acetonitrile) were as described in [22]. The identification peaks were confirmed by the retention time and absorbance spectra of the pure compounds, i.e., *p*-coumaric acid, caffeic acid, syringic acid, vanillic acid, and *t*-ferulic acid. The concentrations of the free phenolic acids were calculated using standard calibration curves with determination coefficients >99% and were expressed in mg kg^{-1} dry weight (DW).

2.6. SDS-PAGE of Gluten Proteins, Separation and Densitometric Analysis of Gliadins, High Molecular Weight Glutenin Subunits (HMW-GS), and Low Molecular Weight Glutenin Subunits (LMW-GS)

Grain samples of 35 g from each cv. were milled to a fine powder with a Knifetec™ 1095 (Foss, Hillerød, Denmark). Gluten proteins (gliadins, HMW-GS, and LMW-GS) were extracted from 30-mg samples and quantified following a previous reported methodology [23].

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a Mini-PROTEAN Tetra Cell (Bio-Rad) using 8% acrylamide gels for HMW-GS, and 12% acrylamide gels for gliadins and LMW-GS. Aliquots of 20 µg of each dried protein were suspended in 20 µL of loading buffer containing 20 g L^{-1} SDS, 0.2 g L^{-1} bromophenol blue, 1 mL L^{-1} β-mercaptoethanol, 0.05 mol L^{-1} Tris HCl (pH 6.8), and 100 mL L^{-1} glycerol, then boiled at 95 °C for 5 min before loading onto the gels. An Amersham Low Molecular Weight Calibration Kit for SDS Electrophoresis (MW 14,400–97,000 Da) (GE Healthcare, Chicago, IL, USA) was used to detect HMW, LMW-GS and gliadin bands. After electrophoretic separation at 40 mA, the gels were fixed in 70 mL L^{-1} acetic acid and 400 mL L^{-1} methanol and stained with Coomassie Brilliant Blue R-250 Staining Solution (Bio-Rad). Image Lab 4.5.1 software (Bio-Rad) was used for relative quantification of the glutenin subunits on each gel. Gliadins were divided into three classes (ω, α/β and γ) based on their molecular weight.

2.7. Bioinformatics and Statistical Analysis

Following sequencing, the FastQ files were processed using a custom script based on the QIIME software suite [24]. Paired-end read pairs were assembled to reconstruct the complete Probio_Uni/Probio_Rev amplicons. Quality control retained sequences with a length between 140 and 400 bp and a mean sequence quality score >20, while sequences with homopolymers >7 bp and

mismatched primers were omitted (Table S1). In order to calculate downstream diversity measures (alpha and beta diversity indices, Unifrac analysis), 16S rRNA Operational Taxonomic Units (OTUs) were defined at ≥99% sequence homology using the Uclust algorithm [25], and OTUs with less than 10 sequences were filtered. All reads were classified to the lowest possible taxonomic rank using QIIME [24] and a reference dataset from the SILVA database [26]. The microbial richness of the samples (alpha-diversity) was calculated with the Chao1 and Shannon indices for 10 sub-samplings of sequenced read pools and represented by rarefaction curves (Figure S1).

One-way and two-way ANOVAs using the SigmaPLot 12.5 software (Systat Software Inc., San Jose, CA, USA) were carried out to detect significant differences in gluten protein components, rhizosphere phyla and family abundances among the Tritordeum and durum wheat cvs. and the agricultural management systems. Bacterial taxa with p-values ≤ 0.05 were selected and identified as the phylotypes or bacterial groups that were significantly affected by the two farming systems. Differences among means for root parameters, grain yield, mineral composition and phenolic acid concentrations were ascertained by ANOVA and the Newman–Keuls test ($p \leq 0.05$) using the Statgraphics Centurion XV software (Manugistics, Rockville, MD, USA).

3. Results and Discussion

3.1. Root Growth and Rhizosphere Microbial Biodiversity

Roots of durum wheat cv. Iride, and Tritordeum cv. Bulel and HTC-444 were analyzed at the three-leaf stage in early autumn. We found differences between the two species in the morphological parameters, i.e., length, surface area, diameter, and tip density, but no significant differences between the cultivation methods, i.e., conventional vs. organic (Table 1). Under both management systems, the Tritordeum cvs. had a significantly shorter root length and surface area than the reference durum wheat cv. Iride (about −54%), and a greater root thickness, as evidenced by average diameter.

Durum wheat cv. Iride showed significantly greater root growth in the conventional compared with the organic management system. The same trend was observed in Tritordeum cv. HTC-444, although the difference was not significant, whereas cv. Bulel showed the opposite trend, with better root growth under organic farming, although again the difference was not statistically significant (Table 1). No significant differences were found with regard to the branching index, neither among cvs. nor between management systems (main effect), although durum wheat cv. Iride values were higher than those for Tritordeum cv. Bulel under organic cultivation.

Regarding the microbial characterization, a total of 677,098 raw pyrosequencing reads were obtained. After filtering and removing chimeras, a total of 561,869 sequences were retained with mean numbers of total sequences per sample (average read of two replicates) ranging from 50,097 to 83,078 (Table S1). The number of OTUs and the bacterial diversity indices, i.e., the Shannon diversity index and the Chao1 estimator of richness (at 97% sequence similarity), showed there to be no significant differences among the samples in the bacterial diversity in the BS and the rhizosphere soil (Figure S1). Sequences were assigned to 15 phyla representing >0.5% of the total abundance (Figure 1; Table S2).

Table 1. Root length, surface area, diameter, tip density, and branching index (no. of forks/total root length) (mean ± SE; n = 3) in durum wheat (cv. Iride) and Tritordeum (cv. HTC-444 and Bulel) cultivated under organic and conventional agricultural systems. Letters indicate statistically significant differences among cultivars within the same farming system (Newman–Keuls test, $p \leq 0.05$).

Farming System	Cultivar	Root Parameters				
		Length (cm Plant^{-1})	Surface Area (cm^2 Plant^{-1})	Average Diameter (μm)	Tips (n Plant^{-1})	Branching Index (n Forks cm^{-1})
Organic	Iride	72.9 ± 8.036 a	11.9 ± 1.854 a	514 ±24.17 b	97.6 ± 27.08 a	1.76 ± 0.385 a
	Bulel	38.5 ± 0.651 b	6.90 ± 0.211 ab	569 ± 14.17 a	30.0 ± 2.835 b	0.77 ± 0.029 b
	HTC-444	29.2 ± 2.554 b	4.74 ± 0.158 b	523 ± 34.48 ab	26.1 ± 2.570 b	1.10 ± 0.105 ab
Conventional	Iride	86.8 ± 33.55 a	12.1 ± 5.017 a	432 ± 9.948 c	103 ± 45.12 a	1.28 ± 0.287 a
	Bulel	32.3 ± 2.657 b	5.05 ± 0.584 b	495 ± 17.77 b	25.1 ± 4.636 b	1.11 ± 0.203 a
	HTC-444	39.3 ± 5.851 b	7.30 ± 0.995 b	592 ± 7.219 a	36.7 ± 12.54 b	1.32 ± 0.228 a
Farming system		ns	ns	ns	ns	ns
Cultivar		*	*	**	*	ns
Farming system × Cultivar		ns	ns	**	ns	ns

ns: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

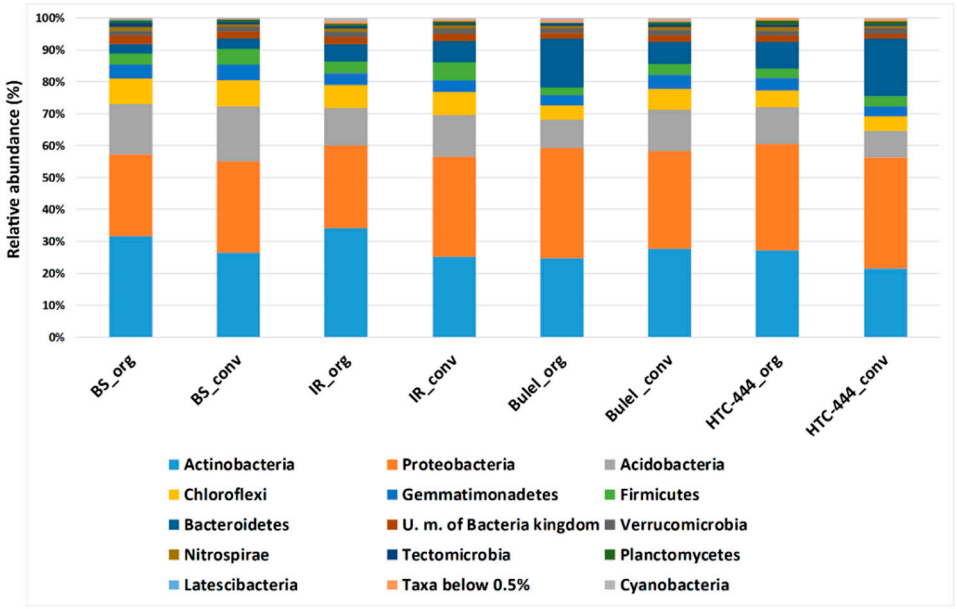

Figure 1. Microbial community composition at the phylum level in bulk soil (BS) and in the rhizosphere soil of durum wheat cv. Iride (IR), and Tritordeum cvs. Bulel and HTC-444 under organic (org) and conventional (conv) farming systems.

Among the most highly represented phyla, Actinobacteria were significantly more abundant under organic than under conventional management in bulk soil (31.60% vs. 26.52%; $p \leq 0.05$), in the durum wheat cv. Iride rhizosphere (34,19% vs. 25,16%; $p \leq 0.05$), and in the Tritordeum HTC-444 rhizosphere (27.17% vs. 21.47%; $p \leq 0.05$). Conversely, Proteobacteria were significantly less abundant under organic than under conventional management in bulk soil (25.63% vs. 28.48%; $p \leq 0.05$), in the Iride rhizosphere (25.77% vs. 31.22%; $p \leq 0.05$), and in the HTC-444 rhizosphere (33.23% vs. 34.74%; $p \leq 0.05$) (Figure 1; Table S3). The rhizosphere of Tritordeum cv. Bulel showed an opposite trend for these phyla, with Actinobacteria significantly less abundant (24.71% vs. 27.76%; $p \leq 0.05$) and Proteobacteria significantly more abundant (34.47% vs. 30.48%; $p \leq 0.05$) under organic than under conventional management (Figure 1; Table S3). It may be argued that, on the one hand, the organic soil is already richer in relevant microorganism groups from which the Iride and HTC-444 rhizospheres can benefit, but that, on the other hand, Bulel roots are more selective in shaping their rhizosphere environments in the two soils/management systems.

Regarding the less abundant phyla, Bacteroidetes were overall found in significantly greater quantities in the rhizospheres of both durum wheat and Tritordeum cvs. than in bulk soil, in accordance with other findings on barley, wheat and other crop species [19,27]. In the Tritordeum cv. Bulel, the Bacteroidetes phylum was also more abundant under organic than under conventional management (15.47% vs. 6.76%; $p \leq 0.05$) (Figure 1; Table S2). This bacterial group is reported in the literature as being a good biological indicator of lower agricultural soil disturbance, so these data suggest that organic management has a lower impact on the soil biota compared with conventional management [28]. The greater abundance of Bacteroidetes in Bulel under organic management is also supported at the family level by analysis of the Flavobacteriaceae and Sphingobacteriaceae, both of which belong to the Bacteroidetes phylum and are significantly more abundant in the organic than in the conventional rhizosphere (6.06% vs. 2.47%, and 7.33% vs. 1.03%, respectively; $p \leq 0.05$) (Table S3). These bacterial groups have important ecological functions, since Flavobacteriaceae contribute to the turnover of soil organic matter and the decomposition of pesticides and have been associated with plant growth promotion and disease protection [29]. Sphingobacteriaceae are involved in degradation processes and the conversion of biomolecules [30]. Oxalobacteraceae is another family belonging to the Proteobacteria phylum found in significantly greater abundance in the organic than in the conventional rhizosphere

of Bulel (9.65% vs. 5.66%; $p \leq 0.05$) (Table S3). Within this family, *Janthinobacterium* is a genus that is able to synthesize an antimicrobial and antifungal compound (violacein pigment) and improve environmental stress response in plants [31].

The increases in these taxa under organic management are probably associated with the increasing trends in the surface area and root length of Tritordeum cv. Bulel (Table 1). The beneficial effect of Bacteroidetes also seems to be confirmed in the Tritordeum cv. HTC-444, although it is associated with greater root length (39.3 vs. 29.2 cm plant^{-1}), root surface area (7.3 vs. 4.74 cm^2 plant^{-1}) and number of root tips per plant (36.7 vs. 26.1) (Table 1) in the conventional *vs.* organic system. Indeed, the Bacteroidetes phylum is more abundant in HTC-444 under conventional than under organic management (17.96% vs. 8.44%, $p \leq 0.05$) (Table S2). The increase in Bacteroidetes in HTC-444 is also supported at the family level with a higher percentage of Flavobacteriaceae in conventional *vs.* organic cultivation (11.50% vs. 3.28%; $p \leq 0.05$) (Table S3). In durum wheat Iride, Bacteroidetes abundance does not seem to be related to root growth, and we found no significant increase in this phylum and related families.

It is worth noting that Bacteroidetes is more abundant in the rhizosphere of Tritordeum compared with durum wheat, whether organically cultivated (Iride 5.60%; Bulel 15.47%; HTC-444 8.42%) or conventionally cultivated (Iride 6.82%; Bulel 6.77%; HTC-444 17.96%) (Figure S2).

A recent meta-analysis of the root microbiome of various crops and their wild relatives has shown an enrichment of Bacteroidetes in the wild plants compared with the corresponding cultivated crop plants, and this trait has a species-specific signature [32]. In barley, approximately 50% of the bacterial species differentially enriched on the roots of the wild plants were found to belong to the Bacteroidetes phylum, within which the family of Flavobacteriaceae exhibited high diversity [32]. Host genotype also has a small but significant effect on the diversity of root-associated bacterial communities. For instance, regarding wild and domesticated barley, the Bacteroidetes phylum and the Flavobacteraceae family show moderately significant increases moving from modern barley varieties to landraces, and from landraces to spontaneous plants [27]. Since Tritordeum is an amphydiploid deriving from the hybridization between durum wheat and a wild barley, it is conceivable that its root rhizobiome also shares similar characteristic with the wild parents. Overall, the cultivation of Tritordeum in an organically managed system could be enhanced by stimulation with a beneficial microbioma to boost plant growth and health.

3.2. Grain Yield, and Nutrient and Phenolic Acid Contents

Tritordeum responded differently to durum wheat in terms of plant growth under conventional and organic farming. Plants of both Tritordeum cvs. Bulel and HTC-444 reached a smaller height than durum wheat cv. Iride under organic management, whereas the opposite was found under conventional management (Table 2). This was probably the reason for the higher lodging rate in cv. HTC-444 under conventional farming, whereas in the organic fields only few plants flattened, with no significant differences among cvs. Lodging is strictly related to stem strength and nitrogen availability and uptake; in this regard, Tritordeum has been shown to have a higher affinity for nitrate uptake at the root level compared with its parent wheat [33], and has a higher nitrate uptake rate at different external nitrogen supply levels [9].

Table 2. Plant height and percentage of lodging at the heading stage, thousand kernel weight (TKW), test weight (TW), and grain yield (mean ± SE; $n = 3$) in durum wheat (cv. Iride) and Tritordeum (cv. HTC-444 and Bulel) cultivated under organic and conventional agricultural systems. Letters indicate statistically significant differences among cultivars within the same farming system (Newman–Keuls test, $p \leq 0.05$).

Farming System	Cultivar	Plant Height (cm)	Culm Lodging (%)	TKW (g)	TW (g L^{-1})	Yield (t ha^{-1} at 14% Humidity)
Organic	Iride	73.9 ± 2.1 a	8.33 ± 1.67 a	48.6 ± 0.59 a	81.7 ± 0.95 a	2.47 ± 0.16 a
	Bulel	65.8 ± 0.9 b	1.67 ± 1.67 a	35.9 ± 0.58 b	75.0 ± 0.41 b	1.02 ± 0.09 b
	HTC-444	68.4 ± 3.5 ab	8.33 ± 6.01 a	37.4 ± 0.06 b	72.8 ± 0.79 b	0.86 ± 0.08 b
Conventional	Iride	78.6 ± 0.3 b	1.67 ± 1.67 b	53.1 ± 0.91 a	81.9 ± 0.54 a	5.35 ± 0.02 a
	Bulel	80.2 ± 0.5 a	3.33 ± 1.67 b	41.7 ± 0.23 b	77.9 ± 0.27 b	3.33 ± 0.10 b
	HTC-444	82.4 ± 1.3 a	13.3 ± 1.67 a	40.3 ± 0.25 b	75.3 ± 0.32 c	2.99 ± 0.12 c
Farming System		***	ns	***	**	***
Cultivar		ns	*	***	***	***
Farming System × Cultivar		*	ns	ns	ns	**

ns: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$.

The grain yield of the two Tritordeum cvs. was about 3 t ha^{-1} under conventional farming, consistent with values recorded by [11] across different Mediterranean countries, but much lower than the 5.3 t ha^{-1} of durum wheat cv. Iride. A similar pattern was observed under organic farming, with the Bulel and HTC-444 cvs. having similar grain yields at 1.02 and 0.86 t ha^{-1}, respectively, compared with the ~2.5 t ha^{-1} of durum wheat. Tritordeum generally had a smaller kernel size than the reference durum wheat, as evidenced by lower TKW and TW in both faming systems, confirming the results for grain quality parameters obtained in previous researches [9,14]. However, grain nitrogen/protein concentration was significantly higher in Tritordeum than in durum wheat, a difference that was significant under organic management and only a trend under conventional management (Table 3). Nitrate reduction by the enzyme nitrate reductase is considered an important rate-limiting step in nitrogen assimilation, and Tritordeum is known to have higher nitrate reductase activity than wheat. This fact, and the observation that protease activity in senescing leaves is higher in Tritordeum than in wheat, suggests that Tritordeum has a higher potential for nitrogen remobilization from vegetative tissues to the grains when approaching maturity [34].

Mineral analyses of grains revealed significant differences among the cvs. for all the elements examined, except *p* content (Table 3). In particular, under organic management, Tritordeum had significantly higher concentrations of Ca, Mg, and S (both cvs.), as well as K (cv. Bulel), and Fe and Zn (cv. HTC-444) compared with durum wheat cv. Iride. Under conventional management, cv. HTC-444 exhibited higher mineral concentrations in general, and cv. Bulel higher S concentrations. As a consequence of yield levels and mineral concentrations, the overall Zn uptake was significantly greater under organic than under conventional management in all the cvs. tested. These results suggest that macro- and micro-nutrient content is negatively related to yield, but there is probably a genetic effect. It is also thought that organic management facilitates mineral uptake, probably due to improved microbial biodiversity and higher abundances of bacterial taxa involved in nutrient bioavailability [35].

Given that, among the antioxidant compounds, phenolic acids have great potential to improve human health [36], it is worthwhile evaluating the effects of different management systems on free phenolic compounds in both durum wheat and Tritordeum. There was a significantly higher concentration of total free phenolic acids in the grains of durum wheat cv. Iride and both Tritordeum cvs. under conventional than under organic management (Table 4) ($p \leq 0.05$). However, when individual compounds were analyzed, significant differences between the management systems were found only for vanillic acid ($p \leq 0.01$), being the most abundant phenol and present in significantly higher concentrations under conventional than under organic cultivation. Regarding varietal comparisons, there were significant differences between durum wheat and Tritordeum in syringic acid and caffeic acid ($p \leq 0.01$ and $p \leq 0.05$, respectively), the former lower and the latter higher in Tritordeum than in durum wheat (Table 4). These data are also consistent with the slightly higher content of total phenolic acids in Tritordeum than in durum wheat, although the difference is not statistically significant, in agreement with a recent study in which cv. Bulel was compared with barley and durum wheat (cv. Saragolla) [14]. The total fraction of soluble phenolic acids in cv. Bulel reported by these authors was very similar to our measure, although, contrary to our results, they found lower values in Bulel than in durum wheat. However, it is well known that the amount of phenolics, particularly the free fraction, is markedly affected by environmental conditions [37], such as farm management and soil type. There is also large intraspecific variation in phenolic contents as evidenced in wheat cvs. by [38]. In this regard, recent literature has reported great variability in phenolic compound concentrations in a collection of Tritordeums having different cytoplasms and chromosome substitutions [39], which is consistent with the differences between cv. Bulel and cv. HTC-444 observed in this study.

Table 3. Mineral concentrations (mean ± SE; $n = 9$) in grains of durum wheat (cv. Iride) and Tritordeum (cv. HTC-444 and Bulel) cultivated under organic and conventional agricultural systems. Letters indicate statistically significant differences among cultivars within the same farming system (Newman–Keuls test, $p \leq 0.05$).

Farming System	Cultivar	Element							
		N (%)	Ca (mg kg^{-1})	Fe (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	P (mg kg^{-1})	S (mg kg^{-1})	Zn (mg kg^{-1})
Organic	Iride	2.10 ± 0.02 c	326 ± 5 d	39.7 ± 2.3 c	5107 ± 208 bc	1353 ± 80 c	5381 ± 246 abc	1438 ± 34 b	55.8 ± 5.3 bc
	Bulel	2.32 ± 0.03 b	406 ± 32 bc	47.0 ± 3.3 bc	6386 ± 429 a	1626 ± 88 ab	5664 ± 343 ab	1899 ± 111 a	58.1 ± 5.2 b
	HTC-444	2.65 ± 0.12 a	452 ± 10 ab	57.5 ± 3.7 ab	5845 ± 81 ab	1699 ± 34 a	6053 ± 95 a	1978 ± 50 a	72.1 ± 3.2 a
Conventional	Iride	2.27 ± 0.05 a	354 ± 22 cd	43.2 ± 3.5 c	4721 ± 267 c	1351 ± 96 c	5161 ± 335 bc	1572 ± 80 b	44.2 ± 4.6 cd
	Bulel	2.26 ± 0.01 a	367 ± 24 cd	46.2 ± 2.6 c	5240 ± 328 bc	1420 ± 93 bc	4633 ± 285 c	1873 ± 123 a	41.8 ± 2.3 d
	HTC-444	2.31 ± 0.05 a	506 ± 36 a	64.63 ± 4.8 a	6028 ± 495 ab	1702 ± 93 a	5823 ± 325 ab	2047 ± 112 a	56.6 ± 3.7 bc
Farming System		ns	ns	ns	ns	ns	ns	ns	**
Cultivar		**	***	***	*	**	*	***	**
Farming System × Cultivar		**	ns	ns	ns	n	ns	ns	ns

ns: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$.

Table 4. Total soluble phenol contents and phenolic acid profiles (mean ± SE; $n = 9$) in grains of durum wheat (cv. Iride) and Tritordeum (cv. HT-444 and Bulel) cultivated under organic and conventional agricultural systems. Letters indicate statistically significant differences among cultivars within the same farming system (Newman–Keuls test, $p \leq 0.05$).

Farming System	Cultivar	Phenolic Acid (μg g^{-1} d.w.)					
		Vanillic	Caffeic	Syringic	*p*-Coumaric	*t*-Ferulic	Total Amount
Organic	Iride	41.5 ± 20.0 b	9.22 ± 2.11 b	21.7 ± 3.4 a	2.71 ± 1.32 a	20.5 ± 4.8 a	95.8 ± 15 b
	Bulel	46.1 ± 16.1 b	13.22 ± 5.1 ab	6.43 ± 2.59 c	1.84 ± 0.82 a	41.2 ± 26.58 a	108 ± 47 ab
	HTC-444	97.1 ± 51.8 ab	15.55 ± 3.8 ab	10.2 ± 2.7 bc	2.04 ± 0.18 a	10.8 ± 1.03 a	135 ± 50 ab
Conventional	Iride	147 ± 38.5 a	7.46 ± 1.65 b	20.1 ± 3.2 a	2.07 ± 1.36 a	10.9 ± 0.03 a	188 ± 43 ab
	Bulel	140 ± 9.7 a	15.96 ± 2.0 ab	10.3 ± 2.9 bc	1.83 ± 0.22 a	10.3 ± 0.43 a	179 ± 13 ab
	HTC-444	166 ± 14.6 a	20.96 ± 3.2 a	16.5 ± 1.0 ab	1.99 ± 0.28 a	9.75 ± 0.26 a	215 ± 15 a
Farming system		**	ns	ns	ns	ns	*
Cultivar		ns	*	**	ns	ns	ns
Farming system × Cultivar		ns	ns	ns	ns	ns	ns

ns: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$.

3.3. Grain Protein Content and Composition

Grain protein content differed significantly across cvs. and management systems. As with grain N concentration (Table 3), under organic management the Tritordeum cv. HTC-444 had the highest protein content (14.8%) followed by Bulel (13.3%) and then the reference durum wheat cv. Iride (12.0%), the differences between the three values being statistically significant. Under conventional management the highest protein contents were in Iride and HTC-444 (both averaging 13.1%), followed by Bulel (12.8%), but the difference was not statistically significant ($p > 0.05$). While these results confirm the general rule that productivity is negatively correlated with grain protein content, attention should be drawn to the fact that the protein content of the Tritordeum cvs. was ≥ 13%, which is a critical threshold for technological quality processing. There was also a significant "cultivar × management" interaction, with the grain protein content of Tritordeum HTC-444 higher under organic than under conventional management, whereas the opposite was the case for durum wheat cv. Iride. Given the negative correlation between grain yield and grain protein concentration in cereals [40], this is probably a consequence of the increased yield under conventional management, which was about double for durum wheat and more than triple for Tritordeum (Bulel + 326% and HTC-444 + 347%).

This trend in protein content was also confirmed for the total gluten proteins extracted, with significantly higher values in cv. HTC-444 (37.5 vs. 32.8 mg g^{-1} flour, organic vs. conventional, respectively) and Bulel (34.2 vs. 31.1 mg g^{-1} flour), compared with durum wheat cv. Iride (30.5 vs. 34.6 mg g^{-1} flour) (Figure 2a). Interestingly, the differences in protein abundances were mainly due to the HMW-GS subunits, which were significantly higher under organic than under conventional cultivation for all the cvs./species analyzed (Figure 2b), while LMW-GS showed the opposite trend in durum wheat cv. Iride and no significant variation between farming systems in Tritordeum cvs. (Figure 2c). Gliadins, the most abundant gluten proteins, were again higher in Tritordeum and lower in Iride under organic management compared with conventional management (Figure 2d).

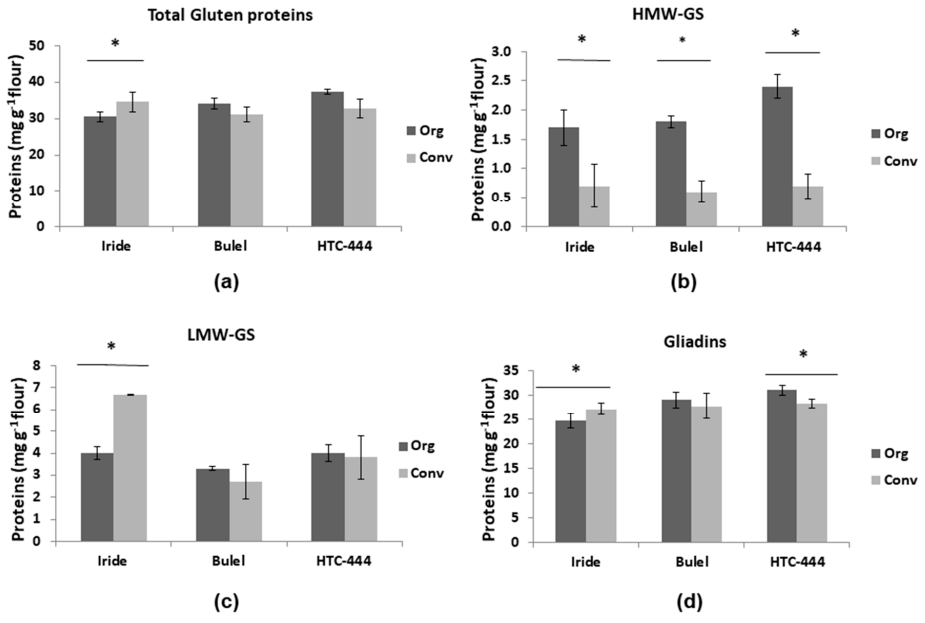

Figure 2. Total gluten content (**a**) HMW-GS (**b**) LMW-GS (**c**) and gliadin (**d**) fractions of durum wheat cv. Iride, and Tritordeum cv. Bulel and HTC-444 under organic and conventional systems. Data are reported as averages of 3 replicates ± standard deviation. Significant differences between organic (org) and conventional (conv) systems for the same cultivar are indicated with asterisks (* $p \leq 0.05$).

In a previous study comparing the gliadin compositions of common wheat and Tritordeum flour and bread, the authors reported significant reductions in ω-gliadins and in peptides containing immunogenic epitopes in Tritordeum [41]. In this study, we observed no differences in ω-gliadin abundances between Tritordeum and durum wheat, but we found lower abundances in cv. Bulel under organic management than under conventional management (Figure 3), which was associated with an increase in γ and α, β gliadins.

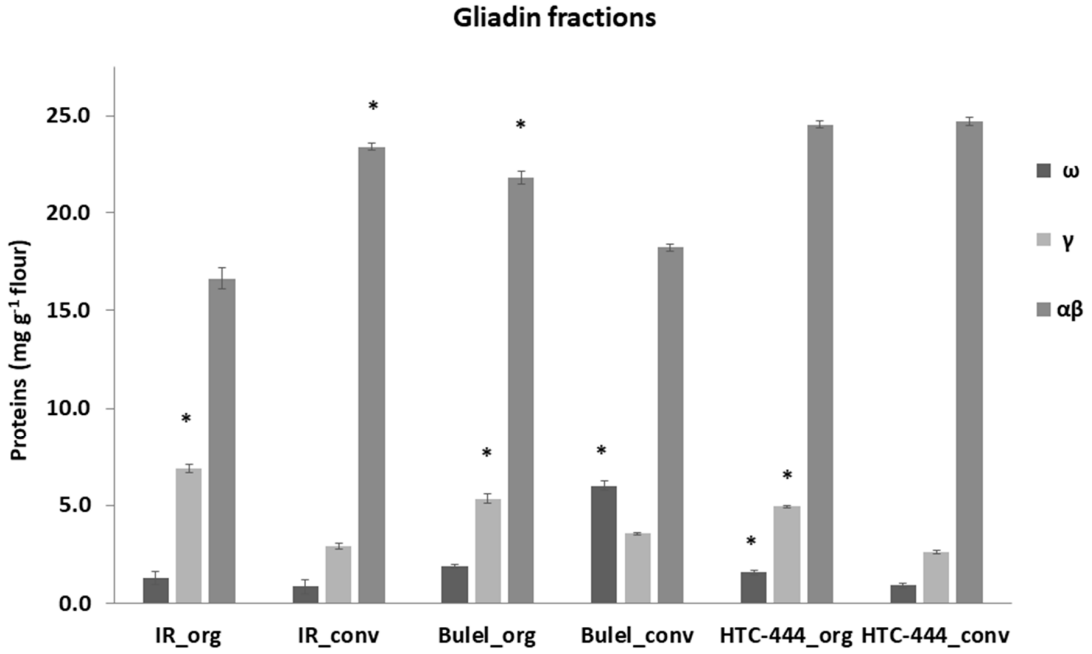

Figure 3. Gliadin subunits (ω, γ, α/β) separated by SDS-PAGE in durum wheat cv. Iride, and Tritordeum cv. Bulel and HTC-444 under organic and conventional systems. Data are reported as averages of 3 replicates ± standard deviation. Significant differences between organic (org) and conventional (conv) systems for the same cultivar are indicated with asterisks (* $p \leq 0.05$).

Gluten protein content and the amounts of its various protein classes, in particular gliadins, are highly affected by environmental factors during both vegetative growth and grain filling of wheat [42]. Our results suggest that gliadin contents and proportions in Tritordeum may also be modulated through cultivation method/management system.

4. Conclusions

Tritordeum combines the nutritional value of barley with the technological qualities of durum wheat, and therefore represents a possible alternative to wheat for the production of bread, pasta, and other food products. Here, the reference durum performed better than the Tritordeum cultivars investigated, under both conventional and organic farming systems, suggesting that further improvement in the yield potential of Tritordeum through breeding and the optimization of agronomic practices is still needed to make it competitive to the main cereal crops. However, Tritordeum does seems to adapt better than durum wheat to organic farming by increasing the below-ground rhizosphere community of the Bacteroidetes phylum, whose many microorganisms are known to have beneficial effects on plant growth. Positive effects on grain quality in terms of improvements in the protein/gluten content are also expected under organic farming, and these can be exploited to obtain flours/semolina for specific bakery/pasta supply chains. The high mineral and antioxidant phenol contents of Tritordeum, particularly under organic management, also have an important nutraceutical value, that will probably be better exploited in wholemeal flours/semolina.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/11/1717/s1: Figure S1: Rarefaction curves generated for 16S rRNA gene sequences obtained from samples. Panels represent the rarefaction curves of aggregates of the 16 samples of bulk soil (BS) and samples of the rhizosphere soil of durum wheat (cv. Iride, IR) and Tritordeum (cv. Bulel, cv. HTC-444) under organic (org) and conventional (conv) management. Table S1: Data filtering report of sample runs. Table S2: Bacterial phyla with relative variations (var) and significance levels (*p*-value) in bulk soil and in the rhizosphere soil of durum wheat (cv. Iride) and Tritordeum (cv. HTC-444, cv. Bulel) cultivated under organic (org) and conventional (conv) agricultural management. Data are presented as percentage abundances of the mean values of 3 biological replicates. Table S3: Bacterial families with relative variations (var) and significance levels (*p*-value) in bulk soil and in the rhizosphere soil of durum wheat (cv. Iride) and Tritordeum (cv. HTC-444, cv. Bulel) cultivated under organic (org) and conventional (conv) agricultural management. Data are presented as percentage abundances of the mean values of 3 biological replicates.

Author Contributions: Conceptualization, G.V., S.F., and R.R.; data curation G.V., A.P., and C.D.C.; formal analysis G.V., M.L., C.P., and C.D.C.; funding acquisition R.R. and T.V.; investigation G.V., M.L., and C.D.C.; methodology G.V., C.D.C., and C.P.; writing—original draft G.V., S.F., and C.D.C.; writing—review and editing G.V., C.D.C., and T.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors wish to thank Agrasys and Tomato Farm for their valuable suggestions and for providing seeds of the two Tritordeum cultivars.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAOSTAT. Available online: http://www.fao.org/faostat/en/#home (accessed on 4 May 2020).
2. Shewry, P.R.; Hey, S.J. The contribution of wheat to human diet and health. *Food Energy Secur.* **2015**, *4*, 178–202. [CrossRef] [PubMed]
3. Kotschi, J. Agricultural biodiversity is essential for adapting to climate change. *Ecol. Persp. Sci. Soc.* **2007**, *16*, 98–101. [CrossRef]
4. FAO. *The Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture*; FAO: Rome, Italy, 2010.
5. Döring, T.F.; Knapp, S.; Kovacs, G.; Murphy, K.; Wolfe, M.S. Evolutionary plant breeding in cereals-into a new era. *Sustainability* **2011**, *3*, 1944–1971. [CrossRef]
6. Tadesse, W.; Sanchez-Garcia, M.; Assefa, S.G.; Amri, A.; Bishaw, Z.; Ogbonnaya, F.C.; Baum, M. Genetic gains in wheat breeding and its role in feeding the world. *Crop Breed. Gen. Genom.* **2019**, *1*, e190005.
7. Martín, A.; Sanchez-Monge Laguna, E.S.M. A hybrid between *Hordeum chilense* and *Triticum turgidum*. *Cereal Res. Commun.* **1980**, *8*, 349–353.
8. Martín, A.; Sanchez-Monge Laguna, E. Cytology and morphology of the amphiploid *Hordeum chilense* × *Triticum turgidum* conv. *Durum. Euphytica* **1982**, *31*, 261–267. [CrossRef]
9. Martín, A.; Alvarez, J.B.; Martín, L.M.; Barro, F.; Ballesteros, J. The development of Tritordeum: A novel cereal for food processing. *J. Cereal Sci.* **1999**, *30*, 85–95. [CrossRef]
10. Agrasys. Available online: http://www.tritordeum.com (accessed on 25 January 2020).
11. Villegas, A.; Casadesù, J.; Atienza, S.G.; Martos, V.; Maalouf, F.; Karam, F.; Aranjuelo, I.; Nogués, S. Tritordeum, wheat and triticale yield components under multi-local Mediterranean drought conditions. *Field Crop. Res.* **2010**, *116*, 68–74. [CrossRef]
12. Atienza, S.G.; Ballesteros, J.; Martín, A.; Hornero-Méndez, D. Genetic variability of carotenoid concentration and degree of esterification among Tritordeum (×*Tritordeum* Ascherson et Graebner) and durum wheat accessions. *J. Agric. Food Chem.* **2007**, *55*, 4244–4251. [CrossRef]
13. Pérez-Gálvez, A.; Mínguez-Mosquera, M.I. Esterification of xanthophylls and its effect on chemical behavior and bioavailability of carotenoids in the human. *Nutr. Res.* **2005**, *25*, 631–640. [CrossRef]
14. Giordano, D.; Reyneri, A.; Locatelli, M.; Coïsson, J.D.; Blandino, M. Distribution of bioactive compounds in pearled fractions of Tritordeum. *Food Chem.* **2019**, *301*, 125228. [CrossRef]
15. Erlandsson, A. Tritordeum. Evaluation of a New Food Cereal. Master's Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden, 2010.
16. Hack, H.; Bleiholder, H.; Buhr, L.; Meier, U.; Schnock-Fricke, U.; Weber, E.; Witzenberger, A. Einheitliche Codierung der phanologischen Entwicklungsstadien mono- und dikotyler Pflanzen—Erweiterte BBCH-Skala, Allgemein. *Nachrichtenbl. Deut. Pflanzenschutzd.* **1992**, *44*, 265–270.

17. Pornaro, C.; Macolino, S.; Menegon, A.; Richardson, M. WinRHIZO technology for measuring morphological traits of bermudagrass stolons. *Agron. J.* **2017**, *109*, 3007–3010. [CrossRef]
18. Milani, C.; Hevia, A.; Foroni, E.; Duranti, S.; Turroni, F.; Lugli, G.A.; Sanchez, B.; Martín, R.; Gueimonde, M.; van Sinderen, D.; et al. Assessing the fecal microbiota: An optimized ion torrent 16S rRNA gene-based analysis protocol. *PLoS ONE* **2013**, *8*, e68739. [CrossRef]
19. Visioli, G.; Sanangelantoni, A.M.; Vamerali, T.; Dal Cortivo, C.; Blandino, M. 16S rDNA profiling to reveal the influence of seed-applied biostimulants on the rhizosphere of young maize plants. *Molecules* **2018**, *23*, 1461. [CrossRef]
20. USEPA, EPA Method 3052. Microwave assisted acid digestion of siliceous and organically based matrices. In *Test Methods for Evaluating Solid Waste*, 3rd ed.; USEPA: Washington, DC, USA, 1995.
21. Adom, K.K.; Sorrells, M.E.; Liu, R.H. Phytochemical profiles and antioxidant activity of wheat varieties. *J. Agric. Food Chem.* **2003**, *51*, 7825–7834. [CrossRef]
22. Dal Cortivo, C.; Conselvan, G.B.; Carletti, P.; Barion, G.; Sella, L.; Vamerali, T. Biostimulant effects of seed-applied Sedaxane fungicide: Morphological and physiological changes in maize seedlings. *Front. Plant Sci.* **2017**, *8*, 2072. [CrossRef]
23. Visioli, G.; Bonas, U.; Dal Cortivo, C.; Pasini, G.; Marmiroli, N.; Mosca, G.; Vamerali, T. Variations in yield and gluten proteins in durum wheat varieties under late-season foliar versus soil application of nitrogense fertilizer in a northern Mediterranean environment. *J. Sci. Food Agric.* **2018**, *98*, 2360–2369. [CrossRef]
24. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Peña, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [CrossRef]
25. Edgar, R.C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **2010**, *26*, 2460–2461. [CrossRef]
26. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, D590–D596. [CrossRef]
27. Bulgarelli, D.; Garrido-Oter, R.; Münch, P.C.; Weiman, A.; Dröge, J.; Pan, Y.; McHardy, A.C.; Schulze-Lefert, P. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe* **2015**, *17*, 392–403. [CrossRef]
28. Li, F.; Chen, L.; Zhang, J.; Yin, J.; Huang, S. Bacterial community structure after long-term organic and inorganic fertilization reveals important associations between soil nutrients and specific taxa involved in nutrient transformations. *Front. Microbiol.* **2017**, *8*, 187. [CrossRef] [PubMed]
29. Kolton, M.; Green, S.J.; Harel, Y.M.; Sela, N.; Elad, Y.; Cytryn, E. Draft genome sequence of Flavobacterium sp. Strain F52, isolated from the rhizosphere of bell pepper (*Capsicum annuum* L. cv. Maccabi). *J. Bacteriol.* **2012**, *194*, 5462–5463. [CrossRef]
30. Wolińska, A.; Kuźniar, A.; Zielenkiewicz, U.; Izak, D.; Szafranek-Nakonieczna, A.; Banach, A.; Błaszczyk, M. Bacteroidetes as a sensitive biological indicator of agricultural soil usage revealed by a culture-independent approach. *Appl. Soil Ecol.* **2017**, *119*, 128–137. [CrossRef]
31. Kanelli, M.; Mandic, M.; Kalakona, M.; Vasilakos, S.; Kekos, D.; Nikodinovic-Runic, J.; Topakas, E. Microbial production of violacein and process optimization for dyeing polyamide fabrics with acquired antimicrobial properties. *Front. Microbiol.* **2018**, *9*, 1495. [CrossRef]
32. Pérez-Jaramillo, J.E.; Carrión, V.J.; de Hollander, M.; Raaijmakers, J.M. The wild side of plant microbiomes. *Microbiome* **2018**, *6*, 143. [CrossRef]
33. Barro, F.; González-Fontes, A.; Maldonado, J.M. Nitrate uptake and reduction by durum wheat (*Triticum turgidum*) and Tritordeum (*Hordeum chilense* × *Triticum turgidum*). *J. Plant Physiol.* **1994**, *143*, 313–317. [CrossRef]
34. Barro, F.; Fontes, A.G.; Maldonado, J.M. Organic nitrogen content and nitrate and nitrite reductase activities in Tritordeum and wheat grown under nitrate or ammonium. *Plant Soil* **1991**, *135*, 251–256. [CrossRef]
35. Baruah, R. Towards the bioavailability of zinc in agricultural soils. In *Role of Rhizospheric Microbes in Soil*; Meena, V., Ed.; Springer: Singapore, 2018.
36. Ozcan, T.; Akpinar-Bayizit, A.; Yilmaz-Ersan, L.; Delikanli, B. Phenolics in human health. *Int. J. Chem. Eng. Appl.* **2014**, *5*, 393–396. [CrossRef]

37. Fernandez-Orozco, R.; Li, L.; Harflett, C.; Shewry, P.R.; Ward, J.L. Effects of environment and genotype on phenolic acids in wheat in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2010**, *58*, 9341–9352. [CrossRef]
38. Li, L.; Shewry, P.R.; Ward, J.L. Phenolic acids in wheat varieties in the HEALTHGRAIN Diversity Screen. *J. Agric. Food Chem.* **2008**, *56*, 9732–9739. [CrossRef]
39. Navas-Lopez, J.F.; Ostos-Garrido, F.J.; Castillo, A.; Martîn, A.; Gimenez, M.J.; Piston, F. Phenolic content variability and its chromosome location in Tritordeum. *Front. Plant Sci.* **2014**, *5*, 10. [CrossRef]
40. Acreche, M.M.; Slafer, G.A. Variation of grain nitrogen content in relation with grain yield in old and modern Spanish wheats grown under a wide range of agronomic conditions in a mediterranean region. *J. Agric. Sci.* **2009**, *147*, 657–667. [CrossRef]
41. Vaquero, L.; Comino, I.; Vivas, S.; Rodríguez-Martín, L.; Giménez, M.J.; Pastor, J.; Sousa, C.; Barro, F. Tritordeum: A novel cereal for food processing with good acceptability and significant reduction in gluten immunogenic peptides in comparison with wheat. *J. Sci. Food Agric.* **2018**, *98*, 2201–2209. [CrossRef] [PubMed]
42. Altenbach, S.B.; Kothari, K.M.; Lieu, D. Environmental conditions during wheat grain development alter temporal regulation of major gluten protein genes. *Cereal Chem.* **2002**, *79*, 279–285. [CrossRef]