Valorization of wheat production in marginal areas: farmer−centric experimentation for variety choice and evolutionary population development

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Highlights

- *During two growing seasons, agronomic and quality traits of seven wheat genotypes, two wheat evolutionary populations and one einkorn mix were assessed.*
- *The high values of test weight observed for the underutilized cereals confirm that hill and mountain environments are optimal for their growth.*
- *GGE-biplot analysis highlighted comparable yield performances of evolutionary wheat populations, with different levels of stability.*
- *The choice of genotypes suited for the growing condition and the target value chain is crucial for creating competitiveness and contributing to the protection of the Apennine area.*

Abstract

In Italy, from 2000 to 2010, 58% of farms in mountain areas were abandoned leading to a 33% decrease in available land for agriculture. This research aimed to restore value and competitiveness to the Apennine area, by proposing a balanced and sustainable agriculture model. Following the needs of farmers, underutilised cereals were selected as the ideal genetic material for the study and development of short local food supply chains. The field experiments were carried out in two organic farms located in the Emilia-Romagna Region (Italy). During two growing seasons, seven wheat genotypes, two wheat evolutionary populations and one einkorn mixture were cultivated under organic farming management. Results related to functional traits are presented along with the main agronomic and technological parameters that were determined. Several nutritional properties are included. Mean yield and stability performance over environments for each genotype were explored using the "Genotype and Genotype by Environment biplot" elaboration. Considering yield performances, "Benco" was closer to the 'ideal' genotype. All the results were evaluated with the farmers, who expressed their own preferences from field observations. The right coupling between environment and genotypes can discourage the abandonment of hilly and mountainous farms by enhancing the economic competitiveness of agriculture in these regions.

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Introduction

Farmland abandonment is not a new phenomenon. Over recent decades, this has occurred in many developed countries as a consequence of changing socio-economic factors (Li and Li, 2017). The reason for abandonment is principally related to the loss of economic viability of certain areas, which are abandoned when they stop generating an income flow, thereby causing a decline of farmland profit (MacDonald *et al.*, 2000). In particular, agricultural lands that are located in disadvantaged areas, such as mountainous or hilly areas, are more vulnerable to abandonment due to adverse farming conditions (Li and Li, 2017). These adverse conditions include low productivity combined with high production costs rendering mountains and hilly farmlands less competitive.

Similar to other European mountainous regions, the critical issues of the Italian Apennine areas are related to topography, remoteness and physical disadvantage. In Italy, mountainous and hilly areas account for about 77% of the national territory (ISTAT, 2022). In these areas, agricultural, silvicultural, and livestock farming activities are predominant and are significant for not only ensuring the permanence of the population in marginal rural settings, but are also for responsible landscape management (Varotto, 2006).

The Emilia-Romagna area is very heterogeneous in terms of landscape morphology, with about 50% made up of the Po Valley, with the remainder being comprised of mountainous and hilly lands. The difference in terms of economic productivity between the two macro areas is remarkable because the lowland area represents one of the most productive areas in Italy, while the mountainous and hilly areas are designated as marginal areas. When comparing the data collected by the 2010 census and 2000 census, respectively, it can be seen that in the mountainous areas of Emilia Romagna, 58% of the farms were abandoned, with a corresponding decrease in of the agricultural surface and utilised agricultural area (UAA) of 33% and 31%, respectively. In hilly areas, the same trend was recorded (36% of farms abandoned, a 14% decrease in the agricultural area, and a 19% reduction in the UAA).

Additionally, from a demographic point of view, there was a marked decrease, caused by depopulation processes. From the data collected between the years 1951 and 2001, the population decreased by 50% and between 2001 and 2008 by 2.5% (ISTAT, 2022). This trend can be reversed, or limited, only if specific policy responses make new developmental opportunities available (MacDonald *et al.*, 2000).

The protection of mountainous areas by the European Union is a difficult objective to achieve, partly because these areas are diverse with different needs. For this reason, the decision was made to allocate EU funds to support the most disadvantaged areas, through the 2014-2020 Rural Development Programmes [Reg. (EU) 1305/2013].

These funds are managed by local authorities (Regions and Provinces) and allow for the following: the implementation of projects that strengthen the competitiveness of the local economic activities, improvements in the quality of life, promotion of the sustainable use of rural resources, and the enhancement of historical and cultural heritage. This approach also includes support for innovative and experimental programs for mountainous areas, focusing on agri-food supply chains, renewable energy, culture, environmental resources and soil conservation.

In this context, the Emilia Romagna Region, using funds allocated by the EU Programmes, has invested in supporting agriculture, which is recognized as a source of employment and a tool for land protection. A large portion of the funds have been allocated for organic farms and those in conversion, providing more incentive to farms in both hills and mountains as they are more suited for this type of agriculture. To restore value and competitiveness to the Apennines (mountainous and hilly environments), programs are needed to achieve a sustainable increase in agricultural production by identifying crops and varieties that can be competitive in these areas. Landraces and ancient wheat varieties could be incorporated into low-input farming systems more effectively than modern varieties designed to maximize production through high inputs (Costanzo *et al*., 2019). In inland areas, which have always been characterized by farming systems with reduced agronomic inputs, ancient varieties could reduce the gap in production when compared to modern varieties. Furthermore, in this context of adverse conditions, the adoption of organic heterogeneous material (OHM), characterized by greater genetic and phenotypic diversity, could be a viable solution to cope with stressors and climatic changes compared to uniform varieties (Döring *et al.*, 2011). The possibility of cultivating an evolutionary population from different crop species (including common wheat) in Europe was initially introduced as a Temporary Experiment by the Commission Implementing Decision 2014/150/EU for conventional and organic farming. Currently and only for organic farming systems, it has been officially approved the possibility of employment of OHM by the Regulation (EU) 2018/848 (30 May 2018).

One possible strategy in cultivating heterogeneous material in cereal production is the use of evolutionary populations obtained by Participatory Plant Breeding (PPB) (Doring *et al.*, 2011).

Following the use of this method in past years, some Emilia-Romagna organic farmers, especially from marginal areas of the Region, started to choose varieties better suited to their needs and conditions of their specific environments. The objective was to select varieties for low-input agriculture that were also acceptable to farmers (Di Silvestro *et al.*, 2012). Furthermore, most of the selection processes took place on farmers' fields and all decisions were jointly taken by both the farmer and the breeder (Ceccarelli and Grando, 2007). In fact, the PPB method is primarily a type of participatory research in which farmers feel involved in the breeding process (Ceccarelli, 2009).

In participatory research, not only researchers and farmers, but also processors and consumers are involved in designing and developing technologies. This method has been used to emphasize favorable interactions when significant genotype and environment interactions exist (Ceccarelli *et al.*, 2000). Moreover, the method is used to evaluate the agronomic characteristics of different genotypes and their adaptability to hilly and mountainous areas (Migliorini *et al.*, 2016).

Following specific requests from farmers in marginal and disadvantaged environments, PPB programs largely use landraces or ancient varieties as the ideal genetic material (Morris and Bellon, 2004). Local varieties, or landraces, could have an important role in marginal areas, because they are well adapted to the environment in which they either evolved or in which they were selected. As such, these landraces are more resilient compared with the modern cultivars, particularly under unfavorable conditions (Morris and Bellon, 2004; Camacho Villa *et al.* 2005; Migliorini *et al.*, 2016; Colombo *et al.*, 2022).

In addition, the PPB model can impact positively on biodiversity, since it provides the possibility of obtaining varieties that are different to most varieties produced by conventional breeding which are genetically identical (Ceccarelli and Grando, 2007). Further to the economical, agronomic and ecological benefits, PPB promotes psychological and moral benefits, due to the progressive empowerment of the farmers' communities (Ceccarelli and Grando, 2007). In recent years, farmers in the Emilia-Romagna region, in collaboration with researchers at the Alma Mater Studiorum - University of Bologna, developed two common wheat populations for lowland and hillside environments. These are, respectively, named "Oroset" and "Appen.Bio" populations, which yet have not been evaluated for potential agronomic performance and quality traits.

The research project Appen.bio aims to restore value and competitiveness to the Apennines, evaluating the suitability of different genotypes for marginal lands and low-input agricultural systems. In this context, underutilised cereals, characterized by high environmental adaptability, represent the ideal genetic material for the study and development of short supply chains, focused on products with characteristics of typicality and marked quality. The agronomic and qualitative characterization of ancient wheat varieties, landraces and especially their evolutionary populations, can represent a winning strategy to restore the economic competitiveness to mountain and high hill farms, thanks to the improved suitability of those genotypes to sub-optimal environmental conditions and limited input availability. The objective of this study was to test how the agronomic and food quality characterization of underutilised cereals (landraces, old varieties, evolutionary populations, niche species such as einkorn) can support a strategy to restore competitiveness to local communities in mountain and high hill farms, in disadvantaged areas.

Materials and Methods

Appen.bio project: the context and the participatory design approach

Following a multi-actor approach and an on-farm trial design, the Appen.bio project (2016-2019, https://www.appenbio.eu/) was set up with the aim of: i) responding to the needs of farmers located in marginal areas, ii) satisfying the needs of producers and food processors to obtain products with specific quality aspects, and iii) creating a short and complete supply chain (Figure 1).

The experimental design specifically focused on the identification of the genetic material to be used in the experimental trials (see section "Genetic material"), also taking advantage of the existing wheat varieties collection set up by the Alma Mater Studiorum - University of Bologna in 2017/18. This collection permitted a preliminary evaluation of numerous field-grown accessions. Moreover, for the first time, the participation in the EU Temporary Experiment permitted official notification and marketing of the evolutionary populations, consisting of heterogeneous material. The involvement of the company Alce Nero Spa (Castel San Pietro Terme, BO, Italy), interested in implementing a short supply chain project in the Bologna Apennines, increased consumer interest in products grown in marginal areas. Within the Appen.bio project, farmers and field technicians were involved in the evaluation of the different genotypes in the two field trials. Specifically, for each crop year, seven evaluators (during visits to farms in the period before the harvest), were involved in filling out a questionnaire that contained questions related to: A) Weed abundance and species composition, B) Lodging percentage, C) Diseases presence, and D) Yield value.

Based on these observations, the evaluators included a feedback qualitative score (between 7 and 9 = satisfactory; between 6 and 4 = partially satisfactory; between 3 and 1 = unsatisfactory) in the questionnaire in relation to their feedback (Figure 2).

Genetic material

The germplasm evaluated included seven wheat accessions, two common wheat populations and one einkorn mix.

The wheat accessions included landraces and old varieties of common wheat (*Triticum aestivum* L.), that were chosen from the varietal heritage wheats of Italy. Landraces are local ecotypes, sometimes of unknown origins, that were widely adopted in the provinces of Emilia-Romagna, Tuscany and Veneto between the mid-1800s and early-1900s. In general, old varieties (released before 1950) were developed using landraces as parental varieties.

The wheat accessions selected included: "Abbondanza", "Andriolo", "Canove", "Benco", "Gamba di ferro", "Autonomia A", "Funo" (Table 1). The selection of these specific genotypes among the great Italian heritage wheats was based on the evaluation of the following characteristics: presence of awns, grain colour, susceptibility to lodging, susceptibility to mildew/rust, cold susceptibility, protein content, nutritional and nutraceutical properties. In addition, varieties were chosen for their high environmental adaptability and productive capacity in both marginal areas and

Figure 1. Participatory design approach to produce knowledge and identify effective solutions.

low-input production systems.

The selection of the genotypes was based on information collected from the Biodiversity Database of the Veneto Region (Italy), from observations conducted on the wheat variety collection of the Alma Mater Studiorum - University of Bologna (Dinelli *et al.*, 2011; Di Silvestro *et al.*, 2012), as well as from bibliographic research about the main characteristics of the varieties traditionally and historically used in the Italian hilly and mountainous areas (Porfiri, 2014; Tellarini, 2017).

In addition, the contribution of the local farmers was fundamental. The latter provided precious advice, attributable to their extensive experience in the cultivation of these genotypes.

The two common wheat (*Triticum aestivum* L.) populations tested were "Oroset" and "Appen.Bio". Both represent evolutionary populations of common wheat developed by the Crop Physiology research team of the Department of Agricultural and Food Science of the University of Bologna and notified to the Temporary Experiment of Commission Implementing Decision 2014/150/EU. The "Oroset" population was obtained from the reciprocal crossings of five old wheat varieties "Andriolo", "Verna", "Frassineto", "Gentil Rosso" and "Inallettabile". Instead, the "Appen.Bio" population was obtained from the reciprocal crossings of seven old varieties "Andriolo", "Canove", "Benco", "Gamba di ferro", "Abbondanza", "Autonomia A" and "Funo".

A mix of monococcus einkorn genotypes (*Triticum monococcum* L.) was also evaluated, selected on the basis of the agronomic characteristics and nutritional properties of the grain (such as antioxidant activity), examined in a preliminary phase. Due to its adaptation to low-fertility soils, resistance to cold, drought and disease, as well as competitiveness with weeds, einkorn wheat can represent a valid crop alternative for agricultural systems operating in marginal hilly and mountainous areas.

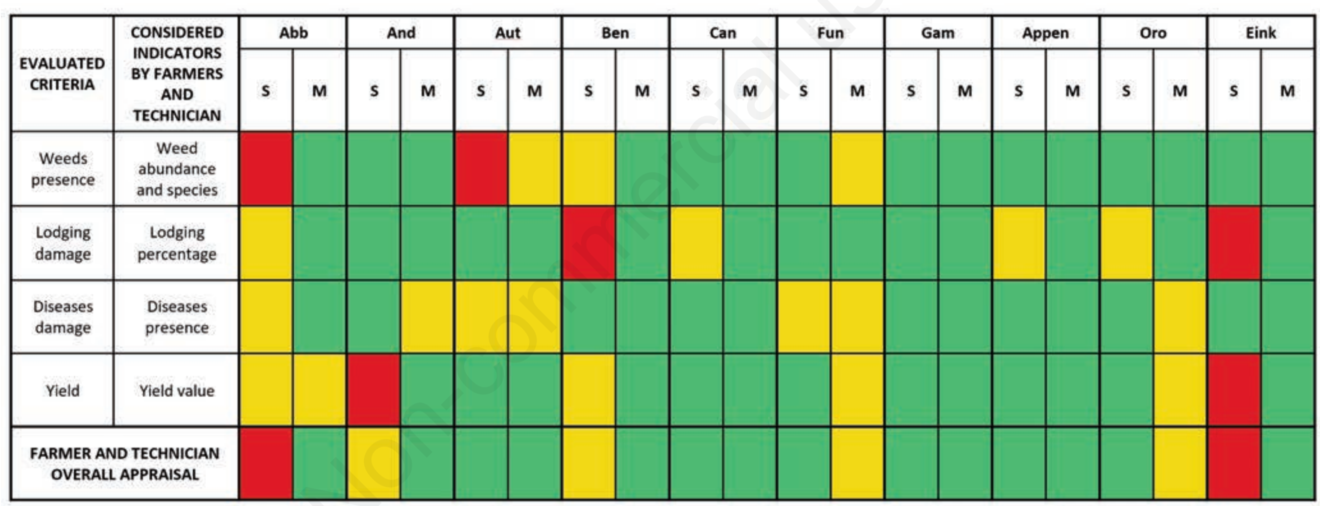

Figure 2. Farmers and field technicians' qualitative average appraisal for the on-farm trials during the two growing seasons of the study. S and M represent the two experimental locations of cultivation (San Lazzaro and Monterenzio, respectively). Genotype: Abb, Abbondanza; And, Andriolo; Aut, Autonomia A; Ben, Benco; Can, Canove; Fun, Funo; Gam, Gamba Di Ferro; Appen, Appen.Bio population; Oro, Oroset population; Eink, Einkorn mix.

Table 1. The landraces and old *Triticum aestivum* L. varieties cultivated in two organic farms located in the Emilia-Romagna Region (Italy) over two consecutive cultivation years (2016/2017-2017/2018).

Genotype	Classification	Year	Breeder	Area of origin	Adaptability
Abbondanza	Old	1950	M. Michahelles	Tuscany hills	Mountains
Andriolo	Landrace	-	-	Tuscany (1800s)	Mountains
Autonomia A	Old	1938	M. Michahelles	Central Italy	Lowlands
Benco	Landrace	-	-	Used all over Tuscany	Mountains
Canove	Landrace	-	-	Veneto	Mountains
Funo	Old	1944	IABO	Emilia-Romagna	Lowlands
Gamba di ferro	Landrace	-	-	-	Mountains

IABO, Istituto Allevamento Vegetale Bologna, Bologna, Italy.

Factors, treatments and design of the experiment

The same field experimental scheme was set up for two growing seasons (2016/2017-2017/2018), in two different organic farms located in the Emilia-Romagna Region (Italy), with different soil and climatic conditions. The first was a high hilly area at the Azienda Morara Andrea organic farm (44°18'26.35"N; 11°22'24.87"E; altitude 333 m a.s.l.), located in the municipality of Monterenzio (Bologna). The second was a low hilly area at the Azienda San Giuliano organic farm (44°27'12.26"N, 11°24'49.48"E; altitude 80 m a.s.l.), located in the municipality of San Lazzaro di Savena (Bologna). All genotypes were grown individually in plots, according to a complete randomized design with two replicates, each plot with dimension of 3.2×6.5 m. Seed material was sown with a plot seeder (seed density of 150 kg/ha) on 14 October 2016 and 26 October 2017, respectively, in Monterenzio and on 3 November 2016 and 27 October 2017 in San Lazzaro, respectively. The harvest, using a plot combine harvester was performed on 3 July 2017 and 6 July 2018 in Monterenzio, respectively. In San Lazzaro, the harvest was performed on 26 June 2017 and 27 June 2018. No fertilization or phytosanitary treatments were carried out during the cultivation cycle, even though organic growing guidelines allow the use of natural origin fertilizers and biopesticides. Meteorological data (rainfall, average, maximum and minimum temperatures) during the two cultivation seasons (Figure 3A,B), was recorded by the Regional Agency for Prevention, Environment and Energy meteorological stations (Arpae, Emilia-Romagna), located in both San Lazzaro (44°27'09.0"N 11°24'12.0"E) and Monterenzio (44°19'03.0"N 11°24'12.0"E). The soil type was clay loamy in Monterenzio and silt loamy in San Lazzaro (Agriparadigma laboratories).

Morphological and bio-agronomic parameters

For each cultivation year, various parameters were monitored in different phenological stages. Plant height was measured from the ground level to the base of the spike. Lodging was registered on all plants within each plot and expressed as a percentage of the total number of plants. The length of the spike was expressed in cm by measuring the distance between the most basal and the most distal spikelet. The presence of weeds was estimated and expressed as a percentage of the total plot surface occupied by weeds. The disease incidence and severity were estimated based on the adapted Cobb's Scale (0-100%). Disease ratings were calculated using a descriptive assessment scale with different classes of scale ratings (*i.e.*, 0-10), in which each rating corresponds to a specific infection percentage over the surface area of tissue under investigation, as previously described by Bosi *et al.*, 2022. At the end of the crop cycle, the main agronomic and commercial parameters (yield, hectolitre weight, 1000-seed weight) were determined. Yield was measured within each plot and expressed as t ha^{-1}. Hectoliter weight (HW) of the kernels was determined by the Infratec 1241 Grain Analyzer (FOSS Analytical A/S, Hillerød, Denmark) based on the manufacturer's guidelines.

Nutritional and functional properties

For the analysis of functional quality parameters, the grain collected from each plot was milled with a laboratory stone mill, to produce wholegrain flour. Each analysis was carried out employing two replicates for each accession per year. All laboratory analyses were performed by adhering to the official methods of both the Association of Official Analytical Chemists (AOAC INTERNATIONAL) and the American Association of Cereal Chemists (AACC Intl.). On the collected flour samples the following analytical determinations were carried out: protein content, total starch, lipid, insoluble dietary fibre (IDF), soluble dietary fibre (SDF), total dietary fibre (TDF), total polyphenol (TP) content, total flavonoid (TF) content, radical, and ferric reducing antioxidant potential (FRAP). All the standards (gallic acid and catechin) and chemical reagents used were of analytical grade and purchased from Merk Life Science S.r.l. (Milan, Italy). The protein fraction present in the various wheat samples was expressed as a percentage of dry weight and determined by Kjeldahl analysis, in accordance with AOAC and AACC methods. Total starch content was determined using the Infratec 1241 Grain Analyzer (FOSS, Hillerød, Denmark) based on the manufacturer's guidelines. The total lipid content of the flour was determined following Folch's methodology (Folch *et al.*, 1957), adapted to cereals according to the official AOAC method. Insoluble and soluble dietary fibre

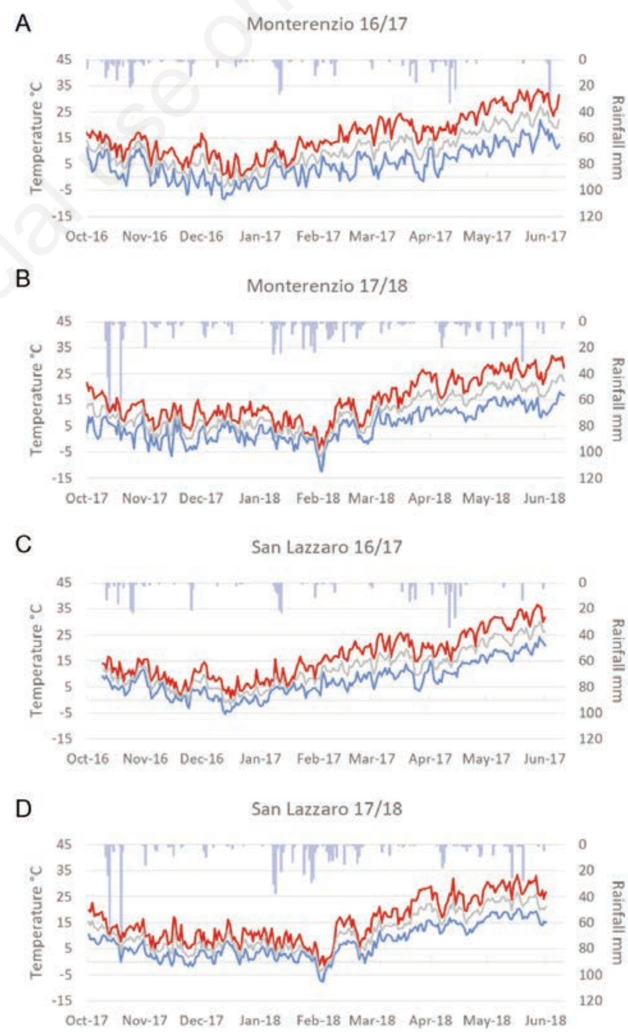

Figure 3. Meteorological data of the 2016/17 and 2017/18 seasons, from ARPAE's meteorological stations located in Monterenzio and San Lazzaro. Red, grey and blue lines represent respectively maximum, mean and minimum daily temperatures (°C), while blue bars represent the daily-based cumulative rainfall (mm).

(IDF and SDF) were extracted and measured according to the instruction protocol provided with the Megazyme Total Dietary Fibre Assay Procedure kit (Megazyme International Company, Wicklow, Ireland), that was based on previously reported methods (Prosky *et al.*, 1988; Lee *et al.*, 1992).

Total polyphenol (TP) content, comprising both free (FP) and bound (BP) polyphenols, were extracted as described previously (Dinelli *et al.*, 2011). Free and bound polyphenols were then measured according to the Folin-Ciocalteau spectrophotometric (765 nm) method using gallic acid as a reference standard (Singleton *et al.*, 1999) and the total calculated. Likewise, the free and bound flavonoids were individually measured using a spectrophotometric (510 nm) colorimetric assay with catechin as a reference standard (Adom *et al.*, 2003) and then summed to produce the total. Ferric reducing antioxidant potential (reduction of Fe^{2+}) was determined using a spectrophotometric (593 nm) method reported previously (Benzie and Strain, 1996). As with the polyphenols and flavonoids, antioxidant activity in the free and bound fraction were summed and expressed as total FRAP.

Statistical analysis

The collected data was statistically analysed by applying the three-way Analysis of Variance (ANOVA), using CoStat software (CoHort Software, California), as previously done by Migliorini *et al.* (2016) and Döring *et al.* (2015). For each of the monitored parameters, the significance of the fixed factors "genotype", "growing site" and "growing season" were respectively studied, as well as the interaction between these independent factors. Year was considered as a fixed factor because of the evolutionary characteristic of the experiment (*i.e.*, seed was used locally in each location from the previous harvest). The significance (at $p<0.05$) of the differences between the means (found in the ANOVA), for each genotype, farm and year was determined using the Student-Newman-Keuls post hoc statistical test. To highlight possible correlations among the different parameters analysed, especially the nutritional parameters, the Pearson Product Moment Correlation Coefficient (r) was calculated. Significance was recorded for values at $p<0.05$. Finally, a Genotype and Genotype x Environment (GGE) analysis was performed for basilar variables (yield and protein content), by applying the "mean *versus* stability" tool (Bosi *et al.*, 2022). To do so, the GGEBiplotGUI package (Frutos *et al.*, 2014) was used, as it is in the R Statistical Software (v4.0.2; R Core Team, 2021). The "mean *versus* stability" tool facilitates the visualization of the mean performance and stability of each genotype, considering the different cultivation environment.

Results

Meteorological and soil data

The meteorological data showed that in the farm at Monterenzio (333 m a.s.l.), in 263 days from sowing to harvest in the 2016/17 season, there was 467.4 mm of precipitation over 82 rainfall days. In the 255 days from sowing to harvest in the 2017/18 season, 789.8 mm over 115 rainfall days was recorded. In the farm at San Lazzaro (80 m a.s.l.), 363.9 mm was recorded over 58 rainfall days in the 236 days between sowing to harvest for the first growing season. Instead, in the second season of 243 days and 715.6 mm over 96 rainfall days was recorded. Overall, the latter was the most wet season. However, more than 25% of the cumulative rainfall was registered in November 2017 in both sites. In contrast, the first season showed a more evenly distributed rainfall from November to May. Minimum temperatures in Monterenzio reached -8.6°C in the first growing season, in a period spanning nearly two months from December 8th to January 30th where minimum temperatures were consistently below 0°C. Instead in the second growing season, the minimum temperatures in the same period were slightly higher. The same was also registered for the winter temperatures at San Lazzaro. Higher temperatures during early summer, at the seed ripening phenological stage, were recorded in San Lazzaro, with maximum temperatures reaching 35.8°C in June 2017 and 33.1 in June 2018. In Monterenzio, maximum temperatures were 30°C on average. Spring temperatures in Monterenzio were lower than 10°C until May, in both 2017 and 2018. However, 2017 was a colder year, with a temperature of 0°C recorded on April 30th. Overall, Monterenzio showed colder winters and received more rainfall, both in frequency and amount of water, than San Lazzaro. San Lazzaro was characterized by warmer and dryer conditions during late spring and early summer. The 2017 season was colder than 2018, especially in spring, even though a freeze was recorded in late February at both sites, with a minimum of -12.4°C and -7.5°C in Monterenzio and San Lazzaro, respectively. The main soil fertility parameters and information about the growing cycles and the main cropping operations are reported in Table 2.

Morphological and bio-agronomic parameters

Over the two-year period, grain yield statistically differed for cropping season, with averages of 2.33 and 2.81 $t\ ha^{-1}$ in 2016/17 and 2017/18, respectively. No significant effect on yield was recorded for the two sites. There was a significant effect on the genotypes. The einkorn mix and the "Abbondanza" variety had

Table 2. Soil parameters, analyzed by Agriparadigma's laboratories, and agronomic operations carried out.

	2016-17		2017-18	
	Monterenzio	San Lazzaro	Monterenzio	San Lazzaro
Soil parameters prior to sowing				
Organic matter (% w/w)	2.6	2.8	4.1	1.6
Total N (‰ w/w)	1.5	1.06	1.7	0.6
C/N ratio	17.3	15.3	14.1	15.7
Assimilable P ($mg\ kg^{-1}$)	-	-	500	580
Exchangeable K ($mg\ kg^{-1}$)	-	-	2300	3000
Agronomic operations				
Sowing date	14th Oct	3rd Nov	26th Oct	27th Oct
Sowing density ($kg\ h^{-1}$)	150	150	150	150
Harvest date	3rd July	26th June	6th July	26th June
Cropping cycle duration (days)	263	236	255	243

lower yields of 1.39 t ha^{-1} and 1.83 t ha^{-1}, respectively, compared to the remaining eight accessions. "Benco" was among the most productive genotypes in terms of yield (3.01 t ha^{-1}). The interaction between cultivation environment and genotype was most strongly significant ($p \leq 0.001$), with year and genotype also showing a significant interaction ($p \leq 0.01$).

The 1000-seed weight was influenced by all three independent factors examined and is reported in Table 3. Climatic conditions of the 2017/18 agricultural season positively influenced this parameter (47.37 g) compared to the previous season (43.37 g). Regarding the environmental factor, in San Lazzaro the average 1000-seed weight was statistically higher (46.22 g) than that measured at harvest in Monterenzio (44.18 g). The 1000-seed weight of the "Benco" genotype (53.78 g) and the "Oroset" population (52.45 g) were among the highest values, with the einkorn mix being among the lowest values (31.99 g). The interactions between the three factors (season, site, genotype) were all highly significant. The hectoliter weight differed between the two growing seasons, while the site factor did not show any significant differences. In 2016/17, the average value for all the genotypes was 80.54 kg hl^{-1}. This value was significantly increased in the following season, with a value of 82.10 kg hl^{-1} recorded. A certain variability was observed between genotypes: the best performances were obtained from the einkorn mix (84.33 kg hl^{-1}), followed by "Andriolo" (82.31 kg hl^{-1}) and "Abbondanza" (82.15 kg hl^{-1}), respectively. The lowest value was recorded for "Gamba Di Ferro" (77.88 kg hl^{-1}). From the data collected in the field before harvesting, the plant height parameter was significantly influenced by all the factors. In the first growing season, the average height of the plants was 118.36 cm. This value was decreased to 112.31 cm in the following season. Differences were also observed between the two sites. The average height measured in San Lazzaro (117.91 cm) differed statistically from that recorded in Monterenzio (112.31 cm). Spike length parameter was significant for growing season and genotype, with no differences recorded between the sites. The length of the spikes in 2016/17 was 10.94 cm, with a 20% decrease (8.80 cm) recorded in the following year (2017/18). Analyzing the "genotype" factor, it can be highlighted that plant height and spike length parameters were correlated with each other ($r=0.506$; $p<0.05$). The largest dimensions were recorded for the genotype "Gamba Di Ferro" (12.03 cm). The smallest dimensions were detected for the einkorn mix (7.68 cm). All factor interactions were found to be significant. It appears that better conditions occurred in the 2016/17 season, with a lodging percentage of 22.75%. Instead, in the 2017/18 season, the lodging percentage more than doubled (48.00%). Site was also statistically significant. In Monterenzio, lodging was lower (29.50%) compared to San Lazzaro (41.25%). In the analysis of the varietal effect, einkorn mix showed a high lodging tendency (59.38%). Of the wheat genotypes, higher lodging percentages were detected in "Benco", "Canove" and "Appen.Bio" population, which exceeded 40%. For both "Abbondanza" and "Funo", lodging percentages were only 2.5%. Weed infestation in the fields was statistically different both between sites and for genotypes. The lowest average percentage was recorded in Monterenzio (9.73%). Common weeds, including the species *Papaver rhoeas* L. and *Avena fatua* L. were found in San Lazzaro with an average infestation percentage of 20.65, more than double that of Monterenzio. In Monterenzio, there was a more diverse but smaller presence of *Papaver rhoeas* L, *Convolvulus* spp. L., *Matricaria chamomilla* L. and *Trifolium spp*. L. Both pathogen incidence and severity were measured on a scale from 0 to 10. All three factors, growing season, site and genotype, were highly significant in terms of the presence of wheat pathogens. In the 2016/17 season, infections were almost absent (0.35/10) (only *Septoria tritici* blotch was detected). Instead, pathogen severity was not relevant (0.10/10). In the following season (2017/18), due to the different climatic conditions, the average pathogen incidence on plants was more evident (0.68/10) (only presence of leaf blotch was detected), as was pathogen severity (0.20/10). However, both pathogen incidence and severity did not negatively affect grain yield and quality. A greater pathogen incidence on wheat was observed in Monterenzio (0.80/10), compared to San Lazzaro (0.23/10). All factor interactions were statistically significant, with the exception of the interaction between growing season and site for pathogen severity. In

Table 3. Morphological and bio-agronomic parameters.

	Yield	1000-seed weight	Hectoliter weight	Plant height	Spike length	Lodging	Weeds	Pathogen incidence	Pathogen severity
Season	***	***	***	***	***	***	Ns	***	**
2016/17	2.33^{b}	43.37^{b}	80.54^{b}	118.36^{a}	10.94^{a}	22.75^{b}	15.00	0.35^{b}	0.10^{b}
2017/18	2.81^{a}	47.03^{a}	82.10^{a}	112.31^{b}	8.80^{b}	48.00^{a}	15.38	0.68^{a}	0.20^{a}
Site	Ns	***	Ns	***	Ns	***	***	***	***
Monterenzio	2.52	44.18b	81.23	112.75^{b}	9.84	29.50^{b}	9.73^{b}	0.80^{a}	0.25^{a}
San Lazzaro	2.62	46.22a	81.41	117.91^{a}	9.90	41.25^{a}	20.65^{a}	0.23^{b}	0.05^{b}
Genotypes	***	***	***	***	***	***	***	***	***
Abbondanza	1.83^{b}	41.33^{d}	82.15^{b}	92.25^{e}	9.73^{cd}	2.50^{c}	36.88^{a}	1.00^{a}	0.50^{a}
Andriolo	2.85^{a}	44.45^{c}	82.31^{b}	119.08^{b}	10.50^{bc}	36.25^{b}	11.88^{cd}	0.25^{cde}	0.00^{c}
Autonomia A	2.47^{a}	47.90^{b}	83.62^{a}	104.50^{d}	8.73^{ef}	31.88^{b}	30.00^{ab}	1.00^{a}	0.38^{ab}
Benco	3.01^{a}	53.78^{a}	80.15^{c}	121.13^{b}	10.63^{bc}	43.13^{ab}	4.75^{d}	1.00^{a}	0.25^{bc}
Canove	2.96^{a}	40.73^{d}	80.37^{c}	112.65^{c}	9.45^{de}	51.25^{ab}	10.00^{cd}	0.38^{bcd}	0^{c}
Funo	2.85^{a}	44.73^{c}	80.12^{c}	93.40^{e}	8.28^{fg}	2.50^{c}	21.25^{bc}	0.63^{b}	0.13^{c}
Gamba Di Ferro	2.51^{a}	47.13^{b}	77.81^{d}	141.60^{a}	12.03^{a}	39.38^{b}	9.38^{cd}	0.00^{e}	0^{c}
Appen.Bio population	2.93^{a}	47.53^{b}	81.65^{bc}	122.75^{b}	10.73^{bc}	50.00^{ab}	10.38^{cd}	0.13^{de}	0^{c}
Oroset population	2.91^{a}	52.45^{a}	80.70^{c}	125.10^{b}	10.95^{b}	37.50^{b}	7.38^{cd}	0.50^{bc}	0.25^{bc}
Einkorn mix	1.39b	31.99^{e}	84.33^{a}	120.13^{b}	7.68^{g}	59.38^{a}	10.00^{cd}	0.25^{cde}	0^{c}
Season × site	*	***	***	Ns	***	***	Ns	***	Ns
Season × genotype	**	***	**	***	***	***	**	***	***
Site × genotype	***	***	**	*	**	***	**	***	***

Yield (t ha^{-1}), 1000 seeds weight (g), hectoliter weight (kg hL^{-1}), plant height (cm), spike length (cm), lodging (%), weeds (%), pathogen incidence and severity (0-10 scale). *$p \leq 0.05$ **$p \leq 0.01$ ***$p \leq 0.001$. Ns, not significant. [a-h]Different letters denote significant differences determined by SNK *post hoc* test.

addition, farmers involved in the research were asked to give their opinion about the agronomic performance during field surveys. Two farmers in total and five field technicians from Emilia-Romagna organic companies were asked to express a qualitative judgment about the agronomic performances of the different genotypes in the different environments (Figure 2). Basically, they appreciated the cultivation of high size genotypes, especially for their good competition with weed species. The principal farmers' interests were about the evolutionary populations and the einkorn mix. The farmer at San Lazzaro appreciated the good agronomic performances of the "Oroset" population, that in her opinion represented the best compromise between good yield and good phytosanitary characteristics. The einkorn mix did not completely satisfy the San Lazzaro farmer's expectation, especially given the low yield, combined with a high lodging incidence. In contrast, the Monterenzio farmer expressed good impressions about the agronomic performances of the "Appen.Bio" population and einkorn mix. The Monterenzio farmer strongly appreciated the high adaptation potential of "Appen.Bio" and einkorn mix to his own marginal environment, where it is quite rare to achieve their production performances without any use of external inputs.

Nutritional and functional properties

The quantification of the main macronutrients and functional micronutrients in harvested wheat and einkorn grains are reported in Tables 4 and 5.

Table 4. Nutritional properties.

	Proteins	Starch	Lipids	IDF	SDF	TDF
Season	***	***	***	**	***	***
2016/17	14.30^{a}	59.25^{b}	5.20	16.20^{a}	5.04^{a}	21.25^{a}
2017/18	13.49^{b}	63.51^{a}	3.59	15.62^{b}	3.78^{b}	19.40^{b}
Site	***	***	Ns	Ns	***	Ns
Monterenzio	13.26^{b}	61.99^{a}	4.44	15.81	4.63^{a}	20.44
San Lazzaro	14.57^{a}	60.77^{b}	4.35	16.02	4.19^{b}	20.21
Genotypes	***	***	***	***	***	***
Abbondanza	13.04^{e}	61.49^{b}	4.04^{c}	17.22^{a}	4.21^{cd}	21.43^{ab}
Andriolo	13.92^{bcd}	63.14^{a}	3.99^{c}	15.23^{cd}	3.72^{e}	18.96^{e}
Autonomia A	13.11^{de}	62.55^{a}	4.48^{b}	15.59^{cd}	4.28^{bcd}	19.87^{cde}
Benco	14.60^{b}	60.50^{b}	4.60^{ab}	15.71^{cd}	4.60^{abc}	20.31^{bcd}
Canove	13.53^{cde}	61.05^{b}	4.59^{ab}	16.27^{abc}	4.57^{abc}	20.84^{abc}
Funo	13.42^{de}	63.36^{a}	4.06^{c}	15.29^{cd}	3.87^{de}	19.16^{de}
Gamba Di Ferro	12.97^{e}	60.91^{b}	4.41^{b}	16.86^{ab}	4.85^{ab}	21.71^{a}
Appen.Bio population	13.92^{bcd}	61.37^{b}	4.26^{bc}	15.92^{bc}	4.54^{abc}	20.45^{bc}
Oroset population	14.26^{bc}	60.27^{b}	4.61^{ab}	16.23^{abc}	4.60^{abc}	20.86^{abc}
Einkorn mix	16.23^{a}	59.17^{c}	4.92^{a}	14.79^{d}	4.90^{a}	19.68^{cde}
Season × site	***	***	***	**	***	***
Season × genotype	***	***	***	Ns	**	**
Site × genotype	***	***	*	**	Ns	*

Proteins, starch, lipids, insoluble dietary fibers (IDF), soluble dietary fibers (SDF), Total dietary fibers (TDF), expressed in g in 100g of dried matter. *p≤0.05 **p≤ 0.01 ***p≤0.001. Ns, not significant. $^{a-h}$Different letters denote significant differences determined by SNK *post hoc* test.

Table 5. Functional properties.

	Free olyphenols	Bound polyphenols	Total polyphenols	Free flavonoids	Bound flavonoids	Total flavonoids	FRAP antioxidant activity
Season	***	***	Ns	***	***	***	***
2016/17	143.36^{a}	111.44^{b}	254.79	122.66^{a}	25.75^{b}	148.41^{a}	0.85^{b}
2017/18	118.71^{b}	137.82^{a}	256.53	80.17^{b}	44.10^{a}	124.26^{b}	1.01^{a}
Site	**	***	***	***	Ns	***	Ns
Monterenzio	127.62^{b}	121.23^{b}	248.85^{b}	106.51^{a}	34.21	140.72^{a}	0.93
San Lazzaro	134.45^{a}	128.02^{a}	262.47^{a}	96.31^{b}	35.64	131.95^{b}	0.94
Genotypes	***	***	***	***	***	***	***
Abbondanza	115.22^{bc}	135.85^{ab}	251.07^{abc}	94.99^{c}	35.56^{bc}	130.55^{de}	1.01^{a}
Andriolo	133.57^{ab}	137.89^{a}	271.46^{a}	110.28^{b}	38.45^{b}	148.73^{b}	1.02^{a}
Autonomia A	121.73^{bc}	120.17^{c}	241.89^{bc}	93.75^{c}	29.67^{de}	123.66^{e}	0.85^{b}
Benco	142.62^{a}	112.06^{d}	254.67^{abc}	110.64^{b}	26.98^{e}	137.62^{cd}	0.83^{b}
Canove	126.19^{bc}	124.66^{c}	250.85^{abc}	81.33^{d}	29.66^{de}	110.99^{f}	0.97^{a}
Funo	135.23^{ab}	125.17^{c}	260.40^{ab}	106.14^{b}	32.38^{cd}	138.52^{cd}	0.94^{a}
Gamba Di Ferro	136.19^{ab}	129.54^{abc}	265.73^{a}	104.83^{b}	34.99^{bcd}	139.82^{c}	1.00^{a}
Appen.Bio population	126.95^{bc}	126.78^{bc}	253.72^{abc}	96.73^{c}	29.67^{de}	126.40^{e}	0.95^{a}
Oroset population	142.69^{a}	129.16^{abc}	271.85^{a}	97.85^{c}	34.01^{bcd}	131.86^{cde}	0.95^{a}
Einkorn mix	129.96^{ab}	105.00^{d}	234.96^{c}	117.59^{a}	57.63^{a}	175.22^{a}	0.80^{b}
Season × site	***	***	***	*	Ns	*	***
Season × genotype	***	**	**	***	***	***	**
Site × genotype	***	*	*	***	***	***	Ns

Free polyphenols (FP), bound polyphenols (BP), Total polyphenols (TP), Free flavonoids (FF), bound flavonoids (BF), total flavonoids (TF), expressed in mg in 100g of dry matter, FRAP antioxidant activity, expressed in mmol Fe^{2+}in 100g of dried matter). *p≤0.05 **p≤ 0.01 ***p≤0.001. Ns, not significant. $^{a-h}$Different letters denote significant differences determined by SNK *post hoc* test.

Large significant differences were found in protein content, for the site, genotype and growing season factors, respectively. Overall, in the 2016/17 cropping season, the average protein content was 14.30 g 100g^{-1}, which was followed by an approximate decrease of 5.5% in the following season. Instead, the differences between the sites were attributable to the different pedo-climatic conditions, including altitude and variability of rainfall. Over both seasons, the protein content of the genotypes harvested in San Lazzaro was higher than that found for the genotypes in Monterenzio. The einkorn mix had the highest protein content (16.23 g 100g^{-1}). A high protein content was also observed for both wheat populations ("Oroset", 14.26 g 100g^{-1} and "Appen.Bio",13.92 g 100g^{-1}). Among the pure wheat varieties, high protein contents were recorded for "Benco" (14.60 g 100g^{-1}) and "Andriolo" (13.92 g 100g^{-1}). The statistical analysis also highlighted significant interactions between site and genotype. Some varieties, such as "Abbondanza" and "Autonomia A", attained an excellent protein content, especially in San Lazzaro. Starch content differed significantly between the two growing seasons, sites and the different genotypes, respectively. Grain produced in the 2017/18 season displayed a 6.7% higher starch content than that of previous season, in which rainfall was significantly below the seasonal average. The same trend was observed for the different growing sites. In Monterenzio, characterized by more consistent rainfall, the starch content was approximately 2% higher, compared to San Lazzaro. However, this pattern was not recorded in all the genotypes, as "Benco" and "Funo" produced more starch in Monterenzio. In the two growing seasons, the genotypes with higher starch contents were "Andriolo" (63.14 g 100g^{-1}) and "Funo" (63.36 g 100g^{-1}). Instead, the einkorn mix reported the lowest value (59.17 g 100g^{-1}). As regards the lipid content of the grain, the statistical analysis revealed significant differences between seasons and genotypes, with no differences for the cultivation area (Table 4). For the 2016/17 and 2017/18 seasons, the average lipid content was 5.20 g 100g^{-1} and 3.59 g 100g^{-1}, respectively, showing a difference of 30% between the two agricultural seasons.

The einkorn mix contained the highest lipid content (4.92 g 100g^{-1}). Of the wheat varieties, the "Oroset" population (4.61 g 100g^{-1}), "Canove" (4.59 g 100g^{-1}) and "Autonomia A" (4.48 g 100g^{-1}) had the highest content, while the lowest lipid content was recorded for "Andriolo" (3.99 g 100g^{-1}). A significant interaction between growing seasons and sites was also highlighted.

The IDF and TDF were significantly influenced by the genotype and the season. Conversely, SDF was influenced by all factors. Overall, the TDF content was higher in the first year. The site significantly influenced the SDF content, with higher values in Monterenzio, while no significant differences were found for IDF and TDF. Regarding genotypes, Table 4 underscored the opposite response in the einkorn mix, which responded more positively to the conditions in San Lazzaro, especially in IDF and consequently in TDF. Between genotypes, the highest TDF values were observed in "Gamba di Ferro" (21.71 g 100g^{-1}), "Abbondanza" (21.43 g 100g^{-1}), "Canove" (20.84 g 100g^{-1}) and the "Oroset" population (20.86 g 100g^{-1}), respectively. The variety with the lowest TDF content was "Andriolo", with 18.96 g 100g^{-1}. Mean yield and protein content, as well as their respective stability, over environments for each genotype were explored using the GGE biplot software (Figure 4). The method is used for the evaluation of the genotypes in relation to an ideal genotype (represented by a blue circle), which is used as a reference (Mitrovic *et al.*, 2012). Considering yield, Benco was closer to the 'ideal' genotype followed by "Canove" and the "Oroset", population, respectively. In contrast, the low yielding genotypes ("Abbondanza" and einkorn mix) were considered undesirable because they were placed far from the ideal genotype. In addition, "Andriolo", "Benco", "Canove" and the "Oroset" population had higher stability, described in the Figure 4A by shorter vector. As regards the protein content, the einkorn mix showed the highest mean value (with low stability), while "Autonomia", "Funo" and "Gamba di Ferro" showed the lowest values, but with higher values of stability (Figure 4B).

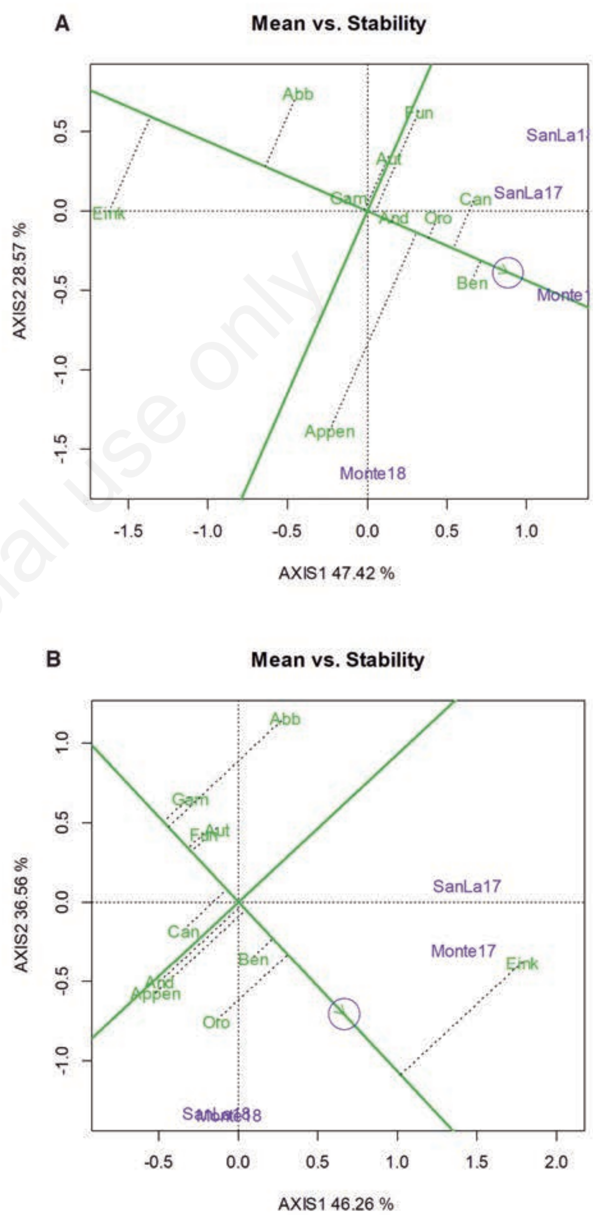

Figure 4. GGE-biplot analysis for "mean performance versus stability" for yield (**A**) and protein content (**B**). Sanla17 and Sanla18 represent the two years of cultivation (2017 and 2018 respectively) in San Lazzaro location while Monte17 and Monte18 represent the two years of cultivation (2017 and 2018 respectively) in Monterenzio location. Genotype: Abb, Abbondanza; And, Andriolo; Aut, Autonomia A; Ben, Benco; Can, Canove; Fun, Funo; Gam, Gamba Di Ferro; Appen, Appen.Bio population; Oro, Oroset population; Eink, Einkorn mix. Green: satisfactory; Orange: partially satisfactory; Red: unsatisfactory.

In the 2016/17 season, an average free polyphenols (FP) content of 143.36 mg $100g^{-1}$ was observed, whereas in the following season (2017/18) the value decreased by about 17%. On average (over the two seasons), San Lazzaro was the site with the highest FP content (134.45 mg $100g^{-1}$), compared to Monterenzio (127.62 mg $100g^{-1}$). From the analysis carried out on the genotypes, the highest FP content was found for the "Oroset" population (142.69 mg $100g^{-1}$) and "Benco" (142.62 mg $100g^{-1}$), followed by "Gamba Di Ferro" (136.19 mg $100g^{-1}$), "Funo" (135.23 mg $100g^{-1}$), "Andriolo" (133.57 mg $100g^{-1}$) and mix (129.96 mg $100g^{-1}$). Furthermore, FP is the only phenolic fraction for which every possible interaction between the factors is highly significant (Table 5). Bound polyphenols (BP) content, on the other hand, show highly significant differences for all the single factors. The content of the bound polyphenols fraction showed an opposite trend to that of the free fraction in the two experimental years, with an average BP content of 111.44 mg $100g^{-1}$, recorded in the first growing season, which increased by about 20% in the 2017/18 season. "Andriolo" (137.89 mg $100g^{-1}$) showed the highest BP value, followed by "Abbondanza" (135.85 mg $100g^{-1}$), "Gamba Di Ferro" (129.54 mg $100g^{-1}$) and the "Oroset" population (129.16 mg $100g^{-1}$). The most significant interaction ($p \leq 0.001$) was growing season x site. Total polyphenols (TP) content did not differ statistically in the two years, as was observed for FP and BP. However, TP content was influenced by site and genotype. The highest TP content was recorded in San Lazzaro, with an average of 262.47 mg $100g^{-1}$, compared to Monterenzio, with an average value of 248.85 mg $100g^{-1}$. There were also significant differences between the genotypes. The highest TP contents were found in the "Oroset" population (271.85 mg $100g^{-1}$), "Gamba Di Ferro" (265.73 mg $100g^{-1}$) and "Andriolo" (271.46 mg $100g^{-1}$), with the lowest contents measured in the einkorn mix (234.96 mg $100g^{-1}$). Free flavonoids (FF) content differed significantly over the two growing seasons. In the 2016/17 season, the average content was 122.66 mg $100g^{-1}$, while in the 2017/18 season the average value decreased by about 30%. The different cultivation areas were also statistically significant. Free flavonoids (FF) content was higher (106.51 mg $100g^{-1}$) in Monterenzio than in San Lazzaro (96.31 mg $100g^{-1}$). The einkorn mix contained the highest FF content (117.59 mg $100g^{-1}$); followed by the wheat genotype "Benco" (110.64 mg $100g^{-1}$), "Andriolo" 110.28 mg $100g^{-1}$), "Funo" (106.14 mg $100g^{-1}$) and "Gamba Di Ferro" (104.83 mg $100g^{-1}$), respectively. The interactions, season x genotype and site x genotype, were significant, underlining a differing adaptability for flavonoid production by the genotypes. In the first year, the average BF content of the varieties was 25.75 mg $100g^{-1}$, which increased by about 70% in the second year. Regarding the single genotypes, similar to the FF content, the BF content of the einkorn mix was significantly higher than wheat (57.63 mg $100g^{-1}$). Instead, the wheat varieties with the highest BF content were "Andriolo" (38.45 mg $100g^{-1}$) and "Abbondanza" (35.56 mg $100g^{-1}$). The statistical analysis did not highlight any interactions between season and site, whereas the interactions between season and genotype and between site and genotype, respectively were highly significant. Total flavonoids (TF) content showed significant differences over the two growing seasons, with a content of 148.41 mg $100g^{-1}$ in the 2016/17 season, which then decreased by 16% in the following season. As regards the site, a higher TF content was observed in Monterenzio (140.72 mg $100g^{-1}$), compared to San Lazzaro (131.95 mg $100g^{-1}$). The einkorn mix contained the highest TF content (175.22 mg $100g^{-1}$), which differed significantly from the values for wheat. Of the wheat genotypes, "Andriolo" (148.73 mg $100g^{-1}$), "Gamba Di Ferro" (139.82 mg $100g^{-1}$) and "Funo" (138.52 mg $100g^{-1}$) contained the highest TP content, respectively. Finally, the statistical analysis of the antioxidant activity revealed significant differences between the average values for the two growing seasons but not for the different sites. In the 2017/18 season, the average antioxidant activity was 1.01 mmol Fe^{2+} 100 g^{-1} of wholemeal flour, 16% more than that in the first season. The genotypes also differed statistically from each other. The antioxidant potential of the flour samples (FRAP) ranged from a maximum of 1.02 mmol Fe^{2+} 100 g^{-1} of wholemeal flour, measured for the "Andriolo" genotype, to a minimum of 0.80 mmol Fe^{2+} 100 g^{-1}, for the einkorn mix. Furthermore, the interaction between year and site was highly significant. To determine possible correlations between the main agronomical, nutritional and nutraceutical traits, the Pearson Correlation coefficients were calculated, and the results are reported in Table 6.

Table 6. Correlation matrix of the principal variables in the study.

Pearson correlation	Plant height	Spike length	Yield	Hectoliter weight	TDF	FP	BP	TP	FF	BF	TF	FRAP	Protein	Starch
Plant height	1													
Spike length	0.506**	1												
Yield	0.083	0.084	1											
Hectoliter weight	-0.333*	-0.505**	-0.088	1										
TDF	0.258	0.545**	-0.392*	-0.430**	1									
FP	0.347*	0.532**	-0.139	-0.465**	0.442**	1								
BP	-0.168	-0.177	0.181	0.056	-0.151	-0.257	1							
TP	0.158	0.306	0.028	-0.346*	0.251	0.634**	0.584**	1						
FF	0.180	0.355*	-0.345*	-0.254	0.305	0.447**	-0.529**	-0.048	1					
BF	-0.051	-0.405**	-0.018	0.379*	-0.447**	-0.297	0.381*	0.056	-0.463**	1				
TF	0.169	0.142	-0.399*	-0.045	0.059	0.314*	-0.352*	-0.018	0.828**	0.114	1			
FRAP	-0.188	-0.093	0.149	-0.044	-0.086	-0.254	0.886**	0.538**	-0.498**	0.323*	-0.347*	1		
Protein	0.317*	0.089	-0.185	0.123	0.135	0.411**	-0.281	0.120	0.325*	-0.172	0.256	-0.359*	1	
Starch	-0.394*	-0.466**	0.385*	0.220	-0.734**	-0.618**	0.473**	-0.141	-0.523**	0.457**	-0.297	0.466**	-0.723**	1

FP, Free polyphenols; BP, bound polyphenols; TP, Total polyphenols; FF, Free flavonoids; BF, bound flavonoids; TF, total flavonoids; FRAP, ferric reducing antioxidant potential. Pearson Correlation Coefficients of principal variables under investigation. **Correlation is significant at 0.01 level (two-tailed); *correlation is significant at 0.05 level (two-tailed), Correlation analysis (Table 6) showed interesting implications, that could be considered in relation to agronomic and nutritional aspects. From an agronomic point of view, a significant and positive correlation emerged between plant height and spike length, with possible important implications related to lodging plant risk. However, these relationships did not appear to negatively affect wheat productivity, as no significant correlations between plant height, ear length and production yield were found. Considering instead the main nutritional and functional parameters, it is interesting to note that while there was a high and significant correlation between polyphenol content (especially in the bound form) and FRAP antioxidant activity, there was a parallel positive correlation between FRAP activity and only bound flavonoid content (Table 6), indicating that probably these compounds, although with antioxidant action, exert chemical activities that are not totally detectable by FRAP assay.

Discussion

One of the main problems facing the Bolognese Apennines and disadvantaged rural areas throughout Europe is the lower productivity typical of hilly and mountainous areas. This has led to the abandonment of a significant proportion of the cultivated land. In addition, high climatic variability requires crops with resilient traits, such as stable yields, consistent quality parameters, strong competitive ability against weeds, and low susceptibility to lodging (Bosi *et al*., 2022). The two years of this trial showed a certain variability between locations and growing seasons: most of the climatic variability is between years, but it is notable that weather extremes were more intense in Monterenzio, the high Apennine site. Interestingly, the year factor affected yield, but the field site wasn't significant in terms of the overall grain production. In our experiment, most of the variability could be attributed to weather conditions, more than location factor.

Good agronomic results were obtained in terms of lower weed infestation and lodging susceptibility in Monterenzio. Moreover, old varieties are more suitable for organic cultivation, due to the greater competitiveness of tall wheat plants against weeds (van der Meulen and Chauhan, 2017).

Overall, from evaluating the agronomic parameters, the einkorn mix confirmed values reported in the literature (Vallega, 1992). Instead, the soft wheat varieties showed lower yields than those obtained by similar landraces and old varieties, cultivated during previous experiments on organic farms located in the plains (Di Silvestro *et al*., 2016). However, it was interesting to note that the "Oroset" and "Appen.Bio" populations registered average yield values (approximately 2.9 t ha^{-1}) higher than other wheat mixtures, cultivated in hilly regions evaluated in a previous study (Migliorini *et al*., 2016).

From information collected from the farmers, a "mean" yield for a modern variety cultivated in the same growing conditions and sites could fluctuate between 2.5 and 4.0 t ha^{-1}. Even if in some case, the yield of the modern genotypes could have been higher than those reported in the present study, the difference in price between food products obtained from local varieties or evolutionary populations (specifically adapted to a limited area) and price of food obtained from modern varieties rewards the farmer for the potential lower yield. In fact, farmers confirmed that a "mean" price for organic flour from modern varieties obtained in their farms, could vary between 2.0 and 2.5 € kg-1, while the price for landraces or population organic flour could reach also 3.0 € kg-1, up to 6.0 € kg-1, depending on the market sector.

The GGE analysis of yield variable (Figure 4A) highlighted that the two wheat populations had better performances than most of the other genotypes cultivated separately. The "Oroset" population showed a very high yield stability among the different growing environments, while in contrast the "Appen.Bio" population registered a low stability. This evidence is probably determined by the different original genetic composition of the two populations, that could influence both the agronomic performances and the time required for the population to reach a high yield stability when data from different environments are compared. Despite these considerations, results on yield stability should be carefully interpreted, because data from more sites and years could improve the evaluation of yield stability of the considered genotypes.

It is assumed that the evaluated populations will evolve naturally, thanks to their characteristic genetic diversity, improving the adaptation capacity of the wheat crop to specific environments (Ceccarelli, 2009). Farmers and technicians' field evaluations showed interest in this opportunity through their qualitative appraisals. They particularly appreciated the evolutionary populations in the evaluation, especially for the potential adaptability when cultivated for several seasons in the target environment. The einkorn mix was not rated agronomically successful in all locations, especially due to its low yields, as confirmed also by the GGE analysis (Figure 4A). Nevertheless, in marginal areas (*e.g.* Monterenzio location), where average yields are lower, this species was also considered to be interesting by the farmers.

The 1000-seed weight, comparing the two test years, seems to have been affected by the low rainfall of 2016/17 season, assuming a marginal average difference of less than 4 g compared to the following season. Considering the hectoliter weight, among the genotypes in the trial, the einkorn mix reported a value of 84.33 kg/hl, significantly higher than the other accessions in the trial. Probably this difference between einkorn and wheat can be attributed to the different size of the kernel.

The height of wheat plants has always been one of the most studied agronomic parameters, together with the yield, and often included in breeding programs. In the 2016/17 season the average difference in plant height respect to the following year (2017/18) was about 6 cm, probably due to a drier season than the 2017/18. Noticeable differences were also observed between the different locations: at San Lazzaro, the genotypes showed an average height of about 5 cm higher than in Monterenzio. The effects of this statistically significant difference can be due to many factors, including: the different altitude and the type of soil that was more calcareous and poorer in primary nutrients at Monterenzio than at San Lazzaro. Analyzing in detail the plant height of the different genotypes, a wide heterogeneity was found. In particular, the average height of "Abbondanza", "Andriolo", "Autonomia A", "Canove" and "Funo" were lower than the data reported in the literature (Migliorini *et al.,* 2016; Di Silvestro *et al*., 2012). Probably, the hilly growing environment influences plant height, reducing it. "Gamba Di Ferro" genotype represents an exception to this evidence, with an average plant height of 141.60 cm, which is above average previous data (Migliorini *et al.,* 2016; Di Silvestro *et al.,* 2012).

Considering the spike length parameter, the differences highlighted in Table 3 within the individual factors was attributed to causes very similar to those that probably influenced plant height, as the two characters are highly correlated to each other and influenced both by the genotype and by the cultivation environment (Liu *et al*., 2022; Johnson *et al*., 1966). As shown in Table 3, no evident correspondence between lodging and yield was observed, suggesting that, for ancient accessions, the lodging is mostly carried out late and does not negatively influence the phenological phases of kernel filling and ripening. On the other hand, significant differences were found in the percentage of lodging for both two agricultural years and for the two locations, confirming that environmental conditions have a high impact on the lodging incidence. The high lodging percentage, registered for einkorn mix, could have been influenced by the different mechanical characteristics of the stem, but also by the sowing density used in this study was the same as that employed for wheat. Einkorn mix lodging incidence could probably be reduced adopting a lower sowing density, compared to that of wheat (Lazzaro *et al.*, 2017).

Another important parameter for crops that grow in organic farming is the percentage of weeds present in the field, considering the possible effects that they could have on agronomic and nutritional characteristics of the crop. In this study, significant differences emerged between the genotypes. "Abbondanza" and "Funo", which had recorded the lowest heights among the varieties under study, were among the most contaminated by weeds. Statistical differences for the site factor were also found; the Monterenzio farm, located at a higher altitude and with a typical plant biodiversity of high hills, had an average percentage of weed infestation that was around 10%, while at San Lazzaro farm, characterized both by a warmer climate, that probably stimulated the germination of weeds, and by more fertile soils, the weed infestation value was almost double that of the Monterenzio farm.

In the two years of cultivation, the diseases detected in both farms did not negatively affect any agronomic or nutritional parameter. The adopted agronomic practices and the organic cultivation system has enhanced the rusticity of all the accessions analyzed in the test. The low pathogen incidence detected in this study did not allow for assumptions about how the high genetic variability, typical of ancient varieties, could represent a genetic basis for new breeding programs aimed at improving the pathogen resistance of new genotypes. Specific trials to study pathogen resistance traits of these genetic materials should be realized in the future.

Overall, evaluating the agronomic parameters analyzed, einkorn mixes often reflected the values reported in the literature for this cereal species. All wheat varieties adapted without problems to the organic farming system, providing in some cases interesting agronomic performances. The results of the "Oroset" and "Appen.Bio" populations have never deviated too much from the averages of all the other evaluated wheat genotypes, considering the agronomic parameters examined. Regarding the results for protein content, statistical significance was observed for both the effect of crop years and the test sites. No statistically significant correlation was found between yields and protein content (Table 6), contrary to what is often reported in the literature (De Santis *et al.*, 2017). The trend for protein content (higher in the first crop year) is the exact opposite of what was observed for yield (higher in the second year). The protein content was shown to vary according to the climatic variables recorded in the two crop years. Some studies state that higher temperatures, the persistence of a dry climate and infrequent rainfall may induce the accumulation of proteins in the grain (Arzani and Ashraf, 2017). The present results corroborated those reported in the literature (Nuttal *et al.*, 2017; Triboi *et al.,* 2006). Of the growing seasons, the first year was the driest, with a lower-than-average regional climatic rainfall, corresponding to the higher protein contents. Furthermore, dryer conditions occurred in San Lazzaro, which similarly resulted in significantly higher protein values than in the high hilly location (Monterenzio). The GGE analysis (Figure 4B) confirmed that the einkorn mix registered the highest protein content among genotypes, but it was characterized from a high variability, considering the different environmental conditions, highlighting that the environmental conditions can have a major influence on protein content for einkorn species. The average protein content of the two wheat populations reached values comparable to those achieved by individual genotypes grown separately, but presented a lower stability than some varieties, like "Benco" or "Canove" (Figure 4). Obviously, GGE results on stability related to yield and protein content should be interpreted with caution as we considered only two years and two locations. The performances of these genotypes should be evaluated over more years at more locations. Among the wheat accessions, the highest values were recorded in "Benco" and, secondarily, in both populations, showing how the combination of different varieties, grown in mixtures adapting over the years, can be a viable cultivation alternative from a production and nutritional point of view.

The starch content was influenced by environmental conditions. Under higher rainfall and humidity conditions, a higher average starch content was registered, in particular, during the 2017/18 growing season and in the Monterenzio site (rainiest year and location during the experimental trial). The lipid content was also influenced by the different weather conditions. Results show how the 2016/17 growing season differed significantly from the following one (2017/18). In this case, lipid accumulation was higher in the first crop year, which was characterized by a spring with less rainfall and drier than the 2017/18 growing season. The different growing areas did not determine any significant effects.

Examining the data reported on dietary fibers and more precisely the trend over the two growing seasons, there was an inversely proportional trend between the starch and the dietary fiber content. Hence, rainfall determined a lower percentage of dietary fiber and a higher starch content. From the collected data, a high and significant negative correlation ($r=-0.734$; $p<0.01$) emerged between the TDF and starch contents (Table 6). Therefore, considering both the positive correlation between yield and starch content, illustrated above, and the negative correlation between starch and fiber content (Table 6), it can be deduced that the increase in starch content enhanced the size and weight of the kernel, positively influencing the yield and negatively influencing the total fiber content (Marcos-Barbero *et al.*, 2021; Amiri *et al.*, 2018; Zi *et al.*, 2018).

Considering the nutraceutical aspects, the growing seasons did not affect TP, but only the relative composition of the two fractions. In fact, due to different levels of rainfall in the two growing seasons, FP were higher in 2016/17, whereas FB were higher in 2017/18. As for the site factor, the TP of the grains harvested in San Lazzaro were higher than those in Monterenzio. In this case, the higher average temperatures during spring and early summer had a positive effect on both phenolic fractions. The highest TP values were obtained from the "Oroset" and "Appen.Bio" populations.

In addition to polyphenols, flavonoids were also quantified. Flavonoids represent a class of polyphenol chemical compounds, which often provide both colors and flavors to the products (Dias *et al.*, 2021). Flavonoids also perform important antioxidant and anti-inflammatory functions in the human body (Di Gioia *et al.*, 2014). The flavonoid fractions were similar in trend to those of the polyphenols, with higher FF in 2016/17 and higher BF in 2017/18, respectively. Total flavonoids content, to the contrary, was significantly higher in the first cropping season and in Monterenzio.

Finally, the FRAP test was performed on the flours, measuring the chelating activity of the phenolic extract. The test measures the reduction and stabilization of transition metals (in particular of Fe^{2+} ions) which are among the causes of the formation of free radicals and consequently of the initiation of the oxidative chain (Di

Loreto *et al.*, 2018). Highly significant correlations were observed between both FRAP and BP (r=0.886; $p<0.01$) and between FRAP and TP (r=0.538; $p<0.01$), showing an increase in antioxidant activity with increasing BP and T, in turn indicating that BP are primarily responsible for antioxidant activity. Similarly, a significant correlation with FRAP was also found for BF (r=0.323; $p<0.05$); thus, suggesting an increase in antioxidant activity.

Summarizing the principal evidence, substantial differences found between the two sites can be attributed to the different altitude, which is related to differences in climate (temperatures and rainfall) and soil fertility. However, from the analysis of the agronomic parameters, it was noted that despite the different limiting conditions of the two locations, the yields did not differ statistically: despite all other agronomic parameters generally being better in the high hill farm. In Monterenzio, the height of the plants was smaller, with a consequent reduction in lodging, the incidence of weeds was more contained, while the hectoliter weight did not differ statistically between the two farms. Landraces and wheat populations have proven to be well adapted for this marginal environment. Less limiting areas, such as San Lazzaro, did not always guarantee better production performances for ancient wheat, but they can negatively influence some agronomic parameters. Farmers' observation, collected during the experimentation, confirmed the previous evidence. The combination of experimental data, quantitative results and farmer's evaluation with the GGE analysis showed a very high potential for the identification of the most suitable genetic material to marginal areas, considering also the stability of production and quality traits.

Conclusions

The objective of this study was to identify tailor-made solutions in fostering food production-consumption in a specific territorial area, through an integrated methodology and by identifying the key actors of in the short food supply chain (farmers, producer, retailers).

The characterization of the genotypes and populations of soft wheat, as well as einkorn, provided interesting agronomic and production results, for the two wheat populations ("Oroset", "Appen.Bio"). Environmental adaptability was also shown by the einkorn mix. Combining ancient genotypes into mixes in marginal areas, could become a strategy to create competitiveness. This competitiveness with other agricultural systems can also contribute to the care and protection of the territory, discouraging the abandonment of the hilly and mountainous areas.

The populations studied in the experiment are quite newly established and therefore the process of adaptation to the environment is still ongoing. Considering the results obtained in only two years were particularly interesting, further studies involving direct evaluations of the genetic material under study by more farmers, the real potential costs and benefits of wheat populations and einkorn mix would be demonstrated. The sharing of experiences and findings between researchers and farmers resulted in a win-win solution for the identification of the better adapted genotypes to the specific environmental conditions of each farm. Beside the scientific results, the farmers' judgment represented a practical indication for varietal choices under specific marginal conditions, highlighted from different preferences that emerged between the two farms for the two common wheat populations and the einkorn mix. An interesting opportunity could be also represented by the application of the employed experimental and research approach of this study to different crop species, with involvement of farmers that can also be based on the creation of local networks, characterized by different local farmer needs, expanding the potential for the overall evaluation of diversified genetic material.

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