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ORIGINAL PAPER

Plant agro-biodiversity needs protection, study and promotion: results of research conducted in Lombardy region (Northern Italy)

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Abstract

The loss of plant agro-biodiversity is a global problem with repercussions on both humans and (agro-)ecosystems. This article presents the data of a census of the herbaceous landraces currently cultivated in Lombardy (Northern Italy), one of the most industrialized regions of Europe, and for which information was previously extremely limited. The census showed that 72 herbaceous landraces are cultivated (conserved on farms) in Lombardy yet most of them are threatened since they are cultivated by a small number of farmers, mostly hobbyists. Only 11% have been the subject of scientific studies while 12.5% are protected since they are registered in the European Register of Conservation Varieties. Lombardy has lost about 78% of its landraces cultivated over the last 70–80 years. The nutritional characteristics of four little-known maize landraces of the Lombardy region recently used for the creation of niche food chains were also analyzed. They have a higher content of protein (about 12.34%) and phytic acid (about 1.35%), compared to a hybrid maize (B73/Mo17), while they are slightly poorer in starch (about 77.85%), Mg and Zn. Some of these landraces, those with coloured kernels due to the high concentration of polyphenols, have high antioxidant activity which makes them interesting for the production of nutraceutical foods. 2D-electrophoretic protein profiles highlighted that the four maize landraces are different one from another. Finally, some actions and tools are suggested to favour the in situ conservation of plant agro-biodiversity.

Keywords Agricultural biodiversity · In situ conservation · Landraces · Nutritional value · Plant genetic resources · *Zea mays*

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Introduction

Biodiversity conservation has been a much debated issue over recent decades, from a local to a global level, as the number of endangered species is increasing alarmingly. In fact, according to the latest report from the International Union for Conservation of Nature (IUCN), 63% of cycads, 40% of amphibians, 35% of dicots, 34% of reptiles, 34% of conifers, 33% of corals, 31% of sharks and rays, 27% of crustaceans, 25% of mammals, 14% of birds, 7.5% of gastropods, 7% of bony fishes and 4% of cephalopods were threatened (critically endangered, endangered or vulnerable) (IUCN 2019). The loss of biodiversity does not only concern wild species, but also those living beings (animals, plants and micro-organisms) that are used directly or indirectly for food and agriculture and which, together, constitute a part of biological diversity defined as agro-biodiversity (FAO 1999). These genetic resources, which are the result of natural selection and processes of domestication and selection performed by mankind since the start of agriculture, have undergone (and are undergoing) heavy losses over the last decades (FAO 2004, 2010). The Food and Agriculture Organization of the United Nations (FAO) estimates that about 75% of global agro-biodiversity has been lost over the last century and that three-quarters of food world-wide is produced by only 12 plant species and five animal species (Hammer et al. 1996; FAO 1999, 2004, 2010; Rischkowsky and Pilling 2007; Esquinas-Alcázar 2010). This loss represents a serious problem that, in addition to impoverishing the planet's biodiversity, reduces the diversity of food available to humans (and other living beings) (Frison et al. 2011), the historical and cultural heritage linked to agri-food traditions that identify specific populations and/or territories, and the availability of genetic resources for genetic crop improvement programs (Yadav and Bidinger 2007; Ceccarelli 2012; Puglisi et al. 2018).

In recent decades there have been several international conferences during which scientists and politicians were able to discuss issues related to the conservation of agro-biodiversity, including the Convention on Biological Diversity (CBD 1992, 2002, 2010; Bragdon 1996; Paton et al. 2008) and the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO 2009), on which the Global Strategy for Plant Conservation is based (Sharrock et al. 2014). This has led to the drawing up of international guidelines and strategies such as, for the European Union (EU), the EU Biodiversity Strategy 2020 (EU Commission 2010) and the 2030 Agenda for Sustainable Development (United Nations 2015) including principles which have been integrated into some European Directives (Directive 2008/62/EC 2008; Directive 2009/145/EC 2009; Directive 2010/60/EU 2010) and hence acquired by EU member states.

Among the various taxa that make up agro-biodiversity that of herbaceous plants requires particular attention due to the high number of species/varieties and their vulnerability. Many of the herbaceous plants used in agriculture are in fact annual species (therophytes) which, if not cultivated by farmers, can face extinction within a few years. To counteract the risk of extinction, various seed banks have been created all over the world (Peres 2016; O'Donnell and Sharrock 2017; Al-Turki et al. 2018) with the objective of preserving ex situ plant genetic resources including landraces (understood as dynamic populations of cultivated plants that have historical origin and distinct identity, and lack formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems (Camacho Villa et al. 2005) still cultivated in open fields or in home gardens (garden-race/garden-crop) (Zeven 1998; Negri 2003) and those whose cultivation has been replaced by that of more productive commercial varieties/hybrids (Srinivasan et al. 2003). In addition to this "static" strategy of conservation, more recently conservation actions have been launched in situ (on farm) that allow selection to act so that genetic changes in populations occur with time ("dynamic" conservation) (Frankel et al. 1995; Hammer et al. 2003; Jackson et al. 2007; Veteläinen et al. 2009). The European Register of Conservation Varieties represents one of the most modern instruments for in situ conservation of landraces adopted by the EU (Spataro and Negri 2013). It envisages the figure of the custodian farmer who is responsible for the production of seed (produced in purity) in the area where the landrace is traditionally cultivated (except for limited exceptions). Italy, too, whose agri-food sector is of great economic importance (USDA 2017), is affected by the loss of landraces (Laghetti et al. 1993; Hammer et al. 1996; Negri 2003; Pacicco et al. 2018), although a significant number of unknown landraces could be still isolated in farms (Montesano et al. 2012). In fact, there is still little information regarding garden-crops as many of the collecting missions carried out in the past were mainly concentrated on major crops grown in fields "easy" to reach. However, Italy is legislating to counteract the problem of landrace loss by following EU guidelines. In particular, with the recent law of 1st December 2015 n. 194 ("Provisions for the conservation and enhancement of biodiversity of agricultural and food interest") (Law 2015/194 2015) Italy established the principles for the establishment of a national system of conservation and enhancement of biodiversity of agricultural and food interest, aimed at protecting local genetic resources of food and agricultural interest from the risk of extinction and genetic erosion. This protection system, which is currently being launched, will collect all the information regarding landraces present in the various Italian regions.

Lombardy is one of the most important regions of Italy, being its fourth largest region (23.8 km^2), hosting over 10 million people (about one-sixth of the total Italian population) and is one of the most productive Italian regions in terms of horticulture and cereals (ISTAT 2010; ISMEA 2014). Moreover, about 36% of Italy's gross domestic product (GDP) is produced by Lombardy, making it the most populous and richest region in the country and one of the richest regions of the Alpine macro-Region (EUSALP) and in Europe (EU Commission 2017). Lombardy Region, which established the procedure for the registration of landraces in the European Register of Conservation Varieties only in 2013 (Legislative Decree 2013/9167 2013), does not yet have a complete list of its herbaceous landraces (currently cultivated) to provide to the Italian national system for the conservation and enhancement of biodiversity of agricultural and food interest. This, in addition to preventing the conservation of agro-biodiversity, does not even allow the study and enhancement of landraces that could be particularly interesting for starting up unique high quality agri-food chains of low environmental impact that could be strategic for smart, sustainable and inclusive growth (Cleveland et al. 1994; Fideghelli and Engel 2009; EU Commission 2010; Newton et al. 2010). For these reason this research aims to:

- Perform a census of, and map, the herbaceous landraces currently cultivated in Lombardy so as to be able to provide information about these landraces to the Italian national system for the conservation and enhancement of biodiversity of agricultural and food interest, and hence protect, study and enhance them.
- Identify, and map, the main subjects (organizations, associations, consortia, universities, research centres, etc.) that deal with the study, conservation and promotion of the plant agrobiodiversity of Lombardy in order to have an overall picture of those active in the sector today.
- Characterize the content of nutritionally-relevant compounds in the kernels of four landraces of Lombardy maize (*Zea mays*) recently registered in the European Register of Conservation Varieties ("Spinato di Gandino", "Rostrato Rosso di Rovetta", "Nero Spi-

noso" (Cassani et al. 2017) and "Scagliolo di Carenno"); because their on-farm conservation can be favoured by knowledge of their nutritional characteristics (currently mostly unknown) by producers and consumers who are the main subjects on which agri-food chains are based.

- Suggest tools and/or improvement actions that may be useful not only to Lombardy Region but also to Italy and other countries that are active or becoming active in safeguarding plant agro-biodiversity and therefore share the results of this case study so that they can be useful beyond Lombardy and represent an opportunity to solicit specific intervention from local, regional, national and international administrations/policy makers.

This research was conducted as part of a three-year project (2016–2018) supported by Lombardy Region and it is a joint work between a research institute, a territorial management body and the farmers who cultivate and preserve landraces.

Materials and methods

Census of lombardy landraces

Information regarding the presence of landraces in the territory of the Lombardy region was collected by bibliographic research and by interviewing elderly farmers. In addition, an online questionnaire was created using Google Form to highlight the presence of farmers who still cultivate landraces, as performed by Last et al. (2014) and Giupponi et al. (2019). The questionnaire consisted of three sections: the first contained a brief description of the research project; the second, sections to be filled in regarding landrace data (traditional name, common and/or scientific name of the species, use, historical information, rarity, agronomic/morphological/ecological/gastronomic characteristics, level of protection); the third, sections to be filled in with the data of the custodian farmer (name, surname, age, name of farm if appropriate, place where the landrace is grown, number of years the landrace has been grown, e-mail, telephone number and address). The compilation of some sections of the questionnaire, those most important for research purposes (traditional name of the landrace, number of years the landrace has been grown, name, surname, telephone number and address information of the custodian farmer), was made compulsory through Google Form functions and, at the end of the questionnaire, authorization was requested for the processing of personal data according to current legislation and research objectives. The questionnaire was sent to over 15,000 contacts throughout the territory of the Lombardy Region and nearby areas. Information was also collected in a similar way regarding the associations and bodies that currently deal with the conservation, study and enhancement of landraces of Lombardy. To reach as many people as possible, information about the initiative was also provided by local media and promoted at conferences and meetings on topics related to agriculture and the conservation of agro-biodiversity. Landrace data, collected through online questionnaires, were checked (so that they corresponded to the characteristics stated by Camacho Villa et al. (2005) and had been cultivated on site for at least 30 years) and/or implemented by contacting those who had completed the questionnaire. In some cases, in order to confirm/identify doubtful species, detailed photos were requested from the custodian farmers. Plant identification was performed using the dichotomous keys of Pignatti (1982) and its nomenclature was adopted. Finally, bibliographic research was carried out in order to collect all the scientific studies (genetic, morphological, phytochemical and nutritional) conducted on the landraces identified to understand the level of scientific knowledge regarding the landraces of Lombardy. This research was performed using data of the latest landrace inventories published for Lombardy as a starting point (Laghetti et al. 1993; Bertolini 2002; Negri et al. 2013) as well as the main scientific research digital databases (Web of Science, Scopus, ScienceDirect, Researchgate).

Plant material and nutritional analysis of maize landraces

The seed samples of the four landraces of maize registered in the European Register of Conservation Varieties ("Spinato di Gandino", "Rostrato Rosso di Rovetta", "Nero Spinoso" and "Scagliolo di Carenno") were collected in November 2016 in the four areas in Lombardy where they are cultivated by custodian farmers: Gandino (Latitude: 45° 48′ N, Longitude: 9° 54′ E, Elevation: 530 m a.s.l.), Rovetta (Latitude: 45° 53′ N, Longitude: 9° 59′ E, Elevation: 650 m a.s.l.), Esine-Piancogno (Latitude: 45° 55′ N, Longitude: 10° 13′ E, Elevation: 670 m a.s.l.) and Carenno (Latitude: 45° 48′ N, Longitude: 9° 27′ E, Elevation: 640 m a.s.l.). The seeds were then sown at the beginning of May 2017 in the experimental fields of Landriano (Latitude: 45° 19′ N, Longitude 9° 15′ E, Elevation: 90 m a.s.l.) in the same agronomic and environmental conditions so as to minimize the variation of the nutritional characteristics of the different seeds due to environmental effects (soil, climate and agronomic techniques). In addition to the four landraces one of the most common commercial hybrids (hybrid B73/Mo17), used in various agronomic and genetic research projects (Eichten et al. 2011; Cassani et al. 2017; Puglisi et al. 2018), was also sown to be used as a control. About 200 seeds, for all genotypes tested, were sown in adjacent rows (the spacing between the rows was 0.70 m and approximately 0.22 m along the rows), under the same agronomic conditions and performing controlled pollination. The experimental fields were in a maize–maize rotation with a standard soil fertilization (240 kg/ha of nitrogen). In early April, the soil was tilled by a disk plow and fine tilled by a harrow. Weed management was carried out by a pre and post emergent herbicides treatment. The fields were not irrigated. About 80 ears of each genotype were shelled (October 2017) and the seeds obtained were mixed to create a single bulk for nutritional analysis.

Nutritional analysis of the seed was performed during 2018 after grinding about 500 g of seeds of each type of corn. In detail, the following parameters were evaluated (each analysis was performed in triplicate):

- Metals content—for the determination of Mg, K, Ca, Mn, Fe, Cu, Zn and P, 0.3 g of maize flour samples were digested by a microwave digestor system (Anton Paar MULTIWAVE-ECO) in Teflon tubes filled with 10 ml of 65% HNO_3 by applying a one-step temperature ramp (at 210 °C in 10 min, maintained for 10 min). After 20 min of cooling time, the mineralized samples were transferred into polypropylene test tubes. Samples were diluted 1:40 with MILLI-Q water and the concentration of elements was measured by ICP-MS (BRUKER Aurora-M90 ICP-MS). An aliquot of a 2 mg l^{-1} of an internal standard solution (72Ge, 89Y, 159 Tb) was added both to samples and calibration curve to give a final concentration of 20 µg l^{-1}. Typical polyatomic analysis interferences were removed by using CRI (Collision-Reaction-Interface) with an H2 flow of 93 ml min^{-1} flown through skimmer cone.

- Dry matter/moisture, ash, crude fiber and ether extract (fat) were analysed, using approximately 50 g of seeds for each genotype, according to AOAC standard methods (AOAC 2000).
- Protein quantification was determined on the basis of the total nitrogen content according to Kjeldahl (Liu and Pan 2017) by using a Buchi KC530 apparatus. A converting factor of 6.25 was used.
- Total starch amounts were determined according to the AOAC 2002.02 by using a Megazyme kit (Wicklow, Ireland), following the manufacturer's instructions. For sample preparation, kernels were ground and about 1 g of each flour was incubated with 20 ml of dimethyl sulfoxide and 5 ml of 8 M HCl for 1 h at 60 °C under vigorous shaking. Then, 50 ml of deionized water were added, pH adjusted to 4.5 with 5 M NaOH, samples were finally filtered through Whatman filter paper and used for the assay.
- Antioxidant activity was carried out using radical conversion assay based on the α-diphenyl-β-picrylhydrazyl (DPPH) reagent (MacDonald-Wicks et al. 2006). For sample preparation, kernels were ground and about 1 g of each flour was extracted overnight at 25 °C under mild shaking with 10 ml of an ethanol/HCl mixture (65 volumes of 95% ethanol and 35 volumes of aqueous 0.3 M HCl). Each of the samples was diluted serially with methanol. Each of these samples was then further diluted with methanol. 200 µl of sample were respectively taken and the volume was made uniformly to 1 ml using the DPPH solution previously prepared: 800 µl DPPH was added to each sample.
- Phytates (phytic acid) content were quantified enzymatically (McKie and McCleary 2016), by using a Megazyme kit (Wicklow, Ireland), following the manufacturer's instructions.
- Polyphenols content was determined according to Folin–Ciocalteau (Singleton et al. 1999). For sample preparation, kernels were ground and about 1 g of each flour was extracted overnight at 25 °C under mild shaking with 10 ml of an ethanol/HCl mixture (65 volumes of 95% ethanol and 35 volumes of aqueous 0.3 M HCl). Aliquots of extract (50 µl) were taken, and then 500 µl of Folin reagent, 2 ml of 15% Na_2CO_3 and 9.5 ml of water were added. After 1 h of incubation at room temperature, the absorbance was read at 765 nm at the spectrophotometer. A calibration curve was prepared, using gallic acid as a standard for quantification.

In addition, SDS-PAGE and 2D-electrophoretic (IEF/SDS-PAGE) protein profiles were compared for the four landraces of maize. Total proteins were extracted from the kernels according to Magni et al. (2007). 1D- and 2D-electrophoretic analysis was performed according to Capraro et al. (2018).

The seed nutritional data of each genotype were used to perform analysis of variance (one-way ANOVA) and principal component analysis (PCA) in order to highlight the differences/similarities among the genotypes and the main variables that differentiated them. For antioxidant activity the opposite numbers values were used. Statistical analysis was performed using R 3.5.1 (R Development Core Team 2018).

Results

Lombardy landraces

The census of the herbaceous landraces currently cultivated in Lombardy provided 237 completed questionnaires and allowed the identification of 72 landraces (Fig. 1) mostly cultivated/conserved in mountain areas (Fig. 2). Most of them belong to the Graminaceae family (Fig. 3) and 60% are *Zea mays*. The Leguminosae represent the second group in terms of number and 84% are bean landraces (*Phaseolus* spp.) which are mainly cultivated in the mountain areas of Lombardy. The Cucurbitaceae are mostly represented by melons (*Cucumis melo*) (40%) and squashes (*Cucurbita maxima*) (30%) cultivated in the plain (Po Valley), while most of the Solanaceae are potatoes (*Solanum tuberosum*) (77%) grown in mountain areas. 70% of the Liliaceae are onion (*Allium cepa*) landraces. More information on the landraces identified is reported in Online Resource 1 and in a specific section of the UNIMONT web site (https://www.unimontagna.it/servizi/agrobiodiversita-vegetale/) which is free to use. There are 36 associations and organizations distributed throughout the Lombardy region that deal with the conservation, study and/or enhancement of agro-biodiversity (Fig. 4). 24 of them (67%) are associations or consortia of farmers that deal, in most cases, with the cultivation and enhancement of a single landrace. Therefore, most of the Lombardy landraces are cultivated and conserved by individual farmers (mostly hobby farmers) who are not affiliated with any association/institution. Analysis of the completed questionnaires showed that a large number (60%) of the farmers who wanted to indicate their age (97), are over 50. This is in accordance with the results of the research carried out in the inland areas of the Basilicata Region (Southern Italy) by Montesano et al. (2012). There are very few scientific studies concerning specific Lombardy landraces and these include only 8 (11%) of the landraces surveyed: "Nustran" and "Curunin" buckwheats (*Fagopyrum esculentum*) (Barcaccia et al. 2016; Capraro et al. 2018), "Nero Spinoso" maize (*Zea mays*) (Cassani et al. 2017; Puglisi et al. 2018), "Spinato di Gandino" maize (Puglisi et al. 2018), "Copafam" bean (*Phaseolus coccineus*) (Giupponi et al. 2018), "Berrettina di Lungavilla" and "Cappello da prete mantovana" squashes (*Cucurbita maxima*) (Orsenigo et al. 2018), "Grano siberiano valtellinese" Tartary buckwheat (*Fagopyrum tataricum*) (Giacomini 1954; Capraro et al. 2018; Giupponi et al. 2019). Only 9 (12.5%) Lombardy landraces are on the European Register of Conservation Varieties: 4 maize ("Spinato di Gandino", "Rostrato Rosso di Rovetta", "Nero Spinoso", "Scagliolo di Carenno"), 2 onions ("Rossa di Breme" and "Dorata di Voghera"), 1 rice ("Vialone nero di S. Alessio"), 1 squash ("Cappello da prete mantovana") and 1 bean ("Borlotto di Gambolò") (Fig. 2).

Nutritional features of maize landraces

Table 1 shows the nutritional analysis values of the Lombardy maize landraces registered in the European Register of Conservation Varieties and of the B73/Mo17 hybrid used as a control, and reports the *p*-values returned by ANOVA test. The five genotypes differ significantly ($p < 0.01$) from each other for most of the characteristics examined. Compared to the control, the seeds of the four landraces have a greater content in moisture, protein, P and phytic acid; they are instead poorer in starch, Mg and Zn. "Nero Spinoso" has the highest amount of polyphenols and Ca. "Spinato di Gandino"

Fig. 1 The 72 herbaceous landraces cultivated in Lombardy: **1** "Nero Spinoso" maize, **2** "Spinato di Gandino" maize, **3** "Rostrato Rosso di Rovetta" maize, **4** "Scagliolo di Carenno" maize, **5** "Quarantino bresciano" maize, **6** "Mais delle Fiorine", **7** "Orobico brembano" maize, **8** "Ottofile pavese" maize, **9** "Rostrato Valchiavenna" maize, **10** "Pop corn" maize, **11** "Taiolone" maize, **12** "Ottofile mantovano" maize, **13** "Ottofile bianco mantovano" maize, **14** "Vialone nero di S. Alessio" rice, **15** "Dellarole" rice, **16** "Rosa Marchetti" rice, **17** "Chinese originario" rice, **18** "Orzo di Vezza", **19** "Orzo di Pedenosso", **20** "Segale di Doverio", **21** "Grano siberiano valtellinese", **22** "Curunin" buckwheat, **23** "Nustran" buckwheat, **24** "Patata di Bossico", **25** "Patata blu di Valtellina", **26** "Patata San Carlo", **27** "Patata bianca di Esino", **28** "Patata bianca di Starleggia", **29** "Patata rossa di Starleggia", **30** "Patata di Schilpario", **31** "Bianco mantovano" pepper, **32** "Peperone di Voghera", **33** "Emma" bean, **34** "Borlotto di Gambolò" bean, **35** "Mangiatutto di Ossimo" haricot, **36** "Bianco di Edolo" haricot, **37** "Cornetto di Loritto", **38** "Cornetto torto", **39** "Dihiplì" bean, **40** "Borlotto nostrano" bean, **41** "Fagiolo di Cevo", **42** "Fagiolo di Garda", **43** "Fagiolo di Zazza", **44** "Fagiolino zio Doro", **45** "Copafom" bean, **46** "Fagiolo di Sussia", **47** "Pisello di Miradolo Terme", **48** "Fava di montagna", **49** "Caffè amaro", **50** "Brutto ma buono" beans, **51** "Copafam" bean, **52** "Paglierina di Sermide" onion, **53** "Dorata di Voghera" onion, **54** "Rossa di Breme" onion, **55** "Cipolla di Brunate", **56** "Hispisighì" onion, **57** "Asparago di San Benedetto Po", **58** "Montina" asparagus, **59** "Moscatello" melon, **60** "Retato di Calvenzano" melon, **61** "Rampeghin piccolo" melon, **62** "Zatta" melon, **63** "Anguria da mostarda a semi rossi", **64** "Anguria da mostarda a semi verdi", **65** "Cappello da prete mantovana" squash, **66** "Bertagnina di Dorno" squash, **67** "Berrettina di Lungavilla" squash, **68** "Ciuenlai" quechua, **69** "Radici di Soncino", **70** "Carciofo di Malegno", **71** "Spinacio nostrano", **72** "Rapa di Lozio"

Fig. 1 (continued)

is the richest in fats, K and Cu while it is the poorest in fiber, polyphenols, Ca, Mn and Fe. "Rostrato Rosso di Rovetta" is the richest in starch (compared to the other three landraces) and the poorest in fats, Mg, K and Zn. "Scagliolo di Carenno" is the richest in protein, Mn, Fe and P, and is the poorest in starch and Cu. The landrace that has the greatest antioxidant activity (i.e. the lowest concentration of the sample required to halve the DPPH) is "Nero Spinoso" followed by "Rostrato Rosso di Rovetta". "Scagliolo di Carenno" is the one with the lowest antioxidant activity and with the highest content in phytic acid. The PCA biplot (Fig. 5) shows that the variables that most affect the ordering of samples along the first axis (PC1) are: the content of Ca, moisture and protein, which decrease along the PC1, and the content of Zn and K whose values increase along the PC1. Along the second axes (PC2) the samples are mainly ordered according to antioxidant activity and Cu content, whose values increase along the axis, and Fe and phytic acid content which decrease along the axis. The SDS-PAGE protein profile (Fig. 6) shows that, from a qualitative point of view, the four samples are quite similar to each other. All have a high fraction of α-zein (molecular weight bands

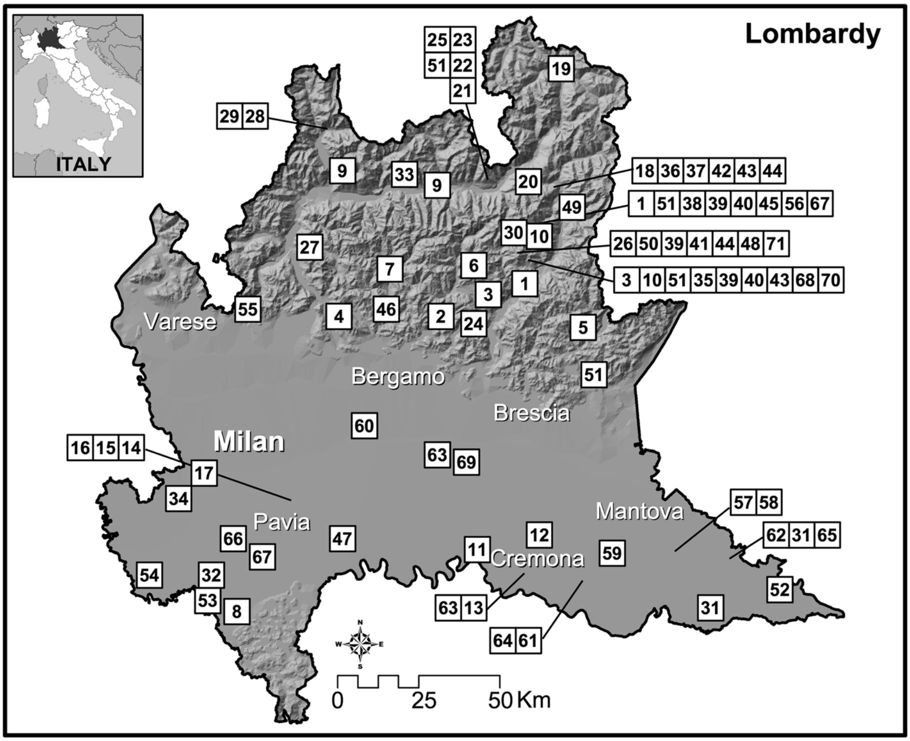

Fig. 2 Localization of the 72 landraces of Lombardy. The numeric identification code of each landrace is the same used in Fig. 1

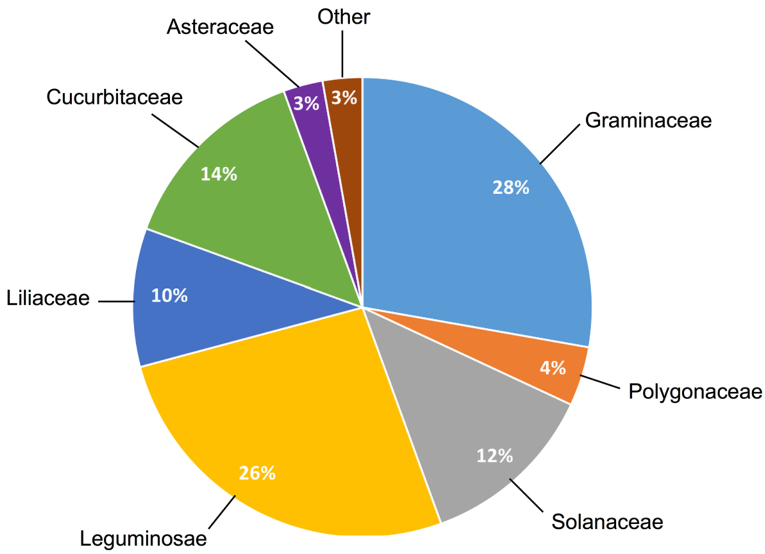

Fig. 3 Subdivision of the herbaceous landraces of Lombardy according to plant family

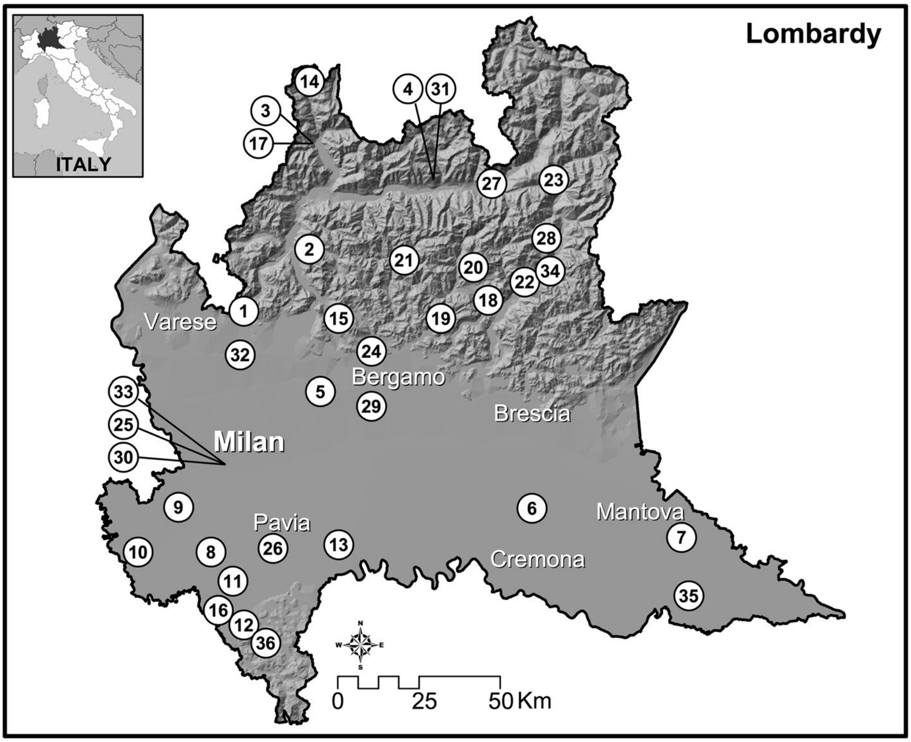

Fig. 4 Location of associations/bodies involved in the study, conservation and enhancement of plant agro-biodiversity in Lombardy: 1 Cipolla di Brunate association, 2 Patata Bianca di Esino consortium, 3 Patata di Starleggia association, 4 Patrimont Valtellina e Valchiavenna association, 5 Ruralp association, 6 L'Antica Terra social cooperative, 7 Consorzio Agrituristico Mantovano consortium, 8 Produttori di Zucca Bertagnina di Dorno association, 9 Produttori Fagiolo Borlotto di Gambolò consortium, 10 Municipality of Breme, 11 Zucca Berrettina di Lungavilla association, 12 Tutela e Valorizzazione Peperone di Voghera (PepeVo) association, 13 Amici di Miradolo association, 14 Giardino Alpino Valcava association, 15 Agricoltori Valle San Martino association, 16 Cipolla Dorata di Voghera association, 17 Comunità Montana Valchiavenna, 18 Rosso Mais association, 19 Mais Spinato di Gandino association, 20 Grani Asta del Serio association, 21 Cerealicoltori Brembani association, 22 Mais Nero Spinoso association, 23 Ge.S.Di.Mont. Research Centre, 24 Lorenzo Rota Botanical Garden, 25 University of Milan, 26 University of Pavia, 27 Pro Specie Rara association, 28 Biodistretto Valle Camonica, 29 CREA Research Center (center for maize cultivation), 30 CREA Research Centre (seed testing and certification), 31 Fondazione Fojanini di Studi Superiori Research Centre, 32 Fondazione Minoprio Research Centre, 33 Lombardy Region, 34 Adamello Regional Park, 35 Asparago S. Benedetto Po association, 36 Promozione Sociale Grani di Tradizione dell'Oltrepò association

within 19–22 kDa) that is the most abundant fraction (more than 70%) of the total zein (Anderson and Lamsal 2011). However, with a more in-depth analysis carried out by 2D-electrophoresis, significant differences become evident (Fig. 7). In particular, "Nero Spinoso" has protein fractions with high molecular weight and protein fractions of molecular weight between 19 and 22 kDa (zein) that are not present in other landraces, while "Spinato di Gandino" and "Scagliolo di Carenno" have a protein fraction (of molecular weight corresponding to the zeins) which differentiates them from other samples.

Table 1 Results of nutritional analysis of the seeds of maize landrace and of hybrid B73/Mo17. *P*-values returned by one-way ANOVA are reported

	Nero Spinoso		Spinato di Gandino		Rostrato Rosso di Rovetta		Scagliolo di Carenno		Hybrid B73/Mo17		*p* value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Moisture (%)	9.70	0.15	8.91	0.71	9.10	0.71	9.08	0.31	7.68	0.34	< 0.001*
Ash (%)	1.76	0.40	1.84	0.06	1.41	0.02	1.77	0.08	1.79	0.05	< 0.001*
Protein (% DW)	12.49	0.59	11.88	1.32	12.28	0.70	12.71	0.37	8.54	0.29	< 0.001*
Fat (% DW)	4.53	0.04	6.52	1.46	4.02	0.31	5.85	0.50	4.63	0.02	0.001*
Starch (% FW)	77.60	0.49	78.80	0.14	79.80	1.13	75.20	1.48	80.65	0.64	< 0.001*
Fiber (% DW)	1.44	0.04	1.23	0.01	1.36	0.14	1.44	0.09	1.35	0.06	0.001*
Antioxidant activity (mg ml^{-1})	0.87	0.03	1.10	0.04	0.93	0.05	1.74	0.08	0.99	0.03	< 0.001*
Phytic acid (% FW)	1.27	0.14	1.27	0.11	1.31	0.03	1.54	0.09	1.03	0.05	< 0.001*
Phenolic content (GAE)	0.16	0.02	0.11	0.03	0.15	0.01	0.12	0.02	0.12	0.02	0.036
Mg (g kg^{-1})	1.74	0.15	1.76	0.07	1.48	0.04	1.88	0.02	1.92	0.09	< 0.001*
K (g kg^{-1})	4.58	0.40	5.26	0.13	3.65	0.12	4.57	0.07	5.03	0.11	< 0.001*
Ca (mg kg^{-1})	67.18	16.00	39.01	3.56	61.88	14.03	58.81	8.94	53.94	4.65	0.168
Mn (mg kg^{-1})	6.33	0.50	5.37	0.03	6.02	0.29	9.40	0.14	9.36	0.05	< 0.001*
Fe (mg kg^{-1})	24.36	2.49	22.36	1.57	24.14	1.23	29.78	1.17	25.17	1.34	0.001*
Cu (mg kg^{-1})	2.14	0.17	2.25	0.23	1.91	0.12	1.33	0.24	1.61	0.31	< 0.001*
Zn (mg kg^{-1})	21.64	1.14	24.82	1.11	19.11	1.24	22.75	0.41	27.96	1.69	< 0.001*
P (g kg^{-1})	4.83	0.13	4.58	0.12	4.24	0.11	4.94	0.05	3.90	0.16	< 0.001*

*Significant (*p*-value < 0.01)

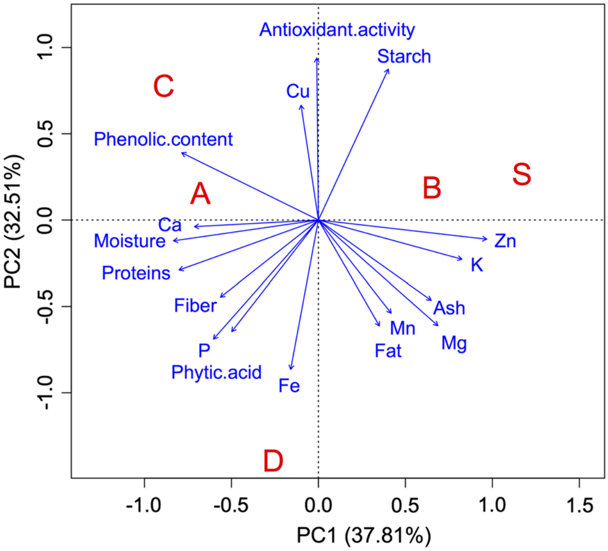

Fig. 5 PCA biplot of maize samples: a "Nero Spinoso"; b "Spinato di Gandino"; c "Rostrato Rosso di Rovetta"; d "Scagliolo di Carenno"; s Hybrid B73/Mo17. The first two axes (PC1 and PC2) explain 70.32% of the total variance

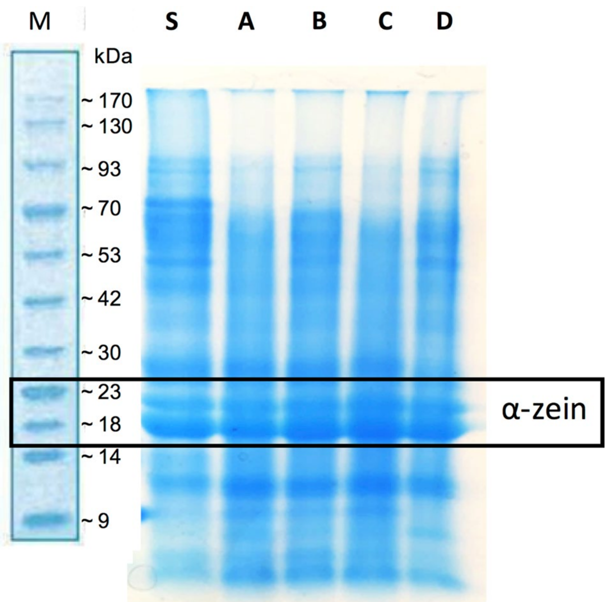

Fig. 6 One-dimension electrophoretic profiles of protein extracted from seeds of "Nero Spinoso" (A), "Spinato di Gandino" (B), "Rostrato Rosso di Rovetta" (C), "Scagliolo di Carenno" (D), Hybrid B73/Mo17 (S). M, molecular weight marker. The α-zein are highlighted in the box

Discussions

The analysis of the data collected in the census of the landraces of Lombardy has revealed a fair number of traditional varieties still present in the territory, although most of them are at great risk of extinction as they are cultivated by just a few farmers (mostly hobbyists). Unfortunately it is difficult to calculate the loss of plant agro-biodiversity in Lombardy due to the lack of data referring to past situations. Such loss can however be estimated by

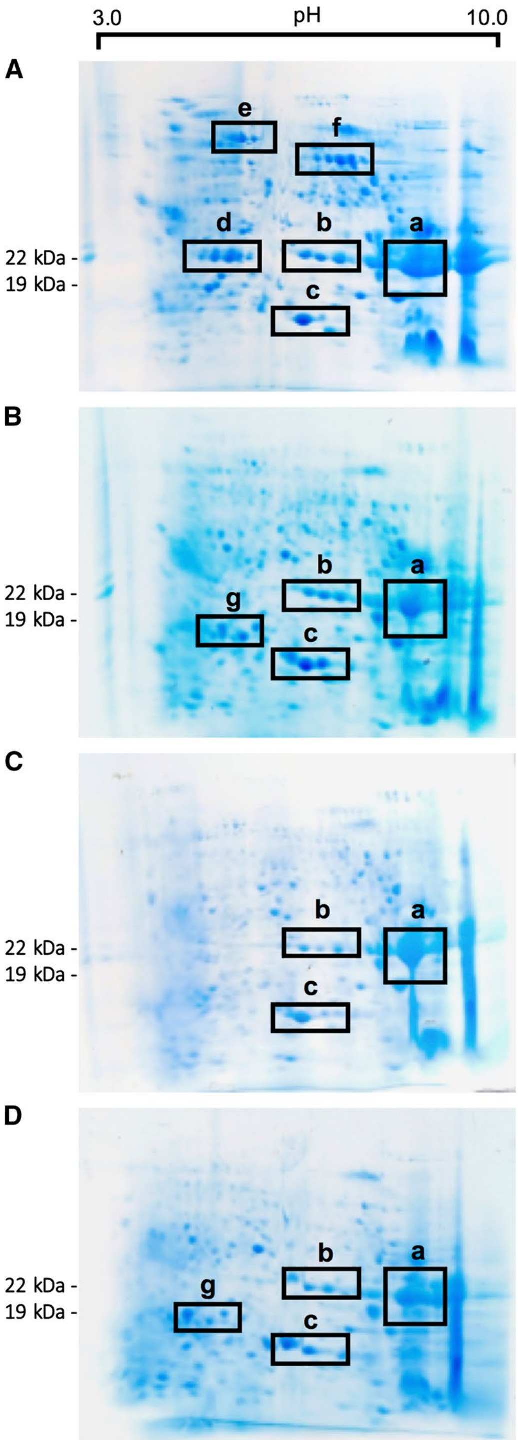

Fig. 7 Two-dimension electrophoretic maps of proteins extracted from seeds of "Nero Spinoso" (**a**), "Spinato di Gandino" (**b**), "Rostrato Rosso di Rovetta" (**c**), "Scagliolo di Carenno" (**d**). Some protein fractions (a, b and c) are present in all the samples while others (d, e, f and g) are present only in some landraces

taking into consideration the data referring to maize landraces. In fact, it is known that, until the 1940s and 1950s, at least 54 landraces were cultivated in Lombardy (Bertolini 2002) while today, based on the data collected in this research, there are just 12. Therefore, as regards Lombardy maize, 78% of the landraces cultivated in situ would have been lost over the last 70-80 years, a value that is in line with FAO estimates on the global loss of plant agro-biodiversity (FAO 2004).

Fortunately, the 54 landraces of Lombardy maize cultivated in the last century have been preserved in seed banks (Bertolini 2002) but this is an exception since most of the other landraces (vegetables and minor cereals) that used to be grown have not been conserved in seed banks and, moreover, their extinction rate could be much higher than that of maize landraces. Analysis of the data has shown a certain variability of the taxa (species, genus, family) to which the various herbaceous landraces belong and this seems due to the fact that Lombardy has a territory with very diversified environments that have favored the cultivation/conservation of different species. In fact all the Cucurbitaceae and the rice landraces are present in the plain (Po Valley) while beans, potatoes, and minor cereals are mostly widespread in mountain environments (Fig. 2), where they find the conditions suitable for their growth or where, more simply, the specific environmental conditions have not allowed the cultivation of more productive species.

Most of the landraces in the census are to be found in mountain (Alps and pre-Alps) and hilly areas since most of the lowland areas have undergone major urbanization and land degradation (Giupponi et al. 2013, 2015) during the last century and the large farms that currently remain in the Po Valley only cultivate selected and highly productive hybrids or commercial varieties. Hence, the mountain territories of Lombardy represent agro-biodiversity hotspots whose heritage should represent a resource for the development of such areas (Giorgi and Scheurer 2015), which are instead still undergoing the phenomenon of the abandonment of the Alps by man (which involves the abandonment of agricultural practices and the loss of landraces) in progress since the middle of the last century (NORDREGIO 2004; Keenleyside and Tucker 2010; Terres et al. 2013).

In order to conserve and enhance agro-biodiversity some farmers have formed associations/consortia although as most of the landraces identified are cultivated/conserved by individual hobby farmers who are not part of any association. Moreover, despite their great commitment, each association manages/enhances its landraces independently and with different strategies, at times without the support of specialized bodies or experts who could advise on the use of modern agronomic tools and techniques in order to avoid seed hybridization and/or genetic erosion, and check that the plant material is healthy. The fact that only a small part (9) of the landraces surveyed has been registered in the European Register of Conservation Varieties can be justified by the fact that the regional law that allows such registration is quite recent (Legislative Decree 2013/9167 2013) and probably still little known by farmers. A better assessment of the effectiveness of this regulatory instrument should therefore be carried out in coming years. The fact is that most of the landraces that today are the basis of successful agri-food chains, including the four maize landraces considered in this study, have been registered in the European Register of Conservation Varieties, which not only means that the landraces themselves are conserved, but also protects the territory where they have been, and currently still are, cultivated. The production of food products which are unique and linked to specific territories could provide employment for young people and counteract the abandonment of rural and mountain areas even though, at the present time, many of those who grow landraces in Lombardy and other areas of Italy are still elderly farmers (Negri 2003; Montesano et al. 2012). Generational change in developed countries like Italy has probably been blocked by the changes in socio-economic models that have been taking place since the second half of the last century, and which have increasingly alienated young people from agricultural practices in response to a strong expansion of the secondary and tertiary sector. Involving young people in modern sustainable agricultural models which also include the cultivation of landraces will require time but above all greater support from organizations that deal with training, conservation of the territory and biodiversity, and socio-economic development (United Nations 2015). Nevertheless, this study highlighted some cases in which custodian farmers were young (under the age of 40) and running successful multi-functional farms.

The nutritional analyses carried out on the four maize landraces considered highlighted the fact that they have characteristics that differentiate them both from commercial maize B73/Mo17 and from each other. It is interesting to note that the landraces with more pigmented kernels ("Nero Spinoso" and "Rostrato Rosso di Rovetta"), due to the greater presence of polyphenols, are those that have greater antioxidant activity. This is in accordance with Zilić et al. (2012), who stated that colored maize kernels have higher levels of health beneficial phytochemicals and antioxidant capacity than unpigmented ones. Hence, the "Nero Spinoso" and "Rostrato Rosso di Rovetta" landraces may be useful for the production of nutraceutical foods, although both these two landraces have a relevant content of phytic acid compared to the commercial hybrid. Phytates are anti-nutritional as they limit intestinal absorption and the bioavailability of some minerals (mainly Zn and Fe) and amino acids in monogastric animals, including humans (Gupta et al. 2015). The higher content of protein and the lower concentration of starch in the landrace kernels (compared to the commercial maize) could be due to the fact that the genetic improvement of the maize varieties performed over the last decades has been aimed at obtaining varieties/hybrids having seeds with a higher starch content as well as plants with high productivity, resistant to corn borer attack (Malvar et al. 2007) and less demanding as regards water (Meseka et al. 2015). The 2D-electrophoretic maps highlighted some differences between the various maize landraces not attributable to climatic, pedological or edaphological causes, since the seeds are from the same cultivation area (Landriano). These differences therefore represent an authentic biodiversity source. In the future it would be interesting to identify all the different protein fractions found and associate them with the phenotypic or nutritional characteristics that characterize the four maize landraces. Furthermore, the production of electrophoretic maps for each landrace that is or will be used in agri-food supply chains could be useful for their traceability in food (Nazzaro et al. 2012) and therefore to counteract falsification. This aspect is, and always will be, of fundamental importance at a global level to support the typical local agri-food sector and therefore to favour the conservation of agro-biodiversity on farms and of all that depends on it: culture, traditions, landscape, agro-ecosystems and local economy.

Conclusion

This study assessed the presence of landraces currently cultivated in one of the most industrialized areas of the Alpine macro-Region and about which information was previously extremely limited. Indeed, only eight herbaceous landraces of Lombardy were reported in the first Italian inventory of in situ maintained landraces (Negri et al. 2013). This research also contributed to knowledge regarding the nutritional characteristics of four landraces of maize from Lombardy (for which information was only fragmentary), recently used for the creation of niche food chains. These data will be used by Lombardy Region for the conservation of its plant agro-biodiversity and will be included in the Italian national system for the conservation and enhancement of biodiversity of agricultural and food interest. Based on the results of this study, some actions are suggested below to help the Lombardy Region and Italy, as well as other developed or developing countries, to protect plant agro-biodiversity:

- Perform a census of (and/or regularly update census data) the landraces cultivated/conserved in situ and those conserved in seed banks in order to identify the genetic resources present in the various territories (Maxted et al. 2013; Pacicco et al. 2018), and create a Red list of landraces (Joshi et al. 2004, 2017);
- establish databases regarding farm diversity, maps of agro-biodiversity which are freely available online and continuously updated thanks to regular monitoring performed in close collaboration with local communities;
- characterize various aspects of the landraces, in particular those concerning agronomic and nutritional characteristics that have major repercussions on production activities and are important in ensuring food security (Frei and Becker 2004). In fact, scientific studies of landraces, as well as being financed more than biodiversity conservation projects, should provide results that can have a more tangible impact on the territory such as the set-up of sustainable and high-quality agri-food chains;
- disseminate information regarding landraces (and products/activities derived from them) as well as the legislative tools for their conservation, in order to make them known to farmers and consumers. At the same time, nutrition education actions should be encouraged to promote understanding of the importance of eating different foods not only from a health, but also from an ethical and conservation, point of view (Negri 2005; Esquinas-Alcázar 2010);
- Produce legislative instruments that allow the conservation of agrobiodiversity and landraces in particular. Specifically, laws should protect not only the single landrace but also the territory of which the landrace is typical, its historical and cultural heritage and the food products that derive from it;
- initiate policies to encourage coordination between farms, agritourisms and various bodies and associations that study and/or maintain landraces and develop strategies that are more inclusive and efficient (Laghetti et al. 2018; Hammer et al. 2018);
- create networks in order to link custodian farmers both with each other and with other actors interested in plant agrobiodiversity such as restaurateurs and managers of agritourisms. This can be done using both traditional tools, such as agrobiodiversity fairs, and innovative ones, such as social networks. The latter, as shown by the recently activated Facebook "Agrobiodiversità vegetale delle montagne lombarde" (https://www.facebook.com/groups/agrobiodiversitaUNIMONT/), can be extremely effective yet are simple to create/manage and have low costs. Such networks should be created at various levels, from the local to the supranational;
- create centres for the collection, analysis and distribution of landrace seeds so that researchers/professionals can control the material that is propagated and provide technical-scientific support to the custodian farmers of each landrace (Wood and Lenné 1997).

In conclusion, it is to be hoped that in future more and more studies will be carried out for the characterization and conservation of agro-biodiversity which, like this, involve territorial management bodies, universities and operators in the agri-food sector in a "team game" since the issue of the loss of genetic resources of agri-food interest is a problem, still too underestimated, to be solved urgently, with collaboration and method (Pacicco et al. 2018), in order to avoid irreversible damage that could affect both man and (agro-) ecosystems. Hence, we hope that the results of this case study can be useful for policy makers operating from a local to a supranational level so that they can act in the most appropriate way to improve the socio-economic conditions of various territories while conserving their agro-biodiversity.

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Compliance with ethical standards

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

References

Al-Turki TA, Al-Namazi AA, Masrahi YS (2018) Conservation of genetic resources for five traditional crops from Jazan, SW Saudi Arabia, at the KACST Gene-Bank. Saudi J Biol Sci. https://doi.org/10.1016/j.sjbs.2018.09.007

Anderson TJ, Lamsal BP (2011) Zein extraction from corn, corn products, and coproducts and modifications for various applications: a review. Cereal Chem 88:159–173

AOAC (2000) Official methods of analysis of AOAC. International 17th edition. Association of Analytical Communities, Gaithersburg, p 43–44

Barcaccia G, Volpato M, Gentili R, Abeli T, Galla G, Orsenigo S, Citterio S, Sgorbati S, Rossi G (2016) Genetic identity of common buckwheat (*Fagopyrum esculentum Moench*) landraces locally cultivated in the Alps. Genet Resour Crop Evol 63:639–651

Bertolini M (2002) Mais in Lombardia: varietà tradizionali. Istituto Sperimentale per la Cerealicoltura Sezione di Bergamo, Quaderni della Ricerca

Bragdon S (1996) The convention on biological diversity. Glob Environ Change 6:177–179

Camacho Villa TC, Maxted N, Scholten M, Ford-Lloyd B (2005) Defining and identifying crop landraces. Plant Genet Res 3:373–384

Capraro J, Magni C, Giorgi A, Duranti M, Scarafoni A (2018) Comparative 1D- and 2D-electrophoretic protein profiles of ancestral and modern buckwheat seeds grown in the Italian Alpine Region. Ital J Food Sci 30:497–503

Cassani E, Puglisi D, Cantaluppi E, Landoni M, Giupponi L, Giorgi A, Pilu R (2017) Genetic studies regarding the control of seed pigmentation of an ancient European pointed maize (*Zea mays*) rich in phlobaphenes: the "Nero Spinoso" from the Camonica valley. Genet Resour Crop Evol 64:761–773

CBD [Convention on Biological Diversity] (1992) Convention on biological diversity: text and annexes. Secretariat of the Convention on Biological Diversity, Montreal https://www.cbd.int/convention/text/. Accessed 11 April 2019

CBD [Convention on Biological Diversity] (2002) Global strategy for plant conservation. Secretariat of the Convention on Biological Diversity, Montreal. https://www.cbd.int/gspc/documents.shtml. Accessed 11 April 2019

CBD [Convention on Biological Diversity] (2010) Conference of the parties 10 decision X/17. Consolidated Update of the Global Strategy for Plant Conservation 2011–2020. Secretariat of the Convention on Biological Diversity, Montreal. https://www.cbd.int/decision/cop/?id=12283. Accessed 11 April 2019

Ceccarelli S (2012) Landraces: importance and use in breeding and environmentally friendly agronomic systems. In: Maxted et al (eds) Agrobiodiversity conservation: securing the diversity of crop wild relatives and landraces. CAB International, Oxfordshire, pp 103–117

Cleveland DA, Soleri D, Smith SE (1994) Do folk crop varieties have a role in sustainable agriculture? Bioscience 11:740–751

Directive 2008/62/EC (2008) Commission Directive 2008/62/EC of 20 June 2008 providing for certain derogations for acceptance of agricultural landraces and varieties which are naturally adapted to the local and regional conditions and threatened by genetic erosion and for marketing of seed and seed potatoes of those landraces and varieties. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32008L0062. Accessed 01 September 2019

Directive 2009/145/EC (2009) Commission Directive 2009/145/EC of 26 November 2009 providing for certain derogations, for acceptance of vegetable landraces and varieties which have been traditionally grown in particular localities and regions and are threatened by genetic erosion and of vegetable varieties with no intrinsic value for commercial crop production but developed for growing under particular conditions and for marketing of seed of those landraces and varieties. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32009L0145. Accessed 01 September 2019

Directive 2010/60/EU (2010) Commission Directive 2010/60/EU of 30 August 2010 providing for certain derogations for marketing of fodder plant seed mixtures intended for use in the preservation of the natural environment Text with EEA relevance. https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1567082342503&uri=CELEX:32010L0060. Accessed 01 September 2019

Eichten SR, Foerster JM, de Leon N, Kay Y, Yeh CT, Liu S, Jeddeloh JA, Schnable PS, Kaeppler SM, Springer NM (2011) B73-Mo17 near-isogenic lines demonstrate dispersed structural variation in maize. Plant Physiol 156:1679–1690

Esquinas-Alcázar J (2010) Protecting crop genetic diversity for food security: political, ethical and technical challenges. Nature 6:946–953

EU Commission (2010) EUROPE 2020 A strategy for smart, sustainable and inclusive growth. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52010DC2020. Accessed 11 April 2019

EU Commission (2017) Region innovation monitor plus. https://ec.europa.eu/growth/tools-databases/regional-innovation-monitor/base-profile/lombardy. Accessed 11 April 2019

FAO (1999) Agricultural biodiversity. Background Paper 1. FAO/Netherlands Conference of multifunctional character of agriculture and land. http://www.fao.org/mfcal/pdf/bp_1_agb.pdf. Accessed 11 April 2019

FAO (2004) Building on gender, Agrobiodiversity and local Knowledge. Food and agriculture organization of the United Nations, Rome. http://www.fao.org/docrep/007/y5609e/y5609e00.htm. Accessed 11 April 2019

FAO (2009) International treaty on plant genetic resources for food and agriculture. Food and agriculture Organization of the United Nations, Rome. http://www.fao.org/3/a-i0510e.pdf. Accessed 11 April 2019

FAO (2010) Second report on the state of the world's plant genetic resources for food and agriculture. Commission on Genetic Resources for Food and Agriculture. Food and Agriculture Organization of the United Nations, Rome

Fideghelli C, Engel P (2009) Biodiversity and local genetic resources: from knowledge to exploitation. Acta Hortic 817:295–310

Frankel OH, Brown AHD, Burdon JJ (1995) Conservation of plant biodiversity. Cambridge University Press, UK

Frei M, Becker K (2004) Agro-biodiversity in subsistence-oriented farming systems in a Philippine upland region: nutritional considerations. Biodivers Conserv 13:1591–1610

Frison EA, Cherfas J, Hodgkin T (2011) Agricultural biodiversity is essential for a sustainable improvement in food and nutrition security. Sustainability 3:238–253

Giacomini V (1954) Il grano siberiano (*Fagopyrum tataricum*) in Valtellina. Ramponi, Sondrio

Giorgi A, Scheurer T (2015) Alpine resources: assets for a promising future. Conclusions from the Forum Alpinum 2014. Mt Res Dev 35:414–415

Giupponi L, Corti C, Manfredi P, Cassinari C (2013) Application of the oristic-vegetational indexes system for the evaluation of the environmental quality of a semi-natural area of the Po Valley (Piacenza, Italy). Plant Sociol 50:47–56

Giupponi L, Corti C, Manfredi P (2015) The vegetation of the Borgotrebbia landfill (Piacenza, Italy): phytosociological and ecological characteristics. Plant Biosyst 149:865–874

Giupponi L, Tamburini A, Giorgi A (2018) Prospects for broader cultivation and commercialization of Copafam, a local variety of *Phaseolus coccineus*, in the Brescia Pre-Alps. Mt Res Dev 38:24–34

Giupponi L, Borgonovo G, Panseri S, Giorgi A (2019) Multidisciplinary study of a little known landrace of *Fagopyrum tataricum* Gaertn. of Valtellina (Italian Alps). Genet Resour Crop Evol 66:783–796

Gupta RK, Gangoliya SS, Singhcorresponding NK (2015) Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. J Food Sci Technol 52:676–684

Hammer K, Knüppfer H, Xhuveli L, Perrino P (1996) Estimating genetic erosion in landraces—two case studies. Genet Resour Crop Evol 43:329–336
Hammer K, Gladis T, Diederichsen A (2003) In situ and on-farm management of plant genetic resources. Eur J Agron 19:509–517
Hammer K, Montesano V, Direnzo P, Laghetti G (2018) Conservation of crop genetic resources in Italy with a focus on vegetables and a case study of a neglected race of *Brassica oleracea*. Agriculture 8:105
ISMEA (2014) Stime di produzione—Cereali autunnali, mais, semi oleosi. http://www.ismeamercati.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/4292. Accessed 11 April 2019
ISTAT (2010) Censimento Agricoltura 2010. http://dati-censimentoagricoltura.istat.it/Index.aspx. Accessed 11 April 2019
IUCN (2019) The IUCN red list of threatened species. Version 2019-1. http://dati-censimentoagricoltura.istat.it/Index.aspxhttps://www.iucnredlist.org. Accessed 11 April 2019
Jackson LE, Pascual U, Hodgkin T (2007) Utilizing and conserving agrobiodiversity in agricultural landscapes. Agr Ecosyst Environ 121:196–210
Joshi BK, Upadhyay MP, Gauchan D, Sthapit BR, Joshi KD (2004) Red listing of agricultural crop species, varieties and landraces. Nepal Agric Res J 5:73–80
Joshi BK, Ghimire KH, Gauchan D, Singh D, Paudel MN (2017) Agrobiodiversity: conservation strategies, methods and action plans. In: Joshi BK, Gauchan D (ed) Rebuilding local seed system of native crops in earthquake affected areas of Nepal, Proceedings of Sharingshop, Kathmandu. NAGRC, BI and Crop Trust, Nepal
Keenleyside C, Tucker GM (2010) Farmland abandonment in the EU: an assessment of trends and prospects report prepared for WWF. Institute for European Environmental Policy, London
Laghetti G, Hammer K, Perrino P (1993) Collecting in north-west Italy. FAO/IBPGR Plant Genet Res Newslett 91:23
Laghetti G, Bisignano V, Urbano M (2018) Genetic resources of vegetable crops and their safeguarding in Italy. Horticult Int J 2:72–74
Last L, Arndorfer M, Balàzs K, Dennis P, Dyman T et al (2014) Indicators for the on-farm assessment of crop cultivar and livestock breed diversity: a survey-based participatory approach. Biodivers Conserv 23:3051–3071
Law 2015/194 (2015) Disposizioni per la tutela e la valorizzazione della biodiversità di interesse agricolo e alimentare. https://www.gazzettaufficiale.it/eli/gu/2015/12/11/288/sg/pdf. Accessed 01 September 2019
Legislative Decree 2013/9167 (2013) Procedura per la presentazione e l'istruttoria delle domande di iscrizione alla sezione delle varietà da conservazione del registro nazionale delle varietà di specie agrarie e ortive. http://www.galdeiduelaghi.org/assets/Uploads/bandi/323/SEO4215-10-2013.pdf. Accessed 01 September 2019
Liu Z, Pan J (2017) A practical method for extending the biuret assay to protein determination of corn-based products. Food Chem 224:289–293
MacDonald-Wicks LK, Wood LG, Garg ML (2006) Methodology for the determination of biological antioxidant capacity in vitro: a review. J Sci Food Agric 86:2046–2056
Magni C, Scarafoni A, Herndl A et al (2007) Combined 2D electrophoretic approaches for the study of white lupin mature seed storage proteome. Phytochemistry 68:997–1007
Malvar RA, Butròn A, Alvarez A, Padilla G, Cartea ME, Revilla P, Ordàs A (2007) Yield performance of the European Union Maize Landrace Core Collection under multiple corn borer infestations. Crop Prot 26:775–781
Maxted N, Magos Brehm J, Kell S (2013) Resource book for preparation of national conservation plans for crop wild relatives and landraces. University of Birmingham, United Kingdom
McKie VA, McCleary BV (2016) A novel and rapid colorimetric method for measuring total phosphorus and phytic acid in foods and animal feeds. J AOAC Int 99:738–743
Meseka S, Menkir A, Obeng-Antwi K (2015) Exploitation of beneficial alleles from maize (*Zea mays*) landraces to enhance performance of an elite variety in water stress environments. Euphytica 201:149–160
Montesano V, Negro D, Sarli G, Logozzo G, Spagnoletti Zeuli P (2012) Landraces in Inland areas of the Basilicata region, Italy: monitoring and perspectives for on farm conservation. Genet Resour Crop Evol 59:701–716
Nazzaro F, Orlando P, Fratianni F, Di Luccia A, Coppola F (2012) Protein analysis-on-chip systems in foodomics. Nutrients 4:1475–1489
Negri V (2003) Landraces in central Italy: where and why they are conserved and perspectives for their on farm conservation. Genet Resour Crop Evol 50:871–885

Negri V (2005) Agro-biodiversity conservation in Europe: ethical issues. J Agric Environ Ethics 18:3–25
Negri V, Donnini D, Gigante D, Landucci F, Pacicco L, Panella L, Torricelli R, Venanzoni R (2013) The first Italian inventory of in situ maintained landraces. http://vnr.unipg.it/PGRSecure/html/national_inventory.html. Accessed 11 April 2019
Newton AC, Akar T, Baresel JP et al (2010) Cereal landraces for sustainable agriculture: a review. Agron Sust Dev 30:237–269
NORDREGIO (2004) Mountain Areas in Europe: Analysis of mountain areas in EU member states, acceding and other European countries. Commissioned report by the European Commission—DG Regional Policy, Brussels. https://ec.europa.eu/regional_policy/sources/docgener/studies/pdf/montagne/mount1.pdf. Accessed 11 April 2019
O'Donnell K, Sharrock S (2017) The contribution of botanic gardens to ex situ conservation through seed banking. Plant Divers 39:373–378
Orsenigo S, Abeli T, Schiavi M, Cauzzi P, Guzzon F, Ardenghi MNG, Rossi G, Vagge I (2018) Morphological characterisation of *Cucurbita maxima* Duchesne (Cucurbitaceae) landraces from the Po Valley (Northern Italy). Ital J Agron 13:338–342
Pacicco L, Bodesmo M, Torricelli R, Negri V (2018) A methodological approach to identify agro-biodiversity hotspots for priority in situ conservation of plant genetic resources. PLoS ONE 13:e0197709
Paton AJ, Brummitt N, Govaerts R, Harman K, Hinchcliffe S, Allkin B, Lughadha EN (2008) Target 1 of the global strategy for plant conservation: a working list of all known plant species progress and prospects. Taxon 57:602–611
Peres S (2016) Saving the gene pool for the future: seed banks as archives. Stud Hist Philos Biol 55:96–104
Pignatti S (1982) Flora d'Italia. Edagricole, Bologna
Puglisi D, Landoni M, Cassani E, Toschi I, Lucchini G, Cesari V, Borlini G, Scapin A, Pilu R (2018) Traditional farmers' varieties: a valuable source of genetic variability for biofortification programs. Maydica 63:1–10
R Development Core Team (2018) R: A language and environment or statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org. Accessed 11 April 2019
Rischkowsky B, Pilling D (2007) The State of the World's animal genetic Resources for food and agriculture—in brief. Food and Agriculture Organization of the United Nations, Rome
Sharrock S, Oldfield S, Wilson O (2014) Plant Conservation Report 2014: a review of progress in implementation of the Global Strategy for Plant Conservation 2011-2020. CBD Technical Series 81. Secretariat of the Convention on Biological Diversity and Botanic Gardens Conservation International, Montréal-Richmond
Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 299:152–178
Spataro G, Negri V (2013) The European seed legislation on conservation varieties: focus, implementation, present and future impact on landrace on farm conservation. Genet Resour Crop Evol 60:2421–2430
Srinivasan CC, Thirtle C, Palladino P (2003) Winter wheat in England and Wales, 1923–1995: what do indices of genetic diversity reveal? Plant Genet Resour 1:43–57
Terres JM, Nisini L, Anguiano E. (2013) Assessing the risk of farmland abandonment in the EU. Final report EUR 25783EN, Joint Research Centre of the European Commission, Luxembourg
United Nations (2015) Transforming our world: the 2030 Agenda for Sustainable Development. A/RES/70/1. https://undocs.org/A/RES/70/1. Accessed 11 April 2019
USDA (2017) Italy exporter guide 2017. Global Agricultural Information Network, report number: IT1771
Veteläinen M, Negri V, Maxted N (2009) European landraces: on-farm conservation management and use. Biodiversity technical bulletin 15. Biodiversity International, Rome
Wood D, Lenné JM (1997) The conservation of agrobiodiversity on-farm: questioning the emerging paradigm. Biodivers Conserv 6:109–129
Yadav OP, Bidinger FR (2007) Utilization, diversification and improvement of landraces for enhancing pearl millet productivity in arid environments. Ann Arid Zone 46:49–57
Zeven AC (1998) Landraces: a review of definitions and classifications. Euphytica 104:127–139
Zilić S, Serpen A, Akillioğlu G, Gökmen V, Vančetović J (2012) Phenolic compounds, carotenoids, anthocyanins, and antioxidant capacity of colored maize (*Zea mays*) kernels. J Agric Food Chem 60:1224–1231

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