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RESEARCH ARTICLE

Characterization of "Mais delle Fiorine" (*Zea mays* L.) and nutritional, morphometric and genetic comparison with other maize landraces of Lombardy region (Northern Italy)

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Abstract The loss of agrobiodiversity is a topic of global impact. On a local scale, Lombardy, in the Alpine macro-Region, has lost more than 78% of its plant agrobiodiversity. Only four maize (*Zea mays* L. subsp. *mays*) landraces of Lombardy are registered in the European Register of Conservation Varieties. However, there are other maize landraces in Lombardy such as "Mais delle Fiorine", which was characterized from an agronomic, morphometric, nutritional and genetic point of view in this research and then compared with the four other landraces already registered ("Spinato di Gandino", "Rostrato Rosso di Rovetta", "Scagliolo di Carenno" and "Nero Spinoso"). "Mais delle Fiorine" resulted richer in starch (81% ± 1.6) and zinc (35.8 ± 9.1 mg Kg^{-1}) and lower in phosphorus (3256.7 ± 204.2 mg Kg^{-1}). The kernels in the five landraces also differ in the mean shape that is obovate without beak. A genetic distinction between "Mais delle Fiorine" and the other varieties was observed, and in particular compared to "Nero Spinoso", while "Scagliolo di Carenno" and "Rostrato Rosso di Rovetta" showed great similarities. As regards agronomical trials, "Mais delle Fiorine" can grow from the Po Valley (90 m a.s.l.) to the mountain environments of the Seriana Valley (also over 900 m a.s.l.) without significant differences in grain yield. In addition, this landrace would seem able to tolerate environments where there is a greater probability of water stress.

Keywords Plant genetic resources · Landraces · Agro-biodiversity · Mountain agriculture · Outline analysis · Genetic distance

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Introduction

The conservation, study and promotion of agrobiodiversity are topics of considerable interest from a local to a global scale. In fact, about 75% of living beings worldwide (animals, plants and microorganisms), used directly or indirectly for food and agriculture (FAO 1999), were lost over the last century and three-quarters of food world-wide is currently produced by

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only twelve plant species and five animal species (Hammer et al. 1996; FAO 1999, 2004, 2010; Rischkowsky and Pilling 2007; Esquinas-Alcázar 2010). Lombardy, one of the largest and richest regions in Italy and in the Alpine macro-Region (EUSALP) (EU Commission 2017), has also lost more than 78% of its agrobiodiversity in the last decades (Giupponi et al. 2020c). Landraces are intended as dynamic populations of cultivated plants that have an 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). Landraces are often maintained in small fields or even in-home gardens (Zeven 1998; Negri 2003) and are therefore frequently threatened with extinction. According to Giupponi et al. (2020c), only 72 herbaceous landraces are cultivated in Lombardy, and most of them are endangered since they are cultivated in mountain areas by a small number of farmers, mostly hobbyists. Among the 72 Lombardy landraces 13 are maize (*Zea mays* L. subsp. *mays*).

In Italy the first information on maize arrived to cardinal Ascanio Sforza by a letter from Pietro Martire d'Anghiera dated 13 November 1494 (Brandolini and Brandolini 2009). During the XVI century several doctors-naturalists planted maize in botanical gardens of Italy and other European countries (France, Germany and Holland). In Italy maize turned from a botanical curiosity into an agricultural cereal only in the second half of XVI century (Messedaglia 1924; Brandolini 1970; Brandolini and Brandolini 2009). Since then, maize has spread widely in Italy and four centuries of cultivation have led to the constitution of many local cultivars and agroecotypes. Most of the agroecotypes belonged to the indurata (flint maize) and indentata (dent maize) groups although there were also agroecotypes of everta group (popcorn) (Brandolini and Brandolini 2009). The first evidence regarding the cultivation of corn in Lombardy dates back to 1556 (Ardenghi 2019) and, from then on, the cereal rapidly spread throughout the region.

Agroecotypes of flint maize for "polenta" production developed over the centuries (Brandolini and Brandolini 2006). Polenta is a traditional dish in Northern Italy. There is proof of the existence of more than 50 landraces of maize (mostly flint maize) until the 1950s in Lombardy (Bertolini 2002), belonging to various racial complexes (eight rows flints, conical flints, microsperma flints, Insubrian flints, early dwarf flints) (Brandolini and Brandolini 2009; Ardenghi 2019). Most of them are no longer grown but are still preserved in the germplasm bank (Maize Research Unit of CREA-MAC) of Stezzano (Bergamo Province, Lombardy).

Of the 13 landraces that are still actively grown in Lombardy only 4 ("Spinato di Gandino", "Rostrato Rosso di Rovetta", "Scagliolo di Carenno" and "Nero Spinoso") are registered in the European Register of Conservation Varieties, which is one of the main tools for the in situ (on farm) conservation of plant agrobiodiversity (Spataro and Negri 2013; Santamaria and Ronchi 2016) in Europe. The inclusion of the above-mentioned maize landraces in the European Register of Conservation Varieties has favoured their study and their promotion. In fact, many agronomic and nutritional characteristics of these varieties are now known (Puglisi et al. 2018; Giupponi et al. 2020c) and this information is useful in small agri-food chains. Moreover, the landraces (besides having an important historical-cultural value) very often also have a high nutritional value (Cassani et al. 2017; Giupponi et al. 2018, 2019) and characteristics that make them more resistant to more severe biotic and/or abiotic factors (Giupponi et al. 2019; Landoni et al. 2020). Their study and conservation therefore represent not only an ethical duty to counteract the loss of agrobiodiversity (Negri 2005) but can also be seen as an opportunity for the creation of unique agri-food chains (Fideghelli and Engel 2009; Frison et al. 2011) to generate income for mountain areas (Giorgi and Scheurer 2015), and for genetic crop improvement programs (Yadav and Bidinger 2007; Ceccarelli 2012; Puglisi et al. 2018).

In addition to the four maize landraces registered in the European Register of Conservation Varieties, in Lombardy there are other maize landraces still studied little or not at all, such as "Mais delle Fiorine" (Fig. 1). This is a conical flint maize cultivated in Clusone (Seriana Valley—Bergamo; Latitude: 45°52′55″N, Longitude: 9°55′31″E; elevation: 650 m a.s.l.) and surroundings until 1977 to produce flour for "polenta" (data of direct testimony). Only in 2014 did its cultivation recommence thanks to a reintroduction project carried out by the "Grani dell'Asta del Serio" association and the germplasm bank of Stezzano (Maize Research Unit of CREA-MAC) where the seeds were stored. In fact, "Mais

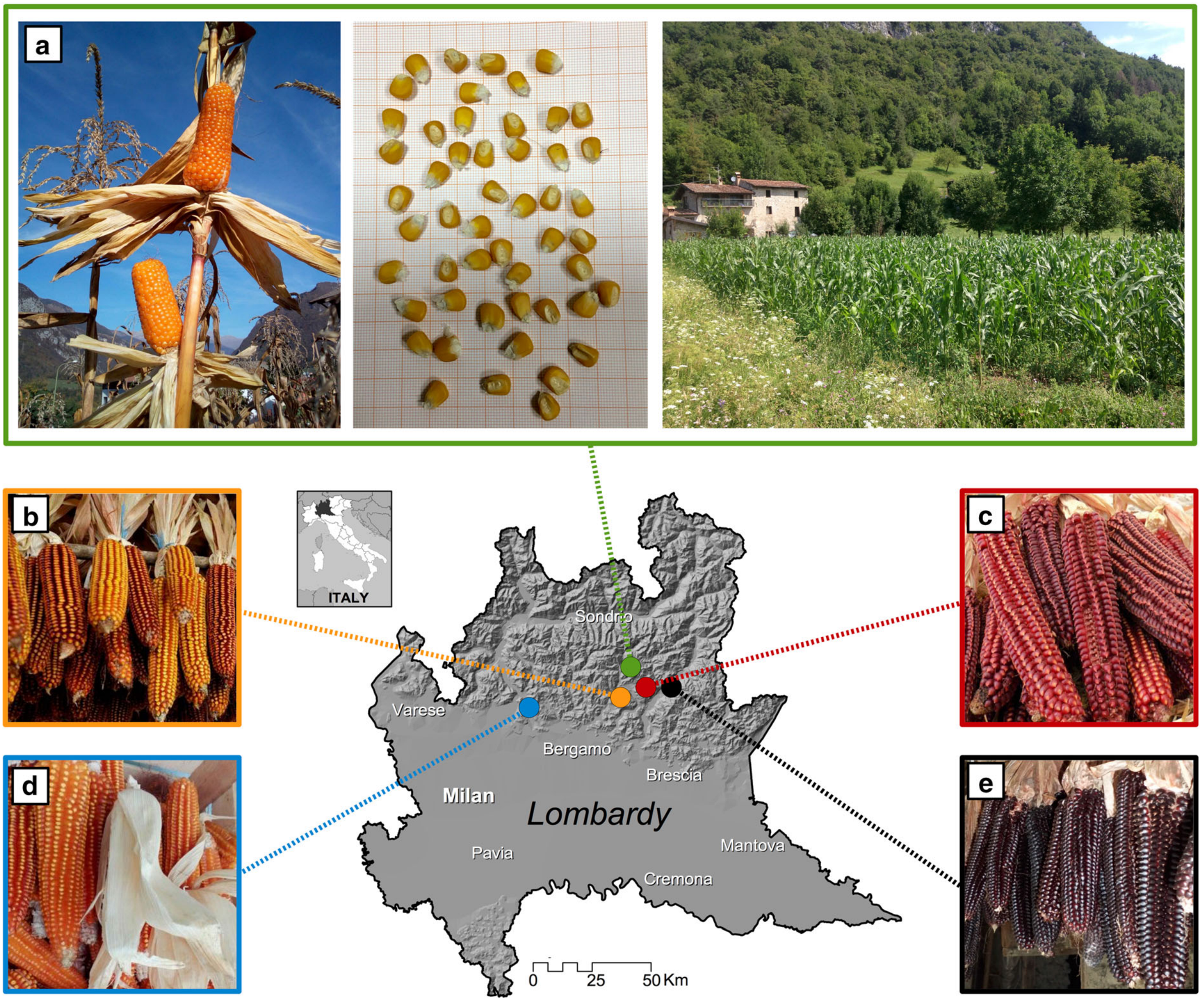


Fig. 1 "Mais delle Fiorine" (ears, kernels and plants in field) **a** and the other four maize landraces of Lombardy registered in the European Register of Conservation Varieties: "Spinato di Gandino" **b**, "Rostrato Rosso di Rovetta" **c**, "Scagliolo di Carenno" **d** and "Nero Spinoso" **e**

delle Fiorine" was collected and catalogued in 1952 (accession number: VA33) (Bertolini 2002) and was then stored in the aforementioned seed bank. "Mais delle Fiorine", compared to the other maize landraces registered in the European Register of Conservation Varieties, has small conical ears and non-beaked kernels. Currently, this landrace is cultivated in Seriana Valley, in Clusone and in some neighbouring areas included in the Orobie Bergamasche Regional Park (Giupponi and Giorgi 2017, 2019), by a few farmers (about ten) who produce flour highly appreciated by consumers. For this reason, the "Grani dell'Asta del Serio" association and the Municipality of Clusone would like to enhance this variety for which, however, there is no scientific data (agronomic, nutritional, genetic, etc.). Lombardy Region is also interested in the characterization of this landrace as it will shortly have to provide the data of its agri-food resources to the national system of conservation and enhancement of biodiversity of agricultural and food interest as established by Italian law 1st December 2015 n.194 ("Provisions for the conservation and enhancement of biodiversity of agricultural and food interest") (Santamaria and Ronchi 2016).

Based on the above considerations, this research aims to characterize "Mais delle Fiorine" from the agronomic, morphometric, nutritional, and genetic points of view. Agronomic data were collected in six experimental fields (five located in the mountain areas of Seriana Valley and one in the Po river Plain) to describe this variety also considering the differences attributable to the growing environment. The same was done for the nutritional analysis of the grain. Finally, the nutritional features, the shape of the kernels, and the genetic data of "Mais delle Fiorine" were compared with those of the other four Lombardy maize landraces registered in the European Register of Conservation Varieties ("Spinato di Gandino", "Rostrato Rosso di Rovetta", "Scagliolo di Carenno" and "Nero Spinoso") to highlight their differences and contribute to the knowledge of Italian/European plant agro-biodiversity.

Materials and methods

Plant material

The seed of the landraces "Spinato di Gandino" (VA 1304), "Rostrato Rosso di Rovetta" (VA1306)," Scagliolo di Carenno" (VA1210), "Nero Spinoso" (VA1269) used in this work were originally obtained from CREA-MAC (Stezzano, BG) and subsequently propagated by "sib pool" in the experimental field of the University of Milan located in Landriano (PV). The seeds of "Mais delle Fiorine" were collected "in situ" in November 2018 from the farmers of the "Grani Asta del Serio" association.

Experimental fields

The seeds of "Mais delle Fiorine" were sown at the beginning of May 2019 in five experimental fields located in the mountain areas of Seriana Valley and in one field located in the Po Plain (Table 1). 150–200 seeds were sown in adjacent rows in each experimental field. The spacing between the rows was approximately 0.70 m and 0.30 m along the rows. The experimental fields were in a maize–maize rotation with standard soil fertilization (about 220 kg/ha of nitrogen) and the maize plants were grown by conventional farming methods (pre-emergence herbicide was applied). The chemical-physical characteristics of the soil of each experimental field are reported in Table 2. The fields were not irrigated.

Agronomic analysis

The following agronomical data were collected in each experimental field where the "Mais delle Fiorine" was cultivated:

- *Plant height* the distance between the base of the male inflorescence (panicle) and ground level was measured considering 50 plants chosen randomly. Plant height was measured in mid-July 2019 during the period of full flowering using a measuring rod.
- *Insertion height of the first ear* measured considering the distance between the ground level and the insertion point of the first ear in the stem. This data was collected considering 50 plants (the same used for plant height).
- *Number of grains per ear* evaluated counting all the grains of 10 ears collected in each experimental field.
- *Ear length* measured considering 10 ears collected randomly in each experimental field.
- *1000-grains weight* assessed by weighing a sample of 100 kernels using an analytical weight scale (Precisa XB 220A, 0.1 mg). The test was performed in triplicate.

Nutritional analysis

A rate of grain (about 500 g) of "Mais delle Fiorine" produced in each experimental field was grinded using a rotor mill (Fritsch Variable Speed Rotor Mill Pulverisette 14), after which the following nutritional analysis were performed in triplicate:

- Moisture content, ash and crude fibre were evaluated according to the AOAC (2000) standard method.
- Protein percentage was calculated considering the total nitrogen content of the flour evaluated according to the Kjeldahl method (AOAC 2000) using automatic analyzer (Kjeltec Auto 1030).
- Fat (oil) quantification was determined on the basis of ether extract content evaluated using the Soxhlet method (AOAC 2000).
- Starch percentage was calculated as follows: Starch (%) = 100 − [Protein (%) + Fat (%)].
- Mg, K, Ca, Mn, Fe, Cu, Zn and P content was evaluated as follows: 0.3 g of maize flour samples were digested by a microwave digestor system

Table 1 Data of the six experimental fields where "Mais delle Fiorine" was grown. Annual solar irradiation was calculated using the Italian solar radiation atlas (ENEA 2019)

Experimental field	Municipality	Latitude N	Longitude E	Elevation (m a.s.l.)	Inclination (°)	Exposure (°)	Annual solar irradiation (MJ m^{-2})
A	Bricconi—Oltressenda Alta (Seriana Valley—BG)	45°55′13′'	9°56′54′'	941	14	216	5026
B	Fiorine—Clusone (Seriana Valley—BG)	45°53′10′'	9°55′32′'	580	2	118	4783
C	Piario (Seriana Valley -BG)	45°53′53′'	9°55′51′'	555	5	280	4703
D	Villa d'Ogna (Seriana Valley—BG)	45°54′27′'	9°55′45′'	530	2	240	4761
E	Cerete (Seriana Valley—BG)	45°51′45′'	9°59′12′'	470	4	28	4649
F	Landriano (Po Plain—PV)	45°19′03′'	9°15′22′'	90	0	–	5088

Table 2 Physico-chemical properties of soil of each experimental field (data source: Minoprio Analisi e Certificazioni s.r.l., 2019)

Parameter	Experimental field					
	A	B	C	D	E	F
Sand (%)	36.1	31.1	28.1	39.1	34.1	56.2
Silt (%)	52.1	54.1	51.1	50.1	60.1	37.2
Clay (%)	11.8	14.8	20.8	10.8	5.8	6.6
pH (H_2O)	6.9	6.9	7.0	7.3	7.2	6.5
Organic matter (%)	7.0	6.2	5.7	10.0	9.1	1.4
Organic carbon (g/kg)	40.8	35.7	33.0	58.16	52.9	8.12
Total nitrogen (g/kg)	4.7	4.0	3.6	5.8	3.1	3.3
C/N	8.7	8.9	9.2	10.0	17.1	2.5
CEC (meq/100 g)	39.9	28.4	30.1	49.2	19.1	10.6
Ca (meq/100 g)	23.32	14.7	17.15	30.42	10.43	6.65
Mg (meq/100 g)	3.58	4.22	3.31	4.39	2.41	1.88
K (meq/100 g)	0.55	0.20	0.47	0.31	0.07	1.10
P available (mg/kg)	81	56	57	102	46	102

The identification code of each field (capital letter) is the same used in Table 1

(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). 2 mg l^{-1} of an internal standard solution 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 ReactionInterface) with an H2 flow of 93 ml min^{-1} flown through skimmer cone. Average values of each metal were expressed as mg/kg grain flour.

Statistical analyses of agronomic and nutritional data

Agronomical and nutritional data were analyzed using one-way ANOVA test (once the assumptions of normality of group data and homogeneity of variances had been verified) to highlight the differences among the six experimental fields. Variables that resulted significant ($p < 0.01$) at the ANOVA test were employed in the canonical correspondence analysis (CCA) to highlight the most important variables that differentiate the fields. CCA was performed considering two data matrices: soil features and other data of the fields (elevation and annual irradiance) matrix and agronomic-nutritional data matrix. Linear and quadratic regression were carried out to identify relationships between elevation of the fields and other variables (irradiance, soil features, agronomic and nutritional parameters resulted significant at the ANOVA test). The significance ($p < 0.05$) of each linear model was evaluated by the *F*-test, and the coefficient of determination (R^2) was calculated to evaluate the goodness of fit of each model. ANOVA, CCA and regression analysis were performed using the "vegan" package of R 3.6.3 (R Development Core Team 2019) software.

Outline analysis of kernels

Fifty kernels for each maize landrace of Lombardy registered in the European Register of Conservation Varieties ("Mais delle Fiorine", "Spinato di Gandino", "Rostrato Rosso di Rovetta", "Scagliolo di Carenno" and "Nero Spinoso") were used for the outline analysis (elliptical Fourier descriptors analysis) (Kuhl and Giardina 1982). The grains were collected from various ears of plants cultivated in Landriano (Po Plain) (Table 1) in 2019 (in the same way as described for the experimental fields and performing controlled pollination) without considering basal and terminal parts of the ears where the kernels are often over/under-developed. Each grain was photographed in dorsal view (Bonhomme et al. 2017) using a digital camera (Canon EOS 2000D). The images were processed using Adobe Photoshop software: the shadows of the grains were removed, and the images were transformed into black and white. The outlines coordinates were extracted with Momocs 1.3.0 (Claude 2008; Bonhomme et al. 2014; Giupponi 2020) in an R environment (R Development Core Team 2019) and converted into Fourier coefficients considering 12 harmonics that gathered at least 99% of the total harmonic power (Bonhomme et al. 2014). In order to control left/right asymmetry the grains were flipped in the same direction, then a landmark was defined at their base as a starting point for importing outline coordinates. The contours were centered, and the outline analysis was carried out without numerical normalization. Principal component analysis (PCA) was carried out on the matrix of coefficients in order to reduce dimensionality and the samples were plotted on the first two axes (principal components). Linear discriminant analysis (LDA) of principal components (PCs) (Giupponi et al. 2020b) was carried out retaining 16 PCs. Mean coefficients per landrace were used to reconstruct the mean shape of the kernels (using 'MSHAPES' function of Momocs) and multivariate analysis of variance (MANOVA) was performed to evaluate the significance of grain shape differences between the five landraces.

Genetic analysis

Leaves from seedlings ($n = 15$) belonging to the 5 varieties were sampled and crushed and then used for DNA extraction using a protocol developed for maize (Dellaporta et al. 1983). DNA samples were amplified using 8 maize simple-sequence repeats (SSR) randomly chosen from MaizeGDB (http://www.maizegdb.org/ssr.php). The primer sequences of the 8 SSR markers were: phi323152 (left end 5′-TCAGGGAGCTCACCTACTACGG-3′; right end 5′-CACGACTGCACCGATTAGC-3′); ZAG105 (left end 5′-GACCGCCCGGGAACTGTAAGT-3′; right end 5′-AGAAAGAAGGTGACGCGCTTTTC-3′); nc009 (left end 5′-CGAAAGCGATCGAGAGACC-3′; right end 5′-CCTCTCTTCACCCCTTCCTT-3′); bnlg1028 (left end 5′-AGAAACGAACACAGCAGCT-3′; right end 5′-TGCATAGACAAAACCGACGT-3′); umc1185 (left end 5′-AGTAAAAGAGGCAAGGACTACGGC-3′; right end 5′-GCGGCGATATATACGAGGTTGT-3′); bnlg594 (left end 5′-CGACGCGCTTTGC-GAGTACCAGTACACA-3′; right end 5′-CTGCGTGCGTCCAGCCTCCACT-3′); phi095 (left end 5′-CCGATCGGCTTTATCACTGTTTTAGC-3′; right end 5′-ATGCACCATTCTAGCACTATAGCAACACT-3′); umc1506 (left end 5′-AAAAGAAACATGTTCAGTCGAGCG-3′; right end 5′-ATAAAGGTTGGCAAAACGTAGGCCT-3′).

PCRs were performed in a final volume of 10 µl, containing 10 ng DNA, 0.1 µM of each primer (forward and reverse), 200 mM of each dNTP, 2.5 mM MgCl2 and one unit of Taq DNA polymerase (Promega, Madison, WI). The reactions were carried out in a thermocycler, programmed for one pre-cycle at 94 °C for 2 min, followed by 35 cycles at 94 °C for 1 min, specific annealing temperature of the primers for 1 min, 72 °C for 1 min, and a final step at 72 °C for 5 min. The amplified fragments were fractionated by electrophoresis using 3% (w/v) agarose gels and stained with ethidium bromide (0.1 μg/μl).

Each band obtained by PCR was considered as an independent locus and numerically coded. A binary data matrix was generated using Microsoft Excel® and used for Principal Coordinate Analysis (PCoA), Analysis of Molecular Variance (AMOVA), and to estimate the Nei Genetic Distance (Pairwise Population Matrix of Nei Genetic Distance) by the program GenALEx v. 6.1 (Peakall and Smouse 2006). The dendogram was obtained by the algorithm UPGMA (Unweighted Pair Group Method Using Arithmetic Averages), using PAST program (Paleontological Statistics, version 3.12).

Results

Agronomic and nutritional features

Table 3 shows the characteristics of "Mais delle Fiorine" for each field and the *p*-values provided by the ANOVA tests. The values of the ten parameters show significant differences between the fields. In detail, the grains of field A have a high starch content and a low amount of protein and zinc. The grains of field B have the highest content of protein, zinc and phosphorus. The highest values of the height of the insertion of the ear and of fat and magnesium content were found in the samples of field C while the plants of field D have the highest value regarding the height of the plants. The kernels of field E have the highest fibre content and the lowest percentage of starch while in the field located in the Po Valley (F) the plants have grown less in height and produced grains poor in fat. As regards the length of the ear, the 1000-grains weight and the number of seeds per ear, the results of the ANOVA test did not show significant differences between the experimental fields (the grains yield of the plants was similar in all fields: about 1000–3000 kg/ha). "Mais delle Fiorine" has a higher starch content (81% ± 1.6) and a lower fibre content (0.91% ± 0.3) in comparison with other maize landraces of Lombardy (Giupponi et al. 2020c). Moreover, its kernels have a higher zinc content (35.8 ± 9.1 mg Kg^{-1}) and lower phosphorus content (3256.7 ± 204.2 mg Kg^{-1}).

Figure 2 shows the CCA biplot. Along the first axis (CCA1), which explains most of the total variance in the dataset (87.14%), the samples are sorted according to elevation (from left to right the elevation decreases). The CCA biplot suggests a relationship between elevation and other variables (such as fat, plant height and ear insertion height) but the results of the regression analysis (Table 4) show a quadratic relationship only between elevation and height of the plants, annual solar irradiation and sand content in the soil. The graphs in Fig. 3 show the relationships between the above-mentioned variables. "Mais delle Fiorine" plants grow less where there is greater solar radiation and sandy soils (fields A and F), therefore where there is a greater probability of water stress for the plants.

Shape of kernels

Figure 4 shows the PCA biplot of the Fourier coefficients calculated for the grains of the five maize landraces of Lombardy and the results of LDA. Along the PC1 the reconstructions of the kernel shape become more acute at the base while along the PC2 the apex becomes less acute. The samples of the five landraces are massively overlapped in Fig. 4a while the results of the LDA of PCs (Fig. 4b) show that "Mais delle Fiorine" differs from other landraces. In fact, the mean shape of its kernels appears different (Fig. 5). While in most landraces ("Scagliolo di Carenno", "Spinato di Gandino", "Nero Spinoso", "Rostrato Rosso di Rovetta") the mean shape is elliptical, in "Mais delle Fiorine" it is obovate as these grains have no beak. Results of the MANOVA test confirmed significant shape differences between the kernels of the five maize landraces ($F_{4,245} = 19.21$; *p*-value < 0.01).

Genetic comparison

With the aim of genetically comparing the five genotypes object of this study molecular markers were used to establish the genetic distance between them. For this purpose, 8 unlinked SSR and 15 individuals for each variety were used to create a pairwise genetic distance matrix for codominant data by GenALEx v. 6.1 program (Peakall and Smouse, 2006). As showed in Fig. 6. PCoA major patterns were built within the multivariate dataset, dendrogram

Table 3 Results of agronomic and nutritional analysis of plants/grains in each experimental field (identification code of each field is the same used in Table 1)

	Experimental field												p-value
	A		B		C		D		E		F		
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	
Agronomic data													
Plant height (cm)	200.60	13.61	216.30	18.96	218.58	21.23	220.80	21.10	218.34	15.83	193.33	30.77	**< 0.01**
Ear insertion height (cm)	85.70	8.50	87.28	13.27	99.80	15.79	98.74	7.57	95.76	10.75	83.50	17.40	**< 0.01**
Ear length (cm)	11.58	1.72	13.56	2.42	11.90	2.66	10.20	2.22	11.22	0.83	11.75	1.41	0.21
Number of grains per ear	373.20	57.22	432.80	103.42	365.00	116.26	272.40	56.40	370.40	94.40	327.80	87.90	0.15
1000-grains weight (g)	269.23	38.72	265.68	28.66	299.64	23.91	312.57	52.38	240.72	40.65	217.61	52.77	0.01
Nutritional data													
Moisture (%)	9.18	0.23	8.63	0.13	10.26	0.13	10.01	0.08	10.07	0.14	9.27	0.15	**< 0.01**
Ash (%)	1.56	0.07	1.68	0.02	1.69	0.06	1.60	0.03	1.59	0.09	1.71	0.04	0.02
Protein (% DW)	9.01	0.16	13.38	0.30	12.52	0.12	10.79	0.12	12.54	0.31	12.95	0.10	**< 0.01**
Fat (% DW)	4.33	0.09	3.90	0.45	4.91	0.11	4.45	0.16	4.36	0.18	3.83	0.10	**< 0.01**
Fiber (% DW)	1.07	0.11	0.72	0.16	0.55	0.22	0.65	0.16	1.26	0.39	1.20	0.08	**< 0.01**
Starch (% DW)	84.03	0.14	80.31	0.80	80.33	0.38	82.51	0.40	80.25	0.06	80.30	0.17	**< 0.01**
Mg (mg Kg^{-1})	1164.56	34.73	1300.10	48.81	1347.06	38.16	1132.70	82.20	1285.76	26.44	1217.22	65.41	**< 0.01**
K (mg Kg^{-1})	3854.84	69.50	4059.86	146.20	3731.88	58.16	3650.25	239.63	4035.93	114.12	3794.14	129.54	0.02
Ca (mg Kg^{-1})	125.74	142.81	95.98	65.38	47.20	7.08	61.35	2.35	56.94	16.89	53.61	51.56	0.70
Mn (mg Kg^{-1})	6.97	2.43	7.69	0.34	7.92	0.19	5.38	0.66	5.85	0.43	6.76	0.37	0.08
Fe (mg Kg^{-1})	9.06	7.85	26.06	6.42	28.23	3.17	12.51	7.57	24.81	9.53	28.47	8.55	0.03
Cu (mg Kg^{-1})	2.77	2.46	4.35	1.50	3.12	0.13	3.17	1.61	3.51	1.70	2.94	1.21	0.79
Zn (mg Kg^{-1})	26.96	1.39	48.91	6.95	35.57	2.39	28.09	3.59	30.61	4.13	44.69	5.48	**< 0.01**
P (mg Kg^{-1})	3070.51	103.61	3509.20	209.68	3270.97	78.29	2967.95	202.61	3312.21	92.42	3409.74	51.43	**< 0.01**

P-values returned by one-way ANOVA are reported and *p*-values < 0.01 are highlighted in bold

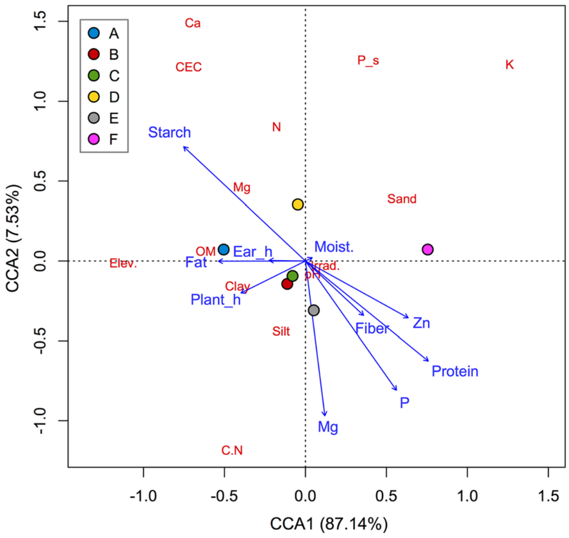


Fig. 2 CCA ordination biplot. The first two axes (CCAs) explain 94.67% of the total variance. The dots (A, B, C, D, E and F) are the experimental fields. Physico-chemical parameters of the fields are highlighted in red while those relating to the agronomic and nutritional characteristics of the plants/grains are highlighted in blue. Key: Ca, calcium in the soil; CEC, cation exchange capacity; Elev., elevation; OM, organic matter; Mg, magnesium in the soil; N, soil nitrogen; Clay, clay content; Silt, silt content; Sand, sand content; C.N, C/N ratio; pH, pH of soil; Irrad., annual solar irradiance; P_s, phosphorus in the soil; K, potassium; Starch, starch content; Ear_h, ear insertion height; Fat, fat content; Plant_h, plant height; Mg, magnesium in the kernel; Moist., moisture; Zn in the kernel; Protein, protein content; P, phosphorus in the kernel; Fiber, fiber content

(UPGMA), and the Nei genetic distance and AMOVA were estimated. A considerable genetic distinction was observed between "Mais delle Fiorine" and all other varieties, and in particular compared to "Nero Spinoso", as shown by PCoA analysis (Fig. 6a) and UPGMA (Fig. 6b) while "Scagliolo di Carenno" and "Rostarto Rosso di Rovetta" showed a great similarity. These data were confirmed by the Nei genetic distance (Fig. 6c) where the distance between "Scagliolo di Carenno" and "Rostarto Rosso di Rovetta" was 0.068 and between "Mais delle Fiorine" and "Nero Spinoso" was 0.750. As expected for open pollinated varieties, the percentages of molecular variance were higher within populations (68%) than among populations (32%) (Fig. 6d).

Discussion

The results of this research provide interesting information concerning the agronomic and nutritional characteristics of "Mais delle Fiorine" and highlight its nutritional, morphometric and genetic differences compared to the maize landraces of Lombardy registered in the European Register of Conservation Varieties. Agronomic analysis has shown that "Mais delle Fiorine" can grow in Lombardy both in the Po Plain (90 m a.s.l.) and in the mountain environments of the Seriana Valley (also over 900 m a.s.l.) without significant differences in grain yield (number of kernels per ear, 1000-grains weight, ear length) (Table 3). In the fields where the climatic and soil characteristics determine less water availability (fields with high solar irradiance and soils with high sand percentage) plants grow less, in accordance with Song et al. (2019), but have a similar productivity. Based on the results of this research, "Mais delle Fiorine" would seem to be able to tolerate environments where there is a greater probability of water stress. This ability, if confirmed by eco-physiological studies (Hussan et al. 2013; Roth et al. 2013), would make "Mais delle Fiorine" an interesting genetic resource for crop improvement programs designed to produce drought-resistant varieties (Meseka et al. 2015; Luo et al. 2019). Moreover, the shape of the "Mais delle Fiorine" caryopsis could represent an adaptation of this landrace to a dry environment. This landrace, differently from the others considered in this study, presents kernels which are not beaked with an obovate shape leaving a smaller space between kernel ranks, hence disfavouring loss of water, as can be seen from the outline analysis. This hypothesis should be verified with specific studies which could also explain why this landrace has caryopsis with morphological characteristics more similar to modern varieties and hybrids than to other corn landraces typical of Northern Italian mountains, many of which are beaked maize (Ardenghi et al. 2018).

Although the yield of "Mais delle Fiorine" was similar in the different experimental fields, some nutritional parameters of the grain were significantly different according to field. This aspect is probably due to the different environmental conditions (climate, soil, biological factors) of the fields. It is known that the growing environment of plants can influence quantity and quality of the metabolites produced

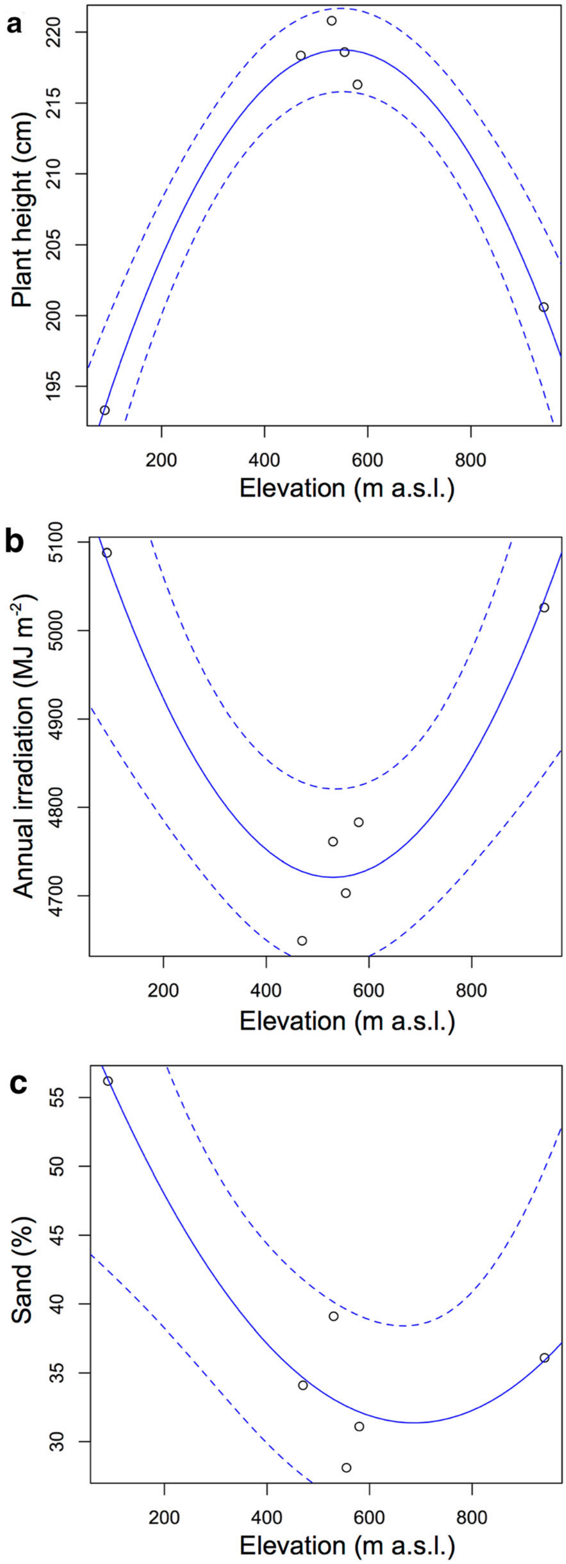
Table 4 Results of the linear and quadratic regression analysis (considering the elevation of the experimental fields as the independent variable)

Variable	Linear			Quadratic		
	R^2	F-value	*p*-value	R^2	F-value	*p*-value
Irradiation	0.234	0.052	0.831	0.879	19.320	**0.019**
Plant height	0.186	0.216	0.666	0.974	96.120	**0.002**
Ear insertion height	0.239	0.033	0.864	0.378	2.521	0.228
Moisture	0.237	0.041	0.848	0.338	0.367	0.719
Protein	0.384	4.112	0.112	0.490	3.403	0.169
Fat	0.057	0.729	0.441	0.116	0.739	0.548
Fiber	0.175	0.254	0.641	0.027	0.934	0.484
Starch	0.379	4.058	0.114	0.497	3.470	0.165
Mg (kernel)	0.210	0.104	0.763	0.263	0.478	0.660
Zn	0.103	1.575	0.278	0.197	0.591	0.608
P (kernel)	0.042	1.222	0.331	0.268	0.470	0.664
Sand	0.323	3.387	0.139	0.806	11.360	**0.040**
Silt	0.197	2.230	0.210	0.544	3.985	0.143
Clay	0.049	0.766	0.431	0.203	0.578	0.613
pH	0.025	0.875	0.403	0.516	3.666	0.157
Organic matter	0.126	1.720	0.260	0.416	2.783	0.207
Nitrogen	0.020	1.102	0.353	0.302	0.421	0.690
C/N ratio	0.090	0.585	0.487	0.191	1.590	0.338
CEC	0.360	3.814	0.123	0.229	1.744	0.315
Ca	0.249	2.653	0.179	0.049	1.131	0.431
Mg (soil)	0.235	2.534	0.187	0.278	1.962	0.285
K	0.031	1.162	0.342	0.728	7.699	0.066
P (soil)	0.157	0.321	0.602	0.065	0.848	0.511

The values of coefficient of determination (R^2) and of F-test are reported. *P*-values < 0.05 are highlighted in bold

(Giorgi et al. 2015; Pavlovic et al. 2019; Giupponi et al. 2020a). In the specific case study it is difficult to understand which factors (or combinations of factors) are responsible for the different chemical composition of the kernels due to the large number of variables. CCA results (Fig. 2) are difficult to render although it would seem clear that elevation is one of the most significant factors and that starch and protein contents are inversely proportional. This could be due to endosperm-specific transcription factors that can regulate the accumulation of starch and proteins in maize seeds (Zhang et al. 2019). To better understand the factors acting on the quality and quantity of nutrients contained in the grain of “Mais delle Fiorine” it would be advisable to study the effect of every single factor (or combination of factors) in a controlled environment.

Comparing the nutritional characteristics of “Mais delle Fiorine” to Lombardy maize landraces registered in the European Register of Conservation Varieties revealed that the former has more starch and zinc and less protein and phosphorus than the others. The high starch content makes it more similar to “modern” commercial varieties/hybrids (B73/MO17) than to traditional cultivars which are generally richer in protein and poor in starch (Berta et al. 2014; Puglisi et al. 2018; Giupponi et al. 2020c). Considering that carbohydrates are the main energy source of human diet (Jéquier 1994) and that their energy is available more rapidly for the organism compared to protein and fat (Kanter 2018), the maize landrace “Mais delle Fiorine” would be more suitable for the production of energetic food, such as functional foods for athletes, than the others considered (Brouns et al. 2002; Helge 2017; Kanter 2018). The high zinc content is another feature that distinguishes “Mais delle Fiorine” from the other Lombardy landraces and that confers a particular nutritional value to it. In fact, zinc is an essential mineral that acts as a cofactor for antioxidant enzymes

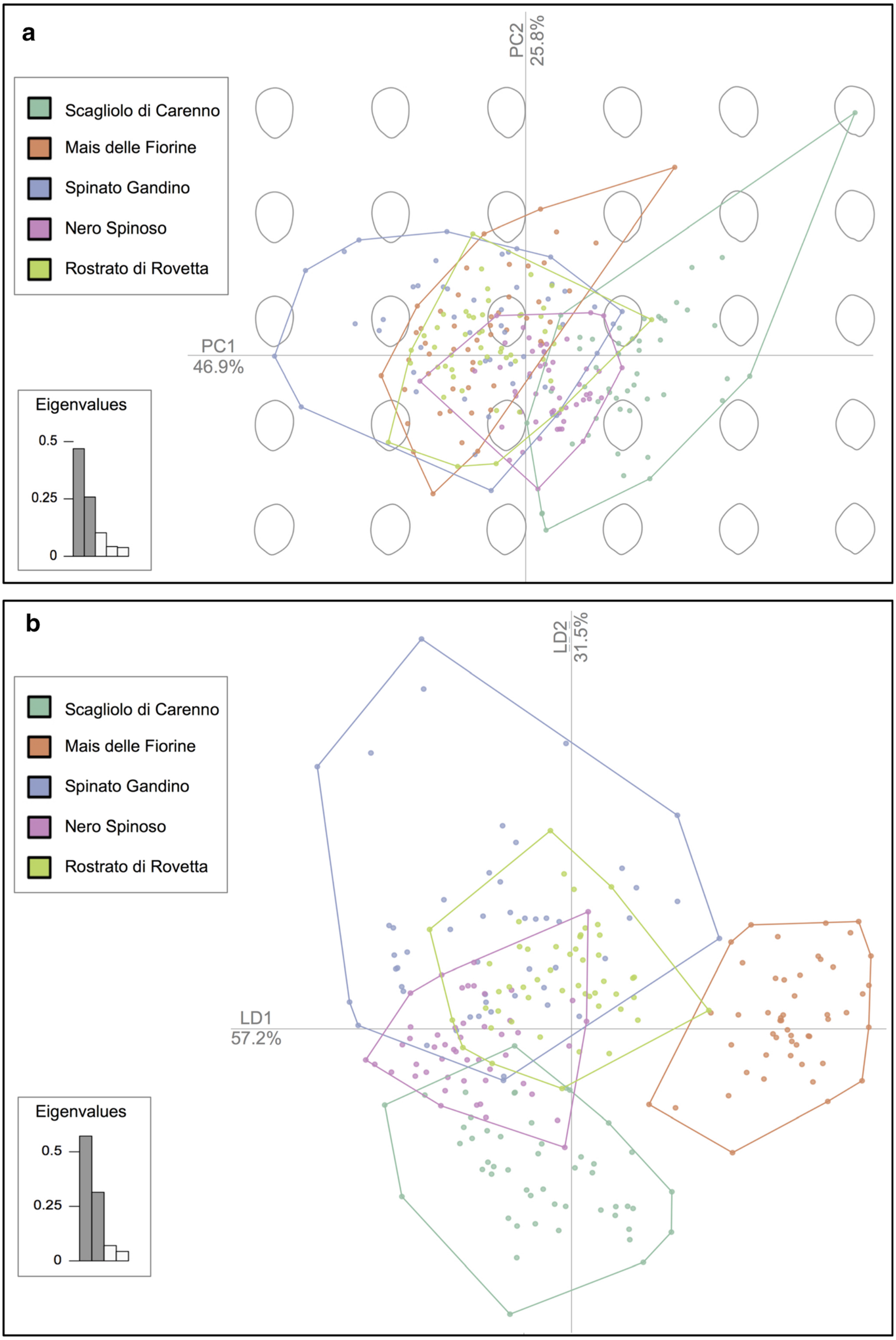


◀**Fig. 3** Quadratic regression with 95% confidence interval bands of plant height **a**, annual solar irradiation **b** and sand content of soil **c**, by elevation. Points represent experimental fields. The values of coefficient of determination (R^2) of the models are reported in Table 4

the lack of which in diet can cause adverse effects on physical growth and cognitive development in humans (infants and children in particular) (Brown et al. 2001; Roohani et al. 2013).

Moreover, the low content in phosphorus gives "Mais delle Fiorine" a high nutritional value. The main storage form of seed phosphorus is phytic acid (PA), an anti-nutritional factor that accumulates as phytate salts, chelating different cations (mainly zinc and iron) and therefore reducing their bioavailability. Monogastric animals (including humans) are not able to degrade PA and its presence decreases the nutritional value of seeds (Raboy 2001; Schlemmer et al. 2009). Iron and zinc deficiencies are widespread in developing countries (Gupta et al. 2015), where diet is mainly based on stable crops. On the other hand, PA appears to play a key role protecting embryo viability from oxidative stress and the germination capacity of low PA seeds tends to decrease after a seed ageing treatment (Doria et al. 2009).

"Mais delle Fiorine" is different from the other maize landraces of Lombardy registered in the European Register of Conservation Varieties, not only for the morphological features of the caryopsis and its nutritional characteristics but also from a genetic point of view. Hence, to better characterize these varieties, genetic analysis by SSRs markers that allow the use of fewer markers for a good genetic characterization (Mir et al. 2013) was performed. Moreover, SSRs do not suffer from the ascertainment bias that Single Nucleotide Polymorphism (SNP) markers show (Hamblin et al. 2007). The peculiarity of "Mais delle Fiorine" is also highlighted by genetic analysis which showed that this variety is genetically distinct from the other varieties studied as shown in Fig. 6. Furthermore, results from this study point to the genetic distinctness of "Nero spinoso" from all the other varieties apart from "Spinato di Gandino" (Nei genetic distance = 0.294). These results indicate that "Mais delle Fiorine" is an open pollinated variety which has maintained its peculiarities over the centuries avoiding cross pollen contaminations most


a
PC2
25.8%
Scagliolo di Carenno
Mais delle Fiorine
Spinato Gandino
Nero Spinoso
Rostrato di Rovetta
PC1
46.9%
Eigenvalues
0.5
0.25
0
b
LD2
31.5%
Scagliolo di Carenno
Mais delle Fiorine
Spinato Gandino
Nero Spinoso
Rostrato di Rovetta
LD1
57.2%
Eigenvalues
0.5
0.25
0

◀ **Fig. 4** PCA biplot **a** resulting from outline analysis of the grains of the five maize landraces and LDA of PCs biplot **b**. Gray figures in the background **a** show reconstructions of kernel shape according to each position in the multidimensional space

likely due to its earliness and ear shape compared to "Spinato di Gandino", "Rostrato Rosso di Rovetta" and "Nero Spinoso" that roughly shared the same cultivation area. However, this should be confirmed by increasing the size of the population and the number of molecular markers.

Conclusion

This research evaluated various features of "Mais delle Fiorine" and the differences compared to other maize landraces of Lombardy. It highlighted that "Mais delle Fiorine" is a rustic landrace able to grow and be productive in different environmental conditions, even where there may be drought stress. Furthermore, the grain of this landrace presents a high nutritional value due to the high content of some nutrients, such as starch and zinc, that differentiate it from the other maize landraces of Lombardy. Moreover, the caryopsis shape and the genetic characteristics differentiate it from the other maize landraces registered in the European Register of Conservation Varieties. These differences represent an authentic biodiversity source as shown by genetic analysis.

This study contributes to implement scientific knowledge of agri-food heritage in Italy, which is one of the European nations richest in plant agrobiodiversity (Laghetti et al. 1993, 2018; Negri 2003; Santamaria and Ronchi 2016; Hammer et al. 2018; Biodiversity International 2019; Giupponi et al. 2020c). Hence, it would be opportune to start initiatives to encourage the conservation and valorisation of maize "Mais delle Fiorine", as has happened for other

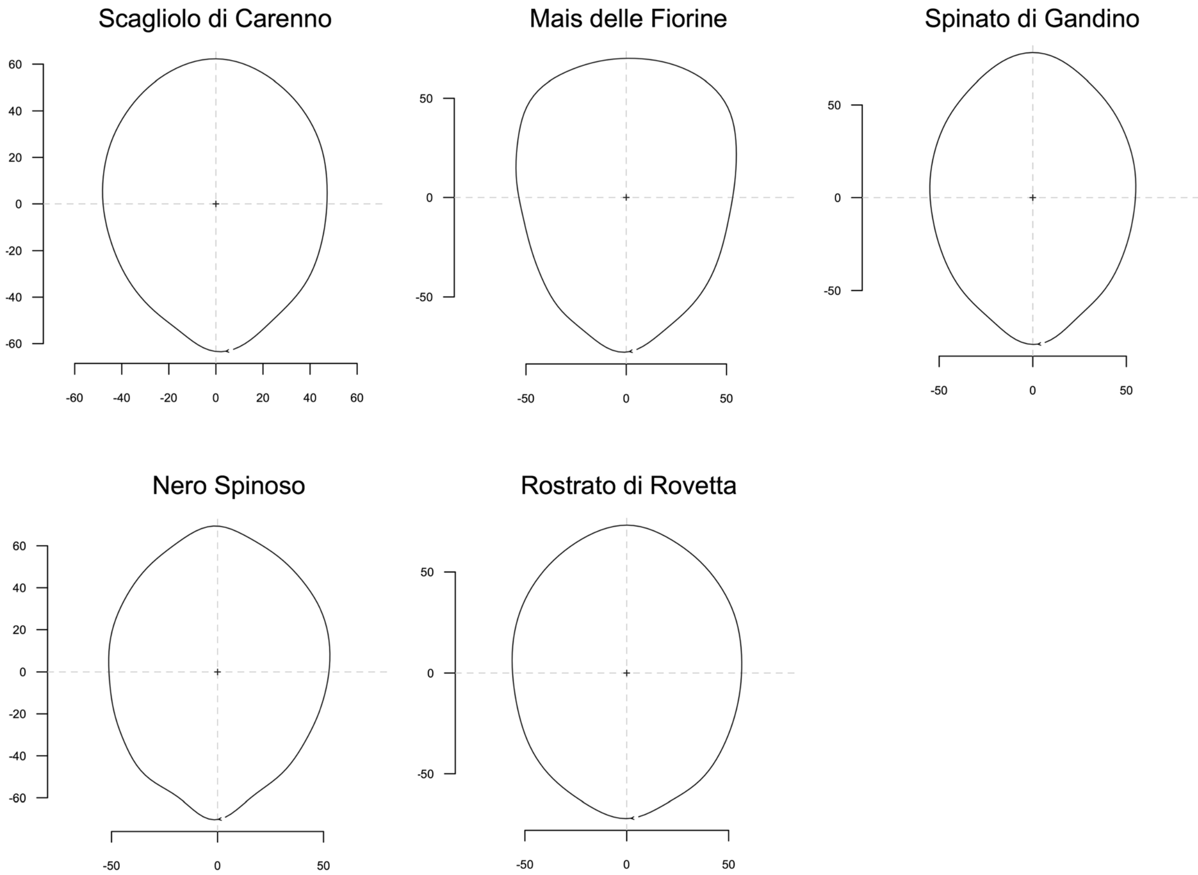
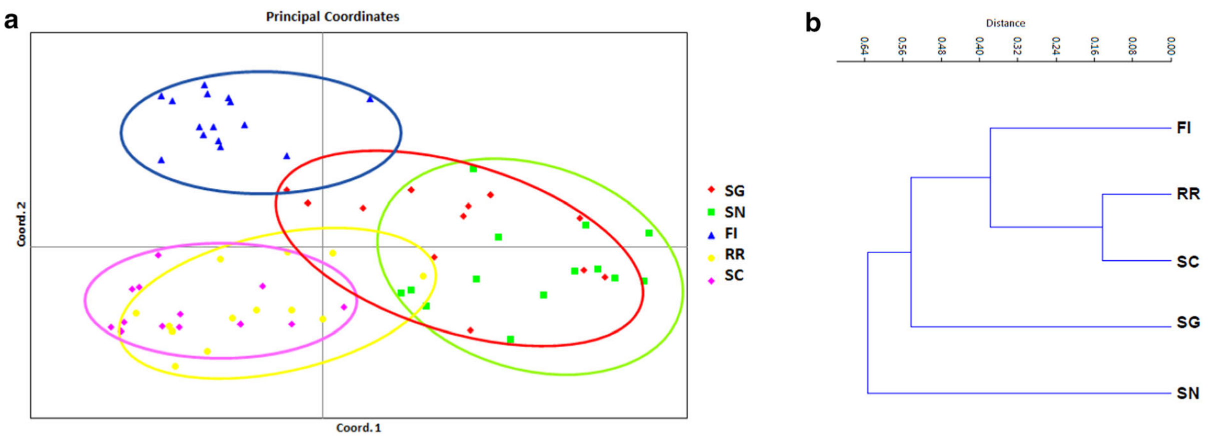


Fig. 5 Mean shape of kernels assigned to the five maize landraces according to elliptical Fourier descriptors analysis



c

Nei Genetic Distance (Pairwise Population Matrix of Nei Genetic Distance).

	SG	SN	FI	RR	SC
SG	0.000				
SN	0.294	0.000			
FI	0.302	0.750	0.000		
RR	0.217	0.381	0.277	0.000	
SC	0.317	0.497	0.247	0.068	0.000

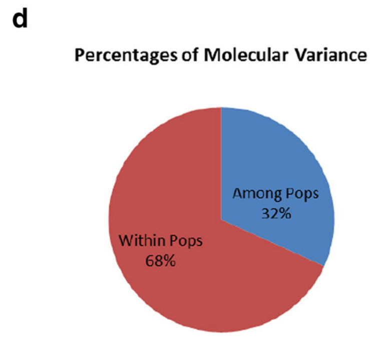


Fig. 6 Genetic comparison between "Mais delle Fiorine" (FI), "Spinato di Gandino" (SG), "Rostrato Rosso di Rovetta" (RR)," Scagliolo di Carenno" (SC) and "Nero Spinoso" (SN) landraces used in this study: PCoA biplot **a**, Unweighted Pair-Group Mean Arithmetic method (UPGMA) grouping **b**, Nei Genetic Distance **c** and AMOVA (Percentages of Molecular Variance) **d**

landraces such as "Mais Spinato di Gandino" (https://www.mais-spinato.com). The latter, after being rediscovered and studied, was registered in the European Register of Conservation Varieties and was the subject of many promotion and valorisation projects by producer associations and municipalities where this landrace is grown. In the case of "Mais delle Fiorine", the already existing association in charge of its conservation in situ, intends to start procedures to register "Mais delle Fiorine" in the European Register of Conservation Varieties using the data produced in this research. It is to be hoped that the characterization, protection and valorisation activities for "Mais delle Fiorine" carried out to date and in the future, will be useful not only for the conservation of this plant genetic resource (FAO 2009, 2010) but also for the creation of economically and environmentally sustainable mountain agri-food value chains (Cleveland et al. 1994; EU Commission 2019) with a low environmental impact coherently with the 2030 Agenda for Sustainable Development goals (United Nations 2015).

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Compliance with ethical standards

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

Ethical approval This research complies with the ethical rules applicable for this journal.

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