Traditional farmers' varieties: a valuable source of genetic variability for biofortification programs

Daniel Puglisi[1*], Michela Landoni[2*], Elena Cassani[1],Ivan Toschi[1],Giorgio Lucchini[1], Valentina Cesari[1], Giulia Borlini[1], Andrea Scapin[1], Roberto Pilu[1]

[1]Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy

[2]Dipartimento di Bioscienze, Università degli Studi di Milano, Via Celoria 26, 20133 Milano, Italy

**These authors contributed equally to this work

Corresponding author: R. Pilu, Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia, Università degli Studi di Milano Via Celoria 2, 20133 Milano, Italy, Fax number: + 39-2-50316521, Phone number: + 39-2-50316549

e-mail: salvatore.pilu@unimi.it,

Abstract

Several studies underlined the superiority from a nutritional point of view of ancient varieties. In the last years the interest for landraces has been growing, for this reason preservation and valorisation of these genetic sources is very important. In particular these varieties are source of precious genetic variability interesting from a scientific point of view to preserve biodiversity but also for biofortification programs aimed to support small rural communities, where the particular maize germplasm has been developed. In this work we characterized from the nutritional point of view 13 ancient Italian varieties and one coming from Spain (Millo Corvo). In this pre-breeding work we demonstrate the nutritional superiority of ancient varieties if compared with modern hybrids. In particular Spinato di Gandino is the best variety for milling properties and for oil, protein, and total phosphorus content; Storo is the best variety for calorific value and for carotenoids and free phosphorus content. Using these varieties in the next future we will start a bio-fortification program aimed to obtain new populations with improved yields and high nutritional value.

KeyWords *flint maize, landraces, biofortification, nutritional value, genetic sources, Zea mays subsp.*

Introduction

Maize (*Zea mays* L. ssp. *mays*) is the main cereal grain cultivated worldwide. It is responsible for providing 15% of the protein and 20% of the calories in the human diet, supplying an energy density of 365 Kcal/100 g and covering a cultivated area of 184.8 million hectares in 2014 (Faostate 2014, Food and Agriculture Organization of the United Nations, Crops Production 2009, Zeppa et al. 2012). Corn can be used in several ways as feed for live-stock, forage, silage and grain, but also for industrial uses. However, human nutrition remains one of the main uses, determining the selection of varieties for producing many typical dishes such as polenta in Italy and pap in South Africa (Zeppa et al. 2012, Cantaluppi et al. 2017).

Maize has a matrix rich in organic compounds and minerals with potential benefits to health. These compounds may in fact act as antioxidants (carotenoids and phenolic compounds), as cofactors for antioxidant enzymes (selenium, zinc) or as indirect antioxidants (betaine, choline, and folate) complementing those found in fruits and vegetables (Hansch and Mendel 2009, Liu-2007). Moreover, consumption of whole grains is preventative against cardiovascular disease, some types of cancer, type 2 diabetes, and obesity (Messias et al., 2015).

Thus, while productivity remains the major target for breeders, focusing on grain quality could reduce deficiencies of some minerals and pro-vitamin A increasing the concentration of functional compounds and the nutritional value, and this will be of particular importance especially for poor populations that use maize as staple food (Messias et al., 2015).

Maize was domesticated about 8,700 BP in Mexico and from there it spread within the Americas (Piperno et al. 2009; van Heerwaarden et al. 2011).

After the discovery of the Americas by Europeans, three main maize sources (corn from the American east coast with higher latitude adaptation, the photoperiod insensitive CATETO types and the Pearl White) played an essential role for the adaptation of maize to Europe (Brandolini and Brandolini et al. 2009; Eschholz et al. 2010).

In Europe, the spread of maize started from Spain and other southern European countries such as Italy, in which it had great success thanks to several favourable environmental and social conditions (Anderson and Cutler 1942; Brandolini and Brandolini 2009). In Italy, the first reports regarding the utilization of corn date from 1600 in the North East where it was adapted to the climatic zones of cultivation (Brandolini 1958; Brandolini and Brandolini 2009). The hybridization during cultivation of these corn sources in different environments led to the establishment of many local varieties that under different photoperiod, temperature, humidity and altitude allowed the constitution and the differentiation of local European varieties and landraces (Brandolini and Brandolini et al. 2009; Eschholz et al. 2010). During this process the work of selection was kept from farmers, in fact they maintained landraces as open pollinated populations, creating a collection of corn plants with high heterozygosity and heterogeneity, which represented a very important source of variability and alleles with high adaptation to the local environments (Lago et. al 2015).

After World War II the introduction of mechanized farming practices and the utilization of dent hybrids which were much more productive, mainly for animal feed, led to the gradual disappearance of local varieties and thus to the loss of the valuable alleles they contained (Brandolini and Brandolini 2009). Fortunately, in more recent years many efforts have been made to recover and preserve the genotypes of the old varieties: in Italy the main maize collections are preserved *ex situ* at CREA Research Centre for Cereal and Industrial Crops located in Bergamo, and in the germplasm bank of the University of Milan located in Landriano (PV).

In this work, after a pre-breeding activity, we studied the nutritional value of fourteen traditional varieties typically used for the production of polenta, comparing these varieties with modern hybrids to find those with valuable properties that could be part of future bio-fortification breeding programs. In particular we assessed their nutritional value for several parameters (calorific value, oil, protein, mineral nutrients, carotenoids content and the repartition between free and total P).

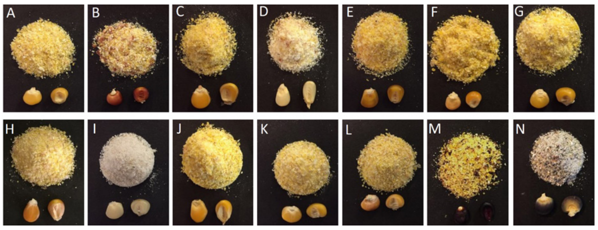
Our results led us to plan a breeding program aimed to obtain neo-synthesis populations with increased nutritional properties for the production of polenta and others typical dishes.

Materials and Methods

Plant Material

We collected 14 ancient varieties: Nostrano dell'Isola, Pignoletto di Tortona, Cinquantino, Bianco Vitreo, Marano, Storo, Cinquantone, Scagliolo, Bianco Perla, Spinato di Gandino, Ottofile Tortonese and Ottofile, maintained in the gene bank of the University of Milan located in Landriano (PV), Italy (N 45°180, E 9°150), Nero Spinoso and Millo Corvo sampled directly from the farmers, at Esine (Italy) and Spain respectively (Fig. 1).

Figure 1. *Panel of ancient varieties with seeds and flour (commonly used for polenta production): Nostrano dell'isola (A), Pignoletto di Tortona (B), Cinquantino (C), Bianco Vitreo (D), Marano (E), Storo (F), Cinquantone (G), Scagliolo (H), Bianco Perla (I), Spinato di Gandino (H), Ottofile Tortonese (K), Ottofile (L), Nero Spinoso (M), Millo Corvo (N).*

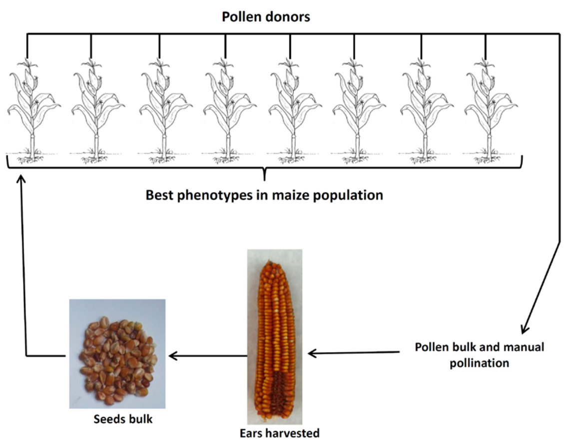


For all the genotypes tested we performed three cycles of massal selection (seasons 2014-2015 and 2016): about 200 seeds were sown in adjacent rows, under the same agronomic conditions, with B73/MO17 as control. The analyses were performed on the materials harvested on 2017: about 70 ears of each variety were shelled and the seeds obtained mixed to create a single bulk used for the determination of the nutritional value.

Conservation and propagation of local varieties in open field

To preserve local varieties we performed controlled pollination to maintain the mechanic isolation of these varieties. Using paper bags we performed sib pool pollination taking pollen from several plants and bulking it (Fig. 2). Then we put the bulked pollen on the ears of different plants of the same population

Figure 2. *Scheme of the sib pool pollination used for the conservation and preservation of 14 traditional varieties.*



Milling

Flour samples were obtained using a ball mill (Retsch MM200, Retsch GmbH Germany), and seeds were ground for 5 min at 21 oscillations s^{-1}

Dry seed weight

Dry seed weight was calculated after weighing 50 seeds per genotype in three replicates, after drying for 48 h at 70 °C.

Bromatological analysis (calorific value, crude protein and ether extract)

Calorific value measures and chemical analyses were performed using approximately 50 g of seeds for each genotype. Gross energy value was determined using an adiabatic calorimeter (IKA 4000, Staufen, Germany). Chemical analyses were performed according to AOAC standard methods (AOAC, 2000), milling and analysing the samples for dry matter, crude protein and ether extract (oil).

Dermination of free phosphorus in seeds

50 mg seed flour were extracted with 2 mL 12.5% trichloroacetic acid (TCA) 25 mM $MgCl_2$ solution (three replicates for each sample). The solutions were mixed and kept in agitation for 30 min at room temperature before being incubated overnight at 4°C. Free phosphorus in the extracts was determined spectrophotometrically through the colorimetrical Chen assay (Chen et al., 1956). Four solutions, containing respectively 0.62, 1.24, 2.48, 3.72 µg/mL atomic P were prepared using a 2 mM Na_2HPO_4 solution: 1980 µL, 1960 µL, 1920 µL and 1880 µL of a freshly prepared Chen's reagent (distilled H_2O, 6 N H_2SO_4, 10% ascorbic acid and 2.5% ammonium molybdate in the ratio 2:1:1:1, v/v/v/v) were added to 20 µL, 40 µL, 80 µL and 120 µL of a 2 mM Na_2HPO_4 solution. 2 mL Chen's reagent was also used as the blank and 1800 µL were added to 200 µL of each extract collected after centrifuge, to reach a final volume of 2 mL. All the solutions were agitated and incubated at 50 °C for 1 h before reading. The absorbance of the reaction mixture was measured at 650 nm. Free P concentration was calculated according to the standard curve.

Determination of ionomic content (P tot, Ca, Fe, Zn) in maize flour

For the determination of elements of interest, 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 of an internal standard solution (72Ge, 89Y, 159Tb) was added both to samples and calibration curve to give a final concentration of 20 µg/L. Typical polyatomical analysis interferences were removed by using CRI (Collision-Reaction-Interface) with an H2 flow of 93 mL/min flown through skimmer cone.

Average values regarding Ca, Fe, Zn were expressed as µg/g seed flour; values regarding P were indicated as mg/g seed flour.

Carotenoids extraction and quantification

3 mL of extraction buffer (acetone, methanol, hexane 1:1:1) were added to 0.25 g seed flour in 15 mL tubes (four replicas for each sample). The samples were vortexed and left in agitation in ice for 30 min, vortexing them again every 10 min. 1 mL nanopure water was added to each sample, then the samples were vortexed and kept in agitation 5 min before centrifuge (3000 rpm for 10 min). 1 mL non-polar phase was collected and filtered through a 0.22 µm syringe filter. The extracts were conserved at -20°C in the dark until reading.

1.8 mL extraction buffer (acetone, methanol, hexane 1:1:1) was added to 200 µL extract (dilution 1:10) to obtain a final volume of 2 mL. The extraction buffer was used as blank. The absorbance was measured spectrophotometrically at 450 nm using glass cuvettes. Carotenoids content was calculated according to the standard curve obtained using five lutein solutions (0.25, 0.5, 1, 2, 4 µg/mg). Standard deviation was calculated.

i

Informatic tools

Microsoft Excel® was used to collect data, SPSS® was used to perform one way ANOVA on sampled data and SIGMA PLOT® was used to obtain graphs.

Results and discussion

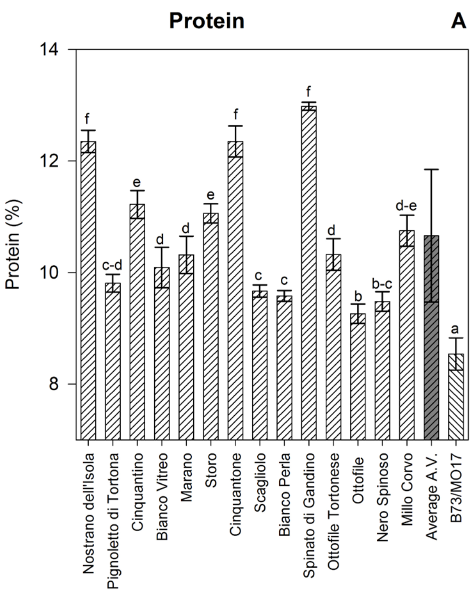
In Italy the use of corn in agriculture dates back to the second half of the sixteenth century, since when the adaptation to different environments together with human selection led to the diversification of hundreds of landraces (Messedaglia 1924; Brandolini and Brandolini 2009).

These varieties were characterized by low yields, when compared with modern hybrids, but had considerable phenotypic and genetic variability that led to yield stability (Liu et al. 2003; Vigoroux et al. 2008; Warburton et al. 2008; Mir et al. 2013). At the end of the II World War these farmer's varieties were replaced by modern hybrids. Nowadays a renewed interest for these traditional varieties is growing, determining the selection of varieties for producing many typical dishes such as polenta in Italy and pap in South Africa (Zeppa et al. 2012, Cantaluppi et al. 2017). Moreover, maize is really important in our diet, in particular for the health benefits due to the ingestion of a matrix rich in organic compounds and minerals (Messias et al., 2015; Hansch and Mendel 2009, Liu 2007). Thus, while productivity remains the major target for breeders, in the future it will be important to focus on grain quality (Messias et al., 2015). For these reasons the study of landraces genetic diversity is a prerequisite for efficient conservation and management and effective use of landraces in breeding programmes (Newell-Mcgloughlin et al., 2008; Cakmak, 2008; Newton et al.,2009).

To pursue this aim we collected 12 local farmers varieties from CREA in Bergamo and sampled two varieties directly from farmers (Millo Corvo in Spain and Nero Spinoso at Esine) (Fig. 1). With the aim to preserve and to standardize, from the genetic point of view, the material stored in the gene bank of the University of Milan we performed three cycles of massal selection (2014, 2015 and 2016). We sowed 200 plants for each genotype and among them in every agronomic season we selected the best 15 phenotypes. From them we collected pollen to perform sib pool pollination using paper bags, maintaining it in isolation to avoid cross contamination (Fig. 2). During the harvest season we selected the 40 best ears both for yield and phenotype and bulked their grains, storing all these varieties in the germplasm bank at 4 °C and 30% of moisture.

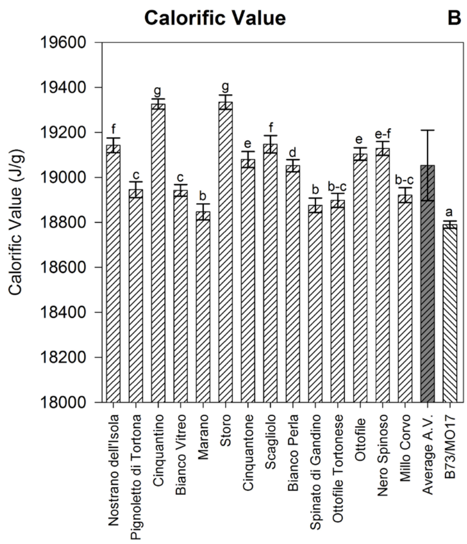
At the end of the selection's process, in 2017, the seeds obtained from about 70 ears of each variety were shelled and mixed to create a single bulk which was used for the determination of nutritional value.

Figure 3A. *Main results of bromatological analysis performed on maize flour: Protein (A); Data regarding, B73/Mo17, DK 440, PR 33A46, NK HELEN controls varieties are taken from Panzeri et al. 2011. Division in homogenous groups is the result of the Tuckey test, performed as a post hoc test after one way Anova (p = 0.05).*



The protein quantification indicated that the protein amount in traditional varieties is higher (10.65 % ± 1.18) than in modern hybrids (9.48 % ± 0.93) with the highest value in Spinato di Gandino (12.98 % ± 0.19), Nostrano dell'Isola (12.35% ± 0.19) and Cinquantone (12.35 % ± 0.27) (Fig. 3A). These results confirm the data from Berta et al (2014) that reported a higher protein content in the Italian variety Ostiglia (9.5 g

Figure 3B. *Main results of bromatological analysis performed on maize flour: Calorific Value (B). Data regarding, B73/Mo17, DK 440, PR 33A46, NK HELEN controls varieties are taken from Panzeri et al. 2011. Division in homogenous groups is the result of the Tuckey test, performed as a post hoc test after one way Anova (p = 0.05).*



100 g^{-1}). Calorific values for different substances and biomasses are well known. For example, wood and cereal straw range from 17 to 19 KJ/g^{-1} and wheat grain is around 17 KJ/g^{-1} (FAO, 2004; Panzeri et al., 2011). Panzeri et al. (2011) found that among the genotypes expressing normal seed phenotypes, the highest calorific values was in 'Scagliolo' that is a traditional population selected for human nutritional purposes. Analysing our data we can confirm this thesis, as we found the highest values in ancient varieties (19052 J/g^{-1} ± 156.6) with the best performances of Storo (19334 J/g^{-1} ± 31.51) and Cinquantino (19326 J/g^{-1} ± 22.47) (Fig. 3B). Oil is confined for the major part in the germ, about 85% of the total kernel oil; the rest is contained in the endosperm. Normal maize provides around 2-6% oil, but some high-oil maize contain more than 6% oil providing a valuable product because of its low levels of saturated fatty acid (i.e. 11% palmitic acid and 2% stearic acid) (Lambert et al., 1997). Corn oil is

Figure 3C. *Main results of bromatological analysis performed on maize flour: Oil (C). Data regarding, B73/Mo17, DK 440, PR 33A46, NK HELEN controls varieties are taken from Panzeri et al. 2011. Division in homogenous groups is the result of the Tuckey test, performed as a post hoc test after one way Anova (p = 0.05).*

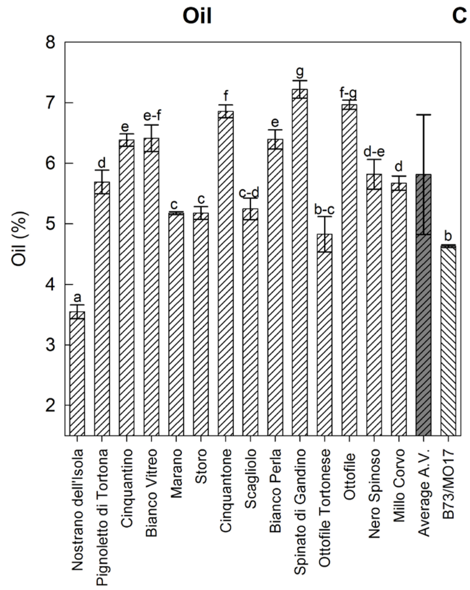
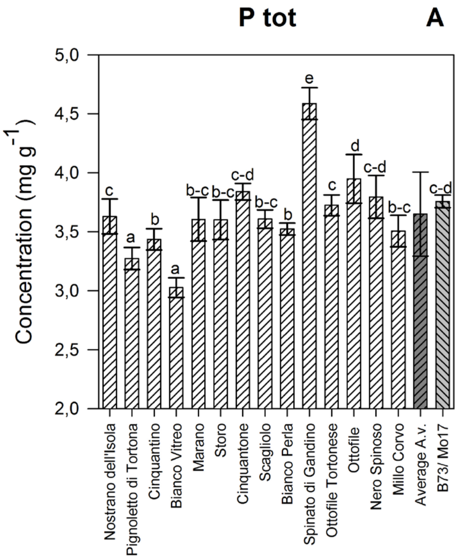


Figure 4A. *Mineral Nutrient and Trace Element Content Determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS): P tot (A). Analysis performed on maize flour. Division in homogenous groups is the result of the Tuckey test, performed as a post hoc test after one way Anova (p = 0.05) .*



also recognized as an excellent source of tocopherols that are important antioxidants and a source of Vitamin E (Dormann 2003). Screening our traditional varieties we saw that also for this parameter the average value (5.81% ± 0.98) is higher than in B73/MO17 hybrid (Fig. 3C). Also in this case Spinato di Gandino gave the best performances (7.22 % ± 0.14). These results are in agreement with those found from Panzeri et al. (2011), proving that traditional varieties could be a valuable source of genetic variation to increase oil in the kernel and that Spinato di Gandino could be considered as a high oil variety. It's well known that also seed weight is an important parameter for the milling industry; in fact maize is dry milled to obtain grit, meal and flour and wet milled to produce starch and other valuable

Figure 3D. *Main results of bromatological analysis performed on maize flour: Dry Seed Weight (D). Data regarding, B73/Mo17, DK 440, PR 33A46, NK HELEN controls varieties are taken from Panzeri et al. 2011. Division in homogenous groups is the result of the Tuckey test, performed as a post hoc test after one way Anova (p = 0.05).*

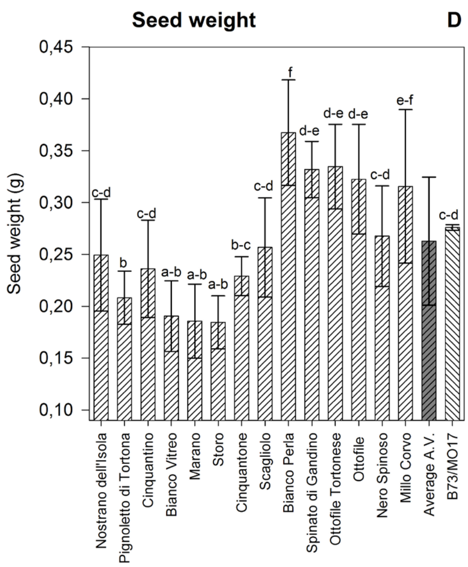


Figure 4B. *Mineral Nutrient and Trace Element Content Determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). P free (B) was determined using Chen assay. Analysis performed on maize flour. Division in homogenous groups is the result of the Tuckey test, performed as a post hoc test after one way Anova (p = 0.05) .*

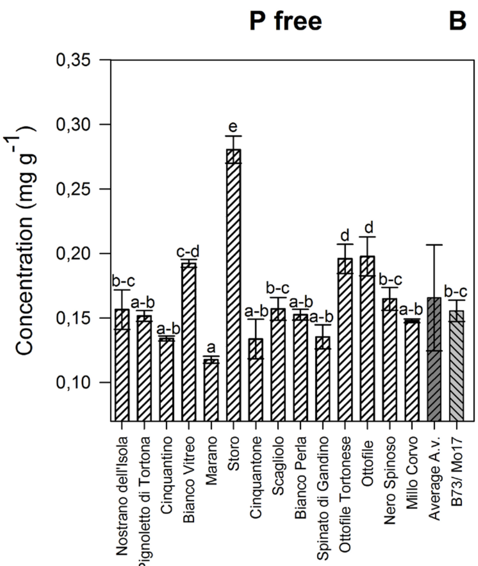


Figure 4C. *Mineral Nutrient and Trace Element Content Determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS): Carotenoids (C). Analysis performed on maize flour. Division in homogenous groups is the result of the Tuckey test, performed as a post hoc test after one way Anova (p = 0.05) .*

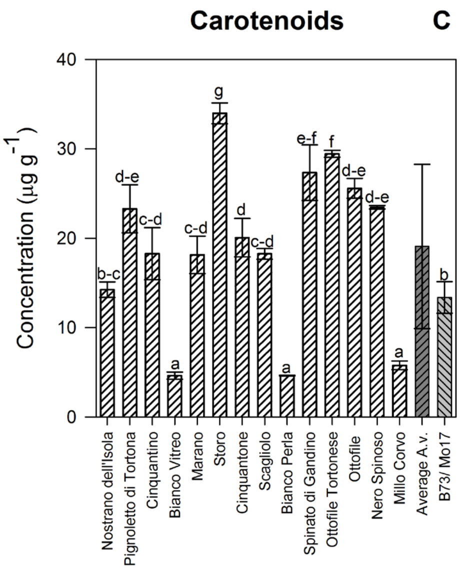
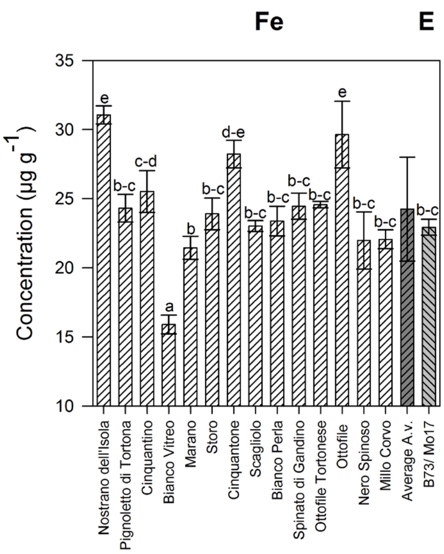


Figure 4E. *Mineral Nutrient and Trace Element Content Determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS): Fe (E). Analysis performed on maize flour. Division in homogenous groups is the result of the Tuckey test, performed as a post hoc test after one way Anova (p = 0.05) .*



by-products such as gluten, germ and bran. From this point of view the best varieties are: Bianco Perla (0.36 g ± 0.05), Spinato di Gandino (0.33 g ± 0.02) and Ottofile Tortonese (0.33 g± 0.04) (Fig. 3D). Spinato di Gandino is among the best varieties also for the total phosphorus (4.58 mg g^{-1} ±0.13) (Fig. 4A). In poor countries free phosphorus deficiencies are a relevant topic, for this reason a geneticist's goal is the reduction in phytic acid content in seeds and the corresponding increase in the level of free phosphorus (Cerino et al., 2010). We analysed the traditional varieties for the free phosphorus, looking for varieties that naturally accumulate high amounts of this element, finding the highest concentration in Storo (0.28 mg g^{-1} ±0.01)(Fig. 4B). Storo proves to be a good variety also for a bio-fortification purpose regarding carotenoids amount in

Figure 4D. *Mineral Nutrient and Trace Element Content Determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS): Ca (D). Analysis performed on maize flour. Division in homogenous groups is the result of the Tuckey test, performed as a post hoc test after one way Anova (p = 0.05) .*

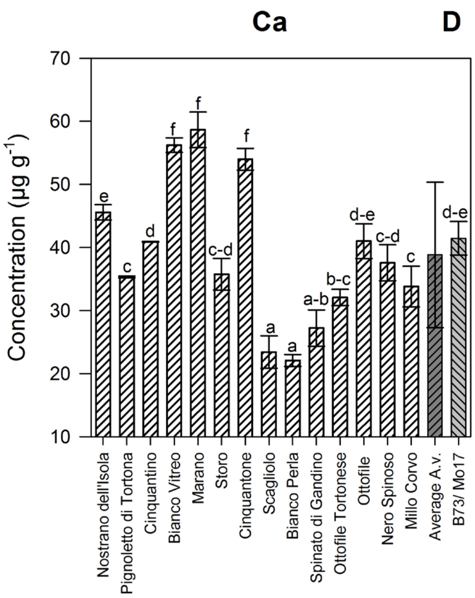


Figure 4F. *Mineral Nutrient and Trace Element Content Determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS): Zn (F). Analysis performed on maize flour. Division in homogenous groups is the result of the Tuckey test, performed as a post hoc test after one way Anova (p = 0.05) .*

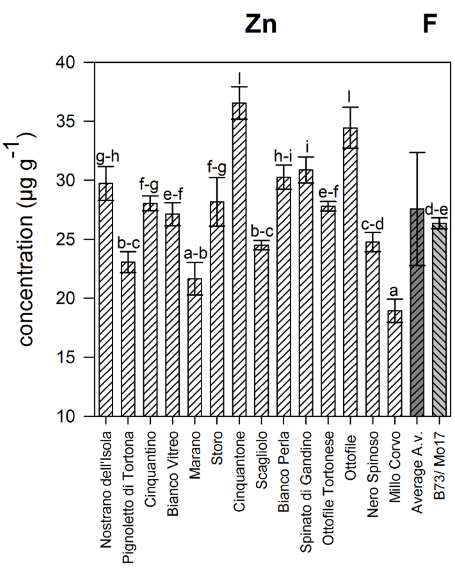
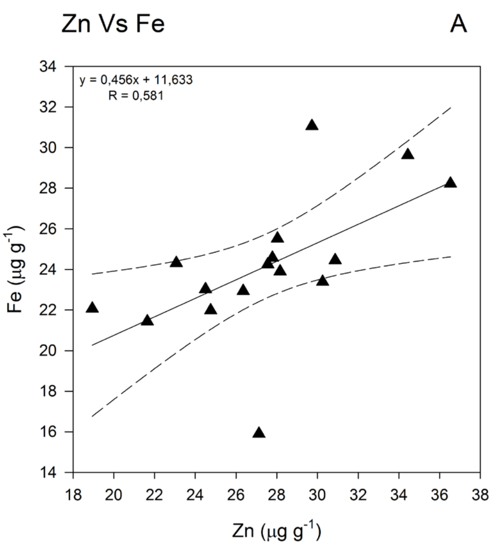
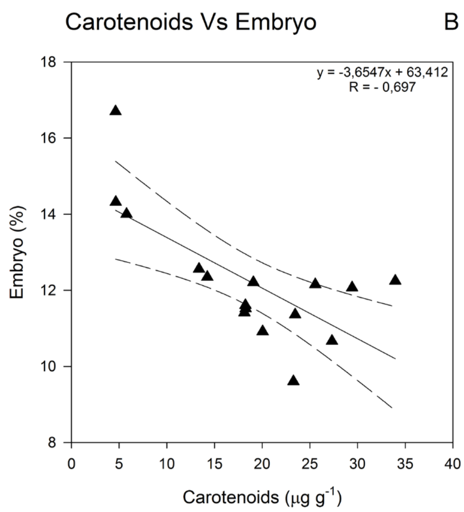


Figure 5A. *Significant correlation among nutrient in traditional maize varieties: Correlation Zn vs Fe (A) . The R showed in the graph is the Pearson coefficient.*



the kernel (33.95 µg g^{-1}) (Fig. 4C) , in fact it is known that globally one third of preschool-age children and 15% of pregnant women are estimated to be vitamin

Figure 5B. *Significant correlation among nutrient in traditional maize varieties: Correlation carotenoids Vs embryo (B). The R showed in the graph is the Pearson coefficient.*



A deficient (Sommer 1982; WHO 2009). Calcium, like zinc, is an important cofactor and molecular signal and is essential in the blood coagulation cascade (Relea et al., 1995). Cinquantone (53.98 µg g^{-1} ± 1.73), Bianco Vitreo (56.23 µg g^{-1} ± 1.15) and Marano (58.65 µg g^{-1} ± 2.81) respectively gave the best results for what concern this element exceeding B73/Mo17 (41.46 µg g^{-1} ± 2.66) (Fig. 4D). Maize presents a broad natural variation for kernel carotenoids, with the best genotypes accumulating 66.0 µg g^{-1} (Harjes et al., 2008). Moreover, we found a significant negative relation between embryo's dimensions and carotenoids content ($R = -0.697$; $P < 0.05$) (Fig. 5B) finding that white varieties have bigger embryo than yellow ones, supporting the thesis of a strong selection of white varieties for human consumption (Cantaluppi et al. 2017). Several cycles of selection of white varieties, for high oil and calorific value, provoked an increase of embryo's dimensions. In developing countries also mineral malnutrition is an important topic in particular iron deficiency is the most common and widespread nutritional disorder in the world. Iron deficiency can retard mental development and learning capacity and impair physical growth (Bouis 2002). Among our varieties Nostrano dell'Isola has the highest amount of iron (31.05 µg g^{-1}) (Fig. 4E); even overtaking the common hybrid B73/Mo17 (22.93 µg g^{-1} ±0.57) and showing to be a valuable source for a breeding program pointed towards an iron increase. Deficiency of zinc, likewise iron one, lowers the intestinal absorption of fat and fat-soluble vitamins including retinol (Ahn and Koo 1995). Maize contains an average of 20 µg g^{-1} zinc, corresponding to approximately 40% of the requirement for non-pregnant women and infants after breast feeding (Cakmak, 2008). Among the varieties in our study Cinquantone (36.53 µg g^{-1}) (Fig. 4F) gave the highest amounts of zinc being the candidate variety for future breeding programs. Moreover in our study we found a significant correlation between iron and zinc in the kernel ($R = 0.581$; $P < 0.05$) (Fig. 5A), we could therefore imagine simultaneous increases of both elements in a breeding activity pointed to increase mineral amount in the grain. This result is in agreement with Welch and Graham 2002 who found a tight correlation between iron and zinc concentration in wheat. Calcium, like zinc, is an important cofactor and molecular signal and is essential in the blood coagulation cascade (Relea et al., 1995). Cinquantone (53.98 µg g^{-1}±1.73), Bianco Vitreo (56.23 µg g^{-1}±1.15) and Marano (58.65 µg g^{-1}±2.81) respectively gave the best results for what concern this element exceeding B73/Mo17 (41.46 µg g^{-1}±2.66).

In conclusion, we demonstrated, from a nutritional point of view, that traditional varieties could be used in breeding programs aimed to bio-fortify maize for different important nutritional elements. From these varieties it could be possible to start a bio-fortification program aimed to obtain new synthesis populations with high nutritional value. The aim of these actions will be oriented to: i) a reduction of nutrient deficiencies in poor countries with a program of participatory plant breeding, and ii) an increase of the added value of the final products by increasing their nutritional characteristics.

Acknowledgements

We wish to thank Dr. Davide Reginelli for his hard work in the field and Dr. Lesley Currah for her editing and suggestions.

Conflict of Interest:

The authors declare that they have no conflict of interest

References

Ahn J, Koo SI, 1995. Effects of zinc, and essential fatty acid deficiencies on the lymphatic absorption of Vitamin A and secretion of phospholipids. J Nutr Biochem 6:595-603

Anderson E. and Cutler HC, 1942. Races of Zea mays: I. Their recognition and classification. Ann. Mo. Bot. Gard. 21:69–88.

Brandolini A, 1958. Il germoplasma del mais e la sua conservazione. Maydica, 3, 4-14

Brandolini A, Brandolini A 2009. Maize introduction, evolution and diffusion in Italy. Maydica 54: 233–242.

Bouis EH, 2002. Plant breeding: a new tool for fighting micronutrient malnutrition. J Nutr 132:491S-494S

Cantaluppi E, Manzi S, Egal A, Puglisi D, Cassani E, Toschi I, Cesari VT, Landoni M, Scapin A, Pilu R, 2017. Nutritional and phenotypical characterization of two South African maize (Zea mays L) varieties sampled in the Qwa-Qwa region. Maydica

Cakmak, I, 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification Plant Soil. 302:1–17.

Cerino F, Cassani E, Landoni M, Doria E, Panzeri D, Lago C, Mesiti F, Nielsen E, Pilu R, 2010. The low phytic acid1-241 (lpa1-241) maize mutation alters the accumulation of anthocyanin pigment in the kernel. Planta 231:1189–1199

Dormann P, 2003. Corn with enhanced anti-oxidant potential Nat Biotechnol 21:1015-1016

Eschholz TW, Stamp P, Peter R, Leipner J, Hund A, 2010. Genetic structure and history of Swiss maize (Zea mays L. ssp. mays) landraces. Genet Resour Crop Evol 57: 71–84.

Faostat—Food And Agriculture Organization of the United Nations, 2014. http://www.faostat.fao.org. Accessed April 5, 2017.

Food and Agriculture Organization of the United Nations (FAO), 2004. UBET Unified Bioenergy Terminology. Available from http://www.fao.org/docrep/007/j4504e/j4504e00.HTM

Food and Agriculture Organization of the United Nations, 2009: Crops production. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID= 567 (last accessed on April 20, 2011).

Hänsch R and Mendel RR, 2009. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Curr. Opin. Plant. Biol. 12:259–266.

Harjes CE, Rocheford TR, Bai L, Brutnell TP, Kandianis CB, Sowinski SG, Stapleton AE, Vallabhaneni R, Williams M, Wurtzel ET, Yan JB, Buckler ES, 2008. Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. Science 319:300-333

Lago C, Landoni M, Cassani E, Cantaluppi E, Doria E, Nielsen E, Giorgi A, Pilu R, 2015. Study and characterization of an ancient European flint white maize rich in anthocyanins: Millo Corvo from Galicia. PloS One 10(5): e0126521 DOI: 10.1371/journal.pone.0126521.

Lambert RJ, Alexander DE, Mollring EL, Wiggens B, 1997. Selection for increased oil concentration in maize kernels and associated changes in several kernel traits. Maydica 42:39–43.

Liu K, Goodman M, Muse S, Smith JS, Buckler E, Doebley J, 2003. Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. Genetics 165:2117-2128.

Liu RH, 2007. Whole grain phytochemicals and health. J. Cereal Sci. 46:207–219.

Messedaglia L, 1924. Notizie storiche sul mais. Quaderno mensile N°7. Sez. Credito Agrario Istituto Federale Credito del Risorgimento delle Venezie, Verona, Italy.

Messias RDS, Galli V, Silva SDDAE, Schirmer MA, Rombaldi CV, 2015. Micronutrient and functional compounds biofortification of maize grains. Crit. Rev. Food Sci. Nutr. 55, 123–39. doi:10.1080/10408398.2011.649314

Mir C, Zerjal T, Combes V, Dumas F, Madur D, Bedoya C, Dreisigacker S, Franco J, Grudloyma P, Hao PX, Hearne S, Jampatong C, Laloë D, Muthamia Z, Nguyen T, Prasanna BM, Taba S, Xie CX, Yunus M, Zhang S, Warburton ML, Charcosset A, 2013. Out of America: tracing the genetic footprints of the global diffusion of maize. Theor Appl Genet 126:2671-2682.

Newton AC, Akar T, Baresel JP, Bebeli PJ, Bettencourt E, Bladenopoulos KV, Czembor JH, Fasoula DA, Katsiotis A, Koutis K, Koutsika-Sotiriou M, Kovacs G, Larsson H, De Carvalho MAAP, Rubiales D, Russell J, Santos TMM, Patto MCV, 2009. Cereal landraces for sustainable agriculture. Sustain. Agric. 2, 147–186. doi:10.1007/978-94-007-0394-0_10

Panzeri D, Cesari V, Toschi I, Pilu R, 2011. Seed calorific value in different maize genotypes. Energy Sources Part A-Recovery 33(18): 1700-1705

Piperno DR, Ranere AJ, Holst I, Iriarte J, Dickau R, 2009. Starch grain and phytolith evidence for early ninth millennium B.P. maize from the Central Balsas River Valley, Mexico. Proc. Natl. Acad. Sci. U. S. A. 106: 5019-5024. Doi: 10.1073/pnas.0812525106 PMID: 19307570.

Relea P, Revilla M, Ripoll E, Arribas I, Villa LF, Rico H, 1995. Zinc, biochemical markers of nutrition, and type I osteoporosis. Age Ageing. 24:303–307.

Sommer A, 1982. Nutritional blindness: xerophthalmia and keratomalacia. Oxford University Press, New York

Van Heerwaarden J, Doebley J, Briggs W, Glaubitz CJ, Goodman M, Gonzalez JDS, Ross-Ibarra J, 2011. Genetic signals of origin, spread, and introgression in a large sample of a maize landraces. Proc. Natl. Acad. Sci. U. S. A. 108: 1088-1092. Doi: 10.1073/pnas.1013011108 PMID: 21189301.

Warburton ML, Reif JC, Frisch M, Bohn M., Bedoya C, Xia XC, Crossa J, Franco J, Hoisington D, Pixley K, Taba S, Melchinger AE, 2008. Genetic diversity in CIMMYT non temperate maize germplasm: landraces, open pollinated varieties, and inbred lines. Crop Sci 48(2):617-624 doi:10.2135/cropsci2007.02.0103.

Welch RM, Graham RD, 2002. Breeding crops for enhanced mineral content. Plant and Soil 245: 205-214.

WHO, 2009. Global prevalence of vitamin A deficiency in populations at risk 1995-2005. In: WHO global database on vitamin A deficiency, World Health Organization, Geneva, pp 1-55

Zeppa G, Bertolino M, and L Rolle, 2012. Quantitative descriptive analysis of Italian polenta produced with different corn cultivars. J. Sci. Food Agr. 92, 412—417.