Comparison among four maize varieties in conventional and low input cultivation

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Abstract

In this work we compared, using a randomized block design, four early maize (*Zea mays* L.) varieties: three traditional varieties (Millo Corvo, Scagliolo, Agostanello) and one modern hybrid (LG 25.38) grown in conventional vs low input farming. We recorded different agronomic parameters and we performed bromatological and ICP-MS analyses, and also quantified carotenoids, anthocyanins and mycotoxins. The analysis of agronomic parameters showed a general trend of better yields from conventional farming. Bromatological analysis did not show significant differences, we found more differences among varieties than between conventional and low input farming. Regarding minerals analysis, with the exception of the iron content, which was significantly higher from low input farming, we found high variability among the genotypes studied. The anthocyanins content, analyzed in the colored variety Millo Corvo, showed a statistically higher value in low imput farming. Finally, in both cultivation methods the level of fumonisins contamination was under the threshold limit. Taken together our data suggest that the effect of the genotypes was considerably higher than the effect of the cultivation method, hence it is the choice of the variety that will determine the nutritional value of the product harvested.

Introduction

Organic and low input farming are rapidly increasing in developed countries like the USA and Europe, but at present only 1.4 percent of the world's agricultural land is under "official" organic cultivation (WILLER and LERNOUD, 2019). Organic certification requirements and farming practices are different worldwide, but generally organic foods are grown without synthetic pesticides, fertilizers, antibiotics and growth hormones. The IFOAM (International Federation of Organic Agriculture Movements) endorses the principles of "health, ecology, fairness, and care" (IFOAM, 2009). Consumers choose organic foods for many reasons, mainly because they are popularly considered healthier, tastier and more environmentally friendly than conventional foods. Despite the widespread perception that organically produced foods are more nutritious than conventional ones, a meta-analysis study (SMITH-SPANGLER *et al.*, 2012) showed that there is no robust evidence to support this perception. In particular these authors concluded that there are no marked health benefits from consuming organic instead of conventional foods, although organic products may reduce the consumer's exposure to pesticide residues and to antibiotic-resistant bacteria. However, a proven fact is that yields in organic farming are lower than those in conventional farming. A study based on the meta-analysis of yield data from 362 datasets, comparing organic and conventional agriculture, showed that the yield of organic crops is on average 80% that of the conventional ones (DE PONTI *et al.*, 2012). However, consumers are willing to pay up to twice as much for organic rather than conventionally produced foods (WINTER and DAVIS, 2006).

In the context of sustainable agriculture and particularly of organic farming, great importance is given to the rediscovery of landraces. A landrace is a dynamic population of a cultivated plant that has an historical origin, without formal crop improvements, is locally adapted and associated with a traditional system. These populations comprise a large number of distinct homozygous lines in the case of self-pollinating crops or, in the case of cross-pollinated crops like maize, are populations with many heterozygous components (NEWTON *et al.*, 2010). Landraces are characterized by high genetic variability in morphological, agronomic and biochemical traits that allow the crop as a whole to be resistant to biotic and a biotic stresses (NEWTON *et al.*, 2010). The rich genetic variability found in landraces and in wild species also concerns nutraceuticals, the content of which was significantly reduced during breeding programs aimed at the development of modern cultivars (GALILI et al 2002; GIUPPONI *et al.*, 2019).

Nutraceuticals are a wide range of different molecules which are advocated as foods or additives to influence human health positively (RUTH and IZZO, 2017). In general, cereal landraces are sources of phytonutrients and micronutrients which have been proven to have a beneficial effect on health, reducing the incidence of aging-related and chronic diseases (DEL POZO-INSFRAN *et al.*, 2006).

Landraces have played a fundamental role in the history of crops worldwide. Farmers cultivated landraces until World War II, when the more productive hybrids were introduced, leading to the gradual disappearance of landraces (BRANDOLINI, 2009). According to FAO, it is estimated that 75% of the genetic diversity of crop plants was lost in the last century (FAO, 1998). The erosion of these sources of variability results in a severe threat to the world's long-term food security (DE PONTI *et al.*, 2012). Fortunately, in more recent years many efforts have been made to recover and preserve the old varieties: in Italy the main maize collection ex situ is preserved at the CREA (Council for Agricultural Research and Agrarian Economy located at Stezzano, BG) (GIUPPONI *et al.*, 2019; CASSANI *et al.*, 2017; PUGLISI *et al.*, 2018).

However, landraces still continue to be important in agricultural production, particularly in marginal environments where modern cultivars lose their competitive advantage (VILLA et al 2006; CANTALUPPI *et al.*, 2017; GIUPPONI *et al.* 2018; GIUPPONI *et al.*, 2019), in specialized production for niche markets (CLEVELAND *et al.*, 1994) and in organic farming. The aim of this paper is to analyze how the cultivation method, low input (mimicking the organic farming) or conventional, can affect different parameters such as yield, nutritional values and mycotoxins contamination in modern and traditional varieties of maize.

Material and methods

Plant material

The early maize varieties studied in this work were three landraces: Millo Corvo (from the germplasm collection of DISAA, Milan, Italy), Scagliolo and Agostanello (from the germplasm collection of CREA, Stezzano, BG, Italy) and one modern hybrid (LG 32.85, from Limagrain company).

Millo Corvo, originally cultivated in the Spanish region of Galicia and now in some spots in the north of Italy, is used to produce a variety of foods. The main feature of Millo Corvo is the distinctive dark-blue/black coloration of the kernels due to the high content of anthocyanins (LAGO *et al.*, 2015).

Scagliolo is a popular Italian variety used for the traditional dish "polenta", it is included in the list of "Variety of Conservation" of the National Register of Varieties of Agricultural and Horticultural Species at MiPAAF (Ministry of Agriculture Food and Forestry) in order to prevent the loss of local traditions and to preserve the genetic variability. This cultivar is able to grow at an altitude of 635 m in the Valsassina area, in the northwest of Italy (LAGO *et al.*, 2014).

Agostanello (accession number VA73) is a landrace typical of the Varese area, in the northwest of Italy, chosen for its earliness. All these three traditional varieties belong to FAO class 200-300 and are cultivated in small fields in the north of Italy.

The hybrid LG 32.85 was chosen because its characteristic traits are very similar to the ones of traditional varieties used in this study, it is an early variety (FAO class 200), with good yield, and suitable for the food supply chain.

Field experimentation

The maize plants were cultivated by low input or conventional farming, in the experimental field of the University of Milan, situated in Landriano (PV), Italy (45°18'N;9°15'E), 88 m a.s.l The experimentation was carried out in the 2016 season, the seeds were sown on April 15th and the ears harvested on September 12th. During the growing season the average temperature was 22,5°C and the total rainfall was 311 mm. Sufficient irrigation was provided periodically as needed to supplement rainfall.

The study was performed on a Haplic Luvisol soil (FAO classification). The characteristics of the soil are reported in Table 1.

The experiment was laid out in randomized blocks. The experimental field was divided into two halves: one for low input (mimicking organic farming) and the other for conventional farming, separated by three rows of maize plants (B73/Mo17 plants). Each variety was cultivated in three plots for low input farming and three plots for conventional farming, for a total of 12 plots for each method of cultivation. The size of each plot was about 10 m^2 (5 m x 2.1 m), with a density of 25 plants per plot, sown in three rows.

In the low input farming method, on the 27th and 53rd

Table 1 - Soil analysis of the experimental field used for this study. SD are shown (n=3).

pH (H2O)	6.5±0.01
pH (HCl)	5.5±0.02
Organic matter	14.2±0.12 g/kg
P available (Olsen)	102±0.96 mg/kg
P available (Bray/Kurtz)	136±1.44 mg/kg
Cation Exchange Capacity	10.6±0.24 cmol/kg
K exchangeable	432±8.16 mg/kg
Mg exchangeable	229±9.40 mg/kg
Ca exchangeable	1331±22.6 mg/kg
Sand	56.2±0.37%
Coarse Silt	14.6±0.52%
Fine Silt	22.6±1.53%
Clay	6.6±0.63%
Estimated water holding capacity	24.3±1.59%

day after sowing (DAS) the inter-row areas were processed by rototiller while, on the 46th and 66th DAS, manual weeding was done on the rows. The rototiller required a total of two work hours, while manual weeding required a total of height work hours. For fertilization, lupine seeds meal at 6,4% of N (LI *et al.*, 2009) was utilized at the maize sixth leaf stage, requiring ½ work hour (Tab.2). We used about 150g/m2 (equivalent to about 9,6 g/m2 N) of lupine seeds meal, interred by rototiller.

In the conventional farming method, on the first DAS a treatment with a pre-emergence herbicide (Clarido) was done, and on the 27th DAS the inter-rows were processed by rototiller. Herbicide treatment took ½ work hour while the rototiller procedure required one work hour. For fertilization, urea was utilized at the maize sixth leaf stage (200 Kg/ha, equivalent to about 9,33 g/m2 N). The same amount of nitrogen was used in the two methods of cultivation (Tab. 2).All the maize was harvested and the seeds were dried to 12-13% of relative humidity.

Agronomic parameters

At maturity we measured some agronomic parameters: plant height, ear height, weight of seeds per ear and single seed weight. For each parameter at least 20 samples were measured. The estimated yield was calculated considering 80,000 plants/ha.

Chlorophyll and Carotenoids quantification in leaves

Mature apical leaves were collected at the flowering stage and the amount of total chlorophyll (a+ b) and carotenoids was quantified as previously reported (ARNON, 1949).

Sample preparation and milling

For each genotype we tested 20 ears (sampled from the plants grown on the two central rows of the three plots of each variety) that were shelled and the seeds obtained mixed to create a single bulk used for bromatological analysis, ionomic content determination, carotenoids and anthocyanins extraction, damaged seeds quantification and mycotoxin analysis.

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

Bromatological analysis (calorific value, dry matter, ash, starch, crude protein, oil and fibers)

The dry matter (DM) of samples was obtained by inserting the samples in preweighed aluminum bags which were dried in a forced-air oven at 80°C for 72 h (AOAC, 2005). All dried samples were ground with a laboratory mill to 0.5 mm (Cyclone Sample Mill, Model 3010-019, pbi International, Milan, Italy) (HEJNA *et al.*, 2020). Calorific value measures and chemical analyses were car-

Table 2 - Agronomic operations carried out in organic and conventional farming.

LOW INPUT			CONVENTIONAL		
DAS	Treatment	Work hours	DAS	Treatment	Work hours
27	mechanical weeding	1	1	herbicide treatment	0.5
46	manual weeding	4	27	Mechanical weeding	1
53	mechanical weeding	1	six leaf stage	fertilization-urea	0.5
six leaf stage	fertilization-lupine	0.5			
66	manual weeding	4			
Total	10.5		Total	2	

DAS: Days After Sowing

ried out 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, 2005), milling and analyzing the samples for dry matter (DM), ash, starch, crude protein, ether extract (oil), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL).

Determination of ionomic content (P, K, Mg, Ca, Fe, Zn, Mn and Cu) in maize flour

For the determination of the elements of interest, 0.3 g maize flour samples were digested by a microwave digestor system (Anton Paar MULTIWAVE-ECO) in Teflon tubes filled with 10 ml of 65% HNO3 by applying a one-step temperature ramp (at 210°C 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 Mn, Cu, Mg, K, Ca, Fe, Zn were expressed as µg/g seed flour; values regarding P were indicated as mg/g seed flour.

Carotenoids extraction and quantification in seeds

Carotenoids extraction was performed as previously reported (PUGLISI *et al.*, 2018). Briefly, 3 ml of extraction buffer (acetone, methanol, hexane 1:1:1) were added to 0.25 g seed flour (three replicas for each sample) and after centrifugation the non-polar phase was collected and filtered.

The absorbance was measured spectrophotometrically at 450 nm using glass cuvettes. Carotenoids content was calculated according to the standard curve obtained using five lutein concentrations (0.25, 0.5, 1, 2, 4 µg/mg).

Extraction and quantification of anthocyanins, flavonols and phenolic acids in Millo Corvo variety

5 mg of flour was boiled with 100 µl of distilled water for 30 minutes and then left in overnight agitation with 1 ml of the extraction buffer (1% HCl, 95% EtOH). After another agitation time of 2 hours with 500 µl of extraction buffer, the supernatants were collected together and centrifuged for 30 minutes. Their absorbance was determined spectrophotometrically at 530nm for anthocyanins, at 350nm for flavonols and at 280nm for phenolic acids (LAGO *et al.*, 2015).

The amount of anthocyanins was calculated as cyanidin 3-glucoside equivalents (ε 26,900 Lm^{-1} mol^{-1}, M.W. 484.82), flavonols content as quercetin 3-glucoside equivalents (ε 21,877 Lm^{-1} mol^{-1}, M. W. 464.38) and the amount of phenolics as ferulic acid equivalents (ε 14,700 Lm^{-1} mol^{-1}, M.W. 194.18). The analyses were conducted four times for each genotype, and the confidence interval (C.I.) at 95% was calculated.

Damaged seeds and husks analysis

For damaged seeds identification, 100 seeds for each genotype were scored by visual inspection. This analysis was performed on three replicates (total 300 seeds for each genotype).

For husks counting, for each genotype 10 plants were randomly chosen and the highest ear was used for husks counting.

Table 3 - Bromatological analysis performed on the four varieties cultivated in low input and conventional farming. DM: Dry Matter, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin.HY: Hybrid; MC: Millo Corvo; SC: Scagliolo; AG: Agostanello. Means (n=3) followed by the same letter are not significantly different between low input vs conventional (Tukey test, p< 0.05). Standard deviation is showed.

Var.	Cultivation	Calorific value (J/g)	DM (%)	Ash (%)	Starch (%)	Protein (%)	Oil (%)	NDF (%)	ADF (%)	ADL (%)
	Low input	18,447 ± 38^a	89.8 ± 0.22^a	1.56 ± 0.06^a	71.2 ± 1.1^a	10.1 ± 0.9^a	4.60 ± 0.04^a	13.3 ± 0.02^a	2.96 ± 0.12^a	1.00 ± 0.04^a
HY	Conventional	19,705 ± 52^b	90.8 ± 0.41^a	1.78 ± 0.05^b	69.3 ± 0.8^a	10.3 ± 0.6^a	5.07± 0.06^b	12.7 ± 0.03^b	4.17 ± 0.07^b	1.63 ± 0.08^b
	Low input	18,724 ± 49^a	91.2 ± 0.67^a	1.78 ± 0.04^a	72.2 ± 0.7^a	13.8 ± 0.4^a	6.41 ± 0.07^a	11.1 ± 0.04^a	3.28 ± 0.03^a	1.11 ± 0.11^a
MC	Conventional	19,055 ± 56^b	91.3 ± 0.32^a	1.59 ± 0.02^b	66.3 ± 1.1^b	13.9 ± 0.8^a	5.46 ± 0.05^b	12.1 ± 0.06^b	3.01 ± 0.02^b	1.03 ± 0.09^a
	Low input	18,789 ± 72^a	91.0 ± 0.56^a	1.73 ± 0.03^a	73.1 ± 0.5^a	12.6 ± 1.1^a	6.17 ± 0.03^a	13.3 ± 0.09^a	2.89 ± 0.08^a	1.30 ± 0.07^a
SC	Conventional	19,020 ± 68^b	90.9 ± 0.38^a	1.69 ± 0.05^a	68.5 ± 0.4^b	12.5 ± 1.2^a	5.65 ± 0.07^b	11.9 ± 0.04^b	3.00 ± 0.06^a	1.22 ± 0.07^a
	Low input	18,789 ± 84^a	90.9 ± 0.59^a	1.62 ± 0.07^a	73.0 ± 0.9^a	11.7 ± 0.6^a	5.72 ± 0.04^a	11.1 ± 0.05^a	3.53 ± 0.08^a	1.59 ± 0.03^a
AG	Conventional	19,918 ± 101^b	90.5 ± 0.34^a	1.53 ± 0.08^a	68.5 ± 0.8^b	11.7 ± 0.8^a	5.27 ± 0.02^b	11.1 ± 0.07^a	3.35 ± 0.10^a	1.50 ± 0.08^a

Table 4 - Data collected regarding husks and damaged seeds number and fumonisins content in the four varieties cultivated in low inputand conventional farming

	Cultivation	HY	MC	SC	AG
Husks number	-	8.5 ± 0.36a	8 ± 0.85a	15 ± 0.56b	14 ± 0.86b
Damaged seeds%	Low input	3.4 ± 0.12a	12.2 ± 0.51a	3.8 ± 0.26a	1.8 ± 0.92a
	Conventional	6.6 ± 0.26b	7.4 ± 0.62b	4.2 ± 0.76a	1.2 ± 0.81a
Fumonisins (ppm)*	Low input	0.93 ± 0.22a	2.77 ± 0.18a	0.45 ± 0.16a	0.27 ± 0.14a
	Conventional	3.75 ± 0.47b	2.27 ± 0.33a	0.18 ± 0.10b	0.85 ± 0.19b

HY: Hybrid; MC: Millo Corvo; SC: Scagliolo; AG: Agostanello.For the husks, means followed by the same letter are not significantly different (n=10). For the damaged seeds (n=300) and fumonisins content (n=3) the comparison was performed between cultivation methods, low inputvs conventional, and means followed by the same letter are not significantly different (Tukey test , $p< 0.05$). For each mean value SD is shown.*Recommended maximum limit for fumonisins in unprocessed maize used as human food: 4 ppm.

Mycotoxin analysis

We analyzed the content of total fumonisins using the Elisa test kit I'screen FUMO (Tecna s.r.l.), a competitive immunoenzymatic assay for the quantitative determination of these mycotoxins.

For each sample analyzed, 50 g of flour was used for the quantitative assay, according to manufacturer's instruction. The analyses were conducted three times for each genotype.

Informatic tools

Microsoft Excel® was used to collect data, SPSS® was used to perform one-way ANOVA on sampled data. Tukey's Test was used to observe the difference between the two cultivation methods.

For PCA analysis we used the paleontological statistics software package for education and data analysis (PAST) (HAMMER *et al.*, 2001).

Author Contributions

Conceptualization, Michela Landoni, Annamaria Giorgi and Roberto Pilu; Data curation, Andrea Scapin, Giulia Borlini and Monika Hejna; Formal analysis, Andrea Scapin, Alessia Follador, Luciana Rossi and Roberto Pilu; Funding acquisition, Roberto Pilu; Investigation, Elena Cassani, Giulia Borlini, Alessia Follador and Martina Ghidoli; Methodology, Luca Giupponi, Monika Hejna and Roberto Pilu; Supervision, Roberto Pilu; Validation, Michela Landoni and Elena Cassani; Writing – original draft, Andrea Scapin; Writing – review & editing, Michela Landoni, Luca Giupponi, Monika Hejna, Luciana Rossi, Annamaria Giorgi and Roberto Pilu.

Results and discussion

Low input vs conventional: agronomic aspects

We have grown in low input vs conventional farming four early varieties of maize: one modern hybrid (HY) and three European landraces, Scagliolo (SC), Millo Corvo (MC) and Agostanello (AG) (Fig. 1).

The experimental design was randomized blocks, each variety was cultivated as reported in Materials and Methods.

We studied some agronomic parameters, in particular: plant height, ear height, seeds weight per ear, single seed weight and chlorophyll content in leaves (Fig. 2). The plants grown in conventional farming were taller than those in low input farming, in particular statistically significant differences between the two cultivation methods were found for HY and SC. The average height of the hybrid was 2.7 m for conventional farming and 2.38 m for low input farming (Fig. 2A).

Also, the ear height was higher in conventional than in

Fig. 1 - Mature seeds of the four varieties studied. From left to right: commercial hybrid (HY), Millo Corvo (MC), Scagliolo (SC), Agostanello (AG).

low input farming even if a statistically significant differences was found only for SC, where the ear height was about 0.9 m in low input farming and 1.1m in conventional farming (Fig. 2B). One explanation could be found in the type of fertilization chosen. In fact, we used urea in conventional farming, a very effective fertilizer for maize while we used organic matter (lupine seeds) for low input farming.

For single seed weight, the differences between low input and conventional farming were not statistically significant (Fig. 2C).

For seeds weight per ear, the measured values were higher in conventional than in low input farming. HY was the most productive variety, with an average production based on weight per ear of 106.18 g in conventional farming and of 97.69 g in low input farming (Fig. 2D). Considering a density of eight plants/m^2, equivalent to 80,000 plants ha^{-1}, the estimated yields were higher in conventional than in low input farming for each variety. A small difference of yield between the two methods, always in favor of the conventional method.

In particular, the increase of yield in conventional far-

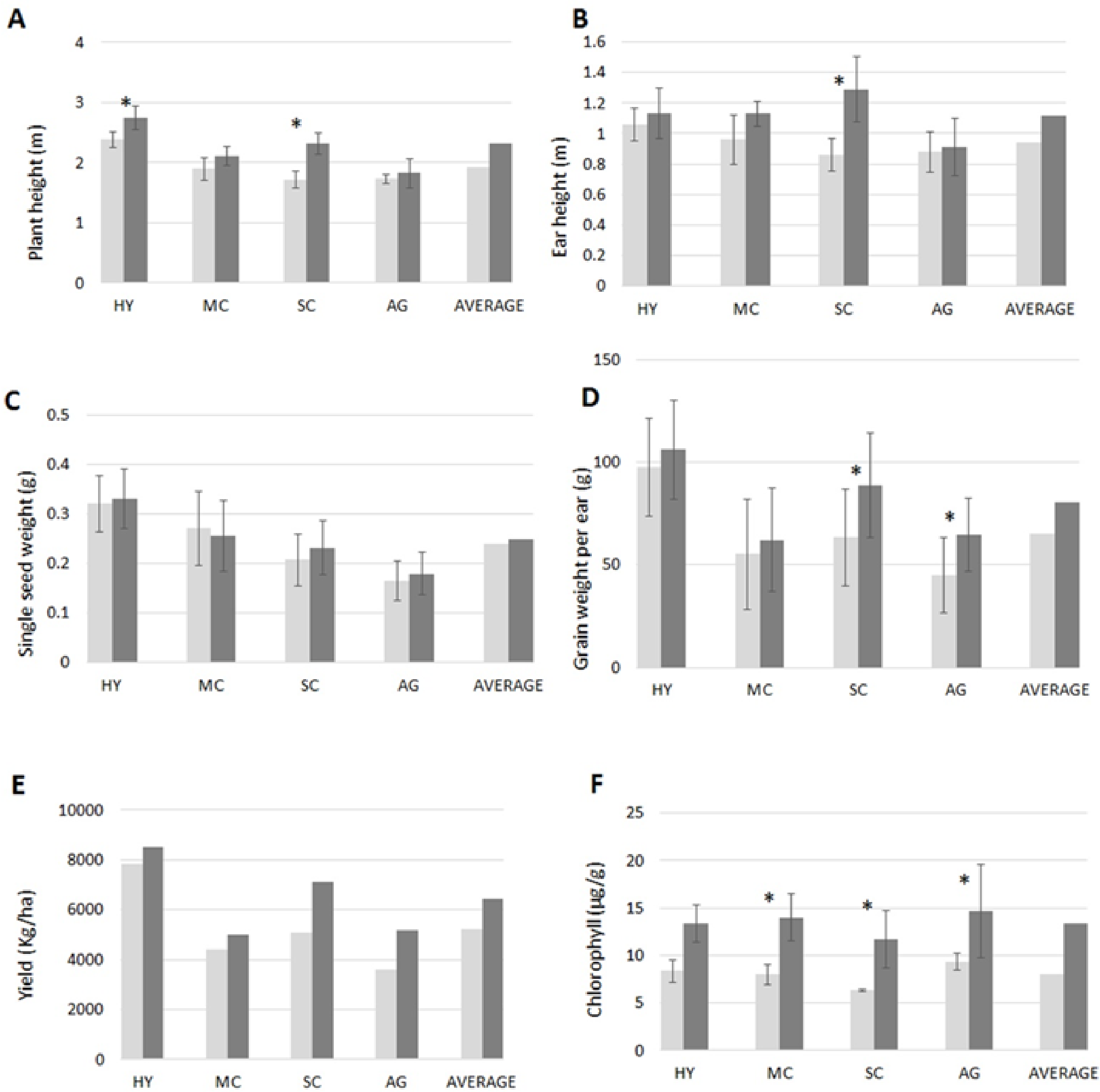

Fig. 2 - Agronomic parameters measured: plant height (A), ear height (B), single seed weight(C), grain weight per ear (D), estimated yield (kg/ha) (E), chlorophyll content (F). HY: Hybrid; MC: Millo Corvo; SC: Scagliolo; AG: Agostanello.: Light grey:low input farming, dark grey: conventional farming. Error bars represent Standard Deviation (SD). * represents significant difference by Tukey's Test (p=0.05).

ming in comparison to low input farming was 8% for HY, 11.26% for MC, 28.54% for SC and 30.28% for AG. The average increase of yield in conventional vs low input farming was +18.76%. HY showed the highest yield (about 8,000 Kg ha^{-1} in both conventional and low input farming) while SC was the most productive landrace (5,000 Kg ha^{-1} in low input farming and 7,000 Kg ha^{-1} in conventional farming) (Fig.2E). The yield is one of the most important parameters taken into consideration when organic and conventional farming methods are compared. A meta-dataset of 362 organic vs conventional comparative studies on crop yields, covering 43 countries worldwide and 67 crops, showed that organic yields of individual crops are on average 80% of con-

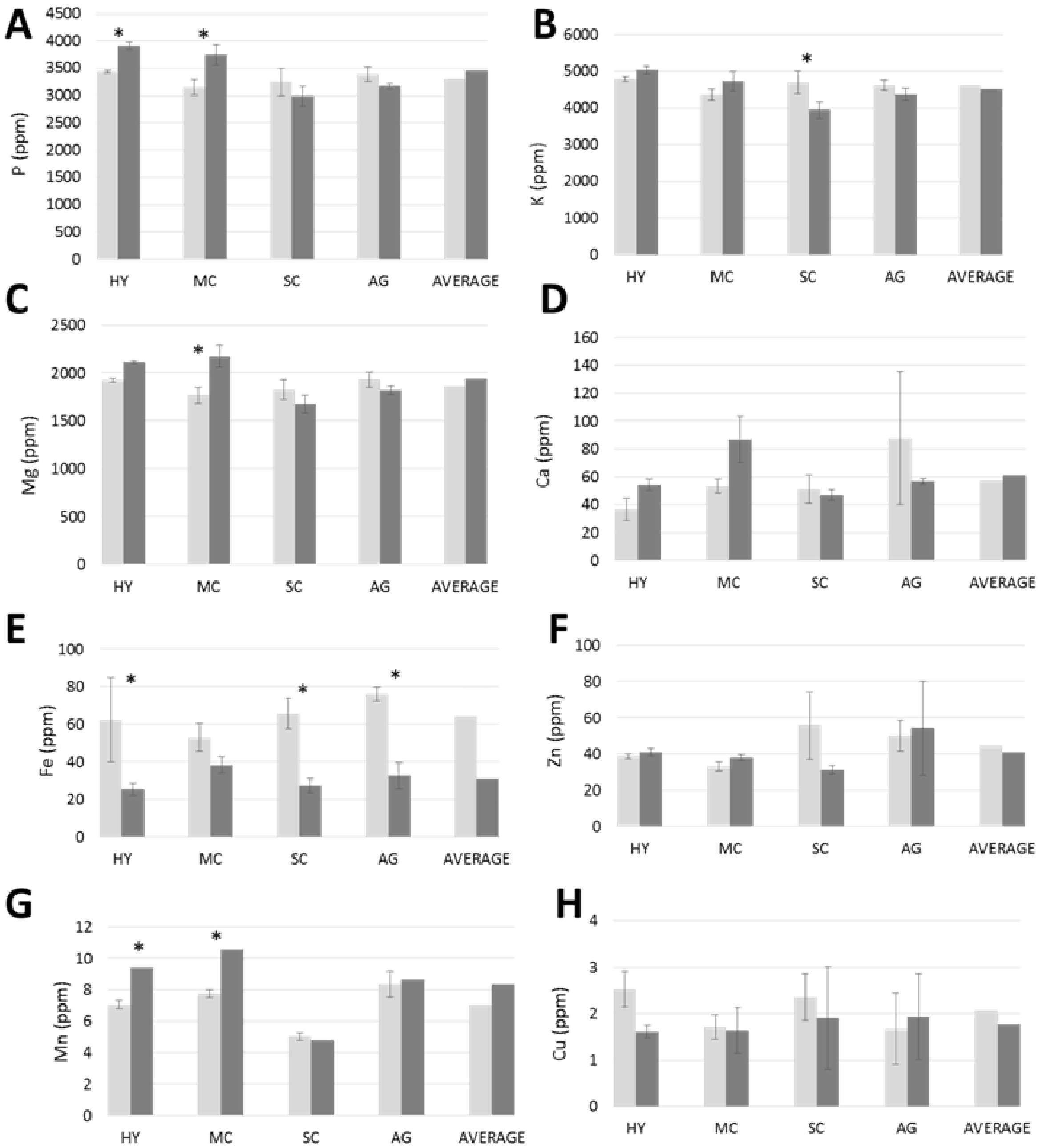

Fig. 3 - Mineral content of the flours obtained from the four varieties. HY: Hybrid; MC: Millo Corvo; SC: Scagliolo; AG: Agostanello. Light grey:low input farming, dark grey: conventional farming. Error bars represent Standard Deviation (SD). * represents significant difference by Tukey's Test (p=0,05).

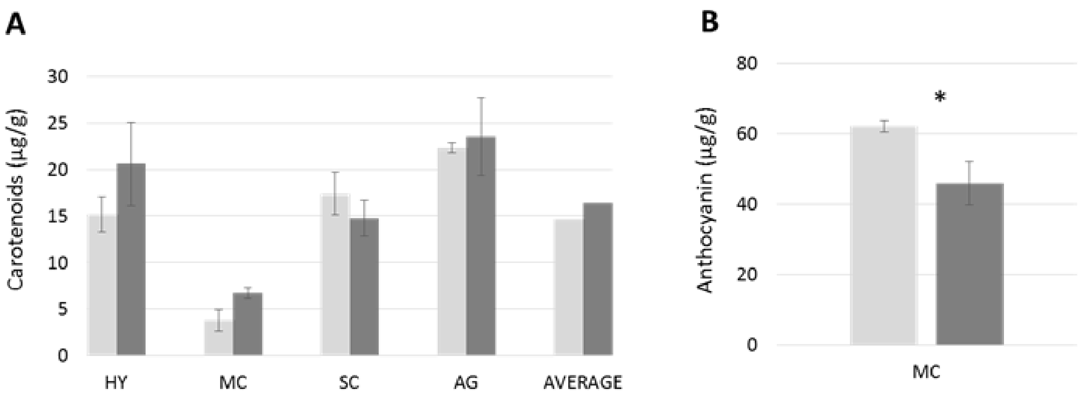

Fig. 4 - Carotenoids (A) and anthocyanins (B) content of the flours obtained from the four varieties.HY: Hybrid; MC: Millo Corvo; SC: Scagliolo; AG: Agostanello. In B we showed only the data of MC because in the other varieties anthocyanins are present only in traces. Light grey:low input farming, dark grey: conventional farming. Error bars represent Standard Deviation (SD). * represents significant difference by Tukey's Test (p=0,05).

ventional yields, but variation is substantial (standard deviation 21%) (DE PONTI *et al.*, 2012). In our work, we found that the average yields for the four varieties grown in low input farming was about 81% in comparison with conventional farming (Fig. 2E), in agreement with the data reported by De Ponti and colleagues (DE PONTI *et al.*, 2012). We did not use pesticides in either cultivation method, the only differences in agronomic operations were the N fertilization (lupine seeds vs urea) and the method used to control the weeds (manual vs mechanical) (Tab.2).

Hybrids are the result of breeding programs aimed at improving the yield and thus the higher yield of the hybrid in comparison to landraces is expected. Conventional breeding programs are realized in conditions of high inputs of inorganic fertilizers and crop protection and thus hybrids lack some traits required for cultivation under organic low-input conditions (LAMMERTS *et al.*, 2011). Landraces have been shown to be able to resist or to tolerate stresses (NEWTON *et al.*, 2010), and thus appear be more suitable for marginal, low input conditions (CECCARELLI, 1994) as in organic farming. However, in our experiment the hybrid performed better than the landraces in both methods of cultivation with no statistically significant reduction of yield in low inputvs conventional method (Fig. 2E). The reduction of yield in low input farming was particularly marked for SC and AG varieties (Fig. 2E), and it was associated with a statistically significant reduction of chlorophyll content (Fig. 2F). In the global yield assessment, it should be also taken into account that the lower yield in low input conditions is associated with a higher number of work hours necessary for the agronomic operations required for this method of cultivation (Tab.2).

The chlorophyll content in leaves (chlorophyll a+b), was higher in plants grown in conventional than in low input farming for all the four varieties. The average content in conventional farming (13.41 mg g^{-1}) was significantly higher than the content recorded in low input farming (7.99 mg g^{-1}) (Fig. 2F).Observations in the open field confirmed these data, with the leaves of plants grown in conventional farming greener than the ones grown in low input farming (data not shown). The agronomic operations we performed in conventional farming included the fertilization with urea that has been reported to be correlated with chlorophyll accumulation, with a rapid increase of pigment concentration after fertilization (YANG *et al.*, 2017). The higher level of chlorophyll in the plants grown in conventional farming, could be the reason for the increased vigor, measured as increased ear and plant height.

Low input vs conventional: nutritional aspects

Micro and macronutrients (Fe, Mn, Zn, Cu, P, K, Mg and Ca) were analyzed in the flour obtained from the milled seeds of the four varieties. No significant differences were observed comparing low input and conventional farming for Ca (Fig. 3D), Zn (Fig. 3F) and Cu (Fig. 3H) content. Iron was the only element analyzed in which there was a different level in low input vs conventional farming which was consistent in all the varieties studied: its amount was higher in low input farming. The average content was 64.13 µg g^{-1}in low input farming and 30.73 µg g-1in conventional farming (Fig. 3E). For the other minerals (P, K, Mg, Mn), we found 6 statistically significant differences in the four varieties, one in favor of low input farming (K content in SC) (Fig. 3B), and fie in favor of conventional farming (P content in HY and in MC, Mg content in MC and Mn content in HY and MC) (Fig. 3A, 3C and 3G).

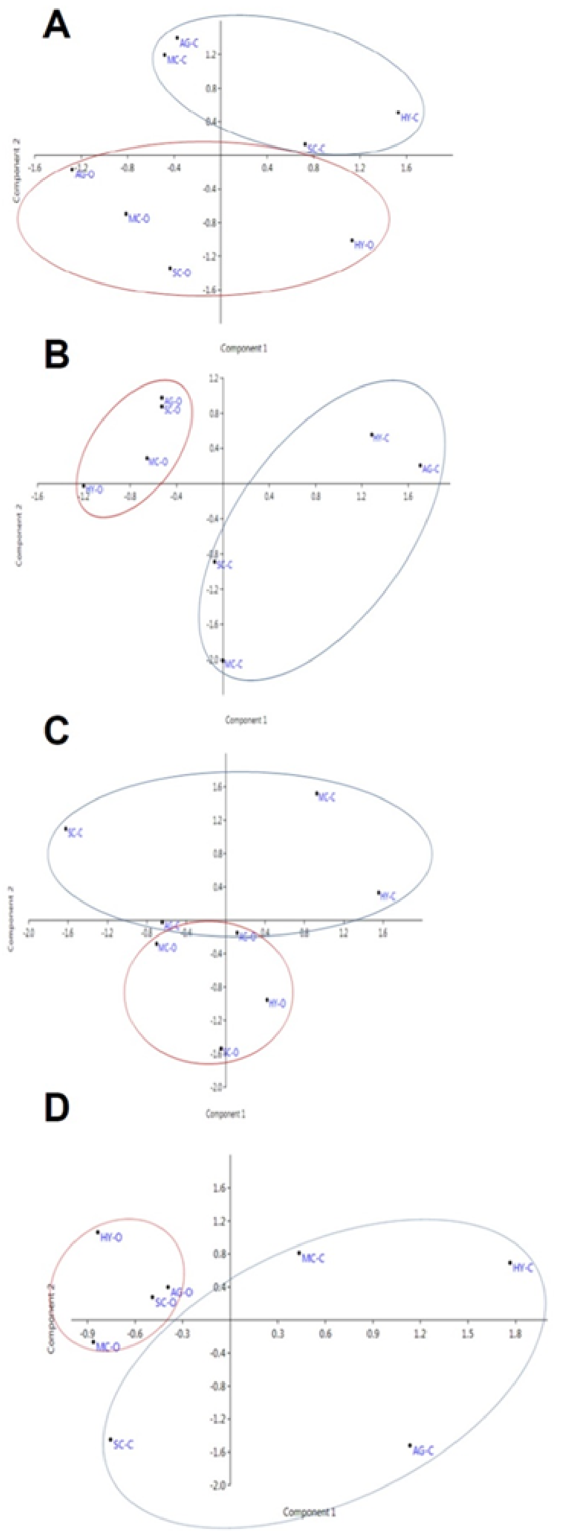

Fig. 5 - PCA analyses.PCA biplot analysis of agronomic parameters of Fig. 2 (A), bromatological data of Table 1 (B), mineral content of Fig. 3 and carotenoids content of Fig. 4A (C) and all the parameters together (D). In D the first two axes (PC1 and PC2) explain respectively 55.7% and 41% of the total variance. HY: Hybrid; MC: Millo Corvo; SC: Scagliolo; AG: Agostanello.-O: low input; -C: conventional.

We quantified by bromatological analysis: calorific value, dry matter, ashes, starch, proteins, oil and fibers (NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin) (Tab.3). Bromatological analysis did not show significant differences between low input and conventional farming as regards dry matter and proteins (Tab.3), while the calorific value was always higher from conventional than from low input farming. For all the other parameters taken into consideration there was a clear difference between the hybrid and the three landraces. Considering the landraces, the values of ashes, starch, oil and ADL were higher in low input than in conventional farming, while considering the hybrid the results were the opposite. This is not surprising considering the big genetic differences which exist between traditional varieties and the modern hybrid, accumulated in about one century of modern genetic improvement. Furthermore, considering protein and oil levels the results showed clearly that both parameters were higher in the three landraces than in the hybrid (Tab.3). Our results are in agreement with previously reported data (PANZERI *et al.*, 2011) that comparing bromatological analyses performed on different maize genotypes showed that Scagliolo had a higher content of proteins and oil than the hybrids analyzed.

Taken together the bromatological analyses showed that the differences were more marked comparing the four varieties than comparing the two methods of cultivation, with the hybrid performing markedly differently from the three landraces.

Among nutraceutical molecules, we studied carotenoids and anthocyanins, two important classes of phytonutrients with antioxidant capacity.

The carotenoid content in the seeds was higher in conventional than in low input farming for all the varieties except SC, even though the differences were not statistically significant (Fig. 4A). The average content among the varieties was 15.94 μg g^{-1} in conventional farming vs 14.66 μg g^{-1}in low input farming and the difference was not statistically significant (Fig. 4A).

The variety with the highest content of carotenoids was AG, with 22.30 μg g^{-1}in conventional farming and 21.70 μg g^{-1} in low inputfarming. The variety with the lowest level of carotenoids was MC (6.69 μg g^{-1}in conventional farming and 3.78 μg g^{-1}in low input farming This variety has been previously reported to lack carotenoids, in fact, without anthocyanins MC seeds would be white (LAGO *et al.*, 2015). The low concentration of carotenoids present in Millo Corvo, derives, most probably, from genetic pollution, because the plants were cultivated under open pollination conditions.

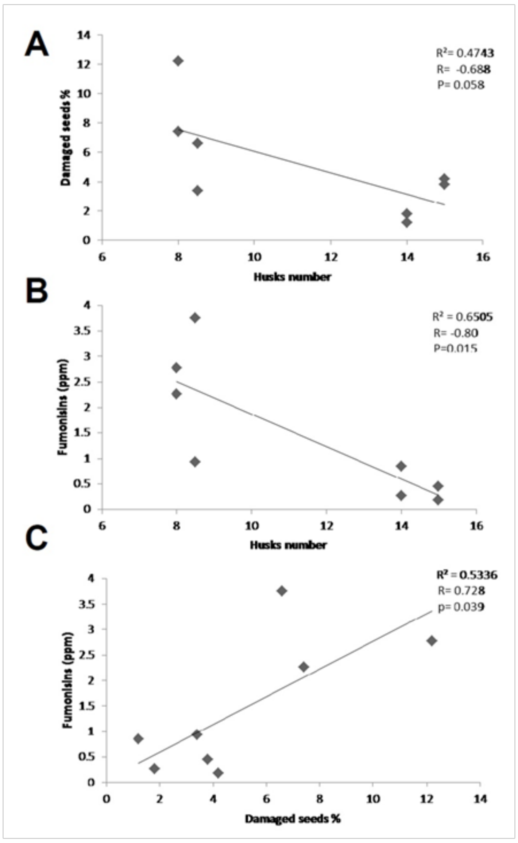

Fig. 6 - Correlation analysis between husk number / damaged seeds % (A), husk number / fumonisins (B) and damaged seeds (%) / fumonisins (C).

We analyzed anthocyanins only for MC variety, because it was the only colored variety among the four analyzed in this experiment. We found a statistically significant difference between the two methods of cultivation: 62.10 mg 100 g^{-1} in low input farming vs 46.02 mg 100 g^{-1} in conventional farming (Fig.4B). Also in this case, no literature data were available concerning anthocyanin content in maize plants grown in conventional vs low input/organic farming. However, our results are in agreement with previously reported data that compared phenols content in strawberries grown in organic vs conventional farming, showed that the anthocyanins level was higher in organically grown plants (CRESCENTE-CAMPO *et al.*, 2012). This paper suggested that this result could be explained considering the role of anthocyanins in the prevention of diseases and in plant protection in general (PILU *et al.*, 2012; LAGO *et al.*, 2014). In low input/organic farming chemicals are not used and thus the plants to resist against external aggressions must produce a greater number of chemical compounds, among them the anthocyanins, for their own defense (WOESE *et al.*, 1997; WINTER and DAVIS, 2006).

Low input vs conventional: PCA analyses

We performed PCA analyses taking into account the agronomic parameters reported in Figure 2, bromatological analysis data of Table3, mineral and carotenoid content of Figures 3 and 4A and all parameters together, with the aim to better highlight the differences between conventional and low input cultivation for the parameters measured in this work. As shown in Figure 5, PCA biplot analysis showed that the varieties cultivated in low input vs conventional farming are grouped in two separated clusters. In particular in Figure 5D, considering all parameters together, it can be noticed that the low input cultivated varieties are more closely grouped in comparison with the conventional ones.

Low input vs conventional: mycotoxins contamination

The number of husks and of damaged seeds were counted to perform the correlation analysis with fumonisins content.

Fusarium infections of maize kernels can result in accumulation of mycotoxins such as fumonisins. Fumonisins contamination in maize grain is of concern because of its causal role in equine leukoencephalomalacia, porcine pulmonary edema, in hepato/nephrotoxity and carcinogensesis in rodents and these chemicals are classified as possible human carcinogens (PILU *et al.*, 2011; SHEPHARD *et al.*, 2013; LANDONI *et al.*, 2020).

The four varieties showed the same number of husks in conventional and low input farming and in particular: 8.5 for the modern hybrid, 8 for MC, 15 for SC and 14 for AG (Tab.4). The percentage of damaged seeds, a measure of corn borer attack, was not statistically different between the two cultivation methods for SC and AG, for the hybrid the percentage was double in conventional farming in comparison with low input farming, while for MC it was higher from low input than from conventional farming (Tab.4).

Fumonisins contamination was quantified and the results are described in Table 4. For all the varieties analyzed the levels are under the threshold of 4ppm, the maximum allowed level for fumonisins in unprocessed maize for human consumption. For the aflatoxins B1 and B2, the content was also under the legal threshold (data not shown).

Correlation analysis showed negative correlations between number of damaged seeds and number of husks (p=0.058) (Fig. 6A) and between fumonisins content and husks number (p=0.015) (Fig. 6B), while a positive correlation was found between fumonisins content and number of damaged seeds (p=0.039) (Fig. 6C). These data could be explained considering that husks exert a mechanical action protecting the ear from corn borer attack, the main vector for fusarium infection.

Literature data concerning the comparison of mycotoxin contamination in cereals in conventional vs organic farming fail to agree. There are studies showing lower (BIRZELE *et al.*, 2002; MEISTER et al, 2009;BERNHOFT *et al.*, 2010), similar (EDWARDS, 2009) and higher (LAZZARO *et al.*, 2015) levels of Fusarium spp. and mycotoxin contamination in organic than in conventional cereal production. In another study, comparing the level of mycotoxins contamination in maize grown in organic vs conventional farming, the conclusion was that environment and varieties are important to determine the contamination, while the method of cultivation did not give a statistically significant difference (RUIZ DE GALARRETA *et al.*, 2014).

Taken together our data suggest that the effect of genotype, on the parameters we measured, was higher than the effect of the cultivation method. The comparison between conventional vslow input cultivation showed only a few statistically significant differences, some of them in favor of conventional farming and some in favor of low input farming. Martí *et al.* reported that the effect of varieties was stronger that the growing system for L-ascorbic acid content in tomato (MARTÌ *et al.*, 2017). In an important work of meta-analysis, that compared 223 scientific papers regarding different crops, the authors did not find significant differences in nutrients in organic vs conventional foods, only phosphorus level was significantly higher in organic farming than conventional produce but this difference was not clinically significant (SMITH-SPANGLER *et al.*, 2012).

As reported in Fig. 5, low input cultivation seems to reduce the differences among varieties, in particular for the factors assessed by bromatological analysis (Fig. 5B) and taking into consideration all the parameters together (Fig. 5D). These data suggest that the more stressful environment present in low input agriculture could lower the phenotypic and chemical differences among varieties. Concluding, our work suggests that in the case of maize the choice of the varieties is determinant especially in low input/organic farming: traditional varieties with high amounts of protein, oil and other phytonutrients could justify the higher effort needed by organic cultivation. Also, with regard to the phytosanitary aspects, early varieties with higher husks number should be chosen to minimize the risk of Fusarium attack and consequently fumonisins contamination.

Further work will be necessary (e.g. more varieties and more environments should be analyzed) to strengthen the data presented in this paper which represents the first attempt to rationalize the maize organic sector, which is in continuous growth worldwide.

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Conceptualization: Michela Landoni and Roberto Pilu; Data curation: Andrea Scapin, Giulia Borlini and Monika Hejna; Formal analysis: Andrea Scapin, Alessia Follador, Luciana Rossi and Roberto Pilu; Funding acquisition: Roberto Pilu; Investigation: Elena Cassani, Giulia Borlini, Alessia Follador and Martina Ghidoli; Methodology: Luca Giupponi, Monika Hejna and Roberto Pilu; Supervision: Roberto Pilu; Validation: Michela Landoni and Elena Cassani; Writing – original draft: Andrea Scapin; Writing – review & editing: Michela Landoni, Luca Giupponi, Monika Hejna, Luciana Rossi, Annamaria Giorgi and Roberto Pilu.

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Conflict of interest

No potential conflict of interest was reported by the authors

References

AOAC, 2005.Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists; Arlington, VA, USA.

ARNON, D., 1949: Copper enzymes in isolated chloroplasts polyphenoloxidase in Beta vulgaris. Plant Physiol. 24, 1-15.

BERNHOFT, A., CLASEN, P-E., KRISTOFFERSEN, A.B., TORP, M., 2010: Less Fusarium infestation and mycotoxin contamination in organic than in conventional cereals. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 27, 842-852.

BIRZELE, B., MEIER, A., HINDORF, H., KRAMER, J., DEHNE, H.,2002: Epidemiology of Fusarium infection and deoxynivalenol content in winter wheat in the Rhineland, Germany. Eur. J. Plant Pathol. 108, 667-673.

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

CANTALUPPI, E., MANZI, S., ABDULKADIR, A.E., PUGLISI, D., CASSANI, E., TOSCHI, I., CESARI, V.T., 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 62. https://journals-crea.4science.it/index.php/maydica/article/

view/1565/1075.

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* L.) rich in phlobaphenes: the "Nero Spinoso" from the Camonica valley. Genet. Resour. Crop Evol. 64, 761-773.

CECCARELLI S. 1994: Specific adaptation and breeding for marginal conditions. Euphytica77, 205-219.

CIOLEK, A., MAKARKA, E., WESOLOWSKI, M., CIERPIALA, R., 2012: Content of selected nutrients in wheat, barley and oat grain from organic and conventional farming. J. Elem. 17: 181-189.

CLEVELAND, D.A., SOLERI, D., SMITH, S.E., 1994:Folk crop varieties: do they have a role in sustainable agriculture? BioScience 44, 740-751.

CRESCENTE-CAMPO, J., NUNES-DAMACENO, M., ROMERO-RODRIGUEZ, M.A., VAZQUEZ-ODÉRIZ, M.L., 2012:Color, anthocyanin pigment, ascorbic acid and total phenolic compound determination in organic versus conventional strawberries (Fragaria ananassa Duch, cv Selva). J. Food Compos. Anal.8, 23-30.

DE PONTI, T., RIJK, B., VAN ITTERSUM, M.K., 2012: The crop yield gap between organic and conventional agriculture. Agric. Syst. 108, 1-9.

DEL POZO-INSFRAN, D., BRENES, C.H., SALDIVAR, S.O.S., TALCOTT, S.T., 2006:Polyphenolic and antioxidant content of white and blue corn (*Zea mays* L.) products. Food Res. Int. 39, 696-703.

EDWARDS, S.G., 2009: Fusarium mycotoxin content of UK organic and conventional wheat. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 26, 496-506.

FAO (Food and Agricultural Organization of the United Nations),1998: The state of food and agriculture, 1998. FAO Agriculture Series, no 31. Rome, Italy. ISBN92-5 104200-4.

GALILI, G., GALILI, S., LEWINSOHN, E., TADMORE, Y., 2002: Genetic, molecular and genomic approaches to improve value of plant foods and feeds. Crit. Rev. Plant Sci. 21, 167-204.

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.

GIUPPONI, L., PILU, R., SCARAFONI, A., GIORGI, A., 2019:Plant agro-biodiversity needs protection, study and promotion: results of research conducted in Lombardy region (Northern Italy). Biodivers. Conserv.https://doi.org/10.1007/s10531-019-01889-3.

GIUPPONI. L., TAMBURINI, A., GIORGI, A., 2018:Prospects for Broader Cultivation and Commercialization of Copafam, a Local Variety of Phaseolus coccineus L., in the Brescia Pre-Alps. Mt. Res. Dev. 38, 24-34.

HAMMER, Ø., HARPER, D.A.T., RYAN P.D., 2001: PAST: Paleontological statistics software package for education and data analysis. Paleontol. Electron. 4. https://palaeo-electronica.org/2001_1/past/past.pdf.

HEJNA, M., MOSCATELLI, A., STROPPA, N., ONELLI. E., PILU, S., BALDI, A., ROSSI, L., 2020:Bioaccumulation of heavy metals from wastewater through a Typha latifolia and Thelypteris palustris phytoremediation system. Chemosphere 241. https://doi.org/10.1016/j.chemosphere.2019.125018.

IFOAM (International Federation of Organic Agriculture Movements), 2009: Principles of organic agriculture. Retrieved 7Th march 2020, from https://www.ifoam.bio/en/organic-landmarks/principles-organic-agriculture.

LAGO, C., CASSANI, E., ZANZI, C., LANDONI, M., TROVATO, R., PILU. R., 2014: Development and study of a maize cultivar rich in anthocyanins: coloured polenta, a new functional food. Plant Breed. 133, 210-217.

LAGO, C., LANDONI, M., CASSANI, E., ATANASSIU, S., CANTA-LUPPI, E., PILU, R. 2014:

Development and characterization of a coloured sweet corn line as a new functional food. Maydica, 59 (3), 191-200.

LAGO, C., LANDONI, M., CASSANI, E., CANTALUPPI, E., DORIA, E., NIELSEN, E., GIORGI, A.,

LAMMERTS VAN BUEREN, E.T, JONES, S.S., TAMM, L., MURPHY, K.M., MYERS, J.R., LEIFERT, C., MESSMER, M.M., 2011:The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: A review. NJAS-Wagen J. Life Sc. 58, 193-205.

LANDONI, M., PUGLISI, D., CASSANI, E., BORLINI, G., BRUNOLDI, G., COMASCHI, C., PILU, R.2020:Phlobaphenes modify pericarp thickness in maize and accumulation of the fumonisin mycotoxins. Scientific Reports, 10 (1),

art. no. 1417,
LAZZARO, I., MORETTI, A., GIORNI, P., BRERA, C., BATTILANI, P., 2015: Organic vs conventional farming: differences in infection by mycotoxin-producing fungi on maize and wheat in Northern and Central Italy. Crop Prot. 72, 22-30.
LI, Z., SCHULZ, R., MÜLLER, T., 2009: Short-term Nitrogen Availability from Lupine Seed Meal as an Organic Fertilizer is Affected by Seed Quality at Low Temperatures. Biol. Agric. Hortic. 26, 337–352.
MARTÍ, R., LEIVA-BRONDO, M., LAHOZ, I., CAMPILLO, C., CEBOLLA-CORNEJO, J., ROSELLÓ, S., 2017:Polyphenol and L-ascorbic acid content in tomato as influenced by high lycopene genotypes and organic farming at different environments. Food Chem. 239, 148-156.
MEISTER, U., 2009: Fusarium toxins in cereals of integrated and organic cultivation from the Federal State of Brandenburg (Germany) harvested in the years 2000–2007. Mycotoxin Res. 25, 133-139.
NEWTON, A.C., AKAR, T., BARESEL, J.P., BEBELI, P.J., BETTENCOURT, E., BLADENOPOULOS, K.V., VAZ PATTO, M.C., 2010:Cereal landraces for sustainable agriculture. A review. Agron. Sustain. Dev. 30, 237-269. https://doi.org/10.1051/agro/2009032.
OSTROWSKA-KOLODZIEJCZAK, A., STUPER-SZABLEWSKA, K., KULIK, T., BUSKO, M., RISSMANN, I., WIWART, M., PERKOWSKI, J., 2016: Concentration of fungal metabolites, phenolic acids and metals in mixtures of cereals grown in organic and conventional farms. J. Anim. Feed Sci. 25, 74-81.
PANZERI, D., CESARI, V., TOSCHI, I., PILU, R., 2011: Seed calorific value in different maize genotypes. Energ. Source Part A 33, 1700-1705.
PILU, R., CASSANI, E., SIRIZZOTTI, A., PETRONI, K., TONELLI, C. 2011: Effect of flavonoid pigments on the accumulation of fumonisin B1 in the maize kernel(2011) Journal of Applied Genetics, 52 (2), pp. 145-152.
PILU, R., BUCCI, A., CASELLA, L., LAGO, C., CERINO BADONE, F., CASSANI, E., LANDONI, M.2012:A quantitative trait locus involved in maize yield is tightly associated to the r1 gene on the long arm of chromosome 10. Molecular Breeding, 30(2),799-807.
PILU, R., 2015: Study and characterization of an ancient European flint white maize rich in anthocyanins: Millo Corvo from Galicia. PLoS One 10. 10(5):e0126521. doi: 10.1371/journal.pone.0126521.
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. https://journals-crea.4science.it/index.php/maydica/article/view/1636.
RUIZ DE GALARRETA, J.I., BUTRÓN, A., ORTIZ-BARREDO, A., MALVAR, R.A., ORDÁS, A., LANDA, A., REVILLA, P., 2014:Mycotoxins in maize grains grown in organic and conventional agriculture. Food Control 52, 98-102.
RUTH, A., IZZO, A.A., 2017:Principles of pharmacological research of nutraceuticals. Br. J. Pharmacol. 174, 1177-1194.
SHEPHARD, G.S., KIMANYA, M.E., KPODO, K.A., GNONLONFIN, G.J.B., GELDERBLOM, W.C.A., 2013:The risk management dilemma for fumonisin mycotoxins. Food Control. 34,596-600.
SMITH-SPANGLER, C., BRANDEAU, M.L., HUNTER, G.E., BAVINGER, J.C., PEARSON, M., ESCHBACH, P.J., SUNDARAM, V., LIU, H., SCHIRMER, P., STAVE, C., OLKIN, I., BRAVATA, D.M., 2012: Are organic foods safer or healthier than conventional alternatives? A systematic review. Ann. Intern. Med. 157, 348-366.
VILLA, T.C.C., MAXTED, N., SCHOLTEN, M., FORD-LLOYD, B., 2006:Defining and identifying crop landraces. Plant Genet. Resour. 3, 73-384.
WILLER, H., LERNOUD, J., 2019: The World of Organic Agriculture 2019. Statistics and Emerging Trends. 20th ed. FiBL and IFOAM. https://www.organic-world.net/yearbook/yearbook-2019.html.
WILLIAMS, P.R, HAMMITT, J.K. 2000:A comparison of organic and conventional fresh produce buyers in the Boston area. Risk Anal. 20, 735-46.
WINTER, C.K., DAVIS, S.F., 2006:Organic foods. J. Food Sci. 71, 117-124.
WOESE, K., LANGE, D., BOESS, C., WERNER BÖGL, K. A., 1997:Comparison of organically and conventionally grown foods- results of a review of the relevant literature. J. Sci. Food Agr. 74, 281-293.
YANG, Y., NI, X., ZHOU, Z., YU, L., LIU, B., YANG, Y., WU,Y., 2017:Performance of matrix-based slow-release urea in reducing nitrogen loss and improving maize yields and profits. Field Crops Res. 212, 73-81.