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 input 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 LERNIOUD, 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 va-
riability 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 Li-magrain 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 m² (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
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 four 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 0.5 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 0.5 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 1 - Soil analysis of the experimental field used for this study. SD are shown (n=3).

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

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

<table>
<thead>
<tr>
<th>DAS</th>
<th>LOW INPUT</th>
<th>CONVENTIONAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Work hours</td>
</tr>
<tr>
<td>27</td>
<td>mechanical weeding</td>
<td>1</td>
</tr>
<tr>
<td>46</td>
<td>manual weeding</td>
<td>4</td>
</tr>
<tr>
<td>53</td>
<td>mechanical weeding</td>
<td>1</td>
</tr>
<tr>
<td>66</td>
<td>fertilizer-lupine</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>manual weeding</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>10.5</td>
<td>2</td>
</tr>
</tbody>
</table>

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 ionic 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 MULTIWAIVE-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 polynatomical 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 mg/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 530 nm for anthocyanins, at 350 nm for flavonols and at 280 nm for phenolic acids (LAGO et al., 2015).

The amount of anthocyanins was calculated as cyanidin 3-glucoside equivalents (€ 26,900 Lm⁻¹ mol⁻¹, M.W. 484.82), flavonol content as quercetin 3-glucoside equivalents (€ 21,877 Lm⁻¹ mol⁻¹, M. W. 464.38) and the amount of phenolics as ferulic acid equivalents (€ 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. 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>
<thead>
<tr>
<th>Var. Cultivation</th>
<th>Calorific value (J/g)</th>
<th>DM (%)</th>
<th>Ash (%)</th>
<th>Starch (%)</th>
<th>Protein (%)</th>
<th>Oil (%)</th>
<th>NDF (%)</th>
<th>ADF (%)</th>
<th>ADL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low input HY</td>
<td>18,447 ± 38°</td>
<td>89.8 ± 0.22°</td>
<td>1.56 ± 0.06°</td>
<td>71.2 ± 1.1°</td>
<td>10.1 ± 0.9°</td>
<td>4.60 ± 0.04°</td>
<td>13.3 ± 0.02°</td>
<td>2.96 ± 0.12°</td>
<td>1.00 ± 0.04°</td>
</tr>
<tr>
<td>Conventional</td>
<td>19,705 ± 52°</td>
<td>90.8 ± 0.41°</td>
<td>1.78 ± 0.05°</td>
<td>69.3 ± 0.8°</td>
<td>10.3 ± 0.6°</td>
<td>5.07 ± 0.06°</td>
<td>12.7 ± 0.03°</td>
<td>4.17 ± 0.07°</td>
<td>1.63 ± 0.08°</td>
</tr>
<tr>
<td>Low input HY</td>
<td>18,724 ± 49°</td>
<td>91.2 ± 0.67°</td>
<td>1.78 ± 0.04°</td>
<td>72.2 ± 0.7°</td>
<td>13.8 ± 0.4°</td>
<td>6.41 ± 0.07°</td>
<td>11.1 ± 0.04°</td>
<td>3.28 ± 0.03°</td>
<td>1.11 ± 0.11°</td>
</tr>
<tr>
<td>Conventional</td>
<td>19,035 ± 56°</td>
<td>91.3 ± 0.32°</td>
<td>1.59 ± 0.02°</td>
<td>66.3 ± 1.1°</td>
<td>13.9 ± 0.8°</td>
<td>5.46 ± 0.05°</td>
<td>12.1 ± 0.06°</td>
<td>3.01 ± 0.02°</td>
<td>1.03 ± 0.09°</td>
</tr>
<tr>
<td>Low input HY</td>
<td>18,789 ± 72°</td>
<td>91.0 ± 0.56°</td>
<td>1.73 ± 0.03°</td>
<td>73.1 ± 0.5°</td>
<td>12.6 ± 1.1°</td>
<td>6.17 ± 0.03°</td>
<td>13.3 ± 0.09°</td>
<td>2.89 ± 0.08°</td>
<td>1.30 ± 0.07°</td>
</tr>
<tr>
<td>Conventional</td>
<td>19,020 ± 68°</td>
<td>90.9 ± 0.38°</td>
<td>1.69 ± 0.05°</td>
<td>68.5 ± 0.4°</td>
<td>12.5 ± 1.2°</td>
<td>5.65 ± 0.07°</td>
<td>11.9 ± 0.04°</td>
<td>3.00 ± 0.06°</td>
<td>1.22 ± 0.07°</td>
</tr>
<tr>
<td>Low input SC</td>
<td>18,789 ± 84°</td>
<td>90.9 ± 0.59°</td>
<td>1.62 ± 0.07°</td>
<td>73.0 ± 0.9°</td>
<td>11.7 ± 0.6°</td>
<td>5.72 ± 0.04°</td>
<td>11.1 ± 0.05°</td>
<td>3.53 ± 0.08°</td>
<td>1.59 ± 0.03°</td>
</tr>
<tr>
<td>Conventional</td>
<td>19,918 ± 101°</td>
<td>90.5 ± 0.34°</td>
<td>1.53 ± 0.08°</td>
<td>68.5 ± 0.8°</td>
<td>11.7 ± 0.8°</td>
<td>5.27 ± 0.02°</td>
<td>11.1 ± 0.07°</td>
<td>3.35 ± 0.10°</td>
<td>1.50 ± 0.08°</td>
</tr>
</tbody>
</table>
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 low input farming. Husks number and damaged seeds percentage were higher in low input farming, while fumonisins content was lower in low input farming (Table 4).

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

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

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 input vs 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.
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 1.1 m 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/m², 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-
Muntingia cultivated in conventional vs 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 conventional yields. 

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).
Maize cultivated in conventional vs low input farming

Conventional 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 input vs 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⁻¹ in 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 five 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. 4](image)

**Fig. 4** - Carotenoids (A) and anthocyanins (B) content of the flours obtained from the four varieties. HY: Hybrid; MC: Millo Corvo; SC: Scaglione; 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).
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 phytomolecules 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 input farming. 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.
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 (CRESCENT-E-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., 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 4 ppm, 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

**Low input vs conventional: PCA analyses**

We performed PCA analyses taking into account the agronomic parameters reported in Figure 2, bromatological analysis data of Table 3, 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/nephrotoxicity and carcinogenesis in rodents and these chemicals are classified as possible human carcinogens (PILU et al., 2011; SHEPHARD et al., 2013; LANDONI et al., 2020).

Fig. 6 - Correlation analysis between husk number / damaged seeds % (A), husk number / fumonisins (B) and damaged seeds (%) / fumonisins (C).
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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 GARRETA 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 vs low 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. Marti et al. reported that the effect of varieties was stronger that the growing system for L-ascorbic acid content in tomato (MARTÍ 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 phytonutritional 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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