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# Mineral contents in grains of seven food-grade sorghum hybrids grown in a Mediterranean environment

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*Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.*

## Abstract

Mineral nutrients play a fundamental role in the biochemical and physiological functions of biological systems. Cereals may especially be an important source of essential minerals in view of their large daily intake both for human health and nutrition. Sorghum, among the cereals, is a major crop being used for food, feed and industrial purposes worldwide. The objective of this study was to determine the mineral contents in grains of seven white food-grade sorghum hybrids, bred and adapted for growth in the central USA and grown in a Mediterranean area of Southern Italy. The seven hybrids were analyzed for grain ash and for minerals contents. Nutritionally, essential macro-, micro- and trace elements content were investigated. The analysis of essential elements was performed by mass spectrometry using a mix solution of internal isotopes standard. The results demonstrated that food-grade sorghum was characterized by high Mg, Fe and Zn content, high K:Na ratio and low Ca:P ratio, compared to other crops, due to the fact that the grain mineral contents of crop species are influenced by the effects of genotypes and environments. Significant variations in the essential elements content were found among the hybrids which allowed us to divide them into three distinct groups on the basis of their mineral profile by cluster analysis. These results are discussed with reference to the importance of minerals in human nutrition and suggest that, like wheat, it is possible to plan research programs for the improvement and selection of sorghum hybrids with high micronutrients content.

**Keywords**: food-grade sorghum; sorghum hybrid; mineral; Mediterranean area.
**Abbreviations:** CD_celiac disease; GFF_gluten-free foods; WFF_wheat-free foods.

## Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] ranks the fifth most important crop in the world in term of total production (FAO, 2011) and constitutes a major source of proteins, calories and minerals for millions of people. Sorghum is a heat and drought tolerant $C_4$ plant and a cereal staple in subtropical and semi-arid regions of Africa and Asia (Kresovich et al., 2005; Dicko et al., 2006; Reddy et al., 2009; Ashok-Kumar et al., 2010). For much of the world, sorghum is an inexpensive source of energy and micronutrients, often the second least expensive after pearl millet [*Pennisetum glaucum* (L.) R. Br.], and a vast majority of the population in Africa and central India depends on sorghum for their dietary energy and micro-nutrient requirements (Parthasarathy-Rao et al., 2006). The United States is the largest producer and exporter of sorghum, accounting for 20% of world production and almost 80% of world sorghum exports in 2001-2003 (USDA-FAS, 2003; Awika and Rooney, 2004). In many developing countries, sorghum has traditionally been used in food products and various food items (Pontieri et al., 2011). Sorghum is considered as a safe food for celiac patients suffering from symptoms associated with an immune reaction to gluten proteins found in all *Triticum* species and closely related cereals such as barley and rye (Kasarda, 2001; Ciacci et al., 2007). Recently, molecular evidence demonstrating the absence of toxic gliadin-like peptides in sorghum was reported, confirming that sorghum can be considered safe for consumption by people with celiac disease (Pontieri et al., 2013). In recent years, sorghum hybrids that produce white grain from a tan-color plant (often called “food-grade” sorghum) have been developed for production of WFF for persons with CD (Tuinstra, 2008). Moreover, new technologies aimed at enhancing the nutritional and functional values of sorghum proteins in industrial-scale processes have been developed (de Mesa et al., 2010). Therefore, sorghum might provide a good basis for GFF for all people, either with or without CD as an alternative to wheat-based foods.

Public and private sector sorghum breeding programs have released many improved sorghum varieties that are adapted to semi-arid and tropic environments including cultivars that meet specific food and industrial requirements (Tuinstra, 2008). Many thousands of additional sorghum accessions and landraces are represented in seed collections around the world, particularly collections in Ethiopia, China, USA, and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Rosenow and Dahlberg, 2000).

There is a need for further characterization of the sorghum collections with respect to food and other quality such as both macro- and micro-nutrient attributes. With reference to the latter compounds, minerals have an essential role in human nutrition (Suzuki and Wada, 1994). Each essential mineral has many diverse physiological functions, so there is increasing interest in understanding the nutritional functions of these essential minerals (Anglani, 1998; Ikeda et al., 2000).

Sorghum is rich in minerals whose bioavailability ranges from low (less than 1% for some forms of Fe) to higher than 90% for Na and K. Compared with barley and rye, sorghum grains exhibit low levels of P, K, Mg, Ca, Na, Zn, Fe, Mn, and Cu (Ragaee et al., 2006). Preliminary studies observed similarity in mineral contents between sorghum and millet, but noted low Ca content in two sorghum varieties differing in grain color (Hulse et al., 1980). Both K and P were reported to be dominant minerals in sorghum grain (Khalil et al., 1984).

The grain mineral content of crop species is affected by both genotype and environment (Zhao et al., 2009; Hussain et al., 2010; Zhang et al., 2010) and genotype-environment interactions complicate the development of improved genotypes for a crop in a targeted environment (Mgonja et al., 2008). The macro- and micro-nutrient contents of improved varieties vary greatly over a wide range of environments; however, some varieties show consistent trends in composition (Ng’uni et al., 2011). Recently, our research group began cultivating white grain, tan-plant “food-grade” sorghum cultivars in Southern Italy (Del Giudice et al., 2008; Pontieri et al., 2010, 2011, 2012). The present study was conducted to analyze the mineral composition of sorghum hybrids grown in temperate Italian environment.

## Results

### *Ash content*

Table 1 shows the seven sorghum hybrid genotypes used in this study. Concerning ash content, the data in Table 2 showed that ash ranged from 1.63% to 2.90% with the HyArch-02 hybrid exhibiting the highest value. These results are in agreement with Moharram and Youssef (1995) mentioned that ash of sorghum grains differ from 1.30% to 3.40%.

### *Macro-elements content in sorghum hybrids*

Sodium, potassium, magnesium, calcium and phosphorus analyses of seven sorghum hybrids are reported in Table 3, and Fig. 1. The content of essential minerals followed the sequence K> P > Mg> Na> Ca in the analyzed samples as confirmed by the percentage of elements with respect to the ash content in Table 6.

Potassium and sodium contents of the samples varied from 3434.46 to 6957.67 mg $Kg^{-1}$ and 489.00 to 840.64 mg $Kg^{-1}$, respectively. In Hy 87341, Hy F-X525, Hy ArchX-02 and Hy F-X715 hybrids potassium content was about 6-fold higher than that of sodium, about 10-fold in Hy X10315 and Hy SP-X303 hybrids, and about 14-fold in Hy X10341. Therefore, the K:Na ratio was similar or higher than the recommended ratio 5.0 (Szentmihalyi et al., 1998) for human diets. The high K:Na ratio suggests that sorghum hybrids could be suitable to ameliorate sodium-related health problems. In fact, diets with higher K:Na ratio are recommended (Arbeit et al., 1992). On the other hand, the sorghum hybrids differed significantly with respect to calcium and phosphorus, and their contents varied from 233.84 to 411.83 mg $Kg^{-1}$ and from 2148.60 to 2963.40 mg $Kg^{-1}$, respectively. A good diet should have a Ca:P ratio over 1.0 (McDowell, 2003). Since the sorghum hybrids recorded a low Ca:P ratio (about 0.14), a large consumption of sorghum flour should be accompanied with calcium supplementation to prevent mineral and osmotic imbalance (Serna-Saldivar and Rooney, 1995).

The magnesium content varied from 1454.92 to 2862.00 mg $Kg^{-1}$, and, in particular, Hy X10341 contained higher amounts of magnesium (2862.00 mg $Kg^{-1}$). The magnesium content in all hybrids was higher than that of corn flour (470 mg $Kg^{-1}$) and wheat flour (250 mg $Kg^{-1}$) as reported in Danish Food Composition Databank (Saxholt et al., 2008). Since sorghum hybrids have higher magnesium contents, they could be considered as a good source of this element.

### *Micro-elements content in sorghum hybrids*

Results obtained for the micro-elements (aluminium, iron, manganese, nickel and zinc) analysis were reported in Table 4, and Fig. 2. The content of micro-elements was in the following order: Fe > Zn > Mn > Al > Ni as confirmed by the percentage of elements with respect to the ash content in Table 6. In this case, it is important to point out the high iron and zinc content in all sorghum hybrids. In particular, the iron content in all hybrids (ranging from 39.36 to 77.03 mg $Kg^{-1}$) was higher than those of corn flour (11 mg $Kg^{-1}$) and wheat flour (~11.8 mg $Kg^{-1}$) as reported in Danish Food Composition Databank (Saxholt et al., 2008). Moreover, the content of aluminum and nickel varied from 9.33 to 19.57 mg $Kg^{-1}$ and 0.46 to 1.27 mg $Kg^{-1}$, respectively. The nickel content in all sorghum samples was higher than those of corn flour (290 µg $Kg^{-1}$) and wheat flour (38 µg $Kg^{-1}$) as reported in Danish Food Composition Databank (Saxholt et al., 2008).

### *Trace elements content in sorghum hybrids*

The mean concentrations of the trace elements are reported in Table 5, and Fig. 3 while their percentage with respect to the ash content are reported in Table 6. Eleven elements (Ag, Ba, Cd, Co, Cr, I, Mo, Pb, Se, Sn and V) were detected in the sorghum hybrids. Barium and lead followed by chromium were the most abundant trace elements. Among the analyzed elements, selenium was the least abundant and its content varied from 2.98 to 14.13 µg $Kg^{-1}$.

**Table 1.** List of sorghum hybrids.

| Hybrid name (Hy) | Source | Kind of hybrid |
|---|---|---|
| Hy X10341 | Richardson Seeds, Ltd (Vega, TX) | $F_1$ |
| Hy X10315 | Richardson Seeds, Ltd (Vega, TX) | $F_1$ |
| Hy X87341 | Richardson Seeds, Ltd (Vega, TX) | $F_1$ |
| Hy F-X525 | Richardson Seeds, Ltd (Vega, TX) | $F_1$ |
| Hy ArchX-02 | Richardson Seeds, Ltd (Vega, TX) | $F_1$ |
| Hy SP-X303 | Richardson Seeds, Ltd (Vega, TX) | $F_1$ |
| Hy F-X715 Ch | Richardson Seeds, Ltd (Vega, TX) | $F_1$ |

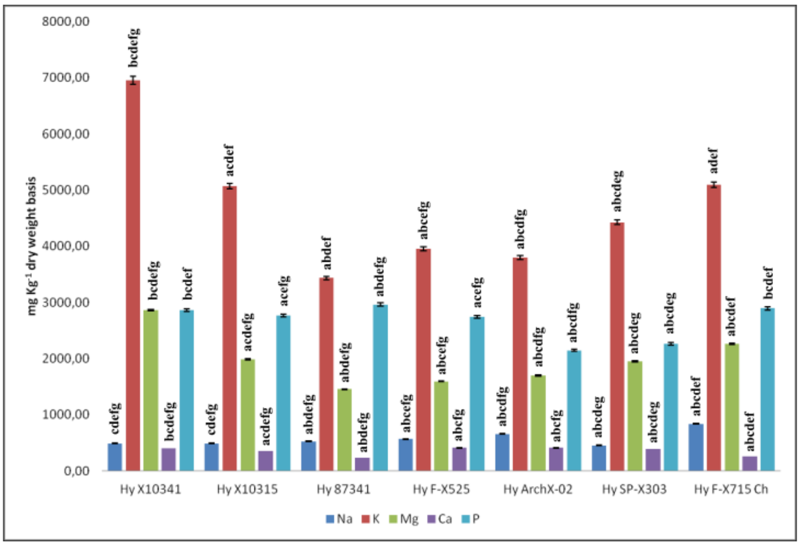

**Fig 1.** Bar graph of nutritionally essential macro-elements content of sorghum hybrids showing the concentrations of macro-elements K, Na, Mg, Ca and P. The content of each macro-element followed the sequence K>P>Mg>Na>Ca in all seven sorghum hybrids analyzed. Values are means of four replicates ± SD (5 replicates). Statistically significant differences: $^{a}$ $p<0.05$ compared to Hy X10341; $^{b}$ $p<0.05$ compared to Hy X10315; $^{c}$ $p<0.05$ compared to Hy 87341; $^{d}$ $p<0.05$ compared Hy F-X525; $^{e}$ $p<0.05$ compared Hy ArchX-02; $^{f}$ $p<0.05$ compared Hy SP-X303; $^{g}$ $p<0.05$ compared Hy F-X715 Ch.

All sorghum hybrids had a variable content of cadmium (ranging from 9.92 to 60.54 µg $Kg^{-1}$) and lead (ranging from 92.62 to 303.89 µg $Kg^{-1}$). Furthermore, significant differences were also detected for cadmium and lead. On the other hand, levels do not exceed the maximum permitted by Regulation (CE) n. 1881/2006 that fixes a limit of some contaminants in food. The maximum limit permitted for both cadmium and lead is 200 µg $Kg^{-1}$. Only Hy X10341 exceeded this limit with lead content of 303.89 ±32.5 µg $Kg^{-1}$.

### *Cluster analysis of mineral profiles in seven sorghum hybrids*

Previous studies reported macro-, micro- and trace elements profiles associated with cultivar or variety clustering (Tejera et al., 2013). To verify the potential use of elements profiles for sorghum hybrids comparisons, cluster analysis was carried out using the amount of macro-element, micro-elements and trace elements as variables for this analysis. The dendrogram in Fig. 4A shows clear differences among the seven sorghum hybrids with three distinct clusters (I, II and III). Samples Hy 87341, Hy F-X525, Hy ArchX-02 and Hy SP-X303 clustered into a well-defined group II, whereas the Hy X10315 and Hy F-X715Ch hybrids clustered in group I and Hy X10341 was in group III). These findings were also confirmed by Principal Coordinate Analysis (PCO) of elements profiles (Fig. 4B), which clearly segregated I, II and III into a well-defined groups.

## Discussion

The main goal of the present study was to compare the mineral composition of seven white food-grade sorghum hybrids, bred and developed in the USA, grown in the Mediterranean environment of Southern Italy. This study has demonstrated either a variation in overall mineral content among the seven sorghum cultivars or a significant variation in the concentration of the different elements analyzed.

Both Table 3 and Fig. 1 show the concentrations of macro-elements K, Na, Mg, Ca and P. The content of each macro-element followed the sequence of K>P>Mg>Na>Ca in all seven sorghum hybrids analyzed. The most abundant mineral was K, followed by P and Mg, a fact that is consistent with the literature data (Afify et al., 2012). Both Table 4 and Fig. 2 show the concentrations of micro-elements Al, Fe, Mn, Ni and Zn. The content of each microelement followed the sequence of Fe>Zn>Mn>Al>Ni in all seven sorghum hybrids analyzed. The most abundant micro-element was Fe, confirming the data reported in the literature (Jambunathan, 1980; Afify et al., 2012). Table 5 and Fig. 3 show the concentrations of trace elements Ag, Ba, Cd, Cr, Co, I, Mo, Se, Pb, Sn and V.

Ash content ranged from 1.63% to 2.90% with the HyArch-02 hybrid exhibiting the highest value. These results are generally in agreement with the data reported in the literature (Afify et al., 2012) but higher than those reported by Pontieri et al. (2009, 2011) for other white sorghum varieties grown in

**Table 2**. Percentage of ash content in different $F_1$ genotypes.

| Hy X10341 | Hy X10315 | Hy 87341 | Hy F-X525 | Hy ArchX-02 | Hy SP-X303 | Hy F-X715 Ch |
|---|---|---|---|---|---|---|
| 2.61 | 2.33 | 1.63 | 2.47 | 2.90 | 2.31 | 2.79 |

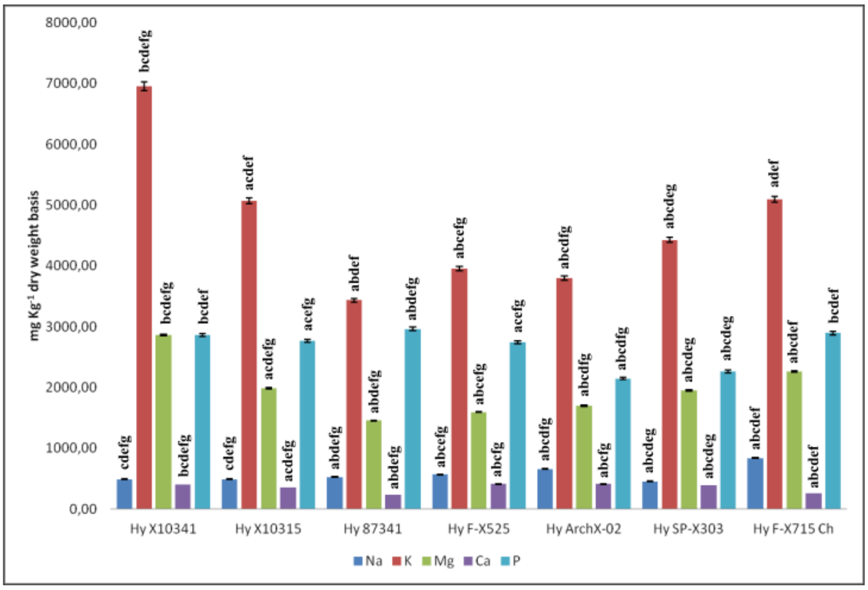

**Fig 2.** Bar graph of nutritionally essential micro-elements content of sorghum hybrids showing the concentrations of micro-elements Al, Fe, Mn, Ni and Zn. The content of each microelement followed the sequence Fe>Zn>Mn>Al>Ni in all seven sorghum hybrids analyzed.Values are means of four replicates ± SD (5 replicates). Statistically significant differences: [a] p<0.05 compared to Hy X10341;[b] p<0.05 compared to Hy X10315; [c] p<0.05 compared to Hy 87341; [d] p<0.05 compared Hy F-X525; [e] p<0.05 compared Hy ArchX-02; [f] p<0.05 compared Hy SP-X303; [g] p<0.05 compared Hy F-X715 Ch.

the Mediterranean area ranging from 0.99% to 1.9%. Since ash is considered to be the mineral component of the plant, our data confirm that the mineral contents of cereal grains are affected by a number of factors including both the genotype and the environment (Anglani, 1998).

Fig. 4A shows a clear difference among the analyzed sorghum hybrids. They clearly divided into three different groups (I, II and III). In the first group, HyX10315 and HyF-X715Ch hybrids were found, whereas, Hy87341, HyF-X525, HyArchX-02 and HySP-X303 hybrids clustered into a well-defined group II. In the group III, only the HyX10341 hybrid revealed a different type of mineral composition as compared to the other six sorghum hybrids analyzed. These findings were confirmed by Principal Coordinate Analysis (PCO) of mineral profiles (Fig. 4B), which clearly segregate I, II and III into a well-defined groups.

Overall, a positive correlation was found among the concentrations of several important elements. Interestingly, the highly significant and positive correlations were found between grain minerals e.g., Fe-Zn (r=0.9956; P= 0.001), Mg-Zn (r=0.9759; P=0.001), Mg-Fe (r=0.9625; P=0.001) and Mn-Zn (r=0.9581; P=0.001) (Table 7), suggesting common genetic factors controlling the accumulation of different minerals or similar physiological mechanisms associated with uptake/translocation of these minerals in the grains. These results point to the potential of simultaneous genetic improvement for two or more grain minerals (Ashok-Kumar et al., 2010).

Essential minerals are vital micro-nutrients for good health and necessary in very tiny amounts in human diet (Suzuki and Wada, 1994; Anglani, 1998). Each essential mineral plays many diverse physiological functions, and various foods can serve as dietary sources. Cereals may be an important source of essential minerals in view of their large daily intake. Sorghum, among the cereals, constitutes a major source of proteins, calories and minerals for millions of people, particularly in semi-arid tropical regions of Africa and Asia. The expanding production of food-grade sorghums for use in gluten-free foods for celiac patients in the US (Taylor et al., 2006) and in Mediterranean countries (Del Giudice et al., 2008; Pontieri et al., 2010, 2011, 2012, 2013), suggest the use of this cereal for healthy nutrition for the entire population as an alternative to foods based on wheat or other grains that contain gluten or as a compliment to existing diets containing wheat. Since the essential minerals for human health are major subject of nutrition, it is important to study the mineral contents in sorghum varieties cultivated in the Mediterranean environment.

With reference to the macro-elements content, the present study shows a K:Na ratio similar or higher than the recommended ratio for the human diet in all seven sorghum hybrids analyzed (Szentmihalyi et al., 1998). It is also well known that an improved K:Na ratio in the diet may benefit bone health, reduce muscle loss, as well as mitigate other chronic diseases such as hypertension and stroke (Arbeit et al., 1992). On the other hand, since the sorghum hybrids recorded a low Ca:P ratio (about 0.14), a large consumption of sorghum flour should be accompanied with calcium supplementation to prevent mineral and osmotic imbalance (McDowell, 2003). The magnesium content in all hybrids was higher than corn flour (470 mg $Kg^{-1}$) and wheat flour (250 mg $Kg^{-1}$) as reported in Danish Food Composition Databank (Saxholt et al., 2008). Since sorghum hybrids have higher magnesium contents, they could be considered as sources of magnesium, which is required for the function of

**Table 3.** Nutritionally essential macro-elements content of sorghum hybrids (mg Kg$^{-1}$)$^{*}$.

| | Hy X10341 | Hy X10315 | Hy 87341 | Hy F-X525 | Hy ArchX-02 | Hy SP-X303 | Hy F-X715 Ch |
|---|---|---|---|---|---|---|---|
| K | 6957.67±67.97$^{bcdefg}$ | 5072.66±49.56$^{acdef}$ | 3434.46±33.56$^{abdef}$ | 3954.84±38.64$^{abcefg}$ | 3799.33±37.12$^{abcdfg}$ | 4427.06±43.25$^{abcdeg}$ | 5093.37±49.76$^{adef}$ |
| Na | 489.09±5.38$^{cdefg}$ | 489.00±5.38$^{cdefg}$ | 527.67±5.80$^{abdefg}$ | 569.51±6.27$^{abcefg}$ | 660.14±7.26$^{abcdfg}$ | 455.09±5.01$^{abcdeg}$ | 840.64±9.25$^{abcdef}$ |
| Mg | 2862.00±15.16$^{bcdefg}$ | 1984.49±10.52$^{acdefg}$ | 1454.92±7.71$^{abdefg}$ | 1596.42±8.46$^{abcefg}$ | 1700.82±9.01$^{abcdfg}$ | 1949.12±10.33$^{abcdeg}$ | 2266.47±12.01$^{abcdef}$ |
| Ca | 404.60±2.71$^{bcdefg}$ | 353.50±2.37$^{acdefg}$ | 233.84±1.57$^{abdefg}$ | 410.76±2.75$^{abcfg}$ | 411.83±2.76$^{abcfg}$ | 390.22±2.41$^{abcdeg}$ | 264.37±1.77$^{abcdef}$ |
| P | 2864.30±27.21$^{bcdef}$ | 2765.40±26.27$^{acefg}$ | 2963.40±28.55$^{abdefg}$ | 2741.30±26.04$^{acefg}$ | 2148.60±20.44$^{abcdfg}$ | 2263.80±21.51$^{abcdeg}$ | 2897.20±27.52$^{bcdef}$ |

Values are means of four replicates ± SD (5 replicates). Statistically significant differences:$^{a}$ p<0.05 compared to Hy X10341;$^{b}$ p<0.05 compared to Hy X10315; $^{c}$ p<0.05 compared to Hy 87341; $^{d}$ p<0.05 compared Hy F-X525; $^{e}$ p<0.05 compared Hy ArchX-02; $^{f}$ p<0.05 compared Hy SP-X303;$^{g}$ p<0.05 compared Hy F-X715 Ch.*Dry weight basis.

**Table 4**. Nutritionally essential micro-elements content of sorghum hybrids (mg Kg$^{-1}$)$^{*}$.

| | Hy X10341 | Hy X10315 | Hy 87341 | Hy F-X525 | Hy ArchX-02 | Hy SP-X303 | Hy F-X715 Ch |
|---|---|---|---|---|---|---|---|
| Al | 12.96±0.96$^{bcdefg}$ | 19.57±1.45$^{acdeg}$ | 9.33±0.69$^{abdefg}$ | 16.69±1.24$^{abcfg}$ | 45.07±3.33$^{abcfg}$ | 19.03±1.41$^{acde}$ | 54.93±4.07$^{ade}$ |
| Fe | 77.03±5.70$^{bcdefg}$ | 57.45±4.25$^{acde}$ | 39.36±2.91$^{abef}$ | 42.89±3.17$^{abefg}$ | 45.07±3.33$^{abcdfg}$ | 56.95±4.21$^{acdeg}$ | 54.93±4.07$^{abcdef}$ |
| Mn | 19.44±0.06$^{bcdefg}$ | 13.75±0.04$^{acdefg}$ | 8.93±0.03$^{abdefg}$ | 11.85±0.04$^{abcefg}$ | 13.03±0.04$^{abcdfg}$ | 14.54±0.05$^{abcdeg}$ | 13.92±0.04$^{abcdef}$ |
| Ni | 1.09±0.07$^{bcdefg}$ | 0.95±0.06$^{acdefg}$ | 0.46±0.03$^{abdefg}$ | 0.57±0.03$^{abcefg}$ | 0.65±0.04$^{abcdfg}$ | 0.77±0.05$^{abcdeg}$ | 1.27±0.08$^{abcdef}$ |
| Zn | 47.05±0.23$^{bcdefg}$ | 32.32±0.61$^{acde}$ | 21.10±0.68$^{abdef}$ | 22.17±0.98$^{abcefg}$ | 25.64±1.07$^{abcdfg}$ | 32.49±0.96$^{acde}$ | 32.46±0.90$^{ade}$ |

Values are means of four replicates ± SD (5 replicates). Statistically significant differences:$^{a}$ p<0.05 compared to Hy X10341;$^{b}$ p<0.05 compared to Hy X10315; $^{c}$ p<0.05 compared to Hy 87341; $^{d}$ p<0.05 compared Hy F-X525; $^{e}$ p<0.05 compared Hy ArchX-02; $^{f}$ p<0.05 compared Hy SP-X303;$^{g}$ p<0.05 compared Hy F-X715 Ch.*Dry weight basis.

**Table 5**. Nutritionally trace elements content of sorghum hybrids (µg Kg$^{-1}$)$^{*}$.

| | Hy X10341 | Hy X10315 | Hy 87341 | Hy F-X525 | Hy Arch X-02 | Hy SP-X303 | Hy F-X715 |
|---|---|---|---|---|---|---|---|
| Ag | 79.03±3.56$^{bcdefg}$ | 5.70±0.26$^{acdef}$ | 7.93±0.36$^{abdef}$ | 114.20±5.14$^{abcefg}$ | 10.70±0.48$^{abcdfg}$ | 3.54±0.16$^{abcdeg}$ | 5.35±0.24$^{adef}$ |
| Ba | 347.04±18.05$^{bcdefg}$ | 423.21±22.01$^{acdefg}$ | 468.40±24.36$^{abdefg}$ | 560.88±29.17$^{abcefg}$ | 672.35±34.96$^{abcd}$ | 669.37±34.81$^{abcd}$ | 706.58±36.74$^{abcd}$ |
| Cd | 60.54±3.81$^{bcdef}$ | 37.12±2.34$^{acdefg}$ | 19.42±1.22$^{abdefg}$ | 9.92±0.63$^{abcefg}$ | 25.94±1.63$^{abcdfg}$ | 23.49±1.48$^{abcdeg}$ | 56.24±3.54$^{bcdef}$ |
| Cr | 172.87±8.31$^{bcdefg}$ | 200.61±9.92$^{acefg}$ | 128.82±6.05$^{abdfg}$ | 194.37±9.01$^{acefg}$ | 129.10±6.05$^{abdf}$ | 254.18±11.14$^{abcdeg}$ | 121.59±5.96$^{abcdf}$ |
| Co | 12.43±0.60$^{bcdefg}$ | 11.19±0.54$^{acefg}$ | 7.12±0.34$^{abdefg}$ | 10.72±0.51$^{acefg}$ | 15.24±0.73$^{abcdg}$ | 15.14±0.73$^{abcdg}$ | 13.76±0.66$^{abcdef}$ |
| I | 14.81±0.71$^{bdefg}$ | 17.09±0.82$^{acdfg}$ | 15.86±0.76$^{bdefg}$ | 20.34±0.98$^{abcefg}$ | 17.36±0.83$^{acdfg}$ | 212.70±10.21$^{abcdeg}$ | 50.60±2.43$^{abcdef}$ |
| Mo | 67.10±3.24$^{bcdefg}$ | 55.48±2.06$^{acdef}$ | 37.92±1.13$^{abdef}$ | 46.14±2.29$^{abcfg}$ | 46.03±2.28$^{abcfg}$ | 74.70±3.95$^{abcdeg}$ | 54.76±2.59$^{adef}$ |
| Se | 2.98±0.33$^{bcdefg}$ | 8.05±0.89$^{adefg}$ | 8.95±0.98$^{adefg}$ | 12.90±1.42$^{abc}$ | 14.13±1.55$^{abcg}$ | 12.58±1.38$^{abc}$ | 11.88±1.31$^{abce}$ |
| Pb | 303.89±11.85$^{bcdefg}$ | 112.92±4.40$^{acdefg}$ | 92.62±3.61$^{abdefg}$ | 209.55±8.17$^{abcefg}$ | 164.03±6.40$^{abcdg}$ | 156.78±6.11$^{abcdg}$ | 126.84±4.95$^{abcdef}$ |
| Sn | 39.47±3.35$^{bcdf}$ | 22.00±1.87$^{adeg}$ | 22.88±1.94$^{adeg}$ | 32.64±2.77$^{abcf}$ | 34.72±2.95$^{bcf}$ | 23.37±1.99$^{adeg}$ | 33.67±2.86$^{bcf}$ |
| V | 11.13±0.94$^{bcdefg}$ | 32.31±2.71$^{acdefg}$ | 21.96±1.84$^{abdefg}$ | 43.76±3.68$^{abcef}$ | 27.86±2.34$^{abcdfg}$ | 38.04±3.20$^{abcdeg}$ | 25.74±2.16$^{abcdf}$ |

Values are means of four replicates ± SD (5 replicates). Statistically significant differences:$^{a}$ p<0.05 compared to **Hy X10341**;$^{b}$ p<0.05 compared to **Hy X10315**; $^{c}$ p<0.05 compared to **Hy 87341**; $^{d}$ p<0.05 compared **Hy F-X525**; $^{e}$ p<0.05 compared **Hy ArchX-02**; $^{f}$ p<0.05 compared **Hy SP-X303**;$^{g}$ p<0.05 compared **Hy F-X715 Ch**.*Dry weight basis.

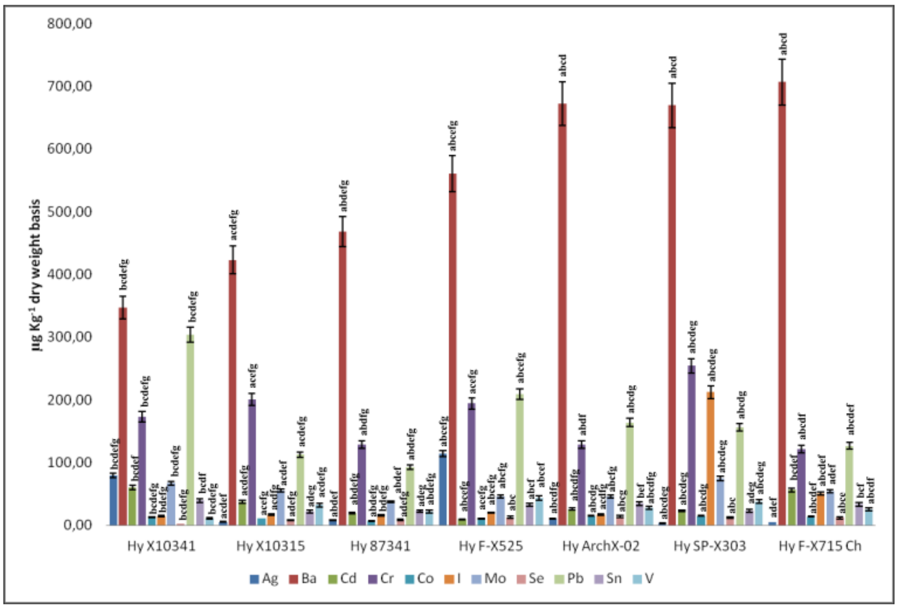

**Fig 3.** Bar graph of nutritionally trace elements content of sorghum hybrids showing the concentrations of trace elements Ag, Ba, Cd, Cr, Co, I, Mo, Se, Pb, Sn and V. Values are means of four replicates ± SD (5 replicates).Statistically significant differences:[a] p<0.05 compared to Hy X10341;[b] p<0.05 compared to Hy X10315; [c] p<0.05 compared to Hy 87341; [d] p<0.05 compared Hy F-X525; [e] p<0.05 compared Hy ArchX-02; [f] p<0.05 compared Hy SP-X303;[g] p<0.05 compared Hy F-X715 Ch.

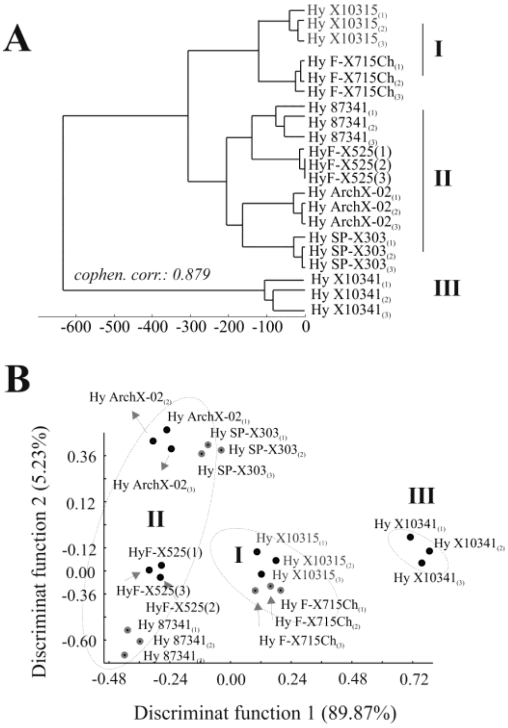

**Fig 4.** Multivariate analyses performed by using three independent analysis from each of seven sorghum hybrids. (**A**), dendrogram corresponding to cluster analysis from element profiles that shows a segregation of group I (Hy X10315 and Hy F- X715Ch hybrids), group II (Hy 87341, Hy F-X525, Hy ArchX-02 and Hy SP-X303 hybrids) and group III (Hy X10341 hybrid). (**B**), principal coordinate analysis (PCO) of same sorghum hybrids.

many enzyme systems in human metabolism (Saxholt et al., 2008).

Regarding the micro-elements, the results reported in the present study show high content of both Fe and Zn in all sorghum hybrids. The latter two elements are essential micro-elements in human nutrition, and their deficiencies are major public health threats worldwide (Ashok-Kumar et al., 2010). Among the micronutrient malnutrition situations afflicting the human population, Fe and Zn deficiencies are of major concern not only because of the serious health consequences resulting from these deficiencies including blindness and anemia, but also because of the number of people affected worldwide, particularly in Africa (Kayodè, 2006; Afify et al., 2011). Programs of genetic enhancement are in progress in the world to develop high Fe- and Zn-containing sorghum cultivars with desired agronomic backgrounds (Ashok-Kumar et al., 2010). With reference to the trace elements such as Ba, Cd, Pb, they could be toxic to human health at concentrations higher than a certain limit. However, the concentrations of trace elements in the seven sorghum hybrids analyzed in this study do not exceed the maximum permitted by Regulation (CE) n. 1881/2006 that fixes a limit of some contaminants in food. On the other hand, the content of selenium in low concentrations in sorghum hybrids of this study is an important factor. Selenium it is an essential element for the human health and has a protecting nature in the integrity of cell membranes (Zannoni et al., 2008).

## Materials and Methods

### *Plant cultivar*

The plant cultivars and source employed in this study are indicated in Table 1. The $F_1$ hybrids are excellent food quality grain producers. Grain threshes very free of the glumes in combine harvesting while the caryopses are very round a process well in the food industry. In particular the hybrids present: (a) white translucent grain color; (b) strongly vitreous grain, easy and clean threshing; and (c) high test weight.

### *Experimental site*

Field trials were conducted at San Bartolomeo in Galdo (BN) south of Italy on a clay-loam soil during summer 2012. San Bartolomeo in Galdo is an inland area at the east of the Campania Region, about 530 m above the sea level. Seven $F_1$ hybrids of sorghum (Table 1) were sown on May 6, 2012 in row plots (2 m × 5 m) replicated 3 times in a randomized block design. Before sowing, a complex fertilizer (NPK=12-12-17 and -2-14 unit of MgO-$SO_3$, respectively) was applied during the growing crop cycle. Besides, Urea (N46%) was distributed at stem elongation stage. An herbicide treatment of glyphosate (4 l ha$^{-1}$) was applied to the field to eliminate weeds before planting. After planting, weeds were controlled by hand hoeing as necessary. Plants were grown without supplemental irrigation. The hybrids were harvested starting from the end of August to mid-September.

### *Reagents and standards*

All chemicals and reagents were analytical-reagent or ICP-MS grade. Metal standard solution was purchased from Ultrascientific (North Kingstown, USA). Ultrapure nitric acid was purchased from Sigma-Aldrich (St. Louis, MO), Ultrapure water (18.0 MΩ) was produced by Millipore Direct-Q UV3.

### *Flour sample preparation*

Sorghum samples were milled into flour using a two-roll mill (Chopin mod. Moulin CD1). Then, milled samples to flour, the samples were sieved with a planetary sieve (Buhler), through a 120 μm$^2$ sieve opening. The sample so obtained is the edible part of the seed.

### *Ash determination*

Ash content was determined according to the AOAC (1995, 900.02) method on ashes. Flour samples (about 10 g) were weighed in a capsule previously calibrated at 550°C for 4 h and chilled in a silica gel dryer. Subsequently, samples were first heated by means of a soft flame to volatilize as much organic matter as possible, then transferred to a muffle furnace (Heraeus mod. K1251F) overnight. Then, ash was chilled in a silica gel dryer and weighed soon after reaching room temperature. The ash rate was determined by the ratio between the remnant mass and the original sample mass.

### *Total minerals determination*

The determination of the elements of interest was performed according to Tenore et al. (2012) using quadrupole inductively coupled plasma mass spectrometry, ICP-QMS (820-MS, Bruker Daltonics, Billerica, MA). The operational parameters were: Plasma flow: 18 L min$^{-1}$, Auxiliary flow: 1.8 L min$^{-1}$, Sheath Gas: 0.14 L min$^{-1}$, Nebulizer flow: 0.98 L min$^{-1}$, RF power: 1.40 kW, Pump rate: 4 rpm, Stabilization delay: 20 s, First Extraction Lens: -40 volts, Second Extraction Lens: -166 volts, Third Extraction Lens: -234 volts, Corner Lens: -208 volts, Mirror Lens left: 29 volts, Mirror Lens right: 26 volts, Mirror Lens bottom: 30 volts; CRI parameters: Skimmer Gas: $H_2$ at 50 mL min$^{-1}$, Sample Gas: He at 10 mL min$^{-1}$; dwell time, 10000 μs; no. of scan replicate: 10, no. replicate for sample: 5. High purity He (99.9999% He, SALDOGAS Srl, Italy) and $H_2$ (99.9999% $H_2$, produced by the DBS H2 generator PGH2-300) have been used, in order to minimize the potential problems caused by unidentified reactive contaminant species in the cell. High radio frequency power (1400 W) has helped maintain plasma stability. All chemicals were of the highest commercially available purity grade. Before use, all glassware and plastic containers have been cleaned using 10% ultra-pure grade $HNO_3$ for at least 24 h, and then rinsed copiously with ultra-pure water before use. Calibration solutions were prepared from multielemental standard stock solutions of 20.00 mg L$^{-1}$. Calibration curves were obtained using 9 calibration solutions. Reagent blanks containing ultra-pure water were additionally analyzed in order to control reagents purity and laboratory equipment. Standards and blanks were subjected to the same treatment as the samples. Determination was performed using a mix solution of internal standard ($^{6}$Li, $^{45}$Sc, $^{72}$Ge, $^{89}$Y, $^{103}$Rh, $^{159}$Tb, $^{165}$Ho, $^{209}$Bi) 10 μg L$^{-1}$ on-line aspired with a T union with the sample and standard solution. The following isotopes were analyzed: $^{23}$Na, $^{24}$Mg, $^{27}$Al, $^{39}$K, $^{44}$Ca, $^{51}$V, $^{52}$Cr, $^{55}$Mn, $^{57}$Fe, $^{59}$Co, $^{60}$Ni, $^{65}$Cu, $^{66}$Zn, $^{78}$Se, $^{98}$Mo, $^{107}$Ag, $^{114}$Cd, $^{118}$Sn, $^{127}$I, $^{137}$Ba, $^{206,207,208}$Pb. These 17 ($^{23}$Na, $^{24}$Mg, $^{27}$Al, $^{39}$K, $^{44}$Ca, $^{51}$V, $^{52}$Cr, $^{55}$Mn, $^{57}$Fe, $^{59}$Co, $^{60}$Ni, $^{66}$Zn, $^{78}$Se, $^{107}$Ag, $^{114}$Cd, $^{137}$Ba, $^{206,207,208,}$ Pb) were quantified using a calibration curve while the others have been quantified using a semi-quantitative method, a means response factor from contiguous elements.

**Table 6.** Percentage of elements with respect to the ash content.

| | Hy X10341 | Hy X10315 | Hy 87341 | Hy F-X525 | Hy ArchX-02 | Hy SP-X303 | Hy F-X715 Ch |
|---|---|---|---|---|---|---|---|
| K | 26.66 | 21.77 | 21.07 | 16.01 | 13.10 | 19.16 | 18.26 |
| Na | 1.87 | 2.10 | 3.24 | 2.31 | 2.28 | 1.97 | 3.01 |
| Mg | 10.97 | 8.52 | 8.93 | 6.46 | 5.86 | 8.44 | 8.12 |
| Ca | 1.55 | 1.52 | 1.43 | 1.66 | 1.42 | 1.69 | 0.95 |
| P | 10.97 | 11.87 | 18.18 | 11.10 | 7.41 | 9.80 | 10.38 |
| Al | 0.05 | 0.08 | 0.06 | 0.07 | 0.05 | 0.08 | 0.06 |
| Fe | 0.30 | 0.25 | 0.24 | 0.17 | 0.16 | 0.25 | 0.20 |
| Mn | 0.07 | 0.06 | 0.05 | 0.05 | 0.04 | 0.06 | 0.05 |
| Ni | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Zn | 0.18 | 0.14 | 0.13 | 0.09 | 0.09 | 0.14 | 0.12 |
| Ag | 0.30 | 0.02 | 0.05 | 0.46 | 0.04 | 0.02 | 0.02 |
| Ba | 1.33 | 1.82 | 2.87 | 2.27 | 2.32 | 2.90 | 2.53 |
| Cd | 0.23 | 0.16 | 0.12 | 0.04 | 0.09 | 0.10 | 0.20 |
| Cr | 0.66 | 0.86 | 0.79 | 0.79 | 0.45 | 1.10 | 0.44 |
| Co | 0.05 | 0.05 | 0.04 | 0.04 | 0.05 | 0.07 | 0.05 |
| I | 0.06 | 0.07 | 0.10 | 0.08 | 0.06 | 0.92 | 0.18 |
| Mo | 0.26 | 0.24 | 0.23 | 0.19 | 0.16 | 0.32 | 0.20 |
| Se | 0.01 | 0.03 | 0.05 | 0.05 | 0.05 | 0.05 | 0.04 |
| Pb | 1.16 | 0.48 | 0.57 | 0.85 | 0.57 | 0.68 | 0.45 |
| Sn | 0.15 | 0.09 | 0.14 | 0.13 | 0.12 | 0.10 | 0.12 |
| V | 0.04 | 0.14 | 0.13 | 0.18 | 0.10 | 0.16 | 0.09 |

**Table 7.** Pearson correlation coefficients among mineral elements.

| | Na | K | Mg | Mn | Fe | Ni | Zn | Se | Mo | Cd | Sn | Ba | Pb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Na | --- | -0.1056 | 0.0178 | -0.1404 | -0.2320 | 0.4006 | -0.1708 | 0.4301 | -0.3290 | 0.3090 | 0.4205 | 0.6157 | -0.2333 |
| K | | --- | 0.9785$^{***}$ | 0.9245$^{**}$ | 0.9703$^{***}$ | 0.7886$^{*}$ | 0.9688$^{***}$ | -0.7665$^{*}$ | 0.6396 | 0.8589$^{*}$ | 04931 | -0.4933 | 0.6698 |
| Mg | | | --- | 0.9379$^{**}$ | 0.9625$^{***}$ | 0.8374$^{*}$ | 0.9759$^{***}$ | -0.6744 | 0.6788 | 0.9051$^{**}$ | 0.5514 | -0.3344 | 0.6495 |
| Mn | | | | --- | 0.9574$^{**}$ | 0.7008 | 0.9581$^{***}$ | -0.5559 | 0.7785$^{*}$ | 0.7397$^{*}$ | 0.5666 | -0.2813 | 0.7666 |
| Fe | | | | | --- | 0.7397 | 0.9956$^{***}$ | -0.7201 | 0.7843$^{*}$ | 0.8014$^{*}$ | 0.3891 | -0.4343 | 0.6494 |
| Ni | | | | | | --- | 0.7596$^{*}$ | -0.3657 | 0.5385 | 0.9189$^{**}$ | 0.3759 | -0.0038 | 0.2305 |
| Zn | | | | | | | --- | -0.7106 | 0.7641$^{*}$ | 0.8387$^{*}$ | 0.4293 | -0.3999 | 0.6411 |
| Se | | | | | | | | --- | -0.2685 | -0.5995 | -0.1623 | 0.8922$^{**}$ | -0.4628 |
| Mo | | | | | | | | | --- | 0.4395 | 0.0647 | -0.003 | 0.4287 |
| Cd | | | | | | | | | | --- | 0.4767 | -0.2260 | 0.3379 |
| Sn | | | | | | | | | | | --- | 0.0107 | 0.7616$^{*}$ |
| Ba | | | | | | | | | | | | --- | -0.3701 |
| Pb | | | | | | | | | | | | | --- |

*Significant correlation at $P<0.05$; ** Significant correlation at $P<0.01$; *** Significant correlation at $P<0.001$.

### *Phosphorus determination*

Phosphorus content was determined according to Pulliainen and Wallin (1996). The sample was dry-ashed in the presence of zinc oxide, and total phosphorus content was measured colorimetrically as molybdenum blue. A Shimadzu 1800 spectrophotometer was used for all measurements.

### *Statistical analysis*

Unless otherwise stated, all results have been expressed as mean ± standard deviation (SD) based on 5 replicates. Statistical analysis was conducted with STATISTICA (Stat Soft, Oklahoma, USA). Comparisons between samples were made with a Mann-Whitney Rank Sum Test or a Kruskal-Wallis One Way Analysis of Variance on Ranks, with accompanying Dunn post-hoc test. The normal distribution of data was verified using the Shapiro-Wilks test. Correlations between continuous variables were analyzed by means of Spearman's Rank Correlation. Alpha (α) was equal to 0.05.

In order to identify groups related to the macro-elements, micro-elements and elements in traces, a multivariate cluster analysis was carried out, using the commercial statistical package PAST (http://folk.uio.no/ohammer/past/). Dendrograms were built by clustering with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) applied Euclidean correlation as previously described (Di Maro et al., 2011). Principal coordinates analysis (PCO) was used to find the eigenvalues and eigenvectors of a matrix containing the distances between all data points (Davis, 1986) applying Euclidean correlation. As correlation coefficients Spearman's rho (basically the r value of the ranks) has been used (Ryan et al., 1995).

## Conclusions

This study verified an acceptable mineral composition of the sorghum hybrids grown in the Mediterranean region and demonstrating the validity of additional testing to select the best white sorghum varieties from the point of view of the content of essential macro-, micro- and trace elements. The results also confirmed previous works (Pontieri et al., 2010, 2011, 2012) on the possibility of expanding sorghum cultivation in the Mediterranean for human use. Identification of sorghum cultivars with high essential micronutrients in the grain would help in expanded dissemination of the cultivars to complement the ongoing efforts for combating the micronutrient malnutrition (Ashok-Kumar et al., 2010).

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