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Nutritional and phenotypical characterization of two South African maize (*Zea mays* L) varieties sampled in the Qwa-Qwa region

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Abstract

Zea mays L represents one of the main source of energy in the diet in many African countries, especially in the sub-Saharan regions. White maize varieties, characterized by the lack of carotenoids, are usually widely preferred in Africa for human consumption, and this contributes to the occurrence of Vitamin A deficiency; yellow varieties, often derived from commercial hybrids, are usually destined for animal feeding. In this study we characterized from the phenotypical and nutritional points of view one white and one yellow South African landrace maize cultivar obtained directly from the farmers in the rural region of Qwa-Qwa (Free State Province). Calorific value, oil, protein, starch, minerals, flavonoids and carotenoids content were determined, together with free and phytic phosphorus (P). Both of the varieties showed lower protein and Fe content in comparison to the ones used as control, and the yellow one also had a low content of Zn. The white variety was characterized by a higher free P content but also by a very low level of carotenoids. Our data show that there are no nutritional reasons to prefer the white variety for human consumption, with the exception of the large size of the seeds, which make them particularly adapted for milling; hence the nutritional value of these varieties, and in particular of the white one, should be improved (protein, Fe and carotenoids), contributing in this way to tackle the problem of malnutrition in South African rural areas.

Keywords: South Africa, *Zea mays*, landraces, flavonoids, nutritional value

Introduction

Maize cultivation is popular all over the world where temperatures are suitable for its ciltivation. For instance, 16 of the 22 countries where corn represents the main source of energy in the diet are in Africa (Dowswell et al,1996; Nuss and Tanumihardjo, 2011). Maize consumption in the local african cuisine is comparable to that of rice in Asia. Its flour is used to produce beverages and porridges (Gouse et al, 2006; Nuss and Tanumihardjo, 2011). In South Africa, where Pap (white maize meal porridge) (Figure 1C) is a staple food for a great part of the population (Oldewage-Theron et al, 2005), corn represents the 30% of the daily energy and protein intake (Doria et al, 2015; Shisana et al, 2014).

Denutrition and micronutrient malnutrition or deficiency are still relevant public health problems in South Africa (Steyn et al, 2006; Vorster et al,1997; Acham et al, 2012): more than the 20% of the local population is affected by stunting/underweight (Doria et al, 2015). Iron and zinc intakes are particularly low (Oelofse et al, 2002). More than the 10% of the population is affected by iron and vitamin A deficiency (Doria et al, 2015; Shisana et al, 2014). Vitamin A deficiency can cause anemia and blindness, reduces resistance to infections and increases the risk of death (Gannon et al, 2014). Zinc intake is inadequate for 45.3% of South African children between 1 and 9 years of age (Samuel et al, 2010). Zinc represents a key component in enzymes which are crucial for metabolism and body functions and is also an anti-inflammatory and antioxidant agent working in cell-mediated immune processes (Prasad, 2007); its deficiency in children can cause adverse effects on both physical growth and cognitive development (Black, 1998; Brown et al, 2001; Gibson, 2006).

Thanks to its wide diffusion, maize can greatly help to improve nutrition in several countries, considering its important role in the diet of many people.

Maize seeds are characterized by a high starch content (about 75-80% of their weight), they contain proteins (10-15%) (even though the content of essential aminoacids tryptophan and lysine is low) and lipids (5%) (Panzeri et al, 2011), and they also represent a source of micronutrients and macronutrients (*e.g.*

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Na, Mg, P, K, Ca, Fe, Zn). Phosphorus availability is a relevant issue for seeds' nutritional value, in fact it is present in the kernel in three fractions: free P, phytic P (as a component of the phytate salts) and cellular P (bound to other cellular compounds).

Phytic acid is accumulated mainly in the scutellum (O'Dell et al,1972; Raboy, 1990), and is the main form of phosphate present in the seed, representing about 50-80% of the total amount of phosphorus (Doria et al, 2015), as a mixture of phytate salts of several cations, such as potassium, iron, zinc, magnesium (Raboy, 2002). During seeds' germination, phytic acid is degraded by phytase, leading to the release of free P, myo-inositol, and cations necessary for seedling growth (Badone et al, 2010). Furthermore, phytic acid has a relevant role in protecting the seeds' embryos from ageing-related damage, thanks to its antioxidant activity, avoiding a decrease in their germination capacity (Badone et al, 2010). Despite the potential health benefits due to its antiradical power, phytic acid represents an anti-nutritional factor for monogastric animals (and humans), since it is able to interfere with protein and starch digestion, and to chelate metal cations, reducing their availability in the digestive apparatus (Nuss and Tanumihardjo, 2011), thus contributing to deficiencies of nutrients in the most vulnerable members of the population.

Many phenolic compounds are accumulated in maize seeds; flavonoids and in particular anthocyanins and flavonols are among the main classes. After ingestion, free phenolics are rapidly absorbed by the small intestine and conjugate, leading to a reduced aglycones accumulation in the blood (Scalbert and Williamson, 2000): instead bound phenolics are released only through colonic fermentation (Adom and Liu, 2002; Andreasen et al, 2001). Maize is known to contain a higher amount of phenolics compared to other cereals (Adom and Liu, 2002; Ndolo and Beta, 2014). They are mainly present in the insoluble-bound form, associated with cell wall polysaccharides; the free form represents only a small fraction of the total amount (Bunzel et al, 2001; Lloyd et al, 2000). Phenolics are mainly accumulated in the outermost layers of the grains: Das and Singh (2016) observed that 74-83% of bound phenolics are accumulated in the pericarp, and the remaining fraction is accumulated mainly in the germ.

Anthocyanins, flavonols, and phenolic acids are able to exert positive effects on human health thanks to their antioxidant activity, contributing to reduce the negative effects of several degenerative and chronic diseases (Lago et al, 2014; 2015). Anthocyanins are water-soluble pigments belonging to the class of flavonoids (Escribano-Bailòn et al, 2004); they confer a purple-blue pigmentation to maize seeds and other plant tissues (Lago et al, 2015), but they are present only in traces in the kernels of yellow and white varieties.

Carotenoids can also be accumulated in maize seeds; they are tetraterpenes, conferring a yellow-orange pigmentation to seeds' endosperm, depending on their concentration. The most abundant carotenoids in maize are the xanthophylls lutein (β,ε-carotene-3,3'-diol) and zeaxanthin (β,β-carotene-3,3'-diol), that constitute together 90% of the total amount (Doria et al, 2015). Other compounds belonging to this family can be accumulated in the kernel: the xanthophylls β-cryptoxanthin (β,β-caroten-3-ol), the carotenes, β-carotene (β,β-carotene) and α-carotene (β,ε-carotene), and also pro-vitamin A. This class of molecules plays a role in the prevention of several degenerative diseases (*e.g.* cardiovascular diseases, cancer, and cataracts), and in particular in the prevention of age-related macular degeneration (AMD), one of the main causes of irreversible blindness (Snodderly, 1995; Faulks and Southon, 2001; Ahmed et al, 2005; Kuhnen et al, 2011). In African countries, white maize varieties are usually preferred for human consumption, rather than yellow ones which are often destined for animal feeding. Unfortunately, white varieties are unable to accumulate high amounts of carotenoids due to the presence of recessive homozygous mutations belonging to the ys class (Lago et al, 2015); this also confers on them a lower antioxidant power compared to yellow and pigmented ones (Lago et al, 2015).

In this study, we characterized from the phenotypical and nutritional point of view two South African maize landrace cultivars: a white one used for human consumption, and a yellow one used for animal feeding. The seeds were sampled directly from the farmers in the Qwa-Qwa region, a mountainous area in Free State province, not far from the northern Lesotho border. We analysed the seeds to assess their nutritional value for several parameters (calorific value, oil, protein, starch, mineral nutrients, repartition between free and phytic P, flavonoids, and carotenoids content).

Our results led us to plan a breeding programme aimed at increasing the nutraceutical properties of this staple food, contributing in this way to tackle the problem of malnutrition affecting a considerable fraction of the population in South Africa.

Materials and Methods

Plant material

The maize varieties studied in this article were sampled in South Africa in the mountainous region of Qwa-Qwa, Thibela, Phomolong (28°37'20.81"S;28°53'58.07"E) and cultivated in the experimental field of the University of Milan situated in Landriano (PV), Italy (45°18'N;9°15'E). Flour samples used for the analysis were obtained by grinding seeds, cleaned from the glumes, with a Retsch MM200 (Retsch GmbH Germany) ball mill for 3 min at 21 Hz.

Phenotypical characterization

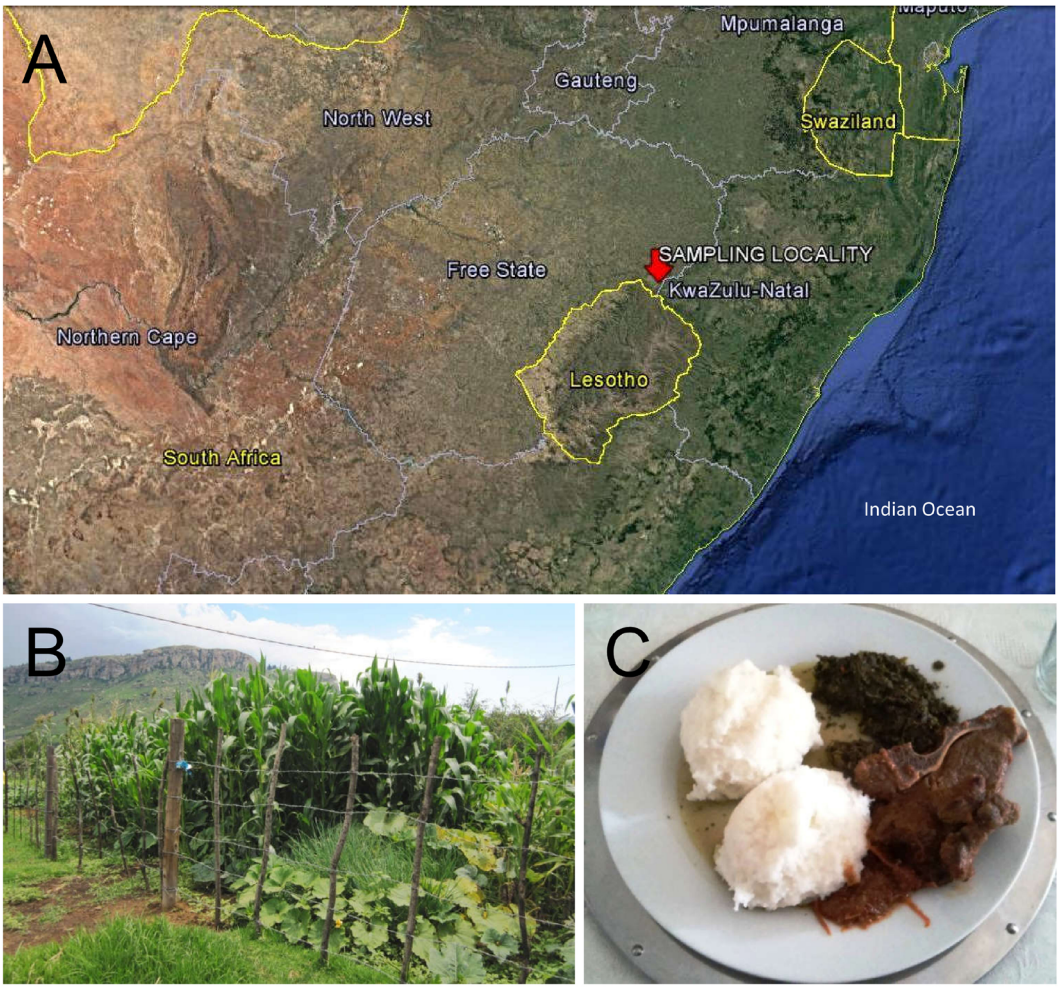

Figure 1 - Sampling site of the white and yellow South African maize cultivars. **A**: The mountain region of Qwa-Qwa (28°37'20.81"S; 28°53'58.07"E); **B**: Vegetable garden where the yellow variety was cultivated; **C**: South African traditional Maize meal porridge, «pap», obtained using white maize flour and water.

To determine the repartition between germ and endosperm 6 seeds for each variety were imbibed overnight in distilled water and the germ was manually separated from the endosperm using a scalpel. The germ and the endosperm were dried separately at 60°C for 24 hours and weighed again to determine their dry weight.

Seeds of both the varieties (n > 50 each) were germinated in the dark after a disinfectant treatment (2% NaClO for 10 min) to determine their germination rate. Plantlets were kept in the dark for 6 days before being exposed to the light, and observed for 15 days to determine the seedlings' tissue- specific pigmentation, both in the dark and in the light.

Twenty-five seeds of both the varieties were sown in the same agronomic conditions at 45° of latitude. The plants so obtained were measured after flowering: plants height was measured at the tip of the flag leaf; the height of the ears was measured at their attachment to the stalks.

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

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

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

For the determination of elements of interest, 0.3 g of maize flour samples were digested by a microwave digestor system (Anton Paar MULTIWAVE-ECO) in Teflon tubes filled with 10 mL of 65% HNO_3 by applying a one-step temperature ramp (at 210°C in 10 min, maintained for 10 min).

After 20 min of cooling time, the mineralized samples were transferred into polypropylene test tubes.

Samples were diluted 1:40 with MILLI-Q water and the concentration of elements was measured by ICP-MS (BRUKER Aurora-M90 ICP-MS). An aliquot of a 2 mg l^{-1} 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^{-1}.

Typical polyatomical analysis interferences were removed by using CRI (Collision-Reaction-Interface) with an H^2 flow of 93 ml min^{-1} flown through skimmer cone.

Average values regarding Na, Mg, K, Ca, Fe, Zn were expressed as µg g^{-1} seed flour; values regarding P were indicated as mg g^{-1} seed flour.

Determination of phytic phosphate in seeds

5 ml extraction buffer (0.4 M HCl + 0.7 M Na_2SO_4) were added to 50 mg seed flour (3 replicates for each sample); the solutions were vortexed and incubated overnight at room temperature. After centrifugation (13,000 rpm for 10 min) 1 ml of a 15 mM $FeCl_3$ 0.2 N HCl solution was added to 1 ml supernatant in plastic screw top 2 ml tubes. The tubes were left in the dry bath at 100°C for 30 min and centrifuged at 13,000 rpm for 10 min to obtain the ferric phytate precipitate; the supernatant was removed. 1 ml 0.2 N HCl was added to wash the pellet, and removed after centrifugation. The samples were digested to completion on a hot plate in H_2SO_4 (400 ml), adding H_2O_2 every three hours until the solution remained clear. All the solutions were diluted adding distilled H_2O to reach a final volume of 2 ml. Phytic phosphorus in the digests was determined spectrophotometrically through the colorimetrical Chen assay (Chen et al, 1956).

The reference standard curve was obtained adding 1,998 µl, 1,996 µl, 1,994 µl, 1,992 µl, 1,990 µl, and 1,972 µl of a freshly prepared Chen's reagent (distilled H_2O, 6 N H_2SO_4, 10% ascorbic acid and 2.5% ammonium molybdate in the ratio 2:1:1:1 v/v/v/v) to 2 µl, 4 µl, 6 µl, 8 µl, 10 µl, and 28 µl of a KH_2PO_4 solution (atomic P 1 µg $µl^{-1}$), respectively. Two ml of Chen's reagent was used as blank. 1,800 µl of Chen's reagent were added to 200 µl of digested solution for each sample. All the solutions were vortexed and incubated at room temperature for 2.5 h before reading the absorbance of the reaction mixture at 650 nm. The concentration of phytic P in the samples was determined considering the measured absorbance, according to the standard curve.

Determination of free phosphorus in seeds

50 mg seed flour were extract with 2 ml 12.5% trichloroacetic acid (TCA) 25 mM $MgCl_2$ solution (3 replicates for each sample). The solutions were mixed and kept in agitation for 30 min at room temperature before being incubated overnight at 4°C. Free phosphorus in the extracts was determined spectrophotometrically through the colorimetrical Chen assay (Chen et al, 1956). Four solutions, containing respectively atomic P 0.62, 1.24, 2.48, 3.72 µg ml^{-1} were prepared using a 2 mM Na_2HPO_4 solution: 1,980 µl, 1,960 µl, 1,920 µl, and 1,880 µl of a freshly prepared Chen's reagent (distilled H_2O, 6 N H_2SO_4, 10% ascorbic acid and 2.5% ammonium molybdate in the ratio 2:1:1:1, v/v/v/v) were added to 20 µl, 40 µl, 80 µl, and 120 µl of a 2 mM Na_2HPO_4 solution. Two ml of Chen's reagent was also used as the blank and 1,800 µl were added to 200 µl of each extract collected after centrifuge, to reach a final volume of 2 ml.

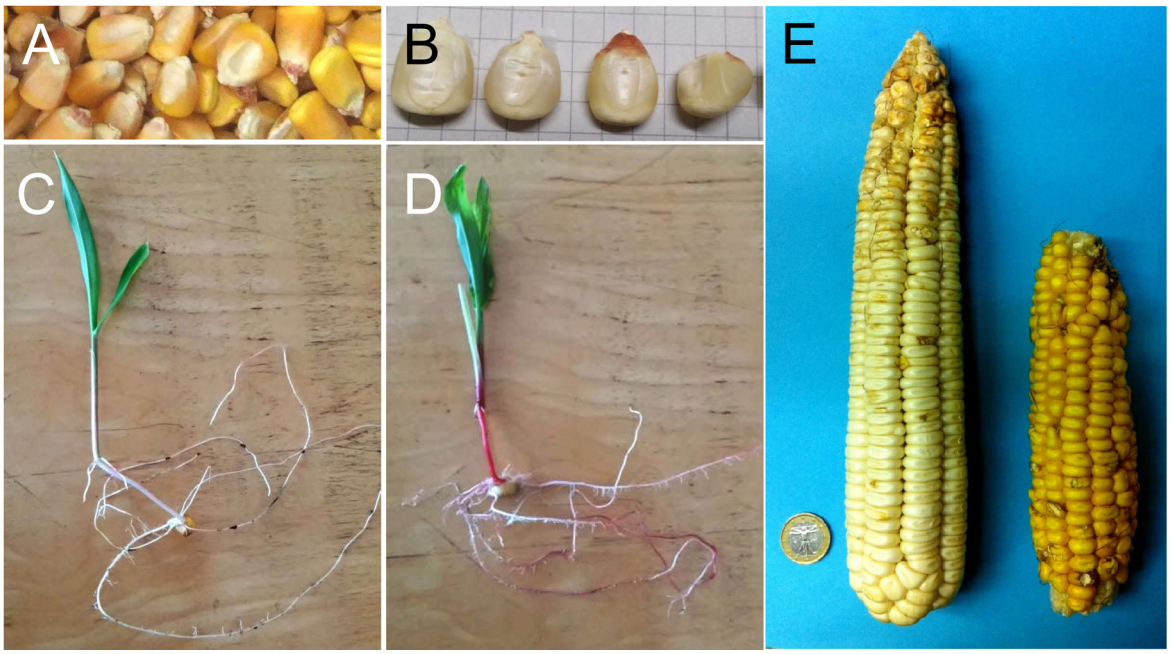

Figure 2 - Seeds and seedlings of the two South African varieties. Seeds of the yellow South African variety (**A**) and of the white one (**B**). Seedlings of the yellow (**C**) and white (**D**) South African varieties after light exposure. **E:** Ears of the white (left) and yellow variety (right).

Table 1 - Measures of calorific value, oil, protein and starch in seeds of the genotypes analysed. Mean values and standard errors of the traits are shown. Data regarding calorific value, oil and protein content in the Scagliolo, B73/Mo17, DK 440, PR 33A46, NK HELEN controls varieties are taken from Panzeri et al, 2011.

Variety	Calorific value (J g^{-1})	Oil (%)	Protein (%)	Starch (%)
S.A. White	18930 ± 34.3	4.06 ± 0.082	8.78 ± 0.165	63.3 ± 1.18
S.A. Yellow	18690 ± 33.8	5.56 ± 0.106	7.44 ± 0.140	68.4 ± 1.27
Scagliolo	19362 ± 31.3	6.02 ± 0.015	13.05 ± 0.293	62.8 ± 1.16
B73/Mo17	18790 ± 15.9	4.63 ± 0.022	8.54 ± 0.290	ND
DK 440	18723 ± 45.5	4.29 ± 0.316	8.6 ± 0.278	ND
PR 33A46	18654 ± 43.2	3.29 ± 0.048	10.71 ± 0.067	ND
NK HELEN	18616 ± 33.6	3.47 ± 0.008	10.01 ± 0.018	ND

All the solutions were agitated and incubated at 50°C for 1 hour before reading. The absorbance of the reaction mixture was measured at 650 nm.

Free P concentration was calculated according to the standard curve.

Flavonoids quantification

About 15 mg seed flour were weighed and transferred into a 2 ml tube (four replicas for each sample); 200 µl distilled water were added, and the samples were boiled at 100°C for 30 min. 1ml of extraction buffer was added to each sample (94.8 ml EtOH 95%, 2 ml distilled water and 3.2 ml 37% HCl were mixed to obtain 100 ml extraction buffer). The solutions were vortexed and left overnight in agitation. The samples were centrifuged at 13,000 rpm for 15 min and the supernatants were collected. 500 µl extraction buffer were added to each pellet; the samples were vortexed and left in agitation for two hours. After centrifugation (13,000 rpm for 15 min), the supernatant was collected and unified with the first one. The whole amount of supernatant collected from each sample was centrifuged again at 13,000 rpm for 30 min before reading. The absorbance was measured spectrophotometrically at 530 nm, at 350 nm and 280 nm respectively for anthocyanins, flavonols and phenolic acids, using the extraction buffer as blank. The anthocyanin content was calculated as cyanidin 3-glucoside equivalents [molar extinction coefficient (ε) 26,900 Lm^{-1} mol^{-1}, MW 484.82], the amounts of flavonols and phenolic acids were calculated as quercetin 3-glucoside (ε 21,877 Lm^{-1} mol^{-1}, M.W 464.38) and ferulic acid (ε 14,700 Lm^{-1} mol^{-1}, MW 194.18) equivalents. The analyses were conducted four times for each genotype, and the confidence interval (CI) at 95% was calculated.

Carotenoids extraction and quantification

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

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

Informatic tools

Microsoft Excel® was used to analyse the collected data.

Results and Discussion

In this paper two South African maize varieties (a yellow and a white one), sampled directly from the farmers in the mountain region of Qwa-Qwa (28°37'20.81"S;28°53'58.07"E), were analysed and characterized from the nutritional and phenotypical points of view (Figure 1A,B). The white variety, characterized by very big ears and large flint dent seeds (Figure 2B,E) is used by the local population for human consumption, and milled to prepare a traditional maize meal porridge called «pap» (Figure 1C), similar to the Italian «polenta». The yellow one was characterized by smaller flint seeds, with a more pronounced dent shape (Figure 2A, 2E); its kernel is manually ground by the local farmers and used as feed for poultry.

Both the varieties were maintained by the local farmers as open pollinated varieties and cultivated in kitchen gardens; unfortunately the two varieties were not always kept in isolation, as demonstrated by the presence of cross contamination.

The average dry weight of the white seeds was 0.655 ± 0.065 g, higher than that of the yellow seeds (0.389 ± 0.06 g) and also, to our knowledge, higher than that of any landrace still cultivated in Europe. Because of their dimensions, white seeds appear particularly adapted for milling, allowing the users to obtain flour with a very fine particle size thanks to the favorable ratio endosperm/pericarp.

The germination rate was higher, but not statistically significant, for the yellow variety (98.18 ± 3.56%) compared to the white (94.54 ± 6.05%). Despite this,

Table 2 - Mineral nutrients quantification through ICP-MS. The South African white and yellow varieties are compared to the B73/Mo17 hybrid and the Spanish Millo Corvo traditional variety. Average values are indicated as $\mu g\ g^{-1}$. Confidence intervals at 95% are shown.

Elements	S.A. White	S.A. Yellow	B73/Mo17	Millo Corvo
Na	13.31 ± 2.87a	11.51 ± 4.22a	12.89 ± 0.79a	10.51 ± 3.86a
Mg	1363.74 ± 149.68a	1241.55 ± 69.33a	1272.33 ± 42.64a	1213.28 ± 105.99a
K	3323.11 ± 330.39ab	3293.76 ± 152.15a	3767.90 ± 134.97b	3195.43 ± 294.11a
Ca	36.80 ± 4.14a	50.45 ± 3.65b	41.46 ± 6.61ab	33.82 ± 8.03a
Fe	15.81 ± 3.10a	18.33 ± 3.75ab	22.93 ± 1.43b	22.07 ± 1.69b
Zn	23.44 ± 6.06a	15.28 ± 1.21b	26.35 ± 1.18a	18.94 ± 2.46ab

the seedlings of the white variety showed a greater vegetative vigour and a more developed root system. (Figure 2C,D).

The seedlings of both the varieties were characterized by the absence of tissue pigmentation in the dark; the yellow variety showed very weak seedling pigmentation after light exposure (Figure 2C). All the observed plantlets of the white variety showed the accumulation of red-purple pigments in both roots and mesocotyl, following light exposure (Figure 2D) suggesting the presence of a *Sn* dominant allele. Sn regulatory gene belongs to the *r1/b1* gene family, that together with the *c1/pl1* gene family, regulates anthocyanin accumulation in plant tissues. *Sn* locus is situated on chromosome 10 near the *r1* locus, and probably originated from an intrachromosomal duplication (Pilu et al, 2003). Even if cultivars adapted to low latitudes are often unable to reach maturity and set seeds at medium-high latitudes because of the longer photoperiod (Petroni et al, 2014), the two South African cultivars, sampled at 28° of latitude, and cultivated in open field conditions in Italy at 45° of latitude, were able to reach maturity. Mature plants did not have high amounts of pigments in their tissues. Plants of the white variety reached 276.6 ± 10.1 cm in height (average height of the ear 197.1 ± 8.1 cm): in fact, low latitude origin maize varieties often reach greater heights when grown at higher latitudes. However, the plants of the yellow variety only reached an average height of 162.6 ± 8.3 cm (height of the ear 111.5 ± 6.9 cm); their limited height, despite their subtropical origin, suggests a high level of homozygosity causing inbreeding depression, probably due to the incorrect conservation of this variety (genetic drift) in recent years. It is highly probable that the yellow variety, even if maintained by the local farmers as a population, derives from a commercial dent hybrid that lost its hybrid vigour after many years of cultivation.

However, the white cultivar is probably an ancient landrace and appears more interesting from the scientific point of view because of its higher variability and its characteristically large seeds, so it is a good candidate for future breeding programmes.

The calorific value, indicated as $J\ g^{-1}$, and the percentage of oil and protein in the two South African varieties was found to be comparable to that shown by colorless modern hybrids (Panzeri et al, 2011; Table 1), which are known for their low nutritional value in comparison with several ancient landraces. In fact the Scagliolo cultivar (an Italian traditional flint maize) was found to show higher values, in particular for its protein content (Panzeri et al, 2011; Table 1). Berta et al (2014) also reported a higher protein content in the Italian variety Ostiglia (9.5 g $100g^{-1}$), and a starch content of 68.7 g $100g^{-1}$, comparable to that in the yellow South African variety (68.4 g $100g^{-1}$).

The content of micro and macronutrients (Na, Mg, P, K, Ca, Fe, and Zn) in the two varieties was quantified by ICP-MS (Tables 2 and 3) using the B73/Mo17 colorless hybrid and the traditional Spanish Millo Corvo pigmented variety as controls. Among the minerals analysed, no significant differences were observed between the two varieties for Na, Mg, and K content (Table 2).

The yellow variety was characterized by a higher content of Ca (50.45 ± 3.65 $\mu g\ g^{-1}$) compared to the white variety and to the Millo Corvo seeds used as control; even though it was somewhat higher, its Ca content was not significantly higher than thatof B73/Mo17 hybrid (Table 2).

Zinc is an essential mineral to assure the functioning of many enzymes and transcription factors, and also an anti-inflammatory and antioxidant agent working in cell-mediated immune processes (Prasad, 2007; Haase et al, 2008; Tokuji et al, 2009): its deficiency can cause adverse effects on both physical growth and cognitive development (Black, 1998; Brown et al, 2001; Gibson, 2006). Unfortunately, 45.3% of South African children have an inadequate zinc intake (Samuel et al, 2010), but our results show that the white South African variety found in Qwa-Qwa, used for human nutrition, has a significantly higher zinc content in the kernel (23.44 ± 6.06 $\mu g\ g^{-1}$) compared to the yellow one that is fed to animals (15.28 ± 1.21 $\mu g\ g^{-1}$); despite this, Zn content in the white variety was not particularly high as it was similar to that of one of the varieties used as control (Table 2), and lower than that one reported by Berta et al (2014) for the variety Ostiglia (33.5 ± 1.1 $\mu g\ g^{-1}$).

Iron concentration was low in both the South African varieties, especially in the white one, compared to the ones used as control (Table 2) and to the value reported for the Ostiglia variety: 26.3 ± 1.5 $\mu g\ g^{-1}$ (Berta et al, 2014); this appears particularly worrying considering that iron deficiency affects more than 10%

Table 3 - Phosphorus quantification in whole seed flour. Total P was quantified through ICP-MS. Free and phytic P repartition was determined. Average values are indicated as mg g^{-1}. Standard Deviation is shown.

	SA White	SA Yellow
Total P	3.48 ± 0.12	2.91 ± 0.05
Phytic P	2.58 ± 0.3	2.39 ± 0.1
Free P	0.53 ± 0.07	0.32 ± 0.02

of the South African population (Doria et al, 2015; Shisana et al, 2014).

To better characterize the two South African varieties from the nutritional point of view, the total amount of phosphorus and its repartition between free and phytic forms were also quantified (Table 3). The total content of phosphorus quantified through ICP-MS was found to be higher in the white variety (3.48 ± 0.12 mg g^{-1}) than that observed in the yellow variety (2.91 ± 0.05 mg g^{-1}). Free P reached 0.53 ± 0.07 mg g^{-1} in the white cultivar and only 0.32 ± 0.02 mg g^{-1} in the yellow, corresponding respectively to 15 and the 11 percent of the total P amount (Table 3). Phytic P content was similar in the two South African varieties: 2.58 ± 0.3 mg g^{-1} and 2.39 ± 0.1 mg g^{-1}, respectively in the white and in the yellow one; the remaining amount of P in the two varieties is represented by the cellular phosphorus. Both the varieties, especially the white one, contained a higher amount of free P and a lower amount of phytic P compared for example, to that measured by Pilu et al (2005) in the B73 colorless inbreed line (0.29 mg g^{-1} and 3.52 mg g^{-1}). Considering that free and phytic P in the seeds are accumulated mainly in the germ (O'Dell et al, 1972; Raboy, 1990), we initially supposed that the higher content of free P in the white variety could be due to a higher ratio germ/endosperm; instead our results showed that the germ represented only 10.5% of the total weight in the white seeds, and 14.4% in the yellow; hence free P concentration must actually be higher in the white variety.

Many compounds can exert an antioxidant activity in seeds, protecting tissues from oxidative stresses due to biotic or abiotic stress conditions: the presence of high amounts of phenolic compounds and carotenoids in seeds directly contributes to higher antioxidant power (Lopez-Martinez et al, 2009; Zilic et al, 2012; Lago et al, 2015). In this paper we quantified spectrophotometrically the amount of anthocyanins, flavonols and phenolic acids in the South African varieties, using the Millo Corvo pigmented variety, able to accumulate anthocyanins in the seeds' aleurone layer (Lago et al, 2015) as the coloured control, and the B73 inbred line as the colourless control (Table 4). As expected for colourless varieties, both the South African ones showed a very low anthocyanin content in the seed flour, expressed as cyanidin-3-glucoside equivalents, comparable to that of the B73 colourless inbreed line and lower than that measured in the coloured variety Millo Corvo (Table 4). No significant differences were observed between the two SA varieties and the ones used as controls for their flavonols content (indicated as quercetin 3-glucoside equivalents) (Table 4). Among the phenolic compounds, ferulic acid seems to be very important for health, as it can be beneficial for cancer prevention (Virgili and Marino, 2008; Tokuji et al, 2009); both the South African varieties showed a content of phenolic acids, expressed as ferulic acid equivalents, similar to that of the B73 inbreed line (113 ± 0.2 mg $100g^{-1}$): 94.71 ± 21.07 mg $100g^{-1}$ for the white variety and 130.54 ± 26.58 mg $100g^{-1}$ for the yellow, much lower (by nearly a half) than that observed in Millo Corvo (216.63 ± 29.05 mg $100g^{-1}$ ferulic acid equivalents) (Table 4). In fact a higher anthocyanin content, such as the one observed in Millo Corvo, is often related to a higher content of others flavonoids sharing a part of the same biosynthetic pathway (Lago et al, 2014; 2015).

Carotenoids are known to exert antioxidant (Handelman, 2001) and anti-angiogenic (Kuhnen et al, 2009) actions, contributing to the prevention of degenerative diseases, such as cardiovascular diseases, cancer, age-related macular degeneration (AMD) and cataract (Faulks and Southon, 2001; Ahmed et al, 2005; Kuhnen et al, 2011). They are hydrophobic C40 isoprenoids synthesized in amyloplasts conferring a yellow-orange pigmentation to the seeds, depending on their concentration. Those accumulated in maize endosperm are mainly lutein and zeaxanthin (Kirk and Tinley-Basset, 1978; Kurilich and Juvik, 1999; Tokuji et al, 2009; Zilic et al, 2012). More than 30 loci are involved in their biosynthesis and the main class of mutations reducing or depleting carotenoids in maize kernel is ys (Chander et al, 2008); as a consequence of these mutations, seeds' endosperm appears pale or white (Lago et al, 2015). White maize varieties are worldwide consumed and appreciated, in particular in many developing countries, even though they are well known to be lacking in vitamin A (derived from carotenoids) which is essential for human health, and thus contributing to the occurrence of vitamin A deficiency (VAD) in those populations (West et al, 2002). A inadequate consumption of carotenoids may cause blindness, growth retardation and anemia, increasing infectious morbidity and mortality (Sommer and Davidson, 2002; Zilic et al, 2012).

Unfortunately the white South African variety that is used for human consumption, showed a low carotenoids content (1.09 ± 0.4 µg g^{-1}), as expected, which was found to be similar to the average value (4.95 ± 0.62 µg g^{-1}) observed in three flint maize varieties having a white endosperm (the Italian Bianco Perla and Bianco Vitreo, and the Spanish Millo Corvo) (unpublished data of our group), suggesting the presence of a recessive homozygous mutation belonging to the white endosperm class (y).

The yellow South African variety contained a higher amount of carotenoids (22.57 ± 2.5 µg g^{-1}), corresponding to the average value observed in 12

Table 4 - Flavonoids spectrophotometrical quantification. Anthocyanins, flavonols and phenolic acids were quantified as mg cyanidin-3-glucoside equivalents, quercetin 3-glucoside equivalents and ferulic acid equivalents respectively per 100 g of dry seed flour. The analyses were conducted four times for each genotype. Data regarding Millo Corvo and B73 controls varieties are taken from Lago et al, 2015. Confidence interval at 95% are shown.

Compound	S.A. White	S.A. Yellow	Millo Corvo	B73
Anthocyanins	6.98 ± 4.46ab	6.25 ± 0.63a	83.45 ± 11.44c	3.00 ± 1.00b
Flavonols	41.72 ± 16.07a	66.19 ± 9.20a	74.21 ± 17.83a	66.00 ± 10.00a
Phenolic acids	94.71 ± 21.07a	130.54 ± 26.58a	216.63 ± 29.05b	113.00 ± 0.20a

Italian flint landraces characterized by a yellow endosperm: 21.94 ± 5.74 μg g^{-1} (unpublished data of our group). Our results are in agreement with the content of carotenoids (lutein and zeaxantin) in maize seeds reported by Mangels et al (1993), between 0.05 and 23 μg g^{-1}.

Finally, a breeding programme has been planned to ameliorate the nutritional profile of the two cultivars which are already adapted to South African growing conditions (climate, photoperiod).

Pigmented maize varieties, carrying the dominant alleles of the regulatory genes of the anthocyanins and carotenoids biosynthesis will be used as pollen donors in a breeding programme based on pedigree selection, to obtain enriched varieties, characterized by a higher antioxidant power compared to the original ones and contributing to tackle the VAD problem.

Particular attention will be focused on the white variety which is used for human consumption: plants will be selected with the aim of increasing protein and Fe content, while maintaining the large size of the seeds that makes this variety particularly adapted for milling.

The breeding programme will be also conducted in South Africa, re-distributing the seeds to the local farmers in poorer communities, thus involving them in participatory plant breeding.

Conclusions

In this work we characterized for the first time, from the phenotypical and nutritional points of view two maize varieties cultivated by South African farmers in the rural region of Qwa-Qwa: a white variety, used for human consumption, and a yellow one destined for animal feeding. The yellow variety shows a low variability and is probably derived from a commercial hybrid, sown for many years by the local farmers. Both the varieties showed low oil and protein content compared to the Scagliolo Italian flint variety used as control, and low iron content compared to the B73/Mo17 hybrid and to the Millo Corvo cultivar. The white variety was characterized by a higher Zn content, but also by a lower content of Ca in comparison with the yellow one. The total content of P and free P was found to be higher in the white variety, while their content of flavonols and phenolic acids was similar, and was lower compared to the pigmented Millo Corvo variety. As expected, the white variety was also found to lack carotenoids. Despite its low nutritional value, the white variety appears interesting because of the large dimensions of the seeds that makes them particularly well adapted for milling. Protein, carotenoids and Fe content will be increased, together with flavonoids content, through a breeding programme aimed to obtain improved varieties that could be considered as everyday functional foods for the local population.

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