Visualisation of the distribution of minerals in red non-tannin finger millet using PIXE microanalysis

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Finger millet (*Eleusine coracana*), a small grained tropical cereal widely cultivated in Africa and India, has recently received considerable interest on account of its nutritional value, potential health benefits and role in food security (Chandrashekar, 2010; Shobana *et al*., 2012). It has been reported to have an unusual and nutritionally beneficial mineral composition, with higher levels of Ca and K than any other cereal (Shobana *et al*., 2012). Calcium is thought to play a vital role in reducing the prevalence of rickets, which is still high in developing countries, despite sufficient UV light for vitamin D production (Pettifor, 2004). Positive K status has been found to reduce the risk of diabetes, cardiovascular and renal disease (He & McGregor, 2008), which is particularly important in developing countries increasingly suffering under the double burden of under- and over-nutrition (Shrimpton & Rokx, 2013). Another millet, pearl millet has been successfully biofortified with increased Fe and Zn contents (Rai et al., 2011). Biofortified pearl millet has been found to have improved Fe and Zn bioavailability (Cercamondi, 2013) and increased absorption (Kodkany, 2013).

In general, knowledge of the distribution of essential minerals in grains is limited (Cvitanich *et al*., 2011). Such information, however, is crucial for understanding the molecular mechanisms responsible for the micronutrient accumulation. Proton induced x-ray emission spectrometry (PIXE) is a non-destructive technique , which not only provides a visual map of the mineral distribution, but also provides quantitative data of the mineral concentrations (Ryan, 2011). The small size of finger millet makes it possible to map the entire kernel. To our knowledge there are no publications where the mineral distribution of an entire cereal kernel has been visualised and quantified using PIXE. Thus, the primary aim of this study was to determine if the mineral distribution of the entire finger millet kernel could be mapped using PIXE. Mapping an entire kernel would also provide unique insight on mineral distribution in this important cereal.

Whole kernels of two non-tannin red finger millet varieties, Tadesse-KNE#1098 and Padet-KNE#409, from the Ethiopian Agricultural Research Institute were obtained free from the pericarp. Tannin (Price et al., 1978), total phenolic (Kaluza *et al*., 1980) and phytate (Frubeck et al., 1995) contents were determined. Mineral contents were analysed by ion coupled plasma-optical emission spectrometry (ICP-OES, SPECTRO Analytical Instruments, Kleve, Germany). The mineral distribution in the grains were mapped by PIXE (Materials Research Department, iThemba LABS, South Africa). The grains were embedded with a commercial resin (Epofix^{TM} Struers), cut longitudinally and coated with a thin layer of carbon. The whole kernel's cut surface (approx. 2 mm^2) was raster scanned using a proton microbeam, with an energy of 3.0 MeV whilst a smaller area (0.8 mm^2) was scanned using an energy of 1.5 MeV. The beam was focused to a minimum spot size of 3 µm^2 with an approximative current of 100 pA. Scanned areas were typically analysed in a square pattern, of up to 128 x 128 pixels, with a dwell time of 10 ms/pixel. For elemental mapping, PIXE and proton backscattering spectra were acquired simultaneously in event-by-event mode, using a Si (Li) X-ray detector shielded with either a 25 µm Be filter (irradiations with 1.5 MeV protons) or a 125 um Be filter (irradiations with 3.0 MeV protons). The PIXE count rate was kept below 1000 counts/second to circumvent pulse pile-up and to achieve satisfactory counting statistics. Samples were irradiated with a total charge between 0.5 and 4 µC. The accumulated PIXE spectra were analysed using GeoPIXE II software (Ryan, 2001). The set-up used a thick sample description and the sample matrix was assumed to be comparable to cellulose.

While significantly different, the phytate, phenolic, tannin and mineral contents (Table 1) of the two finger millet varieties were similar. The phytate contents also fell within previously reported ranges for finger millet (242-1310 mg/100 g) (Mamiro *et al.,* 2001; Shashi *et al*., 2007). The mineral contents fell within the previously reported ranges: Ca (240-532 mg/100 g), Zn (0.9-2.9 mg/100 g), Fe (3.3-20 mg/100 g), P (130-325 mg/100 g) and Mg (66-183 mg/100 g) (Ravindran, 1991; Mamiro *et al.,* 2001; Shashi *et al*., 2007; Glew *et* al., 2008; Shobana *et al*., 2012).

Table 1: Mineral, total phenolic, phytate and condensed tannin contents (dry basis) of finger millet

Variety	**Ca***	**Zn***	**Fe***	**P***	**Mg***	**Phytate ****	**Total phenolics****	**Condensed tannins****
			(mg/100 g)			**(mg/100 g)**	**(mg catechin equiv. /100 g)**	
Padet	600 (78)^{a}	2.4 (0)^{b}	3.8 (1)^{a}	298 (3)^{a}	92 (1)^{b}	604 (21)^{b}	183 (54)^{b}	540 (36)^{b}
Tadesse	533 (70)^{a}	1.9 (1)^{a}	4.5 (3)^{b}	297 (6)^{a}	82 (2)^{a}	548 (33)^{a}	144 (46)^{a}	360 (37)^{a}

() – Values in parentheses are 1 standard deviation of *3 and **4 analyses
^{abc}- values in the same column with different superscripts differ significantly ($p<0.05$)

PIXE analysis did not reveal any significant aluminium contents or patterns (results not shown). This indicated the mineral contents were not altered due to soil or dust contamination or inclusions (Cary *et al*., 1994). Thus, the mineral distributions were a true representation of what is actually present in the kernel.

The elemental maps of both the finger millet varieties were very similar, as is clear in the comparison of the K maps (Figure 1). PIXE analysis, however, did reveal that the high levels of Ca (Table 1) were located mainly in the seed coat (≈750-3750 mg/100 g) and to a lesser extent in the endosperm (≈250-750 mg/100 g), with no Ca in the germ (Figure 1).

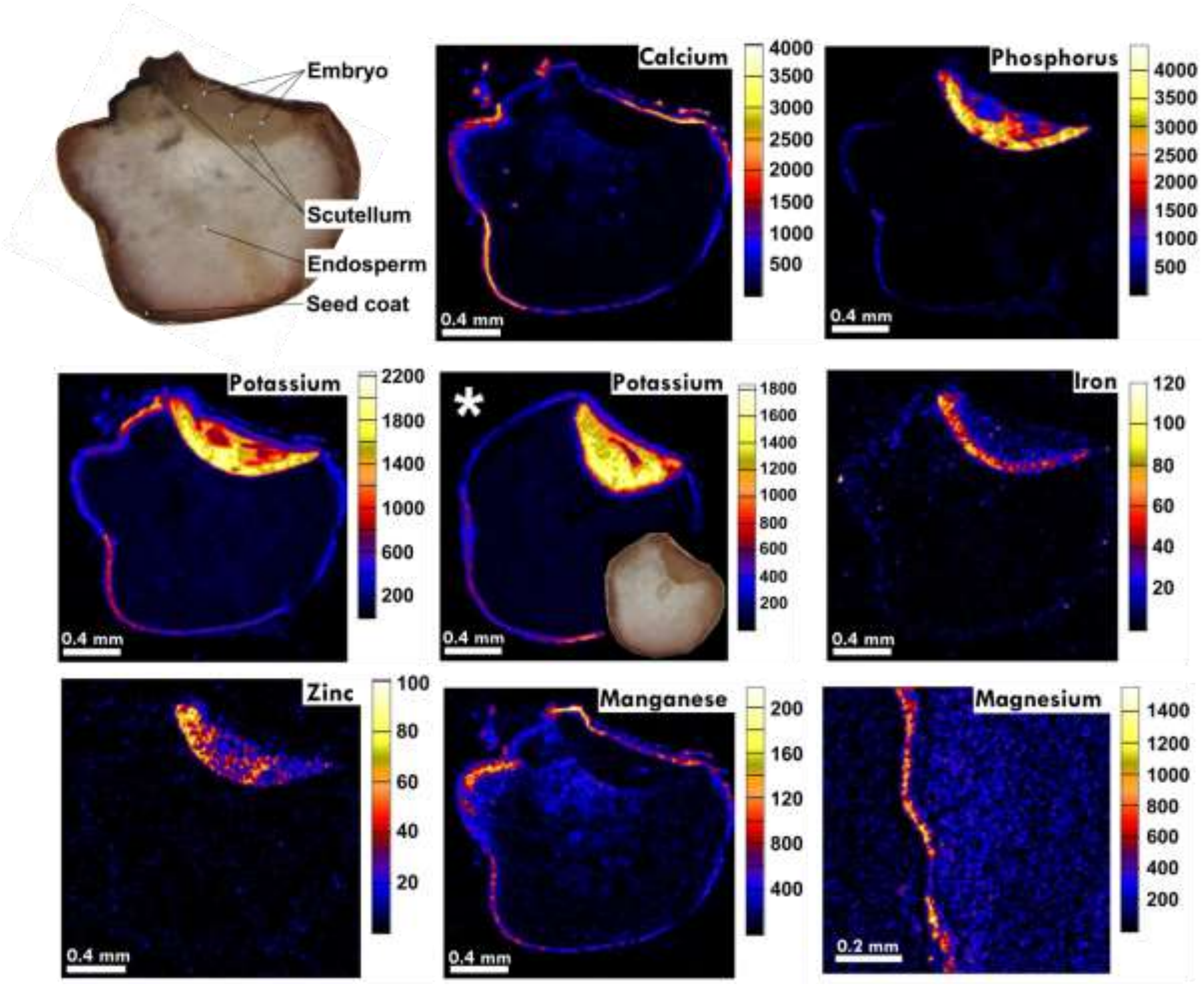

Figure 1: Quantitative visualisation, with concentration scales units mg/100 g, of the mineral distribution in a representative grain of red non-tannin Tadesse finger millet cut longitudinally. *Elemental map of K for Padet finger millet.

Phosphorus was located mainly in the scutellum (≈1500-4400 mg/100 g) with very low concentrations sparsely scattered through the endosperm. Phytate is the main storage form of P (Oatway, 2001) and importantly, the Ca in the endosperm of the finger millet would be less likely to be bound by phytate (O'Dell *et al.,* 1972) and probably be more bioavailable. The high concentrations of K in the scutellum (≈1300-2200 mg/100 g), embryo (≈600-1600 mg/100 g), and seed coat (≈500-1600 mg/100 g), together with the even distribution at relatively high concentrations (≈300 mg/100 g) in the endosperm, agreed with the reported high K concentrations in finger millet (Shobana *et al*., 2012).

Iron was located mainly in the scutellum (≈30-110 mg/100 g), but also scattered unevenly through the rest of the grain (≈0-30 mg/100 g). Zinc was located throughout the germ (scutellum and embryo) (≈20-100 mg/100 g) and like Fe, scattered through the rest of the grain, but more evenly (≈0-20 mg/100 g). The Mn was mostly located in the seed coat (≈40-200 mg/100 g) and similar to Ca, almost no Mn was located in the germ. Lastly, higher concentrations of Mg were observed in the seed coat (≈40-1500 mg/100 g) compared to the endosperm (≈10-70 mg/100 g). Due to the fact that the analysis of the Mg content was performed with a 1.5 MeV proton beam, which maximises the detection limit for light elements, the scanned area for the case of Mg was smaller than for all other maps. Consequently no information was obtained on the Mg distribution in the whole kernel.

PIXE analysis can successfully visualise and quantify the elemental distribution in an entire finger millet kernel. To our knowledge, this quantitative elemental map of finger millet by PIXE is the first of an entire cereal kernel. While there are PIXE elemental maps of parts of some cereal species, these maps are not quantitative (Singh et al., 2013). Apparently, the only PIXE quantitative elemental map, of a whole edible grain, is that of the pseudocereal buckwheat (Pongrac *et al*., 2012). The elemental mapping of finger millet has provided novel information, in particular that its content of Ca is in fact high and is distributed throughout the endosperm where phytate levels are low. This has important nutritional implications as Ca is deficient in the diet of many people, especially of those dependent on plant foods (Pettifor, 2004). Further, these findings demonstrate that mineral distribution mapping obtained by PIXE with a focused beam could be of value in research aimed at biofortifying staple cereal grains with essential minerals.

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