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# Genetic variability in *Musa* fruit provitamin A carotenoids, lutein and mineral micronutrient contents

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ABSTRACT

Bananas and plantains (*Musa* spp.) are a staple food for millions of impoverished people and as such are an important source of vitamins and micronutrients. To evaluate the potential of *Musa* spp. to meet dietary micronutrients requirements, we have screened 171 different genotypes for fruit provitamin A carotenoids (pVACs) contents, and a subset of 47 genotypes for macro- and micro-mineral (iron and zinc) contents using standardised sampling and analytical protocols. The results indicate that there is substantial variability in mean fruit pulp pVACs contents between cultivars, and that cultivars with a high fruit pVACs content are widely distributed across the different genome groups but only at a low frequency. The introduction of such high pVACs cultivars has much potential for improving the vitamin A nutritional status of *Musa*-dependent populations at modest and realistic fruit-consumption levels. In contrast, fruit pulp mineral micronutrient contents (iron and zinc), were low and showed limited inter-cultivar variability, even for genotypes grown under widely-differing environments and soil types. Results are discussed within the framework of the development of strategies to improve the nutritional health and alleviation of micronutrient deficiencies within *Musa*-consuming population groups.



## 1. Introduction

Humans need a wide selection of essential nutrients for normal growth and development. If these are not met, not only do population mortality and morbidity rates increase, but the capacity of individuals to develop and to work normally is also affected. Recent reports from WHO/World bank indicate that micronutrient deficiencies still afflict alarmingly high proportions of the world's population and that literally billions of people in developing countries are affected. To address these issues, the Consultative Group on International Agricultural Research (CGIAR) formed 'HarvestPlus', an initiative coordinated by the International Centre for Tropical Agriculture (CIAT) and the International Food Policy Research Institute (IFPRI) (<http://www.harvestplus.org/>) that "seeks to reduce the effects of micronutrient malnutrition (especially vitamin A, iron and zinc deficiencies) by harnessing plant breeding to develop staple food crops that are rich in micronutrients". The scale of the problem though is enormous. Vitamin A (vit A, retinol) deficiency is recognised to be a public health problem in 118 countries, with close to 20 million pregnant women being vit A-deficient. In addition an estimated 100–140 million pre-school children are vit A-deficient, of which 250,000–500,000 become blind every year and 50% die within 12 months of becoming blind. Iron (Fe) deficiency is the most common micronutrient deficiency in the world, affecting up to 1/3rd of the world's population and the WHO estimates that most pre-school children and pregnant women in developing countries are Fe-deficient (Genc, Humphries, Lyons, & Graham, 2005; Welch & Graham, 1999, 2004). The lack of a standardised procedure to measure Zinc (Zn) deficiency prevents reliable estimates of the number of Zn-deficient people being made, but around 20.5% of the world's population is thought to be at risk (Wuehler, Peerson, & Brown, 2005).

Reducing these vitamin and mineral deficiencies is an essential part of the overall effort to fight hunger and malnutrition. Two options to increase intakes are to provide doses of micronutrients in

*Abbreviations:* CARBAP, Centre Africain Régional de Recherches sur Bananiers et Plantains; CGIAR, Consultative Group on International Agricultural Research; Fe, iron; pVACs, provitamin A carotenoids; NIST, National Institute of Standards and Technology; RAE, retinol activity equivalents; RDA, recommended daily allowance; t-AC, all-trans α-carotene; t-BC, all-trans β-carotene; t-BCE, equivalents all-trans β-carotene; vit A, vitamin A; Zn, zinc.

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the form of pills, capsules or syrups (supplementation), or to add micronutrients to processed foods (food fortification). However diversifying the diet and increasing the micronutrient content of staple crops grown in developing countries are generally regarded as more sustainable approaches. Increasing crop micronutrients contents can be realised through the exploitation of existing micronutrient-rich germplasm or through breeding crops for increased micronutrients levels (biofortification). Traditionally, crop-breeding programs have focused primarily on traits such as yield and disease resistance as it is technically difficult and expensive to screen progeny for enhanced micro-nutrients contents. Nonetheless, with the increase in the world's population, biofortified crops are increasingly seen as a low-cost, sustainable way to reach people with poor access to health-care systems and/or formal markets.

With an annual production of around 100Mt, bananas and plantains (*Musa* spp.) are the world's fourth most important food crop. The fruits are not only consumed raw, but are also processed in a wide range of manners and at all stages of ripening and development. As such they provide a starchy staple across some of the poorest parts of the world, including Africa and Asia, and in some regions consumption may be as high as 400 kg per person per year (<http://faostat.fao.org/>). This dependence of millions of poor on bananas, plantains and their derived food products means that they are an important source of dietary minerals and vitamins. Therefore, the introduction or promotion of micronutrient-rich cultivars can have significant long-term beneficial impacts on the incidence of the micronutrient deficiencies and health of the inhabitants of these regions.

Most dietary vit A is obtained from plants as so-called provitamin A carotenoids (pVACs) which are broken down in the body to yield vit A (Fraser & Bramley, 2004; Yeum & Russell, 2002). Naturally orange-coloured foods are often an indication of a high carotenoids content and orange-fleshed banana and plantain fruits have been shown to contain high levels of pVACs (Davey, Keulemans, & Swennen, 2006; Englberger et al., 2003). However despite these results, there has been no systematic survey of the degree of genetic diversity for either fruit pVACs or for mineral macro- and micronutrients contents within the *Musa* germplasm pool.

In order to assess the degree of diversity in *Musa* fruit pVACs and mineral micronutrients contents, we have screened fruit of banana and plantain cultivars obtained from all the major genome groups using standardised sampling, analytical and growing conditions (Davey et al., 2006, 2007). The aim of this work was thus to establish 'baseline' or 'reference' values for the mean fruit micronutrient (pVACs, Fe and Zn) contents of a wide range of individual *Musa* genotypes and to provide an idea of the potential of new and existing *Musa* cultivars to contribute to an improved nutritional intake of populations in regions where this crop is an important staple.

## 2. Materials and methods

### 2.1. Cultivars and cultivation conditions

Fruit samples were obtained from germplasm collections maintained by CARBAP (Centre Africain Régional de Recherches sur Bananiers et Plantains) at Njombé in Cameroon, from individual registered farms in Eastern Uganda, from collections on the islands of Maui and Haiku, USA, and from the Banana Genebank, Davao City, Philippines. Two Cambodian cultivars were collected from home gardens, in the Kien Svay district, Kandal Province in Cambodia and fruit of the commercial 'Cavendish' banana were purchased from a local Belgian supermarket. All fruits were healthy and undamaged. Details of the collection sites and environmental conditions are summarised in Table 1.

### 2.2. Fruit sampling

Standardised sampling protocols to account for all the variability in pVACs contents present within the fruit of any one bunch were used. This involved collecting fruit from the middle of hands situated at the top (proximal), middle and bottom (distal) end of each bunch, and where possible from bunches harvested at the same time (Davey et al., 2007). The fruit maturity stage was estimated according to the peel colour essentially as described by Dadzie & Orchard, 1997 (Dadzie & Orchard, 1997). According to this scale, stage 1 is unripe/immature, stage 3 starting to ripen, stage 5 ripe, stage 7 fully ripe and stage 9 overripe. Unless noted otherwise, fruit were harvested at the immature green stage (stage 1). An overview of the collection site, genome group, the number of fruits, maturity stage and other important descriptors for each individual cultivar is given in Supplementary table 1.

### 2.3. Fruit transport

All fruits obtained from CARBAP were sliced and frozen immediately after harvest and lyophilised before shipping to the laboratory in Leuven (Belgium) in sealed, polyethylene bags in the dark. All other fruits were transported fresh in padded boxes with free air circulation, and as far as possible, maintained at temperatures of around ±8 °C.

### 2.4. Processing of fresh fruit

Upon arrival, fresh fruits were immediately weighed, sliced lengthwise and photographed next to a standardised colour chart (chart B) (IPGRI-INIBAP/CIRAD, 1996), and then sliced again laterally into quarters. Samples of the peel and the flesh pulp were separately snap frozen in liquid nitrogen for lyophilisation (Labconco,

**Table 1**
Overview of the sample collection sites and cultivation environments.

| Country | Organisation | Site | Province | Longitude | Latitude | Altitude |
|---|---|---|---|---|---|---|
| Cameroon | CARBAP (Centre Africain Régional de Recherches sur Bananiers et Plantains) | CARBAP, Njombé | Littoral | 4 35' N | 9 39' E | 80 m |
| Uganda | Bioversity International, Kampala | Kawanda Agricultural Research Institute | Lewengo | 0 25' N | 13 32' E | 1190 m |
| Hawai'i USA | Pacific Consulting Services, Haiku | National Tropical Botanical Gardens | Hana, Island of Maui | 21 N | 156 W | 400 m |
| | | University of Hawai'i Extension Agricultural Station | Kona, Island of Maui | 21 N | 156 W | 400 m |
| | | Ko'olau Forest Reserve | Island of Maui | 21 N | 156 W | 330 m |
| | | Maui Nui Botanical Gardens | Island of Maui | 21 N | 156 W | 0 m |
| | | Alex Bode's Collection | Haiku, Island of Maui | 21 N | 156 W | 100 m |
| Philippines | Davao National Crop Research and Development Centre | Banana Genebank, at the Bureau of Plant Industry, | Bagao Oshiro | 7 N | 125.5 E | 114 m |
| Cambodia | Bioversity International | Home Gardens, Kien Syay district, | Kandal province | 4° 28' N | 111° 57' E | 15 m |

FreeZone 4.5l, Model 77510, Beun De Ronde, Belgium). Diagonally opposite quarters of lyophilised fruit pulp/peel tissues were pooled and homogenised to a fine powder by grinding in liquid nitrogen in a pestle and mortar (Davey et al., 2007). The pooled powders were then stored in sealed Falcon tubes at −20 °C in the dark.

### 2.5. Micronutrients analysis

Provitamin A carotenoids analysis: Carotenoids were extracted on ice from lyophilised powders under reduced light, using cooled THF:MeOH (1:1, v/v) containing 0.1% BHT (w/v), essentially as previously described (Davey et al., 2006). All chromatographic analyses were carried out on a Waters Alliance, 2690 Separations System, fitted with a thermostatted autosampler, a pulse dampener, a 996 UV–vis photodiode array detector and a column heater (Waters, Mass, USA). Detection was carried out in the range 300–600 nm, at a frequency of 2 Hz and a spectral resolution of 2 nm. The system was controlled, and data collected and integrated using the Millenium 4.0 software packet. Individual carotenoids species were resolved by either $C_{18}$ RP-HPLC using a 150 × 4.6 mm, Waters ODS-2 3 μm particle size column (Millipore, Brussels, Belgium), essentially as according to (Davey et al., 2006, 2007), or a 150 × 4.6 mm, 3 μm, $C_{30}$ RP-HPLC column (YMC Europe, Gmbh, Germany), essentially as according to (Howe & Tanumihardjo, 2006). In the latter case, individual carotenoid species are resolved using a gradient of tert-butyl methyl ether in methanol, containing 0.1% triethylamine as modifier and 0.25% BHT as stabiliser. Peak quantitation was carried out at 450 nm and individual carotenoid species identified on the basis of their characteristic absorption spectra and their retention times relative to known standards (Azevedo-Meleiro & Rodriguez-Amaya, 2004; Howe & Tanumihardjo, 2006; Schierle, Pietsch, Ceresa, Fizet, & Waysek, 2004). β-apo8'-carotenal, at a final concentration of 0.004 μg/ml was used as internal standard. Concentrations of all-trans α-carotene (t-AC), were calculated using molar absorption coefficients calculated from the all-trans β-carotene (t-BC) standard curve at 450 nm using a compensation factor of 0.925 to correct final absorption values, as previously described (Davey et al., 2006). Quantitatively, the other most important carotenoid species present in *Musa* fruit pulp was lutein, and whilst lutein has no vit A activity, it still has important health properties (Calvo, 2005). Lutein concentrations were calculated using a standard curve constructed with genuine commercial standards (Sigma–Aldrich, Belgium), in extraction buffer.

Total carotenoids contents in the same extracts were determined at 450 nm by microtitre plate spectrophotometry using a quartz 96-well microtitre plate, and UV–vis microtitre plate spectrophotometer with appropriate software (Davey et al., 2006). Concentrations were calculated by the external standards techniques using standard curves of freshly-prepared 0.1–10 μg/ml t-BC (Sigma–Aldrich, Belgium), in extraction solvent (Davey et al., 2006; Schierle et al., 2004). All extracts were analysed in triplicate and analyses took place within 12 h of extraction.

Analysis of mineral micronutrients contents: Mineral contents were analysed by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The majority of analyses took place in the laboratories of Waite Analytical Services, at the University of Adelaide in Australia, using ~500 mg aliquots of powdered, lyophilised material. Analyses were carried out on a Spectro CIROS Radial Inductively Coupled Plasma Optical Emission Spectrometer (ICPOES), using proprietary protocols involving digestion of samples with $HNO_3/H_2O_2$ in 50 ml polypropylene tubes, and dilution of extracts to 25 ml prior to analysis. A small number of samples were also analysed at the Laboratory of Soil and Water Management, KU Leuven. Here 100 mg lyophilised, powdered samples were digested overnight in 60–70% ultrapure nitric acid, with heating to 140 °C. After evaporation of extracts just to dryness, samples were reconstituted in 5 ml of nanopure water for analysis using a Perkin–Elmer Optima 3300 DV, ICP-OES. In both cases analyses were calibrated using NIST (National Institute of Standards and Technology) standards (8436 Durum wheat, 1576a and 1573), and/or materials obtained from the "Wageningen International Plant Exchange Programme".

### 2.6. Vitamin A and retinol activity equivalents

Individual pVACs compounds have different vit A activities, due to differences in their chemical structures. The vit A activity of t-BC has been determined to be approximately 50% of that of vit A (retinol) itself, meaning that one retinol equivalent (RE) is equivalent to 2 μg of t-BC. However, more recent data actually suggests that 6 μg of dietary t-BC is required to have the same effectiveness as 1 μg of purified t-BC. Therefore, an overall conversion factor of 12:1 is used to calculate the vit A activity or 'retinol activity equivalent' (RAE) of ingested t-BC (Yeum & Russell, 2002). Other pVACs such as t-AC, and β-cryptoxanthin have only one half of the biological activity of t-BC, meaning that it is necessary to ingest 24 μg of t-AC or β-cryptoxanthin to achieve one RAE (Fraser & Bramley, 2004; Trumbo, Yates, Schlicker-Renfro, & Suitor, 2003). The relative vit A activities of 13-*cis*-β-carotene and 9-*cis*-β-carotene have been estimated as 53% and 38% of t-BC, respectively (Schieber & Carle, 2005). The conversion factors for other possible isomers of t-BC and t-AC are not known, therefore for all these compounds (if present) we utilised a conversion factor of 24:1, per μg of ingested carotenoid. On the basis of these conversion factors, it is possible to calculate RAEs and thus the net vit A nutritional value of the fruit from each cultivar.

## 3. Results and discussion

The carotenoids and pVACs contents of fruit pulp obtained from 171 cultivars representing all the major genome groups in *Musa* were analysed. However fruit mineral micronutrients contents were only determined for a subset of 47 cultivars because initial results indicated that as previously reported, very little variation was present (Davey et al., 2007).

### 3.1. Variations in Musa fruit pVACs contents

#### 3.1.1. Population fruit total pVACs contents

The mean total carotenoids content (determined spectrophotometrically), the total pVACs content (determined by HPLC) and the mean lutein content (HPLC) per genotype for all fruit from all samples analysed are summarised in Supplementary table 2. Total pVACs contents were calculated as the sum of the concentrations of t-AC, t-BC and *cis*-carotenoids (c-BC) following HPLC analysis and are based on at least duplicate extractions per fruit sample. Results provide the mean values and variations for all analyses carried out on fruit of that genotype and therefore also provide an estimate of the variability in carotenoids contents within that genotype.

As shown in Supplementary table 2, the fruit mean total pVACs content per genotype was 30.5 nmol/gdw (1636 μg/100 gdw), but values ranged from 0 (undetectable) to 211 nmol/gdw (11,337 μg/gdw). The overall mean variation in pVACs contents per genotype was +/−4.9 pmol/gdw, corresponding to an average variation of +/−20.5% per cultivar. This relatively high value indicates the degree of biological variability present between individual fruits and plants of each cultivar (Davey et al., 2007), but since the majority of genotypes analysed had low pVACs contents, the mean percentage variation is proportionally higher in these

varieties. The inter-cultivar degree of variability in mean fruit pVACs contents reported here is substantially higher than has previously been reported for *Musa* or indeed for many other plant species. Importantly, these results indicate that there is a sufficiently large degree of genetic variation within the *Musa* germplasm pool to potentially be able to breed for this trait (Hulshof, Kosmeijer-Schuil, West, 2000; Morris et al., 2004; Ortiz-Monasterio et al., 2007).

The values for the fruit pulp total carotenoids contents as determined by spectrophotometry at 450 nm, generally agreed well with the HPLC-derived values. This is to be expected as *Musa* fruit pulp carotenoids consist primarily of pVACs with only small amounts of other carotenoid compounds (Davey et al., 2006). Deviations between the HPLC and spectrophotometric measurements mostly occurred in those cultivars with low pVACs contents, when the influence of other non-pVAC carotenoids such as lutein becomes proportionally larger (Supplementary table 2).

### 3.2. Impact of genome group

The mean fruit pulp pVACs content of cultivars per genome group is summarised in Fig. 1. It can be seen that in general the few tetraploid cultivars analysed, the plantains (AAB), and a collection of cultivars classified as 'miscellaneous' (genome groups only represented by a single cultivar), or classified as 'unknown' genomes (cultivars whose genome group is uncharacterised), tended to have the highest mean pVACs contents.

Clearly these mean values can only provide a broad indication of the possible importance of genome composition, and should be interpreted with caution as the numbers of genotypes analysed within each genome group varies widely and the cultivars may not be entirely 'representative' of that group. Nonetheless, Fig. 1 confirms field reports that plantains always have an orange fruit pulp colour and that this is generally associated with higher carotenoids content. More importantly however, the results indicate that overall there do not appear to be clear divisions between the genome classes, and that the genetic basis for high fruit pVACs contents is widely distributed within the *Musa* germplasm pool.

### 3.3. Musa pVACs profiles

The vit A nutritional value of banana and plantain fruits depends not only on the concentration of pVACs, but also on the relative proportions of the individual pVAC compounds present. In *Musa* fruit pulp, over 90% of the total pVACs invariably consisted of t-AC and t-BC alone, the remaining <10% of the peaks comprising *cis*-carotenoids – mainly 13-*cis* t-BC and probably also 13-*cis* t-AC as determined on the basis of their characteristic Vis-absorption profiles and relative retention times (Davey et al., 2006; van Jaarsveld, Marais, Harmse, Nestel, & Rodriguez-Amaya, 2006). The only other carotenoid species consistently detected in fruit pulp extracts were small amounts of lutein and a few unidentified minor compounds (Davey et al., 2006). This is quite different to the situation in crops such as maize in which the pVACs represent only around 10–20% of the total carotenoids content (Ortiz-Monasterio et al., 2007) or in wheat where the major carotenoids are lutein and zeaxanthin (Leenhardt et al., 2006).

High t-BC contents are a preferred trait as t-BC is the pVAC with the highest vit A nutritional value. Across the sample group analysed here, the proportion of t-BC varied between 0 and 100% of the total pVACs content, with an average of 44.4%. However only 3 genotypes (2%) out of 171 had pVACs profile in which the content of t-BC was greater than 80% and none of these genotypes with a high percentage t-BC also had high total pVACs contents. This contrasts with the results reported by Englberger et al. from the analysis of some rare Micronesian cultivars where the cultivars with the highest pVACs contents consistently also had high proportions (75–100%) of t-BC (Englberger et al., 2003, 2006). This is possibly a characteristic of the unusual Fe'i-type cultivars of the Australimusa (rather than the majority of Eumusa) section found in Micronesia. Due to their limited distribution and unusual growth characteristics though, only one Fe'i-type cultivar, 'Aata', was available for

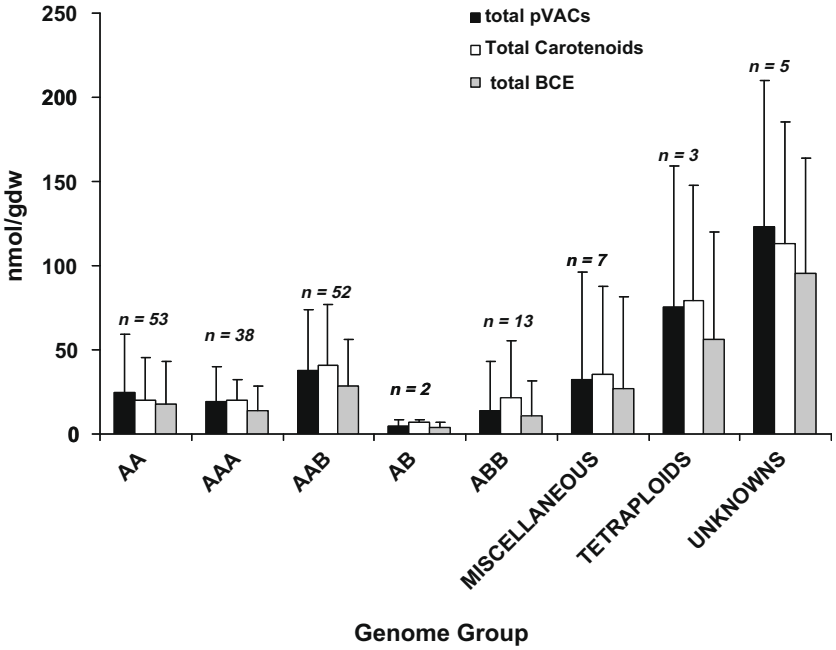


**Fig. 1.** Mean total carotenoids, total pVACs and total all-trans β-carotene equivalents (BCE) contents per genome class across the sample group of 171 *Musa* genotypes analysed. Total carotenoids determined by spectrophotometry, total pVACs and BCE's by RP-HPLC. 'Miscellaneous' refers to a selection of unusual genoptypes represented by only one variety. 'Tetraploids' represents a collection of 3 (different) tetraploid varieties.

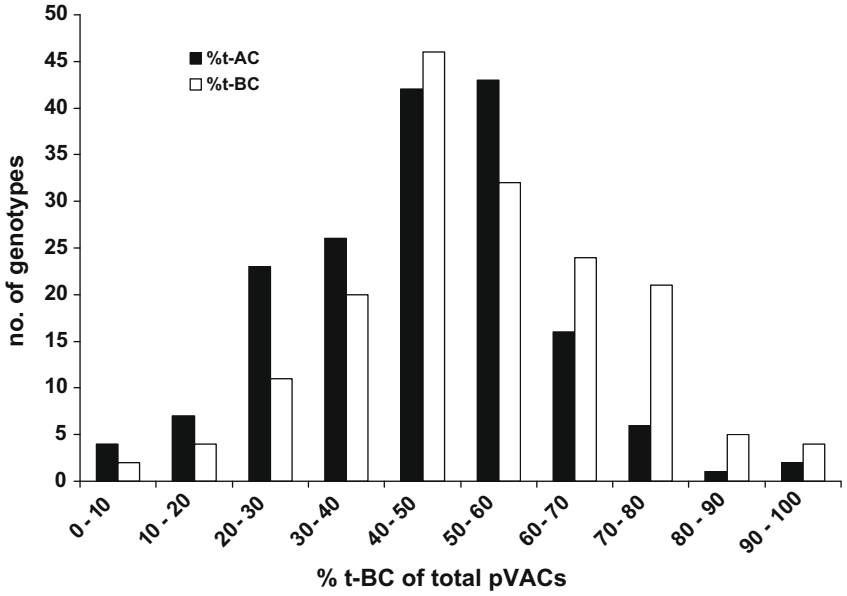


**Fig. 2.** Distribution of the proportions of percentage t-BC and percentage t-AC of the total pVACs content, within 171 cultivars of the sample group.

analysis here, outlining the need for further investigation of this group in the future (Fig. 2).

Since t-AC has only 50% of the vit A activity of t-BC, the relative proportions of t-AC and t-BC affect the overall vit A nutritional value of the fruit. Here we show that the mean proportions of t-AC and t-BC per cultivar display a statistically normal distribution across the sample group. In other crops, the accumulation of t-AC and t-BC has been shown to be regulated by the relative activities of lycopene β-cyclase and lycopene ε-cyclase (Botella-Pavia & Rodriguez-Concepcion, 2006). Interestingly, analyses of individual fruit from any one genotype shows that the percentage proportions of individual pVACs are not only stable, but also are characteristic for that genotype (Fig. 3). If these percentage proportions are also stable across environments, then this means that there is a potentially simple conversion of total carotenoids values (e.g. derived from spectrophotometric measurements) to t-BC equivalents (BCEs), once HPLC analyses for that cultivar have been carried out.

### 3.4. High pVACs cultivars

The distribution of mean fruit pVACs contents per genotype across the sample group clearly shows that fruit pVACs concentrations are non-normally distributed, with most cultivars (64%) having a total pVACs content of less than 20 pmol/gdw (1074 μg/100 gdw) (Fig. 4). In fact, the number of cultivars having pVACs contents higher than 20 pmol/gdw decreases exponentially,

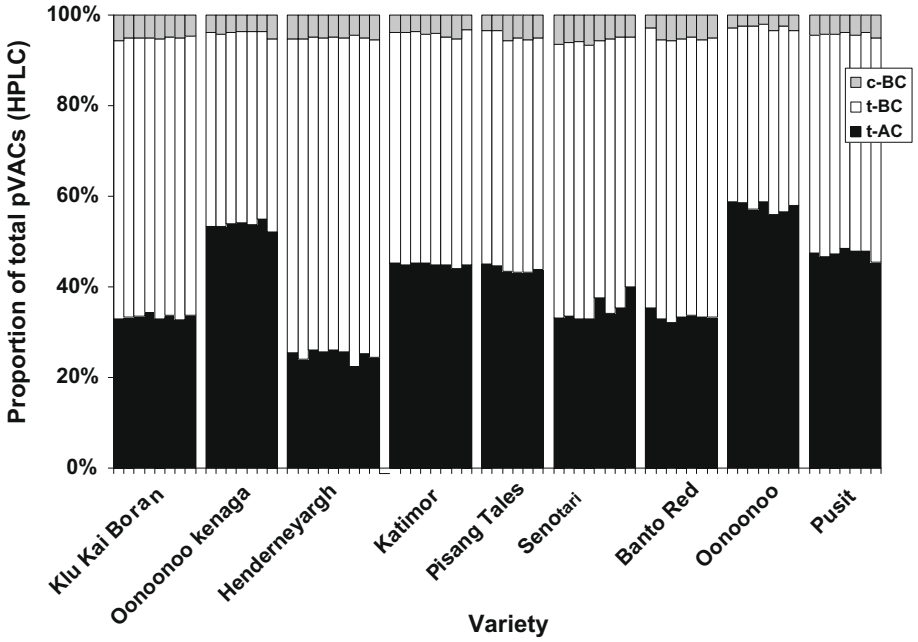


**Fig. 3.** Overview of the variation in the proportions of individual pVAC species within individual fruit of a nine *Musa* cultivars.

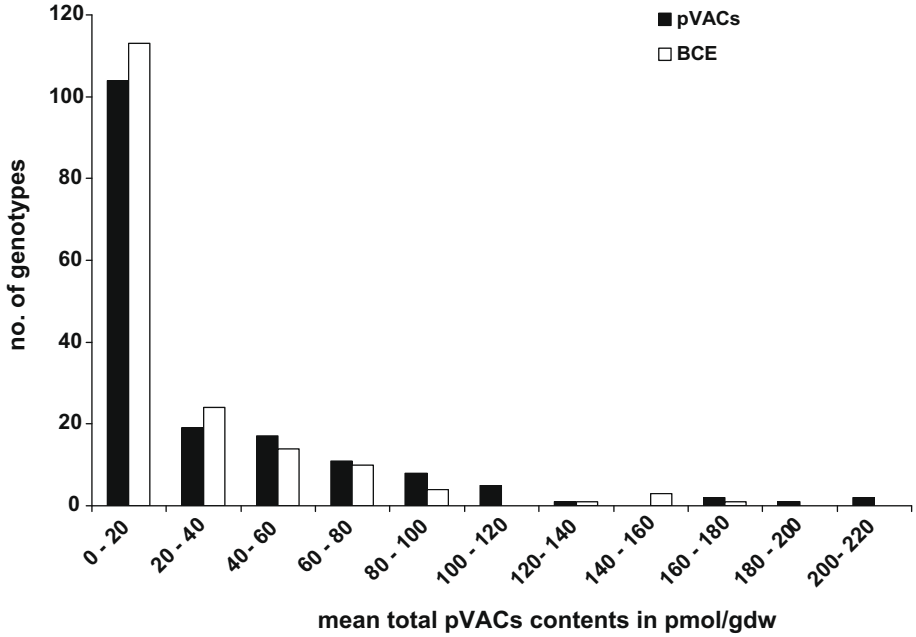


**Fig. 4.** Distribution of fruit mean total pVACs and BCE contents within the sample group of 171 *Musa* cultivars.

and only 25% of the cultivars had values higher than 40 pmol/gdw (2148 μg/100 gdw) whilst the cultivars with the top 10% highest pVACs contents, cover a range from 100 to 220 pmol/gdw.

Results from other crop species indicate that carotenoids and pVACS contents are quantitative traits, determined by the activity of multiple gene products (Santos & Simon, 2002, 2006). The results presented here support this and would further suggest that the combination of alleles responsible for high fruit pVACs contents is not widely distributed within the *Musa* germplasm pool. This may be due to either preferential selection by farmers for traits such as disease resistance or white flesh (and thus low pVACs), or due to the cosegregation of high pVACs alleles with alleles contributing to undesirable characteristics such as poor or abnormal agronomic performance (Gold, Kiggundu, Abera, & Karamura, 2002a, 2002b). For example, constitutive overexpression of the phytoene synthase cDNA in transgenic tomatoes leads to an accumulation of carotenoids, but the diversion of flux within the isoprenoid pathway also results in a 30-fold reduction in gibberellin A1 and a dwarf-plant phenotype (Fray et al., 1995). A similar diversion of flux into fruit pVACs contents might also be expected to impact on the cultivar's growth characteristics, leading for example to poor suckering and slower cycling. Whilst this still remains to be demonstrated, Englberger and co-workers reported that the Fe'i cultivar 'Utin lap' which has the highest pVACs contents measured to date grows very slowly (Englberger et al., 2006). Most plantains, which are characterised by orange flesh and a higher fruits carotenoids content also have long intervals between cycles due to a slow sucker growth related to a shortage in gibberellins (Swennen & Wilson, 1983).

In this study, the cultivars with the highest fruit pVACs contents were 'Bantol Red' (unknown), 'Pusit' (unknown), 'Iholena Lele' (AAB), 'Henderneyargh' (AAS), 'Katimor' (AAB) and 'Chek Porng Mean' (unknown), with values of 211–164 pmol total pVACs/gdw, or BCEs of 2800–1500 μg t-BCE/100 gfw. However, it is noteworthy that for all these cultivars, the fruits were analysed at ripening stages 6 or 7, whereas the majority of samples in the study were harvested and lyophilised at maturity stage 1 (Table 1). It is known that in certain *Musa* cultivars, orange pulp colour increases during ripening, and for example Englberger et al. reported that differences in the carotenoids content of the 'Karat' could have been partially due to differences in ripening (Englberger et al., 2003). Our own work suggests that the impact of ripening is cultivar-specific, with pVACs contents remaining essentially unaltered, increasing or even slightly decreasing depending on the genotype (Davey et al. - unpublished data). In addition fruit ripeness could influence the efficiencies of carotenoids extraction due to pulp softening and the breakdown of cellular structure in ripe fruit. Amongst the cultivars analysed at maturity stage 1, the cultivars with the highest pVACs and BCE contents were 'Paput Wung' (AA), 'Batard' (AAB), 'Topala' (AA) and 'Sepi' (ABB), with values of 116–97 nmol/gdw or 1750–850 μg BCE's per 100 gfw.

The highest measured BCE values of 2808 μg BCE/100 gfw is some 24-fold higher than that of the commercial 'Cavendish'-type cultivars typically found in the Northern hemisphere (116 μg/100 gfw). However these values are still significantly lower than the BCE's reported by Englberger et al. for the Micronesian Fe'i cultivars 'Utin Iap' and 'Utinwas' (BCE's of 8,508 and 8100 μg/100 gfw, respectively), and for the Pacific plantain (AAB) cultivar 'Mangat and Seipahn', with a BCE content of 6503 μg/100 gfw (Englberger et al., 2006). Again, the Pacific plantains are quite distinct from the other plantains measured here, having probably having originated in Papua New Guinea/Melanesia, rather than in Asia or the Malay Archipelago (Lebot, Aradhya, Manshardt, & Meilleur, 1993).

### 3.5. Musa vit A nutritional value

Ultimately, our interest in these results lies in the potential contribution that *Musa* fruit can make to alleviating dietary vit A deficiencies in the regions where they are cultivated and consumed. The US Recommended Daily Allowance (RDA) for vit A is the daily dietary intake required to meet the nutrient needs of 97–98% of healthy individuals in a specific age/sex/physiological group. For children up to the age of 8 years this has been set at 400 retinal activity equivalents (RAEs)/day, whilst 700 RAE is required for non-pregnant adult females (Food & I. o. M., 2001b). Within the HarvestPlus program, micronutrient target levels are based on the 'population-weighted Estimated Average Requirements' (EAR) for that nutrient. The EAR is an approximation of the median of the distribution of

nutrient requirements for individuals of the target population and as such provides the most valid, single-point comparison for the estimates of the probable contribution of a food to the overall nutrient needs of a population (Tarasuk, 2006). The EARs for vit A have been set at 250 μg RAE/day for children, whilst for all other members of the population the EAR is 500 μg RAE/day. In addition, since it is unlikely that *Musa* fruits will be the sole source of vit A in the diet, target levels for crop biofortification are calculated based on the biofortified crop providing 50% of the EAR.

From the values presented in Supplementary table 2, and knowing the proportions of t-AC, t-BC and c-BC, we can calculate the contribution to the daily vit A RDAs and EARs for children and adults made per unit of fresh fruit. From these values, we can see that as little as 100 gfw of pulp from the cultivars with the highest pVACs contents is sufficient to provide ~95% of the EAR for children and 47% of the EAR for adults, indicating that there are already existing, edible and commercial *Musa* cultivars with sufficiently high pVACs contents to immediately have a noticeable impact on population vit A health status at modest and realistic consumption levels.

### 3.6. Variations in Musa fruit mineral elements contents

#### 3.6.1. Mean fruit mineral micro- and macro-nutrient compositions

The mean fruit pulp concentrations of 10 different mineral macro-and micro-nutrients per cultivar are summarised in Supplementary table 3, and an overview of the accuracy and precision of the methodology used in Supplementary table 4. Here, only the results relating to iron (Fe) and zinc (Zn) contents as the two most important mineral micronutrients associated with dietary deficiencies will be discussed in any detail.

For the 47 cultivars analysed, Fe values ranged from 6.5 to 19.5 μg/gdw (ppm) with an average of 9.7 μg/gdw This represents a 3.0-fold range of variation and falls within the ranges reported for some other crops (White & Broadley, 2005). Values for fruit pulp Zn contents range from 3.9 to 11.4 μg/gdw, representing a range of variability of 2.9-fold, values which are again very similar to previously reported results obtained for Hawai'ian-grown *Musa* cultivars of 1.9–10.4 μg/gdw, as well as for cultivars obtained from Cameroon (Davey et al., 2007; Wall, 2006). Whilst the degree of variability is slightly greater than that measured in previous work (Davey et al., 2007), the degree of variation in fruit micronutrient contents is still small considering that fruit samples were obtained from very different locations (Table 1) with very different soil types. In the absence of leaf mineral analyses for the same samples this indicates a lack of genetic diversity in the mechanisms of Fe and Zn uptake, sequestration and transport (Frossard, Bucher, Machler, Mozafar, & Hurrell, 2000; Schmidt, 2003). However, since the soil composition also impacts on plant tissue mineral contents, these results could also suggest that cultivation and mineral-fertilisation conditions are more or less optimal for these cultivars. This does not detract from the fact that certain cultivars are still able to accumulate up to 3-fold higher levels of Fe and Zn in their fruit under the same 'optimal' growth conditions.

The distribution of fruit Fe and Zn contents within the 47 genotypes of the sample group, is statistically 'normally' distributed (data not shown) which is similar to the results observed in other angiosperm species as a whole (White & Broadley, 2005). In accordance with the overall low degree of variation, there are very little differences in mean Fe/Zn contents across the *Musa* genome groups (data not shown).

### 3.7. Fe/Zn nutritional values

The US RDAs for Fe are based on the prevention of Fe deficiency and maintenance of adequate Fe stores in individuals eating a mixed diet. The Fe RDA ranges up to 18 mg/day for pregnant adult females, with the RDA for all age groups of men and postmenopausal women being set at 8 mg/day. The Zn RDA is around 3 mg/day for infants, increasing up to 8 mg/day for males and 11 mg/day for adult females (Food & I. o. M., 2001a; White & Broadley, 2005). The EARs for Fe have been set at 8 mg/day and for Zn at 3 mg/day. Within the group of *Musa* cultivars analysed here, the highest fruit Fe concentrations were found in the cultivars 'Manameg Red', 'Grande Naine' and 'Chek Porng Mean', with values of 12.7–19.5 μg/gdw. For fruit Zn contents 'Ebang' (AAB), 'Grande Naine' and again 'Chek Porng Mean' had the highest values, ranging from 9.9–11.4 μg/gdw. However, even if these minerals were fully bioavailable to the consumer, it is clear that considerable quantities of fruit would have to be consumed to meet the RDA values for these micronutrients. In fact, bioavailability for Fe is estimated to be only in the region of 10% of the ingested values, whilst that of Zn is in the region of 40%, meaning that *Musa* by itself is unlikely to be able to contribute significantly to the daily Fe and Zn requirements of the population at normal consumption levels.

## 4. Conclusions

With vit A, Fe and Zn deficiencies collectively affecting an estimated 50% of the world's population, the challenge of current nutritional health policies is to achieve a sustainable increase in dietary intakes of these micronutrients in the afflicted areas. Food fortification and supplementation are two approaches that have been used (West, 2000), but they have generally proved difficult to implement, particularly in developing countries. For these reasons, breeding for crops with enhanced nutritional content (biofortification) or the introduction of non-indigenous cultivars with high micronutrients contents, are now thought to be the most promising strategies. The success of these approaches however depends on the existence of a sufficient degree of genetic variation for the target traits in the available germplasm pool.

Despite the economic and social importance of bananas and plantains there has been no systematic survey of the micronutrients contents of *Musa* fruit to date. The results presented here demonstrate that within the *Musa* germplasm pool, there is substantial genetic diversity in fruit vit A nutritional contents. This diversity can be exploited to identify cultivars potentially suitable for direct introduction in afflicted regions, and/or for use in breeding programmes to increase the vit A contents of this important staple. However in the latter case, the high sterility of most banana and plantain varieties means that breeding for increased vit A content is likely to be a lengthy process (Swennen & Vuylsteke, 1993; Tenkouano & Swennen, 2004). However, before the widespread introduction of new, non-indigenous species can take place, agronomic trials will have to be carried out as results with plantain breeding have indicated that there exists an inverse relationship between the intensity of orange colouring of fruits (linked to pVACs content) and suckering growth (Swennen, unpublished data). In other words yellow-white fleshed hybrids produced good suckers with short cycling times, whilst orange-fleshed hybrids produced small suckers and had large intervals between cycles. It remains to be seen whether these two traits can be uncoupled from each other, or whether breeding for fruits with a high pVACs content automatically has an adverse effect on the agronomical performance of the cultivar. Worldwide there are well over a thousand banana cultivars or varieties recognised which indicates that more germplasm screening is likely to identify new and novel sources of vit A-rich cultivars. However, in contrast to other crops (Frossard et al., 2000; White & Broadley, 2005), the degree of genotypic variation in *Musa* fruit Fe/Zn contents is clearly limited and combined with the inherently low Fe/Zn concentrations in fruits,

our results suggest that there is little potential for Fe/Zn biofortification in *Musa* spp. via a classical breeding approach unless new, additional sources of micromineral- (Fe/Zn) accumulating germplasm can be identified.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2008.12.088.

## References

Azevedo-Meleiro, C. H., & Rodriguez-Amaya, D. B. (2004). Confirmation of the identity of the carotenoids of tropical fruits by HPLC-DAD and HPLC-MS. *Journal of Food Composition and Analysis, 17*(3–4), 385–396.

Botella-Pavia, P., & Rodriguez-Concepcion, M. (2006). Carotenoid biotechnology in plants for nutritionally improved foods. *Physiologia Plantarum, 126*(3), 369–381.

Calvo, M. M. (2005). Lutein: A valuable ingredient of fruit and vegetables. *Critical Reviews in Food Science and Nutrition, 45*(7–8), 671–696.

Dadzie, B. K., & Orchard, J. E. (1997). *Routine post-harvest screening of banana/plantain hybrids: Criteria and methods. INIBAP Technical Guidelines* (Vol. 2). International Plant Genetic Resources Institute.

Davey, M. W., Keulemans, J., & Swennen, R. (2006). Methods for the efficient quantification of fruit provitamin A contents. *Journal of Chromatography A, 1136*(2), 176–184.

Davey, M. W., Stals, E., Ngoh-Newilah, G., Tomekpe, K., Lusty, C., Markham, R., et al. (2007). Sampling strategies and variability in fruit pulp micronutrient contents of West and Central African bananas and plantains (*Musa* species). *Journal of Agricultural and Food Chemistry, 55*(7), 2633–2644.

Englberger, L., Aalbersberg, W., Ravi, P., Bonnin, E., Marks, G. C., Fitzgerald, M. H., et al. (2003). Further analyses on Micronesian banana, taro, breadfruit and other foods for provitamin A carotenoids and minerals. *Journal of Food Composition and Analysis, 16*(2), 219–236.

Englberger, L., Schierle, J., Aalbersberg, W., Hofmann, P., Humphries, J., Huang, A., et al. (2006). Carotenoid and vitamin content of Karat and other Micronesian banana cultivars. *International Journal of Food Sciences and Nutrition, 57*(5–6), 399–418.

Food and Nutrition Board, I. o. M. (2001a). *Iron. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc* (pp. 290–293). Washington D.C: National Academy Press.

Food and Nutrition Board, I. o. M. (2001b). *Vitamin A. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc* (pp. 65–126). Washington D.C: National Academy Press.

Fraser, P. D., & Bramley, P. M. (2004). The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research, 43*(3), 228–265.

Fray, R. G., Wallace, A., Fraser, P. D., Valero, D., Hedden, P., Bramley, P. M., et al. (1995). Constitutive expression of a fruit phytoene synthase gene in transgenic tomatoes causes dwarfism by redirecting metabolites from the gibberellin pathway. *Plant Journal, 8*(5), 693–701.

Frossard, E., Bucher, M., Machler, F., Mozafar, A., & Hurrell, R. (2000). Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *Journal of the Science of Food and Agriculture, 80*(7), 861–879.

Genc, Y., Humphries, J. M., Lyons, G. H., & Graham, R. D. (2005). Exploiting genotypic variation in plant nutrient accumulation to alleviate micronutrient deficiency in populations. *Journal of Trace Elements in Medicine and Biology, 18*(4), 319–324.

Gold, C. S., Kiggundu, A., Abera, A. M. K., & Karamura, D. (2002a). Diversity, distribution and farmer preference of *Musa* cultivars in Uganda. *Experimental Agriculture, 38*(1), 39–50.

Gold, C. S., Kiggundu, A., Abera, A. M. K., & Karamura, D. (2002b). Selection criteria of *Musa* cultivars through a farmer participatory appraisal survey in Uganda. *Experimental Agriculture, 38*(1), 29–38.

Howe, J. A., & Tanumihardjo, S. A. (2006). Carotenoid-biofortified maize maintains adequate vitamin A status in Mongolian gerbils. *Journal of Nutrition, 136*(10), 2562–2567.

Hulshof, P. J. M., Kosmeijer-Schuil, T., & West, C. E. (2000). Quick screening of maize kernels for provitamin A content. *Journal of Food Composition and Analysis, 20*(8), 655–661.

IPGRI-INIBAP/CIRAD (1996). *Descriptors for banana (Musa spp.)* (p. 54). Montpellier, France: IPGRI.

Lebot, V., Aradhya, K. M., Manshardt, R., & Meilleur, B. (1993). Genetic-relationships among cultivated bananas and plantains from Asia and the Pacific. *Euphytica, 67*(3), 163–175.

Leenhardt, F., Lyan, B., Rock, E., Boussard, A., Potus, J., Chanliaud, E., et al. (2006). Genetic variability of carotenoid concentration, and lipoxygenase and peroxidase activities among cultivated wheat species and bread wheat varieties. *European Journal of Agronomy, 25*(2), 170–176.

Morris, W. L., Ducreux, L., Griffiths, D. W., Stewart, D., Davies, H. V., & Taylor, M. A. (2004). Carotenogenesis during tuber development and storage in potato. *Journal of Experimental Botany, 55*(399), 975–982.

Ortiz-Monasterio, J. I., Palacios-Rojas, N., Meng, E., Pixley, K., Trethowan, R., & Pena, R. J. (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *Journal of Cereal Science, 46*(3), 293–307.

Santos, C. A. F., & Simon, P. W. (2002). QTL analyses reveal clustered loci for accumulation of major provitamin A carotenes and lycopene in carrot roots. *Molecular Genetics and Genomics, 268*(1), 122–129.

Santos, C. A. F., & Simon, P. W. (2006). Heritabilities and minimum gene number estimates of carrot carotenoids. *Euphytica, 151*(1), 79–86.

Schieber, A., & Carle, R. (2005). Occurrence of carotenoid *cis*-isomers in food: Technological, analytical, and nutritional implications. *Trends in Food Science and Technology, 16*(9), 416–422.

Schierle, J., Pietsch, B., Ceresa, A., Fizet, C., & Waysek, E. H. (2004). Method for the determination of beta-carotene in supplements and raw materials by reversed-phase liquid chromatography: Single laboratory validation. *Journal of AOAC International, 87*(5), 1070–1082.

Schmidt, W. (2003). Iron solutions: Acquisition strategies and signaling pathways in plants. *Trends in Plant Science, 8*(4), 188–193.

Swennen, R., & Vuylsteke, D. (1993). Breeding black Sigatoka resistant plantains with a wild banana. *Tropical Agriculture, 70*(1), 74–77.

Swennen, R., & Wilson, G. F. (1983). The stimulation of sword sucker development by applied gibberellin (Ga3) in plantain (*Musa* Sp. Aab). *Fruits, 38*(4), 261–265.

Tarasuk, V. (2006). Use of population-weighted estimated average requirements as a basis for daily values on food labels. *American Journal of Clinical Nutrition, 83*(5), 1217S–1222S.

Tenkouano, A., & Swennen, R. (2004). Progress in breeding and delivering improved plantain and banana to African farmers. *Chronica Horticulturae, 44*(1), 9–15.

Trumbo, P. R., Yates, A. A., Schlicker-Renfro, S., & Suitor, C. (2003). Dietary reference intakes: Revised nutritional equivalents for folate, vitamin E and provitamin A carotenoids. *Journal of Food Composition and Analysis, 16*(3), 379–382.

van Jaarsveld, P. J., Marais, D. W., Harmse, E., Nestel, P., & Rodriguez-Amaya, D. B. (2006). Retention of beta-carotene in boiled, mashed orange-fleshed sweet potato. *Journal of Food Composition and Analysis, 19*(4), 321–329.

Wall, M. M. (2006). Ascorbic acid and mineral composition of longan (*Dimocarpus longan*), lychee (*Litchi chinensis*) and rambuan (*Nephelium lappaceum*) cultivars grown in Hawaii. *Journal of Food Composition and Analysis, 19*(6–7), 655–663.

Welch, R. M., & Graham, R. D. (1999). A new paradigm for world agriculture: Meeting human needs – Productive, sustainable, nutritious. *Field Crops Research, 60*(1–2), 1–10.

Welch, R. M., & Graham, R. D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany, 55*(396), 353–364.

West, C. E. (2000). Meeting requirements for vitamin A. *Nutrition Reviews, 58*(11), 341–345.

White, P. J., & Broadley, M. R. (2005). Biofortifying crops with essential mineral elements. *Trends in Plant Science, 10*(12), 586–593.

Wuehler, S. E., Peerson, J. M., & Brown, K. H. (2005). Use of national food balance data to estimate the adequacy of zinc in national food supplies: Methodology and regional estimates. *Public Health Nutrition, 8*(7), 812–819.

Yeum, K. J., & Russell, R. M. (2002). Carotenoid bioavailability and bioconversion. *Annual Review of Nutrition, 22*, 483–504.