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Sampling Strategies and Variability in Fruit Pulp Micronutrient Contents of West and Central African Bananas and Plantains (*Musa* Species)

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The variability in fruit micronutrient contents in a selection of Central and West African *Musa* varieties cultivated under standardized field conditions was studied. Analysis of the within-fruit, within-hand, and within-plant as well as the between-plant variations demonstrated that both provitamin A carotenoids (pVACs) and mineral micronutrient (Fe, Zn) contents vary significantly across all sample groups. The variations in pVACs contents appear to be at least partly related to differences in the developmental status of the fruit, but the observed trends were genotype-specific. The mean pVACs concentrations per genotype indicated that there is substantial genetic variation in the fruit pVACs contents between *Musa* cultivars, with orange-fleshed plantain varieties (AAB) having generally higher fruit pVACs contents than dessert bananas (AAA). It was not possible to identify consistent trends between the sampling position and fruit Fe/Zn contents. Once the within-bunch micronutrient variability has been accounted for, the mean variations in fruit micronutrient contents between individual plants of a variety generally fell to within acceptable limits. Results are discussed within the framework of standardizing sampling and developing strategies to screen for the nutritional values of new and existing *Musa* varieties.

KEYWORDS: Banana; biofortification; Fe; HarvestPlus; micronutrient; *Musa*; nutrition; plantain; provitamin A carotenoids; vitamin A; Zn

INTRODUCTION

Micronutrient deficiencies are a major cause of premature death and ill health for large sections of the world's population, but even mild levels of micronutrient malnutrition may damage cognitive development, decrease disease resistance in children, and reduce the likelihood that mothers survive childbirth. "HarvestPlus" is a challenge program of the CGIAR, coordinated by the Centro Internacional de Agricultura Tropical (CIAT) and the International Food Policy Research Institute (IFPRI), that aims to address micronutrient deficiencies through the development or breeding of nutritionally enhanced (biofortified) staple food crops to "improve food security, production, and quality of life" (*1*). The three main micronutrients targeted within this program are vitamin A (vit A, retinol), iron (Fe), and zinc (Zn).

Apart from well-known functions in vision, vit A has also been implicated in several other physiological processes including spermatogenesis, fetal development, immune response, and growth, and studies suggest that around 1 million children under the age of 5 die each year from the effects of vit A deficiency (*2*). The active form of vit A in the body is *all-trans* retinol, but there are about 50 naturally-occurring compounds with vit A activity (*3*, *4*) including the plant-derived provitamin A carotenoids (pVACs), which are the focus of this publication.

Severe Fe deficiency causes the deaths in pregnancy and childbirth of over 60,000 women annually (*2*), but even moderate Fe anemia can lead to a substantial reduction in work capacity, the impairment of intellectual performance, behavioral

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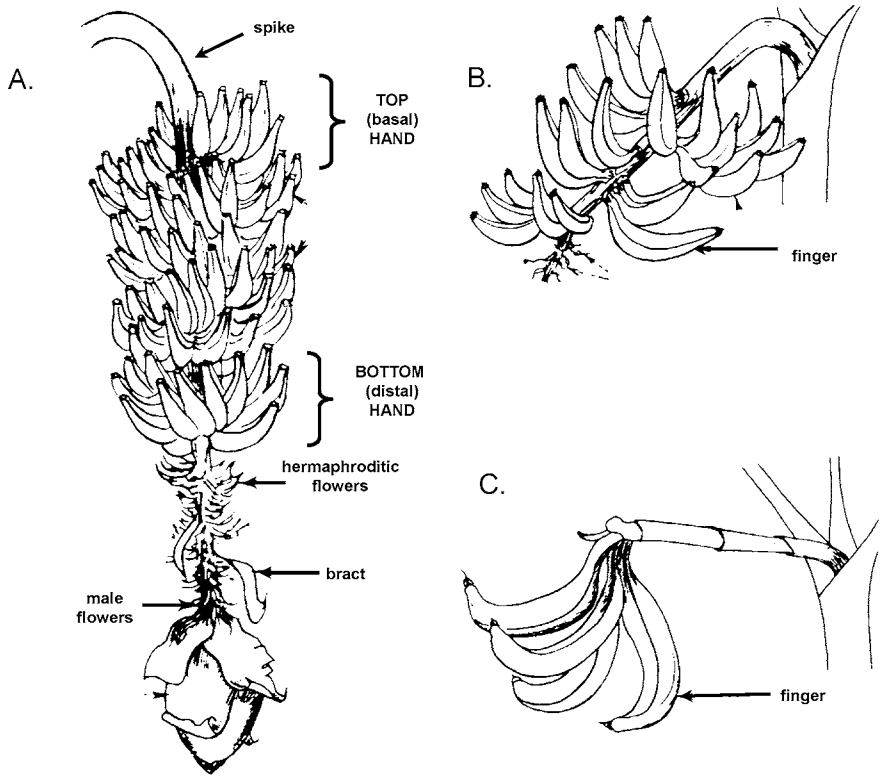

Figure 1. Schematic representation of the morphology of a *Musa* fruit bunch, indicating sampling positions used: (A) "French" type (Cavendish varieties and Yangambi-5); (**B**) "false-horn" (Batard, Mbouroukou-1, Mbourorkou-3); (**C**) "horn". Reprinted and adapted from with permission from ref *36*. Copyright 1983 CIRAD.

changes, and a reduced resistance to infections. It is believed that Fe deficiency affects more than 2 billion people worldwide (for reviews see refs *5*−*8*). In severe cases, Zn deficiency is manifested by retarded growth, depressed immune function, dermatitis, skeletal abnormalities, and diarrhea (*8*, *9*). However, the lack of standardized procedures for measuring Zn deficiency means that the number of people who are actually Zn deficient is unknown, but current estimates put this figure in the region of several billion. Surprisingly, the incidence of Zn and Fe deficiency has actually increased over recent decades, a phenomenon that is believed to be partially linked to the loss of dietary biodiversity arising from the introduction of modern, cereal-based monocultures (*10*−*12*).

Plantains and bananas (*Musa* spp.) are major staple foods of sub-Saharan Africa, with annual production being in the region of 26 M tonnes, of which only some 0.3 M tonne is exported (*13*). As such, these fruits are an important source of dietary minerals and vitamins, and the wider use of micronutrient-rich *Musa* varieties has the potential to have a significant long-term beneficial impact on the population health in these areas. In particular, the occurrence of banana and plantain varieties with naturally orange fruit flesh is an indication that these varieties could be an important source of dietary pVACs (*14*). Currently, however, there is little or no published information on the variability in fruit micronutrient contents or on sampling protocols suitable for establishing baseline micronutrient levels in *Musa* genotypes, and little is understood about the sources of biological variation and how pVACs concentrations vary during plant growth and development in general (for recent reviews see refs *15* and *16*). As part of a screening program to assess the degree of biodiversity in the fruit micronutrient contents of bananas and plantains cultivated in West and Central Africa, we wanted to establish a reliable system for the sampling and analysis of available genotypes. This in turn would allow us to establish "reference values" for the fruit micronutrient contents of individual cultivars and to simultaneously gain some insight into the factors that influence the fruit micronutrient contents. Because of the large number of samples to be analyzed, we first optimized methods of sample preparation, extraction, and analysis to establish robust and rapid protocols suitable for application within a screening program. These results are presented in a recently published paper (*17*), whereas in this paper our results on the variation of pVACs and mineral micro- and macronutrients both within fruit, within-hands, and within-bunches as well as between-plants of several widely consumed *Musa* genotypes are presented.

MATERIALS AND METHODS

Varieties and Fruit Samples. All fruit samples were obtained from the *Musa* germplasm collection maintained by Centre Africain Régional de Recherches sur Bananiers et Plantains (CARBAP), Njombé, Cameroon. Fruits were obtained from healthy, nondiseased individual plants of the same age cultivated under standard field conditions. Each genotype (variety) was represented by at least five identical plants (clones) planted at a density of one plant per 6 m^2.

Fruits were harvested when the fingers of the first hand on the bunch showed signs of ripening or yellowing or when the finger tips turned black. Maturity stage was estimated according to the peel color as described by Dadzie and Orchard (*18*). According to this scale (stages: 1, unripe; 3, starting to ripen; 5, ripe; 7, fully ripe; and 9, overripe), all fruits except Cavendish were harvested at stage 3 and transported in padded boxes with free air circulation, maintained as much as possible at temperatures of around 8 °C. The commercial Cavendish variety used was purchased from a local Belgian supermarket at the fully ripe yellow stage (stage 5). All fruits were hand-delivered to the laboratory in Leuven within 48 h of harvest and directly processed on arrival. Three plantain varieties (AAB genome type) (Batard, Mbouroukou-1, and Mbouroukou-3) and two dessert banana (AAA genome) varieties, a commercial 'Cavendish'-type and a popular local

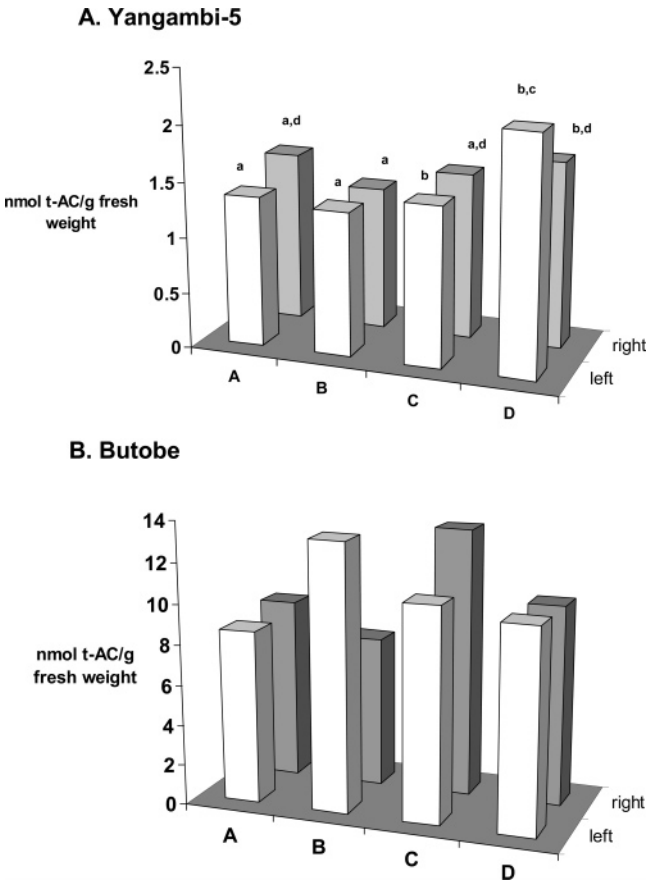

Figure 2. Within-fruit variations in pulp *all-trans* α-carotene (t-AC) contents of the two *Musa* varieties Yangambi-5 (**A**) and Butobe (**B**). One individual finger was peeled and sliced laterally once and then transectionally three times from stem to tip to generate a total of eight portions. The provitamin A carotenoids (pVACs) contents of triplicate (Yangambi-5) or duplicate (Butobe) aliquots of each section were analyzed by RP-HPLC. For pVACs contents of Yangambi-5, values that are statistically significantly from each other ($P = 0.05$) are indicated by different letters. A–D refer to the consecutive sections (quarters) of fruit pulp from base to apex in each half of the fruit that was analyzed.

dessert banana in Central and West Africa, Yangambi-5 (*19*), were analyzed. In addition, a limited set of analyses was carried out on fruit from the East African highland cooking (Matooke) banana variety 'Butobe' (ABB genotype).

Sampling and Extraction. Upon arrival, fruits were weighed, sliced once lengthwise, and photographed next to a standardized color chart (see chart B in ref *20*). They were then sliced once again laterally and the peel and the flesh pulp separately snap-frozen in liquid nitrogen either for storage at −80 °C or for frozen storage at −20 °C in sealed plastic bags in the dark or for lyophilization (Labconco, FreeZone 4.5l, model 77510, Beun De Ronde, Belgium). For analysis, diagonally opposite quarters of the frozen or lyophilized fruit pulp were pooled and homogenized to a fine powder by grinding in liquid nitrogen in a pestle and mortar and stored in sealed tubes in the dark at −20 °C. All extractions were carried out in triplicate according to procedures specifically developed in our laboratory for the analysis of *Musa* tissues (*17*). In brief, ∼50 mg aliquots of lyophilized fruit tissue powder were transferred to a 2 mL, screw-capped polypropylene reaction tube (Greiner, Wemmel, Belgium), containing 500 μL of extraction solvent with 0.25% BHT and three to five acid-washed, sterilized glass beads. Tubes were incubated at 85 °C for 10 min, cooled on ice, and homogenized for 30 s at setting 6.0 in a reciprocal shaker (FastPrep FP120, Hybaid Ltd., Middlesex, U.K.) to ensure complete tissue disruption. Samples were then centrifuged for 15 min at 14000 rpm, and the supernatant was transferred to a fresh 2 mL reaction tube on ice. The pellet was extracted twice more with 500 μL of extraction solvent, and the final combined supernatants were centrifuged at 14000 rpm for 30 min and filtered through disposable 0.45 μm filters prior to HPLC or spectrophotometric analysis.

Carotenoids Analysis. The pVACs contents of aliquots of the filtered, combined extracts were analyzed by RP-HPLC according to the method given in ref *17*. Total carotenoids contents were analyzed in duplicate by microtiter plate spectrophotometry at 450 nm, using a quartz microtiter plate (*17*). In both cases, concentrations were calculated by the external standards techniques using standard curves of freshly prepared 0.1−10 μg/mL *all-trans*-β-carotene (t-BC) (Sigma-Aldrich), in extraction solvent (*17*, *21*).

Analysis of Mineral Micronutrient Contents. Aliquots of lyophilized plant tissue powder were digested with 1 mL of high-purity 60−70% nitric acid overnight at room temperature. Samples were then evaporated just to dryness in a heating block at 140 °C (Tecator Digestion System 40), 1 mL of 60−70% nitric acid was again added, and the sample was again evaporated just to dryness, before reconstitution in 1.0 mL of nanopure water for analysis. Mineral micronutrient concentrations were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a Perkin-Elmer Optima 3300 DV. Reference materials included samples from the Wageningen International Plant Exchange Programma (IPA), a plant sample from INRA (France), two NIST samples (1576a and 1573), and two internal plant standards.

RESULTS

Fruit Sampling. In banana and plantain, fruits develop on a single "spike" or raceme, arranged helicoidally around the central axis ("stalk") of the spike in 5−20 clusters or "hands", with each hand containing up to 20 fruits or "fingers". At the top (the proximal or basal end) of the spike, fruits are persistent and are harvested for consumption. At the lower (distal) end of the spike, the fruits are deciduous. A schematic representation of the types of bunch morphology found in banana and plantain, together with the fruit sampling protocol adopted in this work, is given in **Figure 1**.

Variations in Fruit Pulp Provitamin A Carotenoids Concentrations. *Within-Fruit ("In-Finger") Variation.* A single

Table 1. Mean "In-Hand" (between Fingers of a Single Hand) Percent Variations in Fruit Pulp pVACs Contents As Determined by RP-HPLC and in Total Carotenoids Concentrations As Determined by Spectrophotometric Analysis of the Same Extracts[a]

				pVACs content (HPLC)				spectrophotometry	
				mean % variation in within-hand t-AC content		mean % variation in within-hand t-BC content		mean % variation in within-hand total carotenoids content	
variety	plant	no. of hands analyzed	no. of fruits analyzed	av/plant	av/variety	av/plant	av/variety	av/plant	av/variety
Batard	1	3	9	2.0	2.0	5.4	5.4	3.2	3.2
Mbouroukou-3	1	3	9	24.8	16.7	25.3	17.9	21.8	15.3
	2	3	9	8.5		11.3		8.1	
Grande Naine	1	9	27	45.2	45.2	42.8	42.8	nd	nd

[a] Individual fingers from the left side, middle, and right side of each hand were each analyzed in triplicate.

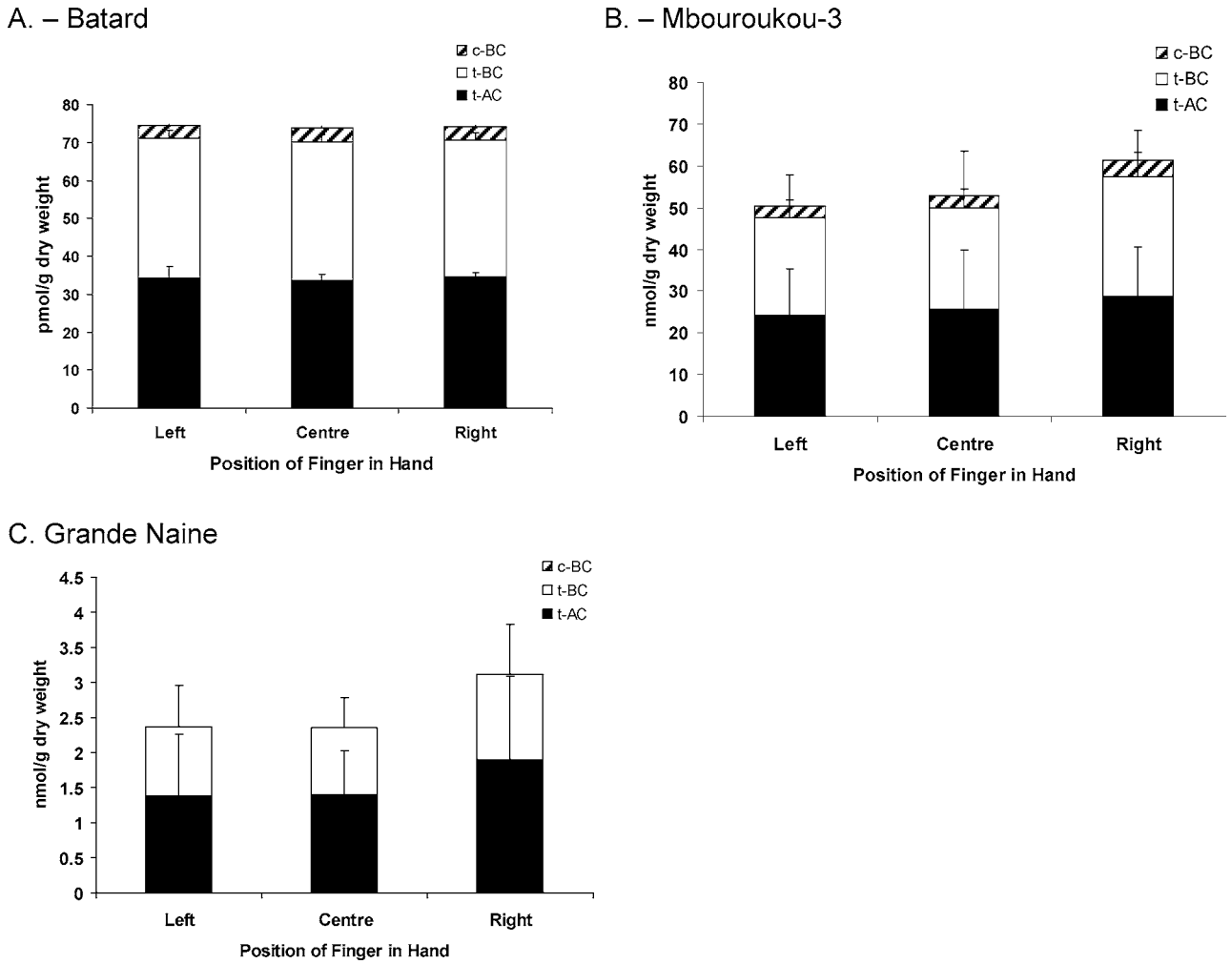

Figure 3. Influence of finger position within each hand on the pulp mean pVACs contents and variability in mean pVACs contents. Individual fingers were obtained from the left side, center, and right side of each hand. Each hand was obtained from either the proximal end, middle, or distal end of each bunch. t-AC, *all-trans*-α-carptene; t-BC, *all-trans*-β-carotene; c-BC, *cis*-β-carotene.

Table 2. Mean Between-Hands (Within-Bunch) Percent Variations in Fruit pVACs Contents As Determined by RP-HPLC and Mean Within-Bunch Total Carotenoids Concentrations As Determined by Spectrophotometric Analysis of the Same Extracts[a]

				pVACs content (HPLC)				spectrophotometry	
				mean % variation in mean within-plant t-AC content		mean % variation in mean within-plant t-BC content		mean % variation in mean within-plant total carotenoids content	
variety	plant	no. of hands sampled	no. of fruits analyzed	av/plant	av/variety	av/plant	av/variety	av/plant	av/variety
Batard	1	3	9	7.0	7.0	8.4	8.4	9.4	9.4
Mbouroukou-1	1	3	3	17.8		8.8		8.4	
	2	3	3	16.9	17.2	6.4	13.1	8.3	10.2
	3	3	3	17.0		24.0		14.0	
Mbouroukou-3	1	3	9	24.8	16.7	25.3	17.9	26.9	19.2
	2	3	9	8.9		10.4		11.5	
Yangambi-5	1	3	3	26.7		26.0		12.5	
	2	3	3	40.5	33.6	41.8	35.9	22.9	20.6
	3	3	3	33.6		40.0		26.4	
Grande Naine	1	9	27	47.3	47.3	44.9	44.9	nd	nd

[a] Mean in-hand carotenoid concentrations for the varieties Batard and Mbouroukou-3, were determined from the analyses of three individual fingers from each hand, as described in the footnote to **Table 1**, and represent the mean percent variation in mean carotenoid contents for all hands analyzed. Hands were sampled from three positions in each bunch. Between-hands carotenoid values for the varieties Mbouroukou-1 and Yangambi-5 were determined from the triplicate analysis of a single finger taken from the center of each individual hand, from three separate positions (proximal end, middle, distal end), on each plant. nd, not determined.

fruit (finger) from both a locally consumed dessert banana (Yangambi-5) and the East African highland cooking (Matooke) banana Butobe was peeled and sliced once longitudinally and then three times laterally to generate a total of eight equal-sized sections (left A−D, right A−D). These sections were powdered, and replicate aliquots were analyzed by RP-HPLC (*17*). Overall analytical reproducibilities were good, with the mean *all-trans*-α-carotene (t-AC) and *all-trans*-β-carotene (t-BC) contents varying by ±6.6 and ±6.8% for triplicate extractions in Yangambi-5 and by ±7.9 and ±6.4%, respectively, for duplicate

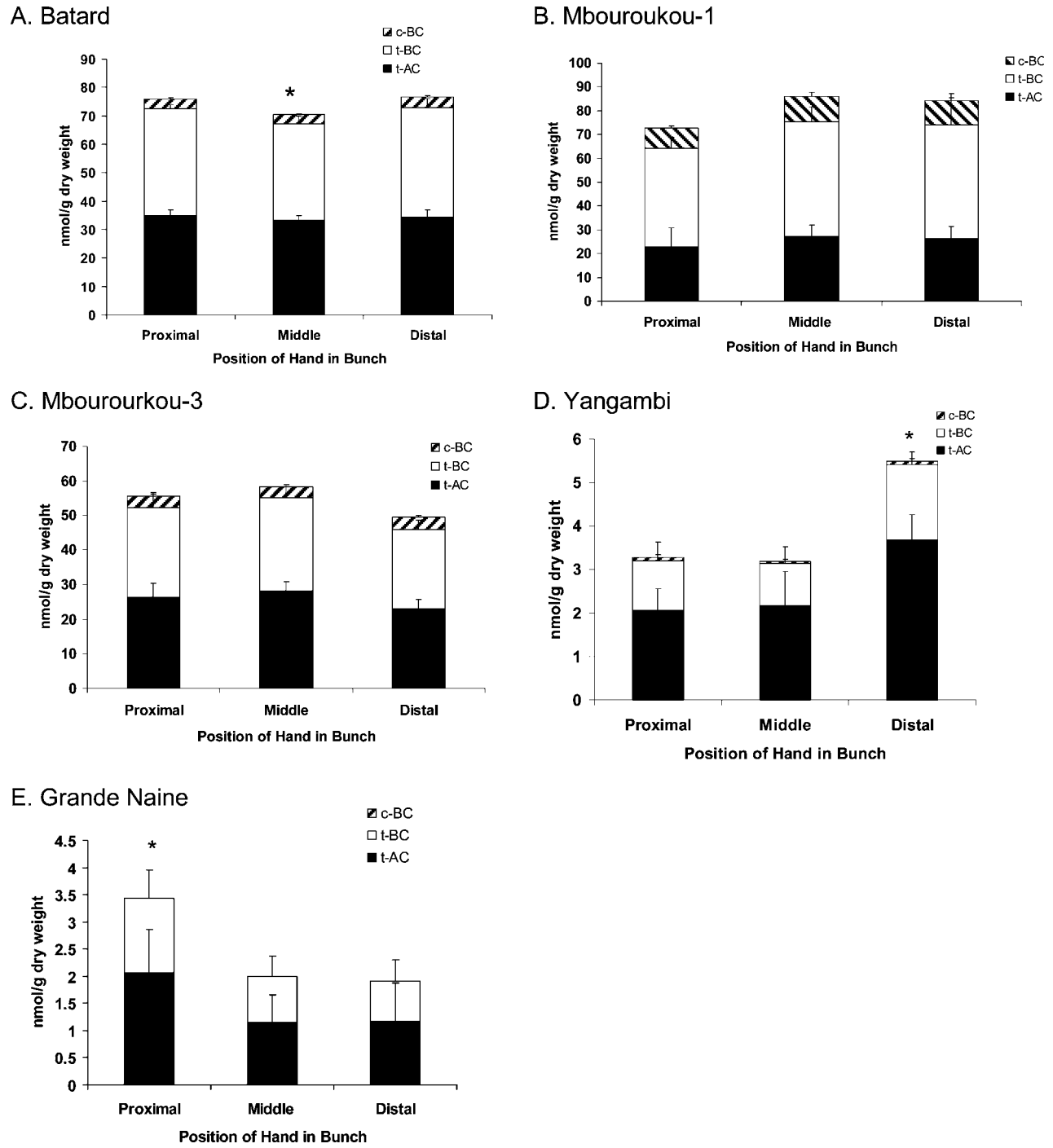

Figure 4. Influence of hand position within the bunch on the concentrations of mean fruit pulp pVACs contents in five banana and plantain varieties. Each hand was obtained from either the proximal end, middle, and distal end of each bunch. t-AC, *all-trans*-α-carotene; t-BC, *all-trans*-β-carotene; c-BC, *cis*-β-carotene. Total pVACs contents that are significantly different ($P = 0.05$) are indicated with an asterisk.

analyses in Butobe. The contents of *cis*-isomers of the pVACs were consistently small and will not be discussed here.

As shown in **Figure 2**, there are substantial and statistically significant differences in the t-AC concentrations along both axes of the fruit. t-BC contents varied in an identical manner (data not shown), so that the relative proportions of these two major pVACs did not alter with the sampling position. In fact, the proportions of t-AC and t-BC remained constant for each individual genotype, across all samples analyzed. Within each fruit, the mean concentrations of t-AC and t-BC per section varied by between ±17.8 and ±23.9% and by ±20.3 and ±21.6% for Yangambi-5 and Butobe, respectively.

Within-Hand Variations. Single fruits were collected from the left side, center, and right side of individual hands harvested from either the proximal end (top), middle, or distal (bottom) end of a single bunch and from different bunches (i.e., different plants) of the same variety. Fruits were processed as described under Materials and Methods and pVACs concentrations analyzed in triplicate by RP-HPLC and total carotenoids by spectrophotometry. Results are summarized in **Table 1**.

For the plantain variety Batard, the mean within-hand variations in fruit pulp t-AC and t-BC contents were only ±2.0 and ±5.4%, respectively, whereas the total carotenoids content as determined by spectrophotometry at 450 nm varied by ±3.2%. In the plantain Mbouroukou-3, fruits from two individual plants were analyzed, with the mean within-hand

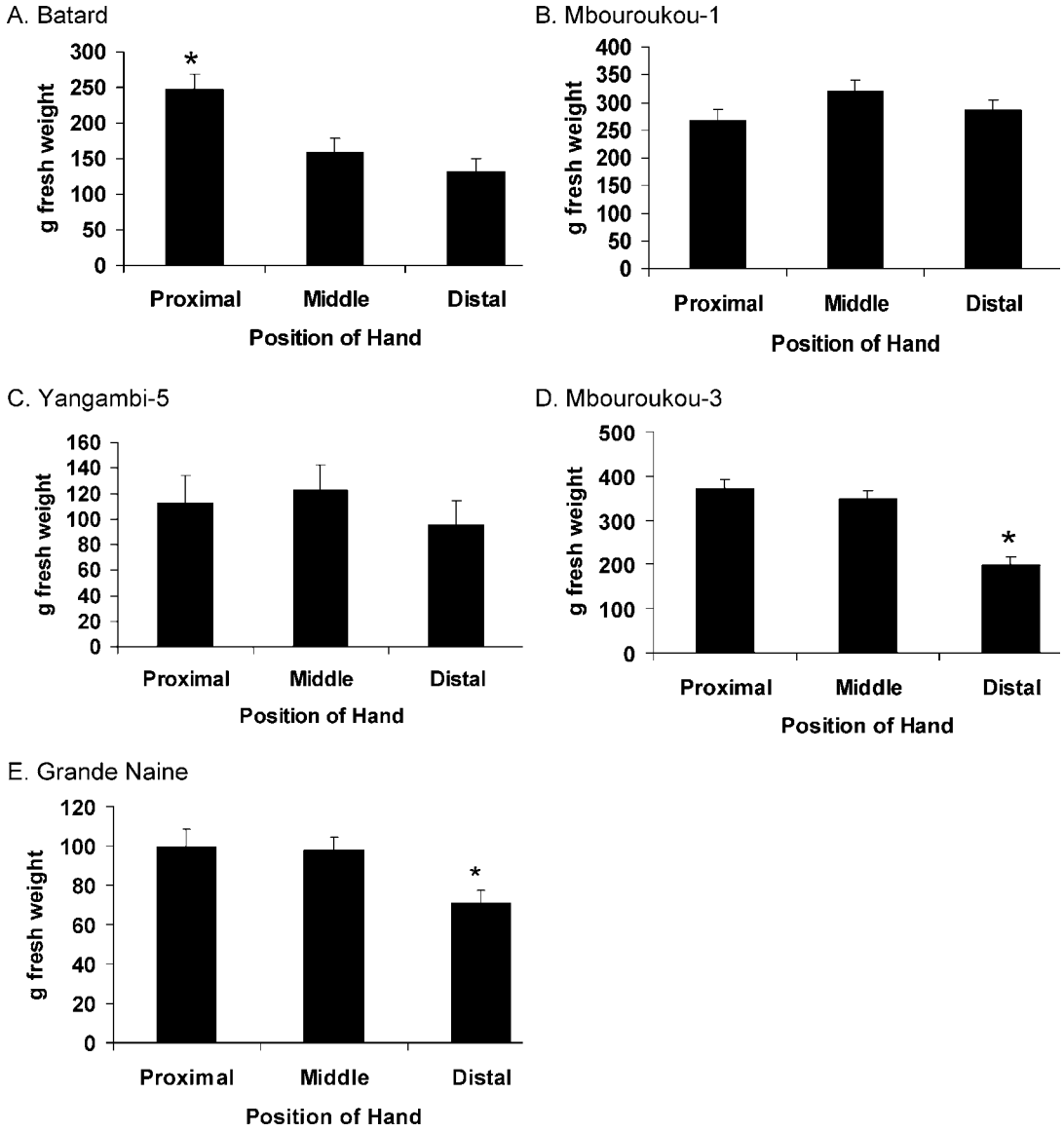

Figure 5. Influence of hand position within the bunch on mean fruit fresh weights per hand. Each hand was obtained from the proximal end, middle, and distal end of each bunch of each variety. Values that are statistically significantly different ($P = 0.05$) are indicated with an asterisk.

variations in pulp t-AC and t-BC contents varying by ±16.7 and ±17.9%, respectively (total carotenoids, ±15.3%), although the variability in mean in-hand pVACs and total carotenoids content was greater in plant 1 (~25%) than in plant 2 (~10%). Finally, in Grande Naine, the mean within-hand variations in pVACs contents were even higher at ±45 and ±43%, respectively. However, the absolute variations within hands were comparable across all of these varieties, ranging from ±0.92 nmol/g dry weight for Grande Naine to ±2.5 nmol/g dry weight for Batard and ±3.4 nmol/g dry weight in Mbouroukou-3.

To help decide which fruit (finger) could be considered to be the most "representative" within any one hand, we also looked at the variability in carotenoids contents according to the position of the finger within the hand (i.e., left, center, or right) (**Figure 3**). In the case of Batard, the mean pVACs contents of fruit from any one position in the hand varied by ±3−9%, whereas in both Mbouroukou-3 and Grande Naine, variabilities were much higher, with maxima of up to ±60%. Importantly, we could not consistently identify any one finger position where the variability, or the absolute pVACs concentration, was significantly different in any of the varieties analyzed (**Figure 3**).

Between-Hands (Within-Bunch) Variations. Fruits from individual hands obtained from the proximal end, middle, and distal end of bunches from up to three plants per *Musa* variety were analyzed using both RP-HPLC and spectrophotometry. Results showing the mean between-hands percent variation in carotenoids contents per variety are summarized in **Table 2**. Once again, the degree of variability encountered was genotype- and in one case (Mbouroukou-3) plant-specific. For Batard, the variation in the mean pVACs contents between individual hands within the single bunch analyzed was only ±7−8%, but variations as high as ±43% were measured between hands obtained from the three bunches of the varieties Yangambi-5 and Grande Naine. In the variety Mbouroukou-3, the mean between-hands variation was around ±17% in each plant.

The developmental status of fruit varies across the bunch because of differences in the time when the flowers of the inflorescence emerge. Therefore, to determine whether pVACs contents significantly change with developmental status, we looked at the influence of hand position (from the top, middle, and bottom) on mean pVACs and total carotenoids status.

In **Figure 4** it can be seen that for Yangambi-5 and Grande Naine there are statistically significant ($P = 0.05$) differences

Table 3. Mean Between-Plant (Between-Bunch) Percent Variations in Mean Plant pVACs Contents per Variety As Determined by RP-HPLC and for Between-Bunch Total Carotenoids Concentrations As Determined by Spectrophotometric Analysis of the Same Samples[a]

			HPLC		spectrophotometry
variety	no. of plants	no. of samples	% variation in mean plant t-AC content	% variation in mean plant t-BC content	% variation in mean plant total carotenoids content
Mbourouku-1	3	9	18.0	11.0	4.3
Mbouroukou-3	2	18	22.5	24.9	58.5
Yangambi-5	3	9	11.0	7.2	11.5

[a] In-plant carotenoid concentrations for the varieties Mbouroukou-1, Mbouroukou-3, and Yangambi-5 for each plant in each variety were calculated as the means of all the mean hand values analyzed in that plant (see footnotes of **Tables 1** and **2** for explanation of legends). Each sample was analyzed in triplicate.

Table 4. Overall Variation in pVACs and Total Carotenoids Contents of Fruit Pulp of Six Banana and Plantain Varieties[a]

			mean pVACs content (HPLC analysis) (nmol/g dry weight)								spectrophotometry (nmol/g dry weight)	
			t-AC content		t-BC content		c-BC content		total pVACs content		total carotenoids content	
variety	plant	no. of fruits	av/plant	av/variety	av/plant	av/variety	av/plant	av/variety	av/plant	av/variety	av/plant	av/variety
Batard	1	9	35.2 ± 0.3	35.2 ± 3.3	37.6 ± 3.6	37.6 ± 3.6	3.6 ± 0.4	3.6 ± 0.4	76.3 ± 7.1	76.3 ± 7.1	84.7 ± 9.8	84.7 ± 9.8
Mbouroukou-1	1	3	23.5 ± 3.6		29.8 ± 2.3		4.5 ± 0.3		57.7 ± 5.5		71.3 ± 6.0	
	2	3	33.8 ± 3.2	29.1 ± 5.6	36.5 ± 2.1	34.3 ± 5.6	6.1 ± 0.7	5.4 ± 1.2	76.4 ± 4.7	68.7 ± 12.2	91.7 ± 7.6	81.1 ± 11.8
	3	3	29.9 ± 4.4		38.5 ± 7.7		5.5 ± 1.7		71.9 ± 14.1		80.1 ± 11.3	
Mbouroukou-3	1	9	14.2 ± 4.5	26.1 ± 12.4	14.6 ± 5.1	25.5 ± 11.6	1.7 ± 0.6	3.3 ± 1.7	30.6 ± 10.2	54.9 ± 25.5	31.3 ± 10.0	53.0 ± 24.5
	2	9	37.3 ± 4.9		35.2 ± 4.7		4.6 ± 0.7		77.1 ± 9.7		74.7 ± 6.5	
Yangambi-5	1	9	2.6 ± 0.7		1.2 ± 0.3		0.3 ± 0.1		4.1 ± 1.1		13.5 ± 1.7	
	2	9	3.0 ± 1.2	2.6 ± 0.9	1.4 ± 0.6	1.3 ± 0.5	0.2 ± 0.1	0.2 ± 0.1	4.6 ± 1.8	4.2 ± 1.5	17.0 ± 3.9	15.4 ± 3.7
	3	9	2.4 ± 0.8		1.2 ± 0.5		0.2 ± 0.1		3.8 ± 1.4		15.6 ± 4.1	
Grande Naine	1	27	1.67 ± 1.1	1.67 ± 1.1	1.05 ± 0.59	1.05 ± 0.59	0	0	2.72 ± 1.59	2.72 ± 1.59	nd	nd
Cavendish[b]	3[c]	3	7.0 ± 0.5	7.6 ± 0.5	5.4 ± 0.3	5.4 ± 0.3	0.8 ± 0.1	0.8 ± 0.1	13.3 ± 0.8	13.3 ± 0.8	29.3 ± 1.4	29.3 ± 1.4
range				21.1		35.8		27.0		28.1		5.5

[a] Results represent the total variability in fruit pulp pVACs and total carotenoids concentrations analyzed per plant and per variety for all analyses carried out in that variety. [b] All results expressed in nmol/g of dry weight. [c] Individual hands purchased from supermarket and therefore almost certainly derived from separate plants.

Table 5. Mean Percent Variations for the Mean In-Hand (Between-Fingers) Fruit Fe and Zn Contents As Determined by ICP-OES[a]

variety	plant	no. of hands analyzed	no. of fruits analyzed	mean in-hand % variation in Fe content	overall variability/ variety (%)	mean in-hand % variation in Zn content	overall variability/ variety (%)
Batard	1	3	9	16.6	16.6	14.3	14.3
Mbouroukou-3	1	3	9	40.1	36.8	13.1	12.4
	2	3	9	33.5		11.7	

[a] Three individual fingers from the left side, middle, and right side of each hand were sampled and each analyzed in duplicate.

in the mean within-hand concentrations of pVACs, depending on whether the hands were obtained from the distal (youngest) or the proximal (oldest) end of the bunch. In Batard, fruit from the middle hand has a small but statistically significant lower pVACs contents, but for Mboroukou-3 and Mbouroukou-1, there was no significant influence of hand position on mean pVACs or on total carotenoids levels.

Because the developmental status has been directly linked to differences in the fresh weight of fruit at harvest, we also looked at the variation in mean fruit fresh weight at harvest as a function of the hand position in the bunch. These results are summarized in **Figure 5**. As shown, Batard, Mbouroukou-3, and Grande Naine all show clear negative trends in fruit fresh weights across the bunch, to the extent that hands at the distal end of the bunch contain fruits that are around 30−45% lighter than fruits in hands from the proximal end of the bunch. In the other varieties, the differences in mean fruit weight are not statistically significant.

Between-Plant Variation. An overview of the between-plant (between-bunch) variations in mean bunch carotenoids concentrations (as the average of the mean in-hand contents) per variety is given in **Table 3**. For the varieties Yangambi-5 and Mbouroukou-1, of which three individual plants and a total of nine different hands were analyzed, the maximal variation in the mean plant pVACs and total carotenoids concentrations was ±7−11.5%, whereas for Mbouroukou-3, the mean variations between the two bunches analyzed were up to ±18% (pVACs) and ±59% for spectrophotometric measurements.

Total Variation within a Variety. The total overall variation in carotenoids concentrations per genotype was determined as the mean of all the analyses carried out for that variety and is summarized in **Table 4**. On the basis of the values in this table, we can see that the mean fruit total pVACs contents (t-AC + t-BC + c-BC) per variety vary between only 76.3 ± 3.6 nmol/g dry weight in Batard (±8.6%), 54.9 ± 25.5 nmol/g dry weight in Mbouroukou-3 (±46.6%), and 2.72 ± 1.59 nmol/g dry weight in Grande Naine (±57%), with the differences in the absolute pVACs contents of fruit derived from individual plants of a variety making the largest contribution to the overall variation. Total carotenoids contents as determined by spectrophotometry agreed well with the HPLC values, with similar values for the mean variations.

Variations in *Musa* Fruit Mineral Elements Contents. The elemental compositions (macro- and microminerals) of aliquots

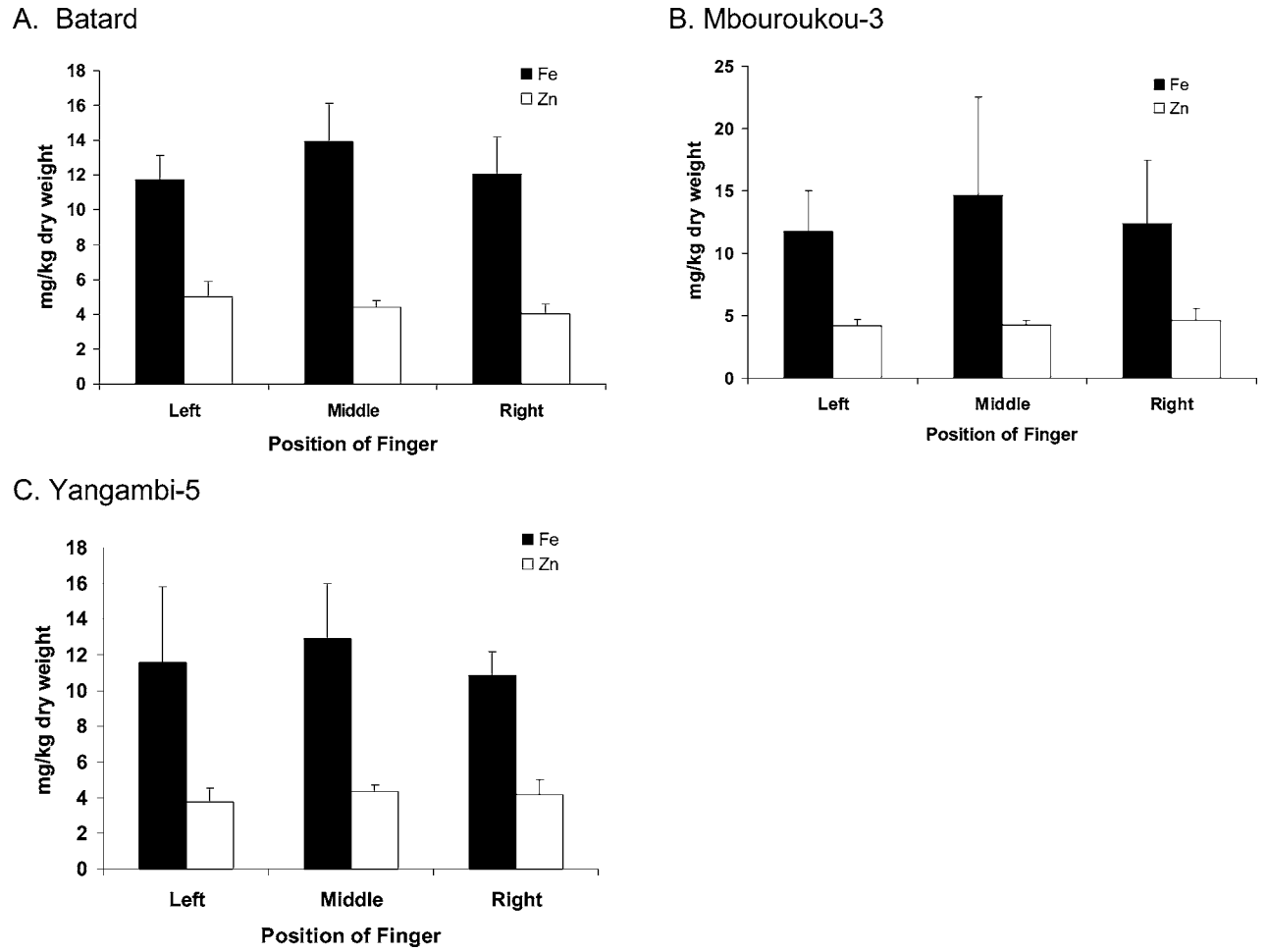

Figure 6. Influence of fruit sampling position within a single hand on content of fruit pulp Fe and Zn contents. Three individual plantain fruits were obtained from the left side, center, and right side of each hand. Each hand was obtained from the proximal end, middle, and distal end of each bunch.

Table 6. Mean Percent Variations in Between-Hands (Within-Bunch) Fruit Fe and Zn Contents As Determined by ICP-OES[a]

				mean % variation in mean hand Fe content		mean % variation in mean hand Zn content	
variety	plant	no. of hands sampled	no. of fruits analyzed	av/plant	av/variety	av/plant	av/variety
Batard	1	3	9	1.3	1.3	7.3	7.3
Mbouroukou-1	1	3	3	39.4		19.6	
	2	3	3	39.2	37.2	33.3	25.8
	3	3	3	32.9		24.4	
Mbouroukou-3	1	3	9	12.0	14.0	8.4	14.0
	2	3	9	15.9		19.5	
Yangambi-5	1	3	3	16.4		19.7	
	2	3	3	10.1	11.9	4.8	14.1
	3	3	3	9.1		17.7	

[a] Mean in-hand Fe/Zn concentrations for the varieties Batard and Mbouroukou-3 were calculated from the analyses of three individual fingers from each hand, as described in the footnote to **Table 1** and represent the variation in the mean Fe/Zn contents of all hands analyzed. Mean between-hand Fe/Zn contents for the varieties Mbouroukou-1 and Yangambi-5 were determined from the analysis of a single finger taken from the center of each individual hand, from three hand positions in each plant.

of the same pooled fruit samples used to determine carotenoids contents were measured using inductively coupled optical emission spectroscopy (ICP-OES). These analyses provided data on the concentrations of the following elements: magnesium (Mg), potassium (K), calcium (Ca), phosphorus (P), sulfur (S), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and boron (B). Of these, only the data for Fe and Zn as the two target mineral micronutrients will be discussed, although the mean fruit tissue concentrations for all of the elements analyzed are presented later. Recoveries for Fe and Zn were within 90 and 105%, respectively, of the certified values of NIST 1576a and 1573.

Within-Hand Variations in Fruit Mean Fe/Zn Contents. As previously observed with the distribution of fruit pVACs concentrations, the variations in the mean within-hand (between-fingers) Fe/Zn mineral contents were genotype-dependent (see **Table 5**). For example, in the variety Batard, mean in-hand Fe and Zn concentrations varied by 16.6 and 14.3%, respectively,

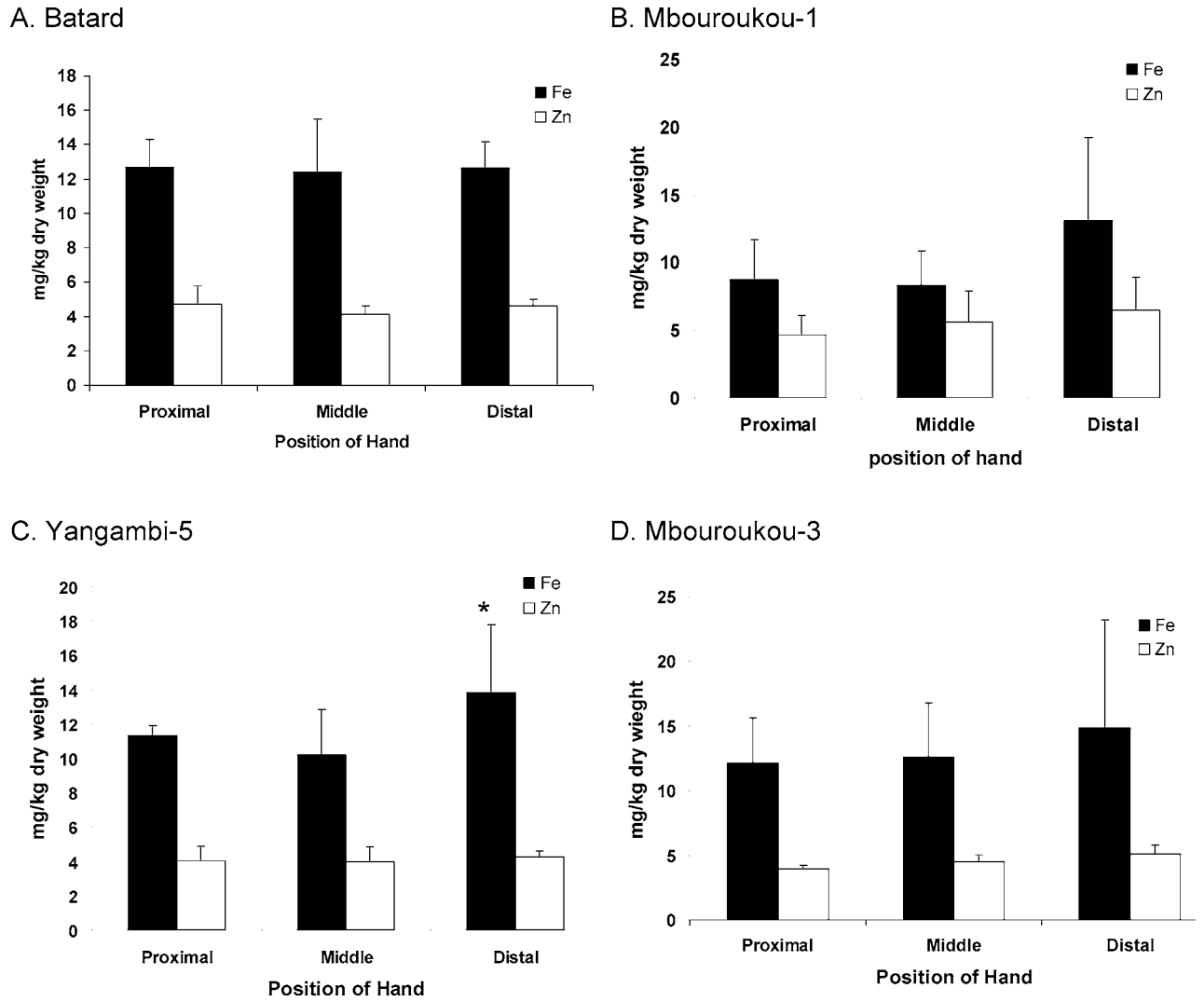

Figure 7. Influence of hand position within the bunch on mean fruit pulp Fe/Zn contents in four Musa varieties. Each hand was obtained from the proximal end, middle, and distal end of each bunch. Values that statistically are significantly different ($P = 0.05$) are indicated with an asterisk.

Table 7. Mean Between-Plant (Between-Bunch) Variations in Mean Fruit Fe and Zn Contents per Variety As Determined by ICP-OES[a]

variety	no. of plants	no. of fruits analyzed	mean between-plant % variation in mean Fe content (mg/kg of dw)	mean between-plant % variation in mean Zn content (mg/kg of dw)
Mbouroukou-1	3	9	26.9	36.5
Mbouroukou-3	2	6	7.0	0.8
Yangambi-5	3	9	11.1	5.2

[a] Mean in-plant fruit Fe/Zn concentrations for the varieties Mbouroukou-1, Mbouroukou-3, and Yangambi-5 calculated as the variability in the mean between-hand values carried out for each individual of each variety (see the footnotes of **Tables 1** and **2**). All values are expressed in terms of mg/kg of dry weight of fruit pulp material.

whereas, again, values for Mbouroukou-3 were higher at ±36.8 and ±12.4% for Fe and Zn, respectively (**Table 5**). The mean analytical variations for each duplicate Fe and Zn measurements were ±24.5 and ±5.0%, respectively, in Batard, and ±18.6 and ±5.5%, respectively, in Mbouroukou-3.

We also looked at the influence of finger position within the hand (left, central, or right) on the variation in fruit mean Fe/Zn concentrations within the varieties Batard, Mbouroukou-3, and Yangambi-5.

Figure 6 shows that the mean Fe concentrations generally appear to be slightly higher in fruit obtained from the middle of the hand but that the differences are not statistically significant. Again we were unable to detect any effect of finger position on the mean concentrations of Zn, and the differences in the variations in absolute Fe/Zn concentrations between varieties were minimal.

Mean Between-Hand (In-Plant or In-Bunch) Variations in Fruit Fe/Zn Contents. The mean between-hand percent variations in fruit Fe/Zn contents are summarized in **Table 6**.

Similar to the results obtained with the analysis of fruit carotenoids and pVACs levels, the lowest variations in mean hand Fe/Zn concentrations were observed within the single plant of the variety Batard. In all other varieties, however, the measured variability was substantially higher, reaching values of up to ±37.2% and averaging ±25.8% for the three plants examined from Mbouroukou-3.

Again, to assess the potential impact of fruit developmental status on Fe/Zn contents, we examined the influence of the hand position within the bunch on mean fruit Fe/Zn concentrations per hand (**Figure 7**).

Similar to the situation found with fruit carotenoids concentrations, results with Mbouroukou-3, Mbouroukou-1, and Yangambi-5 suggest that the concentrations of Fe are higher in fruit obtained from younger (distal) hands. However, the relatively

Table 8. Mean Micro- and Macronutrient Contents of *Musa* Fruit Peel and Fruit Pulp[a]

			macrominerals					microminerals				
sample	no. of fruits	no. of analyses	Mg	K	Ca	P	S	Cu	Fe	Mn	Zn	B
pulp												
Batard	9	18	96.5 ± 11.17	1040.6 ± 195.8	8.1 ± 2.7	81.6 ± 15.7	29.8 ± 1.7	0.36 ± 0.14	1.26 ± 0.21	0.27 ± 0.07	0.45 ± 0.07	0.55 ± 0.07
Mbouroukou-3	18	36	97.7 ± 10.1	1314.5 ± 102.5	5.32 ± 1.98	78.1 ± 9.8	27.1 ± 2.1	0.35 ± 0.10	1.30 ± 0.53	0.41 ± 0.16	0.45 ± 0.07	0.68 ± 0.11
Mbouroukou-1	6	18	114.1 ± 22.5	1650.7 ± 359.9	13.4 ± 16.4	97.6 ± 28.9	33.5 ± 6.7	0.27 ± 0.08	0.99 ± 0.44	0.30 ± 0.14	0.56 ± 0.22	0.30 ± 0.16
Yangambi-5	9	27	110.5 ± 11.3	1728.7 ± 215.8	22.0 ± 4.87	94.3 ± 10.1	38.4 ± 3.9	0.33 ± 0.06	1.17 ± 0.31	0.26 ± 0.05	0.41 ± 0.07	0.34 ± 0.06
Cavendish	3	6	113.2 ± 4.26	1268.3 ± 211.4	26.0 ± 3.6	85.8 ± 8.44	33.1 ± 2.4	0.66 ± 0.32	1.45 ± 0.30	0.91 ± 0.29	0.65 ± 0.09	0.51 ± 0.31
range			1.2	1.7	4.9	1.2	1.4	2.4	1.5	3.5	1.6	2.3
peel												
Batard	1	2	112.3 ± 10.0	5084.3 ± 1668.9	246.6 ± 0.53	135.9 ± 9.13	62.5 ± 0.31	0.17 ± 0.08	1.69 ± 0.04	1.09 ± 0.00	1.55 ± 0.07	1.90 ± 0.10
Mbouroukou-3	nd[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Mbouroukou-1	3	9	111.5 ± 42.8	5756.1 ± 1198.9	123.9 ± 64.7	217.8 ± 44.3	65.2 ± 10.8	0.35 ± 0.11	1.69 ± 0.42	1.73 ± 0.61	2.28 ± 0.46	2.69 ± 0.74
Yangambi-5	3	9	127.7 ± 11.2	5321.8 ± 1113.9	309.2 ± 60.1	122.3 ± 11.4	51.6 ± 6.3	0.56 ± 0.08	7.79 ± 6.14	2.08 ± 0.26	2.83 ± 0.58	1.94 ± 0.12
Cavendish	2	4	106.6 ± 5.83	5002.9 ± 329.3	173.3 ± 22.7	200.7 ± 8.2	59.1 ± 4.9	0.57 ± 0.12	5.48 ± 0.65	2.34 ± 0.62	1.50 ± 0.21	1.95 ± 0.15
range			1.2	1.2	2.5	1.8	1.3	3.4	4.6	2.1	1.9	1.4

[a] All results are expressed in mg/kg of dry weight. [b] nd, not determined.

high degree of variability present between samples means that the differences between hand positions are statistically significant only in the case of Yangambi-5.

Between-Plant (Between-Bunch) Variation. An overview of the measured percent variation between mean bunch Fe/Zn concentrations per variety is summarized in **Table 7**.

For the varieties Yangambi-5 and Mbouroukou-1, three individual plants and a total of nine different hands were analyzed. For these varieties, the mean variation in mean plant (in-bunch) Fe concentrations were ±11.1 and ±26.9%, respectively, whereas the corresponding values for Zn concentrations were ±5.2 and ±36.5%, respectively. The total variation per variety (as the variability across all analyses carried out within any one genotype) was again substantially higher, however (**Table 8**) at ±26.3 and ±20.6% for Fe and ±44.6 and ±38.8% for Zn for Yangambi-5 and Mbouroukou-1, respectively (data not shown). The mean Fe/Zn variability in Mbouroukou-3 was by comparison much lower, and in contrast to the high degree of variability observed in carotenoid levels in this variety.

Mean Varietal Fruit Mineral Micro- and Macronutrient Compositions. Finally, the mean fruit pulp and peel contents of 10 mineral macro- and micronutrients per genotype are given in **Table 8**. As a reference, the results obtained for commercially available fruit from the Cavendish export banana type, which were analyzed at the same time, are also included. The results represent the means and standard deviations for all of the analyses carried out within each variety.

DISCUSSION

From the results presented here, it is clear that substantial variation in fruit pulp pVACs contents exists across all of the sample groups studied, with few common trends being evident apart from the fact that the proportions of the individual pVACs species remain constant for any one genotype. Even within a single fruit (finger) there were statistically significant concentration differences both longitudinally and laterally with an overall variation of around ±20% across the eight sampling points in both fruits analyzed (**Figure 2**). It is thus clear that to compensate for these in-fruit differences, as much of the pulp as is practically possible should be sampled, and for all subsequent analyses we standardized our sampling conditions as pooling diagonally opposite quarters from each individual fruit.

Analyses of fruit from different positions within any single hand demonstrated that there may be considerable variation in the pVACs contents depending on the sampling position. The extent of this variation appears to be genotype-dependent, however, with mean within-hand variabilities ranging from around ±7% in Batard to as high as ±~43% in Grande Naine (**Table 3**; **Figure 3**). The high mean percent variations in pVACs contents of fruit from the variety Grande Naine, however, are mainly due to the inherently low carotenoids content of this variety, as the absolute variations in pVACs contents were comparable in all of the varieties examined. The results also show that it is not possible to identify a finger position within the hand where variability is consistently lower or where pVACs contents were significantly different from other positions in the hand. This suggests that the variability due to hand position in the bunch is greater than the variability due to finger position within the hand. Therefore, our standardized sampling procedures, similar to commercial practices, are to consider the two fruits from the center of each hand to be representative of that hand.

Within the fruit bunch there is a developmental lag as the flowers of the inflorescence emerge. In the dessert banana Gros Michel, this developmental lag between fruits has been reported to be the major source of variability in fruit weight at harvest (*22*, *23*), resulting in a negative gradient in fruit weight from the proximal to the distal end of the bunch. We observed similar results here, with fruit from hands obtained from the distal end of the bunch in Batard, Mbouroukou-3, and Grande Naine being between 30 and 45% lighter than older fruit at the proximal end of the bunch (**Figure 5**). Therefore, if developmental (and ripening) status strongly influences carotenoids concentrations, we would expect to see this also reflected as differences in the mean pVACs and total carotenoids contents of the individual hands throughout the bunch. This does indeed appear to be the case for the Yangambi-5, for which fruit from the distal (youngest) end of the bunch have a significantly higher (130%) mean pVACs concentration than fruit obtained from the proximal end of the bunch (**Figure 4D**). In Mbouroukou-1 and Mbouroukou-3 the measured differences are not statistically significant, but in Grande Naine the opposite trend was found, with the fruit from the oldest, proximal hands having mean pVACs contents that were higher (180%) than those from the distal end of the bunch (**Figure 4E**). In Batard, by comparison, fruit obtained from the center of the bunch had a small, but

statistically significant, lower pVACs contents, but as with the other plantain varieties, fruit carotenoids contents did not vary substantially across the bunch. Not surprisingly, therefore, there are at best only weak correlations between fruit fresh weights (as a marker of development) and the mean pVACs concentrations (data not shown).

These results suggest that differences in fruit development are not the major source of variability in fruit carotenoid contents at harvest. They further suggest that the relationship between pVACs contents and development may be genotype-specific. In this respect it is interesting to note that the plantain varieties (Mbouroukou-1, Mbouroukou-3, and Batard), with the "false-horn" type bunch morphology (**Figure 1**), show much less variation than either Yangambi-5 or Grande Naine, which both have the typical "French" type morphology, together with a greater number of hands and thus a larger range of developmental stages within the bunch. Developmental changes in carotenoids metabolism have been studied in several other fruit species including tomato (*24*, *25*), pepper (*26*), and citrus (*27*, *28*) and, similar to the results reported here, differing trends are seen, even within varieties of the same crop species.

It is also known that carotenoids are involved in plant adaptations to the environment (*24*, *29*−*31*), and our results suggest that bunch morphology may also have an impact on fruit pVACs contents by influencing exposure of the fruit environmental factors or by differences in source-sink relationships. In particular, the more open morphology of the false-horn types could conceivably help to minimize differences in environmental exposure between fruits.

However, despite the large overall variations in mean pVACs contents per genotype (**Table 4**), once the within-bunch variability has been accounted for, the between-plant variations generally fall to within acceptable levels, and the data presented here suggest that mean pVACs concentrations per bunch are fairly consistent for clones which have been cultivated under the same environmental conditions and harvested at the same time. It remains to be seen how reproducible these values will be for the same varieties cultivated under different conditions. Finally, **Table 4** also shows that there is substantial diversity in fruit carotenoids levels within the *Musa* group, with mean fruit total pVACs contents varying by around 28-fold even within this small selection of genotypes. Interestingly, all of the plantain varieties (AAB) have substantially higher carotenoid and pVACs contents than the dessert bananas (AAA), such that the daily vit A requirements of the consumer can be fully met with three to four plantain fruits, of around 190 fruits of the Yangambi-5 variety (data not shown).

Similar to the results found with pVACs, there does not appear to be any particular position within the hand in which fruit Fe/Zn concentrations are more consistent (less variable) or "most representative" for that hand. However, as with the pVACs analysis, the number of samples analyzed for any one hand position may not have been large enough for us to eliminate the impact of variation due to hand position within the bunch. In practice, such an experiment is difficult to carry out, however, because the plants do not flower synchronously, and it is thus difficult to obtain enough fruits of the same maturity levels at any one hand position.

We were also unable to draw firm conclusions about the changes in mean fruit Fe/Zn concentrations across the bunch. Whereas younger fruit from the distal end of the bunch tended to have higher Fe/Zn contents on a dry weight basis, this effect was only statistically significant in Yangambi-5. Thus, again, overall variations in mean Fe/Zn contents per genotype were high and also genotype-dependent. As with the pVACs determinations, however, once the within-bunch variation had been accounted for (i.e., once a mean value for the micronutrient content of the plant had been established), the between-plant variations in Fe and Zn contents were generally acceptable (**Table 7**).

The mean fruit genotype values for Fe and Zn contents varied between 3.9−6.0 and 1.4−2.1 mg/kg of dw, respectively, and agree well with other published results for *Musa* and other plants (see **Table 6** and refs *32*−*35*), but in comparison to pVACs it seems that the degree of genotypic variation in fruit Fe/Zn contents is more limited (∼3-fold). This is not the case in some other crops, however, and substantial genotypic variation has been reported for cereal grain Fe and Zn contents (*33*, *34*). However, plant tissue mineral micronutrient contents are strongly dependent on the soil availability of Fe and Zn, which itself depends on factors such as the soil pH, redox status, cation exchange capacity, water content, plant root architecture, and the presence of mycorrhizal fungi.

To help develop biofortification strategies, it is essential to have some insight into the mechanisms underlying micronutrient accumulation within *Musa* fruit. However, the large degree of variation in fruit micronutrient contents indicates that even establishing baseline levels under standardized growth conditions is not a trivial problem. Perhaps surprisingly it was not possible to identify a position within the bunch that was most representative for fruits of the bunch, and as such it is necessary to collect fruit from hands at the proximal end, the middle, and the distal end of the bunch to obtain representative values for the mean fruit micronutrient contents of that plant. Once this is done, however, the mean between-plant variations per genotype seem to be generally acceptable for plants grown under the same environmental conditions.

ABBREVIATIONS USED

CARBAP, Centre Africain Régional de Recherches sur Bananiers et Plantains; CGIAR, Consultative Group on International Agricultural Research, Fe, iron; pVACs, provitamin A carotenoids; vit A, vitamin A; Zn, zinc.

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LITERATURE CITED

(1) International Center for Tropical Agriculture (CIAT) and International Food Policy Research Institute (IFPRI). *Breeding Crops for Better Nutrition*; Washingtin, DC, 2006; http://www.harvestplus.org/about.html2006.

(2) The World Bank. *Directions in Development. Repositioning Nutrition as Central to Development. A Strategy for Large-Scale Action*; The International Bank for Reconstruction and Development: Washington, DC, 2006.

(3) Fraser, P. D.; Bramley P. M. The biosynthesis and nutritional uses of carotenoids. *Prog. Lipid Res.* **2004**, *43*, 228−265.

(4) Yeum, K. J.; Russell R. M. Carotenoid bioavailability and bioconversion. *Annu. Rev. Nutr.* **2002**, *22*, 483−504.

(5) Dallman, P. R. Iron. In *Present Knowledge in Nutrition*; Brown, M. L., Filer, L. J., Guthrie, H. A., Levander, O. A., McCormick, D. B., Olson, R. E., Steele, R. D., Eds.; International Life Sciences Institute—Nutrition Foundation: Washington, DC, 1990; pp 212−223.

(6) Hess, S. Y.; Thurnham, D. I.; Hurrell, R. F. *Influence of Provitamin A Carotenoids on Iron, Zinc, and Vitamin A Status*; International Food Policy Research Institute (IPFRI) and International Center for Tropical Agriculture (CIAT): Washington, DC, 2005; p 28.
(7) Hurrell, R. F.; Reddy, M. B.; Cook, J. D. Inhibition of nonhaem iron absorption by polyphenolic-containing beverages. *Br. J. Nutr.* **1999**, *81*, 289−295.
(8) Zimmermann, M. B.; Hurrell, R. F. Improving iron, zinc and vitamin A nutrition through plant biotechnology. *Curr. Opin. Biotechnol.* **2002**, *13*, 142−145.
(9) Cousins, R. J.; Hempe, J. M. Zinc. In *Present Knowledge in Nutrition*; Brown, M. L., Filer, L. J., Guthrie, H. A., Levander, O. A., McCormick, D. B., Olson, R. E., Steele, R. D., Eds.; International Life Sciences Institute—Nutrition Foundation: Washington, DC, 1990; pp 251−260.
(10) Welch, R. M.; Graham, R. D. Agriculture: the real nexus for enhancing bioavailable micronutrients in food crops. *J. Trace Elem. Med. Biol.* **2005**, *18*, 299−307.
(11) Welch, R. M.; Graham, R. D. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J. Exp. Bot.* **2004**, *55*, 353−364.
(12) Blanckaert, I.; Swennen, R. L.; Flores, M. P.; Lopez, R. R.; Saade, R. L. Floristic composition, plant uses and management practices in home gardens of San Rafael Coxcatlan, Valley of Tehuacan-Cuicatlan, Mexico. *J. Arid Environ.* **2004**, *57*, 179−202.
(13) FAO. *FAOSTAT—Data*; Rome, Italy, 2006; http://faostat.fao.org/faostat/notes/citation.htm2006.
(14) Englberger, L.; Schierle, J.; Marks, G. C.; Fitzgerald, M. H. Micronesian banana, taro, and other foods: newly recognized sources of provitamin A and other carotenoids. *J. Food Compos. Anal.* **2003**, *16*, 3−19.
(15) Taylor, M.; Ramsay, G. Carotenoid biosynthesis in plant storage organs: recent advances and prospects for improving plant food quality. *Physiol. Plant.* **2005**, *124*, 143−151.
(16) Romer, S.; Fraser, P. D. Recent advances in carotenoid biosynthesis, regulation and manipulation. *Planta* **2005**, *221*, 305−308.
(17) Davey, M. W.; Keulemans, J.; Swennen, R. Methods for the efficient quantification of fruit provitamin A carotenoid contents. *J. Chromatogr. A* **2006**, *1136*, 176−184.
(18) Dadzie, B. K.; Orchard, J. E. Routine post-harvest screening of banana/plantain hybrids: criteria and methods. In *INIBAP Technical Guidelines*; International Plant Genetic Resources Institute: Maccarese, Italy, 1997.
(19) INIBAP. *Musa Germplasm Information System*; INIBAP: Maccarese, Italy, 2006.
(20) IPGRI-INIBAP/CIRAD. *Descriptors for Banana (Musa spp.)*; IPGRI: Montpellier, France, 1996; p 54.
(21) Schierle, J.; Pietsch, B.; Ceresa, A.; Fizet C.; Waysek, E. H. Method for the determination of β-carotene in supplements and raw materials by reversed-phase liquid chromatography: single laboratory validation. *J. AOAC Int.* **2004**, *87*, 1070−1082.
(22) Jullien, A.; Munier-Jolain, N. G.; Malezieux, E.; Chillet, M.; Ney, B. Effect of pulp cell number and assimilate availability on dry matter accumulation rate in a banana fruit [*Musa* sp AAA group 'Grande Naine' (Cavendish subgroup)]. *Ann. Bot.* **2001**, *88*, 321−330.
(23) Jullien, A.; Malezieux, E.; Michaux-Ferriere, N.; Chillet, M.; Ney, B. Within-bunch variability in banana fruit weight: importance of developmental lag between fruits. *Ann. Bot.* **2001**, *87*, 101−108.
(24) Giuliano, G.; Al-Babili, S.; von Lintig, J. Carotenoid oxygenases: cleave it or leave it. *Trends Plant Sci.* **2003**, *8*, 145−149.
(25) Bramley, P. M. Regulation of carotenoid formation during tomato fruit ripening and development. *J. Exp. Bot.* **2002**, *53*, 2107−2113.
(26) Bouvier, F.; Keller, Y.; D'Harlingue, A.; Camara, B. Xanthophyll biosynthesis: molecular and functional characterization of carotenoid hydroxylases from pepper fruits (*Capsicum annuum* L.). *Biochim. Biophys. Acta (Lipids Lipid Metab.)* **1998**, *1391*, 320−328.
(27) Ikoma, Y.; Komatsu, A.; Kita, M.; Ogawa, K.; Omura, M.; Yano, M.; Moriguchi, T. Expression of a phytoene synthase gene and characteristic carotenoid accumulation during citrus fruit development. *Physiol. Plant.* **2001**, *111*, 232−238.
(28) Kato, M.; Ikoma, Y.; Matsumoto, H.; Sugiura, M.; Hyodo, H.; Yano, M. Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in citrus fruit. *Plant Physiol.* **2004**, *134*, 824−837.
(29) Golan, T.; Muller-Moule, P.; Niyogi, K. K. Photoprotection mutants of Arabidopsis thaliana acclimate to high light by increasing photosynthesis and specific antioxidants. *Plant Cell Environ.* **2006**, *29*, 879−887.
(30) Grace, S. C.; Logan, B. A. Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species. *Plant Physiol.* **1996**, *112*, 1631−1640.
(31) Logan, B. A.; Barker, D. H.; Demmig-Adams, B.; Adams, W. W. Acclimation of leaf carotenoid composition and ascorbate levels to gradients in the light environment within an Australian rainforest. *Plant Cell Environ.* **1996**, *19*, 1083−1090.
(32) Hardisson, A.; Rubio, C.; Baez, A.; Martin, M.; Alvarez, R.; Diaz, E. Mineral composition of the banana (*Musa acuminata*) from the island of Tenerife. *Food Chem.* **2001**, *73*, 153−161.
(33) Frossard, E.; Bucher, M.; Machler, F.; Mozafar, A.; Hurrell, R. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *J. Sci. Food Agric.* **2000**, *80*, 861−879.
(34) White, P. J.; Broadley, M. R. Biofortifying crops with essential mineral elements. *Trends Plant Sci.* **2005**, *10*, 586−593.
(35) Wall, M. M. Ascorbic acid, vitamin A, and mineral composition of banana (*Musa* sp.) and papaya (*Carica papaya*) cultivars grown in Hawaii. *J. Food Compos. Anal.* **2006**, *19*, 434−445.
(36) Tezenas de Montcel, H.; De Langhe, E.; Swennen, R. Essai de classification des bananiers plantains (AAB). *Fruits* **1983**, *38*, 461.

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