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

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A B S T R A C T

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