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Variability of carotenoids in a Musa germplasm collection and implications for provitamin A biofortification

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ABSTRACT

Bananas are important staples in tropical and sub-tropical regions and their potential as a source of provitamin A has recently attracted attention for biofortification. A collection of 189 banana genotypes (AAB-plantains, *M. acuminata* cultivars and bred hybrids) was screened to determine variability in fruit pulp provitamin A carotenoid (pVAC) content using high performance liquid chromatography. Total carotenoid content in tested genotypes varied from 1.45 μg/g for hybrid 25447-S7 R2P8 to 36.21 μg/g for *M. acuminata* cultivar ITC.0601 Hung Tu with a mean of 8.00 μg/g fresh weight. Predominant carotenoids identified were α-carotene (38.67%), *trans*-β-carotene (22.08%), lutein (22.08%), 13-*cis*-β-carotene (14.45%) and 9-*cis*-β-carotene (2.92%), indicating that about 78% of the carotenoids in bananas are pVAC. High pVAC genotypes were identified for integration into biofortification strategies to combat vitamin A deficiency in developing countries.

1. Introduction

Carotenoids are a diverse group of multi-functional lipophilic pigments. They are especially important in plants as a component of photosynthetic systems, but also in human nutrition as biological antioxidants and as precursors of vitamin A (Britton, 2008). Vitamin A is a fat-soluble vitamin essential for vision, reproduction, growth and immunity (World Health Organization ((WHO), 2009). Inadequate intake of vitamin A leads to vitamin A deficiency (VAD) with health conditions such as xerophthalmia, anaemia and increased susceptibility to and severity of infections (World Health Organization (WHO), 2009). Over 600 carotenoids have been reported with only over 50 detected in food and in humans, the most predominant being α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin (Arscott, 2013). Carotenoids with provitamin A (pVA) activity such as α-carotene, β-carotene and β-cryptoxanthin have attracted attention for improvement in crop plants (biofortification) to combat VAD (Bai et al., 2011).

Bananas (*Musa* spp.) are an important staple, serving as a source of nutrients and calories for millions of people worldwide, particularly in tropical and sub-tropical regions where it is grown. In 2014, world total production was about 145 MT of which Africa contributed 28% (FAOSTAT, 2017). Over 1000 cultivars exist, mostly derived from intra- or inter-specific hybridization of the wild diploid (2n = 2x = 22 chromosomes) ancestral species *M. acuminata* Colla (A genome) and *M. balbisiana* Colla (B genome) (Heslop-Harrison & Schwarzacher, 2007). Of these, three main banana groups; dessert bananas (AAA genome), plantains (AAB genome) and East African highland bananas (AAA-EA genome) dominate production regions. While dessert bananas are of global importance, small holders mainly grow the latter two banana types as starchy staples, particularly in Africa (Ortiz & Swennen, 2014).

Bananas have a considerable diversity in fruit carotenoid content and have recently gained attention for biofortification in crop improvement programmes. Englberger, Darnton-Hill, Coyne, Fitzgerald, and Marks (2003) first documented high provitamin A carotenoids (pVAC) in Micronesian bananas suggesting its value as a good source of vitamin A. Thereafter, other studies explored and quantified variability in fruit pVAC content in a wider set of varieties (Amorim et al., 2009; Davey, van den Bergh, Markham, Swennen, & Keulemans, 2009; Englberger et al., 2006; Fungo & Pillay, 2011; Heng et al., 2017). These studies reported that the carotenoid profile of bananas includes mainly

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β-carotene, α-carotene and lutein although smaller amounts of β-cryptoxanthin and zeaxanthin have also been reported (Borges et al., 2014; Englberger et al., 2006). Yellow-orange flesh banana cultivars are known to contain higher levels of carotenoids (up to > 20 fold) than cream-white flesh cultivars (Amorim et al., 2009; Englberger et al., 2006; Fungo & Pillay, 2011).

Efficient screening of large numbers of genotypes is critical for identifying trait variation for biofortification in crop improvement programmes. Although previous studies recorded substantial variability of fruit carotenoid content in *Musa* spp. suggesting possibilities for breeding, studies have been limited to a few genome groups, within narrow sets of germplasm with few samples mostly collected from different sites. To our knowledge, few reports exist for carotenoid content in *Musa* spp. where up to 30 genotypes were analysed, despite the vast diversity of *Musa* (Amorim et al., 2009; Borges et al., 2014; Davey et al., 2009; Fungo & Pillay, 2011; Heng et al., 2017). Previous reports on carotenoid quantification also focused mainly on banana cultivars and not so much on hybrids produced by breeding programmes. Moreover, diverse sampling and analytical strategies employed may have contributed to reported variability among cultivars. For example, in the most comprehensive study carried out on 171 banana genotypes by Davey et al. (2009), frozen and lyophilized samples were obtained from several locations, transported and analysed in distant labs. Indeed, carotenoid contents and composition of fruits may vary depending on cultivar/variety, fruit developmental stage and growing conditions (Rodriguez-Amaya & Kimura, 2004).

A breeding programme was initiated at the International Institute of Tropical Agriculture (IITA), Nigeria in the late 1980s to improve plantain cultivars for local consumption (Ortiz & Vuylsteke, 1996). The programme mainly targeted host plant resistance to black sigatoka and to date continued to address other emerging biotic and abiotic constraints as well as fruit quality. Through conventional cross breeding, several improved plantain hybrids bred by IITA (abbreviated as PITA) and advanced breeding lines, have been produced, mainly originating from crosses between plantain cultivars and *M. acuminata* diploid cultivars (Tenkouano & Swennen, 2004; Tenkouano, Pillay, & Ortiz, 2011). Recently, high pVAC content has been added to the breeding targets because of their health benefits, but variability for this trait has not been evaluated in the breeding germplasm.

This study therefore aimed to (1) assess the variability of fruit carotenoid content in 189 genotypes comprising different types of bananas (plantains, *M. acuminata* cultivars and hybrids) present in the IITA germplasm collection in Nigeria; (2) establish their carotenoid profiles; and (3) identify high pVAC genotypes for the development of pVA biofortification strategies in banana.

2. Materials and methods

2.1. Plant material

One hundred and eighty-nine diverse genotypes, comprising plantains (66 genotypes), *M. acuminata* cultivars (64 genotypes) and bred hybrids (59 accessions), maintained in field plots at the IITA, Ibadan research station (3° 54′E and 7° 30′N at 240 m above sea level) were investigated in this study. Plantains (Supplementary Table 1) represented popular farmer preferred varieties within the existing diversity originating from Africa and beyond, categorized into French, False Horn and Horn types based on bunch morphology as described by Adheka et al. (2018). *M. acuminata* cultivars (Supplementary Table 2) were recently acquired from the International Transit Centre (ITC, Leuven, Belgium) and categorized into Papua New Guinea (PNG) cultivars; ssp. banksii cultivars; Indonesia triangle and New Guinea (IndonTriNg) cultivars; Mshare cultivars; assorted cultivars and unclassified cultivars, based on passport data on MGIS database (www.cropdiversity.org) in combination with cytological and molecular groupings (clusters) as described by Christelová et al. (2017). Hybrids (Supplementary Table 3) were advanced lines/clones as well as other breeding lines, previously developed by IITA and Honduran Foundation for Agricultural Research (FHIA) banana breeding programmes. Hybrids were selected for high yield and black sigatoka-resistance and categorized into diploids, triploids and tetraploids. All plants were grown under standard field conditions at a spacing of 3 × 2 m and each variety was represented by a minimum of five plants. For each genotype 2–5 replicate bunches were sampled between August 2014 and February 2018, depending on plant survival and bunch availability. This was a large and diverse set of germplasm in an already existing collection, which made sampling at the same time per season over seasons difficult, but sampling was done as consistently as possible. Bunches were harvested at maturity when ripening was observed at the first hand of the bunch and stored at ambient temperature (25–28 °C) in a dark room for ripening without post-harvest ethylene treatment. However, as the room is enclosed, ethylene released from the fruits facilitated ripening. Healthy and undamaged fruits were sampled from the middle of the second hand of each bunch, at the fresh ripe stage corresponding to stage 5, when fruit colour had turned yellow with green tips and necks (Dadzie & Orchard, 1997). This ensured approximately uniformly ripened fruits for analyses. Sampled fruits were quickly delivered to the Food and Nutrition Sciences Laboratory in IITA Ibadan for carotenoid analysis.

2.2. Chemicals used

Chemical reagents and solvents used in this study were: Hyflosupercel (Celite), anhydrous sodium sulphate (analytical grade) and dichloromethane (laboratory grade) purchased from BDH, Bristol, UK; α-carotene, β-carotene and lutein purchased from Sigma-Aldrich, Steinheim, Germany; acetone (analytical grade) purchased from VWR International, Fontenay-sous-Bois, France; Petroleum ether, methanol (HPLC grade) and methyl *tert*-butyl ether (HPLC grade) procured from Fisher Scientific, Loughborough, United Kingdom.

2.3. Sample processing and carotenoid extraction

Sample preparation, extraction procedures and analysis were carried out under subdued light to minimise light induced degradation of carotenoids. Fruits were first washed with distilled water, air-dried and peeled with sharp stainless-steel knives. Fruit pulp was diagonally cut into halves and each half was further cut into small pieces, mixed thoroughly and wrapped in aluminium foil and immediately analysed.

Banana carotenoids were extracted using acetone as described by Rodriguez-Amaya and Kimura (2004) for cassava, with some modifications. For each sample, 10 g homogenized fruit pulp was thoroughly macerated in a mortar with 3 g of Celite in 50 ml of cold acetone and the solution was filtered with suction through a filter paper in a Büchner funnel. This extraction procedure was repeated 3–4 times until the final residue appeared colourless. The acetone extract was then partitioned to 20 ml of petroleum ether in a separatory funnel. Distilled water was slowly added along the surface of the funnel allowing the two phases to separate without emulsion formation. Following this, the lower aqueous-acetone phase was discarded by washing four times with distilled water and finally washed with 150–200 ml of brine solution (NaCl) to break emulsions formed. The upper organic (petroleum ether) phase was then filtered through anhydrous sodium sulphate into a 50 ml volumetric flask and the volume adjusted to 50 ml with petroleum ether.

2.4. Carotenoid separation and quantification

Separation and quantification of individual carotenoids was carried out on a Waters Alliance e2695 High Performance Liquid Chromatography (HPLC) separation module (Waters Corporation, Milford, MA, USA) equipped with a polymeric YMCTM C30 5 µm

column (4.6 × 250 mm) and a photodiode array detector (PDA). The system was operated by Empower software (Waters Corporation, Milford, MA, USA).

Twenty-five ml of petroleum ether extract (obtained in section 2.3) was concentrated and dried under nitrogen gas and reconstituted in 1 ml dichloromethane:methanol (v/v). The solution was filtered through a 0.22 mm PTFE syringe filter (Millipore) into 2 ml vials (Waters PTFE/silicone septum) for HPLC. Sample injection volumes were 20 µl and flow rate was set at 1.0 ml/min at a temperature of 25 °C. An isocratic elution was performed for 10 min on extracts with 50:50 v/v methanol:methyl *tert*-butyl ether. The UV spectra was observed at 200–600 nm and carotenoids were detected at 450 nm. Identification of lutein, α-carotene, *trans*-β-carotene (*trans*-BC), 13-*cis*-β-carotene (13-*cis*-BC) and 9-*cis*-β-carotene (9-*cis*-BC) were determined using an external standard method based on the calibration curve established from pure standards and verification of absorption spectrum and co-elution with authentic commercial standards (β-carotene, α-carotene and lutein).

Results were recorded in µg/g fresh weight (FW) and recorded values for each sample was a mean of duplicate analysis. Total carotenoids (TC) with provitamin A activity was computed as: pVACs (µg/g) = α-carotene + 13-*cis*-BC + 9-*cis*-BC + *trans*-BC and TC computed as TC (µg/g) = Total pVACs + lutein. Provitamin A content expressed in terms of β-carotene equivalents (BCE) was also computed as BCE (µg/g) = 0.5 (α-carotene + 13-*cis*-BC + 9-*cis*-BC) + *trans*-BC based on Taleon, Mugode, Cabrera-Soto, and Palacios-Rojas (2017).

2.5. Statistical analysis

Statistical analysis was carried out using Statistical Analysis Software (SAS) version 9.4 for Windows. The PROC GLM statement was used for one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test to detect significant differences among means.

3. Results and discussion

3.1. Carotenoid content and profiles of plantains

Table 1 lists the major carotenoids identified and quantified in plantain fruit pulp of 66 genotypes screened (34 French, 28 False Horn and 4 Horn types). Total carotenoid content for all plantains varied from 5.12 to 15.98 µg/g with a mean of 11.74 µg/g. The most abundant carotenoid isolated in plantain fruit pulp was α-carotene (5.44 µg/g) followed by *trans*-BC (3.11 µg/g), 13-*cis*-BC (1.67 µg/g), lutein (1.24 µg/g) and the least was 9-*cis*-BC (0.28 µg/g), representing 46.34%, 26.49%, 14.22%, 10.56% and 2.38% of the total carotenoids respectively.

BCE for all plantains varied from 2.01 to 10.65 µg/g and the mean BCE for French, False Horn and Horn types was 6.89, 6.78 and 6.47 µg/g, respectively (Table 1). The French plantain ITC.0112 Bobby Tannap had the highest BCE (Fig. 1B) while Red plantain had the lowest (Fig. 1E) and this is reflected by a deeper orange colour with a higher TC and BCE concentration (Supplementary Table 4). The top accessions in terms of BCE content (µg/g) for French, False Horn and Horn types were ITC.0112 Bobby Tannap (10.65), ITC.0208 Atali Kiogo (9.74) and ITC.0128 Tshambunu (9.26). Although French plantains had slightly higher values for individual carotenoids lutein, α-carotene, 13-*cis*-BC and 9-*cis*-BC and TC, there was no significant difference in the means for all three plantain types. Data for individual cultivars in various plantain categories are provided in Supplementary Table 4.

Approximately 120 known plantain cultivars exist, comprising selections from existing hybridizations and somatic mutations of a few accessions, with West and Central Africa harboring the greatest variability (Adheka et al., 2018; De Langhe, Pillay, Tenkouano, & Swennen, 2005). Previous studies dealing with carotenoid profiling in banana fruit pulp have only included a few plantain genotypes. Davey et al. (2009) analysed 26 plantain cultivars and recorded BCE ranging from 1.19 to 17.79 µg/g with lowest values and highest values obtained in Niangafelo and Batard respectively. Heng et al. (2017) recently reported TC values of 36.82, 11.65 and 8.55 µg/g for mature fruit pulp from Orishele, Dwarf French plantain and Obubit Ntanga GM, respectively. In our study, these three accessions had TC values of 13.72, 10.29 and 15.60 µg/g, respectively. Ekesa, Nabuuma, Blomme, and Van den Bergh (2015) also recorded a high BCE for ripe fruits of Apantu (82.38 µg/g FW), about 10x higher than what obtained in this study. Differences in growth environment, sampling stage, extraction method and separation as well as quantification procedures could account for such huge cultivar differences between different studies (Saini, Nile, & Park, 2015).

Six French plantains; (ITC.0112 Bobby Tannap, ITC.0519 Obubit Ntanga GM, ITC.1397 French Reversion Red Pseudostem, ITC.0496 Cantebalon, ITC.0109 Obino L'ewai, ITC.0325 Wine Plantain), four

Table 1
Mean and range of carotenoids in 66 plantain cultivars.

Trait	Carotenoid content (µg g^{-1} ± SD)*			
	All plantains (N = 270)	French (N = 133)	False Horn (N = 117)	Horn (N = 20)
Mean				
Lutein	1.24 ± 0.63	1.29 ± 0.61	1.20 ± 0.68	1.09 ± 0.38
α-carotene	5.44 ± 2.29	5.64 ± 2.47	5.28 ± 2.17	4.99 ± 1.71
13-*cis*-BC	1.67 ± 0.95	1.74 ± 1.04	1.65 ± 0.87	1.39 ± 0.58
9-*cis*-BC	0.28 ± 0.16	0.29 ± 0.20	0.28 ± 0.11	0.25 ± 0.10
trans-BC	3.11 ± 1.93	3.05 ± 2.00	3.17 ± 1.86	3.16 ± 1.93
Total pVACs	10.50 ± 3.92	10.72 ± 4.24	10.38 ± 3.63	9.79 ± 3.30
TC	11.74 ± 4.01	12.01 ± 4.32	11.58 ± 3.74	10.88 ± 3.38
BCE	6.81 ± 2.80	6.89 ± 2.99	6.78 ± 2.63	6.47 ± 2.54
Range				
Lutein	0.45–2.33	0.54–2.29	0.45–2.33	0.92–1.60
α-carotene	0.88–8.19	0.88–8.19	2.37–7.38	3.27–6.27
13-*cis*-BC	0.46–4.43	0.46–4.43	0.86–2.58	0.56–1.14
9-*cis*-BC	0.08–0.67	0.08–0.67	0.13–0.38	0.19–0.32
trans-BC	0.23–6.46	0.23–6.46	0.65–5.18	1.59–5.28
Total pVACs	2.91–14-83	2.91–14.83	4.27–14.34	6.64–13.24
TC	5.12–15.98	5.12–15.98	5.23–15.50	7.67–14.28
BCE	2.01–10.65	2.01–10.65	2.46–9.74	4.11–9.26

N: number of bunches sampled, 13-*cis*-BC: 13-*cis*-β-carotene, 9-*cis*-BC: 9-*cis*-β-carotene, *trans*-BC: *trans*-β-carotene, pVACS: provitamin A carotenoids, TC: total carotenoids, BCE: β-carotene equivalents, SD: standard deviation, *means within rows were not significantly different at $P < 0.5$

Fig. 1. Dissected fruit of high and low carotenoid banana genotypes. High content – A: *M. acuminata* cultivar Hung Tu; B: Plantain cultivar Bobby Tannap; C: hybrid 33448-1. Low content – D: *M. acuminata* cultivar No 110; E: Plantain cultivar Red plantain; F: hybrid 25447-S7 R4P11. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

false-horn plantains; (ITC.0208 Atali Kiogo, ITC.0630 Dominico Harton Rojo, ITC.0515 Okoyo Ukom, ITC.0223 Apantu) and one Horn plantain (ITC.0128 Tshambunu) were identified with high BCE (8.72–10.65 μg/g FW) and pVAC (14.28–15.38 μg/g). Plantains constitute a specific banana subgroup, further classified based on bunch morphology. Consumer preferences are limited to the French and False Horn types, while the Horn types are less popular because of their smaller bunch size. In this study, more plantain genotypes were screened than any previous reports, representing about 50% of the existing plantain diversity and interestingly, we identified high pVAC across all three different plantain types.

3.2. Carotenoid content and profiles of *M. acuminata* cultivars

Sixty-four diverse *M. acuminata* cultivars comprising mainly diploids (AA cv) were screened for their carotenoid composition (Table 2). Among these, 18 were PNG cultivars, 7 belonged to the IndonTriNg cluster, 8 were Mshare, 11 were ssp. banksii, 7 belonged to heterogonous subgroups and clusters (assorted) while 13 were unclassified (Supplementary Table 2).

TC of all *M. acuminata* cultivars varied from 0.89 to 30.30 μg/g with a mean of 7.05 μg/g (Table 2). The most abundant carotenoid for all *M. acuminata* cultivars was α-carotene (3.04 μg/g) followed by *trans*-BC (1.87 μg/g), lutein (1.01 μg/g), 13-*cis*-BC (0.90 μg/g) and the least 9-*cis*-BC (0.21 μg/g) constituting 37.06%, 23.04%, 21.55%, 14.51%, 3.85% of the TC respectively. However, carotenoid profiles varied between the six different cultivar categories, with lutein being the most abundant carotenoid in Mshare and assorted category while α-carotene was most abundant in all other categories.

Mean BCE for all *M. acuminata* cultivars was 3.95 and varied from 0.57 to 21.06 μg/g and ANOVA tests indicated significant differences between the mean carotenoid compositions of various cultivar categories (Table 2). Cultivar ITC.0601 Hung Tu (Fig. 1A) had the highest BCE content while ITC.0413 No 110 had the lowest (Fig. 1D). The highest mean BCE (μg/g) was recorded for the ssp. banksii category (7.07), which was not significantly different from that of IndonTriNg (6.18) and PNG cultivars (4.66) but was significantly different from Mshare (1.07), which was the lowest. Comparatively, ssp. banksii diploids had a 5–6-fold higher TC and BCE content than the Mshare cultivars (Table 2). Top accessions for each cultivar category in terms of BCE (μg/g) were ITC.0894 Tainga (14.69) and ITC.0809 Maleb (14.63) for banksii, ITC.0601 Hung Tu (21.06) for IndonTriNg and ITC.0920 Dimaemamosi (10.52) for PNG cultivars. Data for all accessions in various cultivar categories are summarised in Supplementary Table 5.

Generally, this group had a wide variability for all carotenoid traits analysed and this is expected because *M. acuminata* cultivars are genetically diverse, belonging to various subgroups/clusters with diverse geographical origins. While most of these cultivars are AA diploids, a recent classification by Christelová et al. (2017) further groups them into specific clusters based on cytological and molecular characterization using simple sequence repeat markers. According to that classification, the banksii group are diploid AA cultivars of the ssp. banksii background with origins from PNG. The IndonTriNg group also originates from PNG but within the triangle shaped by the Eastern Indonesian islands. Mshare are diploid cooking bananas of the 'Mlali' subgroup found in East Africa and neighbouring islands, and suspected to be the 2n donor of the AAA triploid dessert bananas (Christelová et al., 2017; Hippolyte et al., 2012; Perrier et al., 2009).

One IndonTriNg cultivar (ITC.0601 Hung Tu), four ssp. banksii cultivars (ITC.0894 Tainga, ITC.0809 Maleb, ITC.1187 Tomolo, ITC.0600 Waimara) and three PNG cultivars (ITC.0920 Dimaemamosi, ITC.0932 Gilasalasa, and ITC.0838 Bega) were identified with high BCE content (8.72–21.06 μg/g) and TC content (13.95–36.21 μg/g). These and other high content ssp. banksii diploids have potential for plantain breeding as the banksii are suspected to be the contributors of the A genome in plantains (Perrier et al., 2009; Tenkouano et al., 2011).

Table 2
Mean and range of carotenoids in 64 *M. acuminata* cultivars.

Trait	Carotenoid content (μg g-1 ± SD)*						
	Cultivars all (N = 247)	PNG (N = 67)	ssp. banksii (N = 35)	IndonTriNG (N = 26)	Mshare (N = 33)	Assorted (N = 29)	Unclassified (N = 57)
Mean							
Lutein	1.01 ± 1.40	0.72 ± 0.57^{b}	0.66 ± 0.93^{b}	1.52 ± 3.07^{a}	1.67 ± 1.85^{a}	1.40 ± 1.09^{a}	0.76 ± 0.51^{b}
α-carotene	3.04 ± 3.54	3.73 ± 3.04^{b}	6.05 ± 4.79^{a}	4.68 ± 5.58^{ab}	0.83 ± 0.64^{c}	1.34 ± 1.30^{c}	1.78 ± 1.33^{c}
13-*cis*-BC	0.90 ± 0.81	1.04 ± 0.83^{ab}	1.36 ± 0.95^{a}	1.01 ± 0.75^{ab}	0.41 ± 0.36^{d}	0.62 ± 0.72^{dc}	0.84 ± 0.74^{bc}
9-*cis*-BC	0.21 ± 0.31	0.21 ± 0.20^{b}	0.40 ± 0.59^{a}	0.20 ± 0.22^{b}	0.13 ± 0.13^{b}	0.20 ± 0.33^{b}	0.17 ± 0.20^{b}
trans-BC	1.87 ± 2.33	2.17 ± 2.28^{bc}	3.16 ± 2.55^{ab}	3.23 ± 4.24^{a}	0.38 ± 0.44^{d}	1.09 ± 0.90^{d}	1.36 ± 1.50^{cd}
Total pVACs	6.03 ± 6.09	7.16 ± 5.11^{b}	10.97 ± 7.85^{a}	9.13 ± 9.93^{ab}	1.75 ± 1.07^{c}	3.24 ± 2.42^{c}	4.16 ± 2.70^{c}
TC	7.05 ± 6.42	7.88 ± 5.15^{b}	11.63 ± 7.55^{a}	10.65 ± 12.13^{a}	3.43 ± 2.32^{c}	4.65 ± 2.85^{c}	4.92 ± 2.66^{c}
BCE	3.95 ± 4.11	4.66 ± 3.48^{b}	7.07 ± 5.10^{a}	6.18 ± 6.18^{ab}	1.07 ± 0.66^{c}	2.17 ± 1.57^{c}	2.76 ± 1.98^{c}
Range							
Lutein	0.13–5.91	0.26–1.24	0.13–1.52	0.39–5.91	0.53–3.65	0.54–2.41	0.29–1.50
α-carotene	0.26–16.51	1.06–8.56	0.60–15.88	0.36–16.51	0.26–1.29	0.44–4.30	0.30–3.46
13-*cis*-BC	0.18–2.79	0.40–2.78	0.25–2.79	0.18–1.72	0.25–0.66	0.18–1.50	0.20–2.25
9-*cis*-BC	0.03–1.50	0.08–0.40	0.10–1.50	0.08–0.42	0.05–0.27	0.03–0.50	0.05–0.60
trans-BC	0.10–11.81	0.17–5.62	0.21–7.11	0.36–11.81	0.18–0.76	0.25–2.95	0.10–3.85
Total pVACs	1.56–36.21	2.84–16.65	3.08–23.84	2.36–36.21	2.21–5.00	2.13–9.98	1.56–7.69
TC	0.89–30.30	2.39–15.41	1.91–23.58	1.24–30.30	1.13–2.42	1.00–8.48	0.89–7.41
BCE	0.57–21.06	1.40–10.52	1.36–14.69	0.80–21.06	0.67–1.39	0.63–5.72	0.57–5.37

N: number of bunches sampled, 13-*cis*-BC: 13-*cis*-β-carotene, 9-*cis*-BC: 9-*cis*-β-carotene, *trans*-BC: *trans*-β-carotene, pVACS: provitamin A carotenoids, TC: total carotenoids, BCE: β-carotene equivalents, SD: standard deviation, * means with different letters within rows are significantly different at $P < 0.5$

Cultivars originating from PNG have been reported elsewhere to have higher carotenoid contents (Fungo & Pillay, 2011; Ngoh-Newilah et al., 2009). The only other banana type reported to have higher carotenoid contents than the ssp. banksii and AA cv. IndonTriNg cultivars from PNG are the Fe'i bananas (2006; Englberger et al., 2003), which belong to the Australimusa (now incorporated in Eumusa) section of the genus *Musa* (Christelová et al., 2017). However, the Fe'i bananas with their characteristic red sap, erect bunches and deep orange fruit pulp are only cultivated in the Pacific islands and are not useful for conventional plantain breeding.

3.3. Carotenoid content and profiles of hybrids

Fifty-nine bred banana hybrids (29 diploids, 12 triploids and 18 tetraploids) with diverse genetic backgrounds were analysed by HPLC for their carotenoid composition (Table 3). TC content of hybrids varied from 1.45 to 14.47 μg/g with a mean of 4.96 μg/g. The most abundant carotenoid in hybrids was α-carotene (1.87 μg/g) followed by lutein (1.29 μg/g), *trans*-BC (1.02 μg/g), 13-*cis*-BC (0.66 μg/g) and eventually 9-*cis*-BC (0.12 μg/g) constituting 33.46%, 33.00%, 17.66% 13.34% and 2.54% of the TC respectively. However, except for the *cis*-β-carotene isomers, varying trends in proportions of carotenoids were observed in diploids, triploids and tetraploid hybrids (Table 3). Lutein was the most abundant carotenoid in the diploid category and α-carotene was most abundant in the triploid and tetraploid categories, while 9-*cis*-BC was least abundant in all categories.

Mean BCE (μg/g) was 2.34 for all hybrids and varied from 0.24 to 7.78 but mean was significantly different for diploid (1.47), triploid (2.33) and tetraploid (3.62) hybrids. Except for lutein where the difference was not significant, all other individual carotenoids increased significantly with ploidy levels (Table 3). Tetraploid hybrid 33448–2 had the highest BCE content, while diploid hybrid 25447-S7 R4P11 had

Table 3
Mean and range of carotenoids in 59 banana hybrids.

Trait	Carotenoid content (μg g^{-1} ± SD)*			
	Hybrids (N = 254)	Diploid (N = 117)	Triploid (N = 57)	Tetraploid (N = 80)
Mean				
Lutein	1.29 ± 1.05	1.22 ± 0.90^{a}	1.27 ± 1.33^{a}	1.42 ± 1.02^{a}
α-carotene	1.87 ± 1.79	0.97 ± 0.95^{c}	2.19 ± 1.87^{b}	2.98 ± 1.99^{a}
13-*cis*-BC	0.66 ± 0.55	0.43 ± 0.48^{c}	0.76 ± 0.52^{b}	0.93 ± 0.53^{a}
9-*cis*-BC	0.12 ± 0.13	0.09 ± 0.15^{b}	0.11 ± 0.07^{ab}	0.15 ± 0.11^{a}
trans-BC	1.02 ± 1.35	0.73 ± 1.21^{b}	0.80 ± 0.91^{b}	1.59 ± 1.61^{a}
Total pVACs	3.67 ± 3.26	2.22 ± 2.21^{c}	3.86 ± 3.04^{b}	5.64 ± 3.65^{a}
TC	4.96 ± 3.40	3.43 ± 2.29^{c}	5.13 ± 3.16^{b}	7.06 ± 3.90^{a}
BCE	2.34 ± 2.22	1.47 ± 1.64^{c}	2.33 ± 1.91^{b}	3.62 ± 2.55^{a}
Range				
Lutein	0.18–4.03	0.18–2.88	0.35–4.03	0.60–3.20
α-carotene	0.14–7.84	0.14–3.70	0.28–4.64	0.64–7.84
13-*cis*-BC	0.09–1.74	0.09–1.05	0.19–1.39	0.28–1.74
9-*cis*-BC	0.02–0.66	0.02–0.66	0.04–0.19	0.06–0.32
trans-BC	0.10–5.10	0.10–5.10	0.11–1.76	0.23–3.74
Total pVACs	0.37–12.68	0.37–8.97	0.69–7.99	1.35–12.68
TC	1.45–14.47	1.45–9.67	1.95–9.13	2.52–14.47
BCE	0.24–7.78	0.24–7.03	0.43–4.88	0.79–7.78

N: number of bunches sampled, 13-*cis*-BC: 13-*cis*-β-carotene, 9-*cis*-BC: 9-*cis*-β-carotene, *trans*-BC: *trans*-β-carotene, pVACS: provitamin A carotenoids, TC: total carotenoids, BCE: β-carotene equivalents SD standard deviation, *means with different letters within rows are significantly different at $P < 0.5$

the lowest (Fig. 1C & F). Top accessions for BCE content (μg/g) were 25447-S7 R3P4 (7.03), PITA 21 (4.88) and 33448–2 (7.78) in the diploid, triploid and tetraploid hybrid categories, respectively. Data for all genotypes in various hybrid ploidy categories are summarised in Supplementary Table 6.

The seemingly increasing trend of pVAC content with ploidy in hybrids could be attributed to the use of high pVAC plantains Bobby Tannap, Obino L'ewai and Mbi Egome as parents in crosses to generate tetraploid plantain hybrids. These three plantain accessions were incorporated in plantain breeding schemes owing to their ability to produce seeds from crosses with fertile diploids. Tetraploid hybrids were selected for high yield and sigatoka-resistance from primary crosses made with plantains and the sigatoka-resistant wild diploid *M. acuminata* diploid Calcutta 4 (Vuylsteke, Swennen, & Ortiz, 1993). Triploids were selected from subsequent crosses made from primary tetraploids and improved diploids (Tenkouano & Swennen, 2004). Among these, two triploids (PITA 21 and PITA 26) and two tetraploid plantain hybrids (PITA 1 and PITA 3) were identified with and BCE > 4 μg/g (Supplementary Table 6). Hybrids generally have low pVAC content because parents were not selected with a focus for pVAC enhancement. The fact that relatively high pVAC PITA hybrids were derived from crosses made with plantains highlights the possibility to develop strategies, which accumulate high pVAC alleles in the diploid background for breeding plantains that are more nutritious.

3.4. Carotenoid content and profile of the entire banana collection and prospects for biofortification

Table 4 summarizes the carotenoid composition in all banana genotypes screened in this study as well as the 3 genotype groups discussed in previous sections. Mean TC (μg/g) for all 189 banana genotypes analysed varied from 1.45 for hybrid 25447-S7 R2P8 to 36.21 for cultivar ITC.0601 Hung Tu with a mean of 8.00 μg/g indicating a high variability in carotenoids in bananas. The observed variation in TC is comparable to 0.00–34.56 μg/g with a mean of 5.17 μg/g FW observed by Davey et al. (2009) in 171 diverse banana cultivars. Amorim et al. (2009) also detected a wide variability in TC (1.6–19.24 μg/g) estimated by spectrophotometry in 42 diverse cultivars in Brazil. Similarly, a yellow-orange fruit pulp colour was characteristic of high carotenoid genotypes, while a cream-white fruit pulp colour was characteristic of low carotenoid genotypes (Fig. 1) as also observed by Amorim et al. (2009). These variations form the basis for future research strategies aimed at increasing the nutritional value of bananas through crossing and selection. Chávez et al. (2005) reported average TC contents of 2.46 μg/g ranging from 1.02 to 10.40 μg/g in 1786 genotypes of cassava obtained from diverse geographical locations. High pVAC genotypes were selected and crossed through several cycles leading to the release of biofortified cassava varieties (Ceballos et al., 2017).

Generally, the main carotenoids isolated in the banana collection were α-carotene, *trans*-BC, lutein, 13-*cis*-BC and 9-*cis*-BC (Table 4). The proportions of individual carotenoids in all bananas and respective banana groups analysed is shown in Fig. 2. About 78% of the carotenoids isolated in banana are pVACs: α-carotene and β-carotene (*cis* and *trans*-versions) with α-carotene dominating in most genotypes, while the rest (22%) is made up of the non-pVAC lutein. Noteworthy, plantains recorded the highest proportion (88%) of pVACs while hybrids had the lowest (67%). These results agree with previous studies on carotenoids in banana where the predominant carotenoids isolated were α-carotene and β-carotene with smaller amounts of lutein (2006, 2009; Davey et al., 2007; Ekesa et al., 2015; Englberger et al., 2003; Heng et al., 2017). The high proportion of pVACs in bananas is different from other carotenoid rich staples like maize where Pixley et al. (2013) reported a higher proportion (30–50%) of non-pVACs (lutein and zeaxanthin) and only 10–20% of pVACs (β-cryptoxanthin, β-carotene, α-carotene). Carvalho et al. (2016), screened cassava roots of 23 landraces in Amazonia and isolated β-carotene (26.13–76.72%) as the predominant pVAC. Although variable quantities of lutein was detected, α-carotenes was not detected in the observed cassava genotypes.

Conventional cross breeding programmes for well-known staples like maize and cassava have made significant breeding advancements on carotenoid enhancement following the identification of pVAC dense genetic resources (Ceballos et al., 2017; Manjeru, van Biljon, & Labuschagne, 2017; Pixley et al., 2013). Availability of Next Generation Sequencing Technologies and the existence of high density genotyping platforms have facilitated the development and incorporation of new breeding tools like marker assisted selection to increase breeding efficiency. Quantitative trait loci (QTL) mapping has been used to study the DNA regions related to carotenoid accumulation and identify candidate genes responsible for carotenoid regulation and genome-wide association studies (GWAS) has also been employed in combination with GBS to identify trait linked allelic variations valuable for marker assisted selection in both crops (Manjeru et al., 2017; Rabbi et al., 2017).

Table 4
Mean and range of carotenoids in 189 banana genotypes.

Trait	Carotenoid content (μg g^{-1} ± SD)*			
	All genotypes (n = 771)	Cultivars (n = 247)	Plantains (n = 270)	Hybrids (n = 254)
Mean				
Lutein	1.18 ± 1.07	1.01 ± 1.4[b]	1.24 ± 0.63[a]	1.29 ± 1.05[a]
α-carotene	3.50 ± 3.03	3.04 ± 3.54[b]	5.44 ± 2.29[a]	1.87 ± 1.79[c]
13-*cis*-BC	1.09 ± 0.90	0.90 ± 0.81[b]	1.67 ± 0.95[a]	0.66 ± 0.55[c]
9-*cis*-BC	0.20 ± 0.22	0.21 ± 0.31[b]	0.28 ± 0.16[a]	0.12 ± 0.13[c]
trans-BC	2.02 ± 2.09	1.87 ± 2.33[b]	3.11 ± 1.93[a]	1.02 ± 1.35[c]
Total pVACs	6.82 ± 5.38	6.03 ± 6.09[b]	10.5 ± 3.92[a]	3.67 ± 3.26[c]
TC	8.00 ± 5.56	7.05 ± 6.42[b]	11.74 ± 4.01[a]	4.96 ± 3.44[c]
BCE	4.42 ± 3.64	3.95 ± 4.11[b]	6.81 ± 2.80[a]	2.34 ± 2.22[c]
Range				
Lutein	0.13–5.91	0.13–5.91	0.45–2.33	0.18–4.03
α-carotene	0.14–16.51	0.26–16.51	0.88–8.19	0.14–7.84
13-*cis*-BC	0.09–4.43	0.18–2.79	0.46–4.43	0.09–1.74
9-*cis*-BC	0.02–1.50	0.03–1.50	0.08–0.67	0.02–0.66
trans-BC	0.10–11.81	0.10–11.81	0.23–6.46	0.10–5.10
Total pVACs	0.37–30.30	0.89–30.30	2.91–14-83	0.37–12.68
TC	1.45–36.21	1.56–36.21	5.12–15.98	1.45–14.47
BCE	0.24–21.06	0.57–21.06	2.01–10.65	0.24–7.78

N: number of bunches sampled, 13-*cis*-BC: 13-*cis*-β-carotene, 9-*cis*-BC: 9-*cis*-β-carotene, *trans*-BC: *trans*-β-carotene, pVACS: provitamin A carotenoids, TC: total carotenoids, BCE: β-carotene equivalents SD standard deviation, *means with different letters within rows are significantly different at P < 0.5

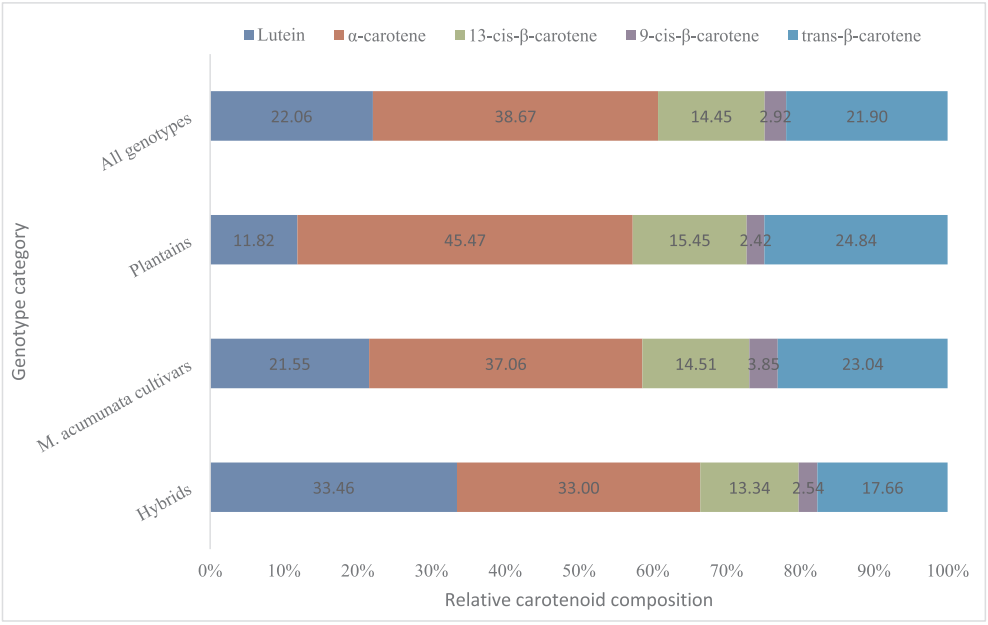

Fig. 2. Relative carotenoid composition (%) of 189 banana genotypes.

Until now, banana breeding has been a long and complex process relying on interploidy crosses and phenotypic selection. Breeding advancements for pVACs is so far limited to the report of the FHIA breeding programme on the development of biofortified plantains in 2009–2011, which resulted in the selection of two sigatoka-resistant high pVAC hybrids SH-4008 and SH 4037 (Aguilar-Morán, 2014). Marker assisted selection has not been effectively deployed for banana improvement, but genomic selection based on prediction models is currently being explored with high predictive values for fruit and bunch traits (Nyine et al., 2018). It is expected that application of new molecular techniques will speed up the breeding process and increase genetic gains for useful traits like pVAC content. Nonetheless, it should be noted that genetic gains in pVAC content should not compromise agronomic, productivity and other desired end-use traits to guarantee widespread farmer acceptance of biofortified bananas.

4. Conclusions

The enrichment of staple crops with essential micronutrients through biofortification is gaining importance as a sustainable means of addressing micronutrient deficiencies. A collection comprising of 189 banana genotypes was analysed for carotenoid content and profiles using HPLC. Total carotenoid content in the collection varied from 1.45 μg/g to 36.21 μg/g with a mean of 8.00 μg/g fresh weight. The main carotenoids were α-carotene (38.67%), *trans*-β-carotene (22.08%), lutein (22.08%), 13-*cis*-β-carotene (14.45%) and 9-*cis*-β-carotene (2.92%). Six French plantains (ITC.0112 Bobby Tannap, ITC.0519 Obubit Ntanga GM, ITC.1397 French Reversion Red Pseudostem, ITC.0496 Cantebalon, ITC.0109 Obino L'ewai, ITC.0325 Wine Plantain), four false-horn plantains (ITC.0208 Atali Kiogo, ITC.0630 Dominico Harton Rojo, ITC.0515 Okoyo Ukom, ITC.0223 Apantu) and one Horn plantain (ITC.0128 Tshambunu) were identified with high BCE (8.72–10.65 μg/g FW) and pVAC (14.28–15.38 μg/g). One IndonTriNg cultivar (ITC.0601 Hung Tu), four ssp. banksii cultivars (ITC.0894 Tainga, ITC.0809 Maleb, ITC.1187 Tomolo, ITC.0600 Waimara) and three PNG cultivars (ITC.0920 Dimaemamosi, ITC.0932 Gilasalasa, and ITC.0838 Bega) had high BCE content (8.72–21.06 μg/g) and TC content (13.95–36.21 μg/g). Top accessions for BCE content (μg/g) in the diploid, triploid and tetraploid hybrid categories, respectively, were 25447-S7 R3P4 (7.03), PITA 21 (4.88) and 33448-2 (7.78).

Identified high pVAC AAB-plantains, banksii spp. and hybrids will be relevant for initiatives seeking to promote banana cultivars and hybrids as a dietary source of provitamin A. Similarly, high pVAC *M. acuminata* diploid cultivars will be explored as parents for hybrid development and genetic analysis of pVAC accumulation in banana. However, the high variation detected within and among genotypes tested calls for further analysis with consistent replications in multiple locations over multiple years to elucidate heritable genetic variation which is stable and relevant for breeding.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2019.100024.

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