

# Pro-vitamin A carotenoid content of 48 plantain (*Musa AAB* genome) cultivars sourced from eastern Democratic Republic of Congo

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## Abstract

**BACKGROUND:** Vitamin A deficiency (VAD) is widespread in sub-Saharan Africa (SSA). Unlike in developed countries, where the main source of vitamin A comes from meat, the diet of poor populations in SSA is largely plant based. It is thus important to identify local / popular plants with higher vitamin A content for combating VAD. Banana (including plantains) is an important staple food crop in this region. The identification and promotion of vitamin A-rich banana cultivars could contribute significantly to the alleviation of VAD in areas heavily dependent on the crop. We assessed pro-vitamin A carotenoid (pVACs) content in the fruit pulp of 48 local plantains from eastern Democratic Republic of Congo, to identify cultivars that could help reduce VAD, especially among young children and women of reproductive age.

**RESULTS:** Mean pVACs content varied from 175–1756 µg/100 gfw in ripe fruits. Significant increases ( $P < 0.001$ ) in total pVACs content occurred after ripening in all cultivars except 'UCG II'. Retinol activity equivalents (RAE) in ripe fruits ranged from 12–113 µg/100 gfw. Fifteen plantain cultivars, including 'Adili II', 'Nzirabahima', 'Mayayi', 'Buembe', and 'Sanza Tatu' (associated with RAE values of 44 µg/100 gfw and above) can be considered as good sources of pVACs. Modest consumption (250 or 500 gfw) of the fruit pulp of the five best plantain cultivars at ripening stage 5 meets between 39–71% and 44–81% of vitamin A dietary reference intake (DRI) respectively, for children below 5 years old and women of reproductive age.

**CONCLUSION:** The 15 best plantain cultivars (especially the top 5) could potentially be introduced / promoted as alternative sources of pro-vitamin A in banana-dependent communities, and help to reduce cases of VAD substantially.

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**Keywords:** children below 5 years; dietary reference intake (DRI); pro vitamin A; retinol activity equivalents (RAE); vitamin A deficiency; women of child-bearing age

## INTRODUCTION

Vitamin A (retinol) and its derivatives (retinal and retinoic acid) are essential for cell differentiation, proliferation, and signaling, and they thus play an important role in vision, reproduction, maintenance of epithelial tissue, and immune functions.<sup>1–3</sup>

Vitamin A deficiency (VAD) is a major public health problem in many developing countries due to an over-reliance on starchy staples and processed foods that are typically deficient in vitamin A, with preschool children and pregnant women being the most vulnerable groups.<sup>4,5</sup> Vitamin A deficiency causes serious chronic diseases such as night blindness, keratomalacia (i.e. dryness with ulceration and perforation of the cornea), loss of vision, bronchopulmonary dysplasia, growth retardation, shortening and thickening of bones, atrophy of the testes, fetal reabsorption, immunodeficiency, and increased morbidity and mortality from infectious diseases.<sup>1–3,6,7</sup> It has been estimated that VAD affects over 190 million children below 5 years of age, especially in Africa and Southeast Asia.<sup>4,5</sup> Over the period 1995 to 2005, up to 1.2 million deaths among children aged 1 to 4 years worldwide were

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linked to VAD, amongst other factors.<sup>5,8</sup> In 2007, the prevalence of VAD in the Democratic Republic of Congo (DR Congo) and Burundi was established at 42.2% (25.2 out of 59.8 million) and 42.6% (3.38 out of 7.94 million) respectively, and has been classified as a 'severe public health problem' by the United Nations Standing Committee on Nutrition.<sup>9</sup>

Humans cannot synthesize vitamin A, and so rely on their diet for its supply, which can be from animal origins in the form of preformed vitamin A (retinol) and / or plant origin in the form of pro-vitamin A carotenoids (pVACs).<sup>10–12</sup> Preformed vitamin A from animal sources (retinol) is more easily absorbed and metabolized than the pVACs from plant foods, which have to be converted into retinol in the human body to be used.<sup>13–15</sup> But foods of animal origin are not always available for millions of people across the world.<sup>16</sup> White-fleshed roots and tubers (e.g. cassava, sweet potato, taro, and yam), banana, and rice dominate people's diets in most households in developing countries, and these are often low in pVACs. The bioavailability of pVACs from different plant sources also varies considerably.<sup>17,18</sup> Furthermore, the structure of pVACs is paramount; for instance, the retinol activity equivalent (RAE) obtained upon the intake of a single unit of all-*trans*  $\beta$ -carotene (t-BC) is twice that of all-*trans*  $\alpha$ -carotene.<sup>14</sup> It is thus important not only to identify and incorporate plant sources that have high levels of pVACs but also those that naturally contain a high proportion of t-BC.

Commonly known plant sources that are rich in pVACs include yellow and orange fruits such as mangoes and papayas, orange-fleshed sweet potatoes, and dark-green leafy vegetables.<sup>16,19</sup> However, consumption is often limited in resource-poor communities, due to the high perishability (coupled

to a lack of storage facilities), seasonality, limited accessibility, and high cost of the fruits and / or vegetables. For example, a 2007 survey carried out in North Kivu province, eastern DR Congo reported that only 55% of children aged 6 to 35 months had consumed foods rich in vitamin A in the previous 24 h, which is lower than the national average of 66%.<sup>20</sup>

Approaches that have been used to meet vitamin A dietary reference intake (DRI) include supplementation (i.e. administering concentrated doses of micronutrients to at-risk populations) and food fortification (i.e. adding micronutrients to processed food).<sup>21,22</sup> Although supplementation and food fortification were found to be effective in several studies,<sup>5</sup> both have generally proven difficult to implement in developing countries,<sup>23</sup> as they are mainly urban based and often fail to reach the rural communities most in need. These communities most often have a limited access to medical facilities and cannot afford fortified foods. With plant sources accounting for over 80% of vitamin A intake in low-income countries,<sup>10,24</sup> the promotion of a diverse diet that includes vitamin A-rich local food crops is now thought to be a more sustainable strategy. In the case of DR Congo, 58% of breastfed children 6–23 months of age, and 38% of those not breastfed, consume diets with three or less food groups, thus having low dietary diversity.<sup>20</sup> Similarly, a study in Butembo, North Kivu found that 48% of children aged 2–5 years had poorly diversified diets.<sup>25</sup>

In large parts of highland East and Central Africa, the food system is banana-based, with daily consumption levels of highland banana (AAA-EA) fruits in countries such as Uganda, Rwanda, and Burundi varying between 0.3 and 1.6 kg per person.<sup>26–29</sup> The carotenoid content in the fruit pulp of banana has been shown to vary between and within cultivars with geographical location, time of harvest, and ripening stage<sup>30–36</sup> (Table 1). The identification

**Table 1.** Range of pro-vitamin A content for various banana cultivars and genome groups, and other crops as reported in the literature. Data are reported as listed in the respective publications. For Mbabazi (2015),<sup>36</sup> values per gdw were converted into values per 100 gfw using moisture data

Reference	Crop/ banana cultivar (group)	Range of total pro-vitamin A content ( $\mu\text{g}/100\text{ gfw}$ )
Englberger <i>et al.</i> (2003) <sup>34</sup>	Micronesian bananas	515 to 6360 $\mu\text{g}/100\text{ gfw}$
Englberger <i>et al.</i> (2003) <sup>35</sup>	Southeast Asia banana cultivars	300 to 4960 $\mu\text{g}/100\text{ gfw}$
Englberger <i>et al.</i> (2006) <sup>37</sup>	Micronesian Fe'i cultivar 'Utimwas' (boiled)	9000 $\mu\text{g}/100\text{ gfw}$
	Boiled Pacific plantain (AAB) cultivar 'Mangat en Seipahn'	8207 $\mu\text{g}/100\text{ gfw}$
Davey <i>et al.</i> (2009) <sup>30,38</sup>	171 <i>Musa</i> cultivars covering essentially all genome groups	0 to 3457 $\mu\text{g}/100\text{ gfw}$
Ekesa <i>et al.</i> (2013) <sup>39</sup>	Selected cooking (2) and plantain (2) cultivars from eastern DR Congo	76–182 $\mu\text{g}/100\text{ gfw}$
Mbabazi (2015) <sup>36</sup>	Dessert bananas (Sukari Ndizi and Gros Michel)	6.3 to 14.4 $\mu\text{g}/\text{gdw}$ (~270 to 620 $\mu\text{g}/100\text{ gfw}$ )
	East African Highland banana (AAA-EA) cultivars	9.6 to 19.9 $\mu\text{g}/\text{gdw}$ (~413 to 856 $\mu\text{g}/100\text{ gfw}$ )
	Plantain cultivar (AAB) 'Gonja Nakatansese'	37 $\mu\text{g}/\text{gdw}$ (~1591 $\mu\text{g}/100\text{ gfw}$ )
	Transgenic 'Sukari Ndizi' with genes enhanced for pVACs production	12.6 to 17.3 $\mu\text{g}/\text{gdw}$ (~542 to 744 $\mu\text{g}/100\text{ gfw}$ )
Fungo <i>et al.</i> (2010) <sup>40</sup>	Various East African Highland Banana (AAA-EA) cultivars 'Sukari ndizi'	146.4–513.7 $\mu\text{g}/100\text{ gfw}$ 50.6 $\mu\text{g}/100\text{ gfw}$
Institute of Medicine (US) Panel on Micronutrients (2002) <sup>14</sup>	Carrots	11 427 to 14 693 $\mu\text{g}/100\text{ gfw}$
	Tomato	3454 $\mu\text{g}/100\text{ gfw}$
	Spinach	9890 $\mu\text{g}/100\text{ gfw}$
	Green cabbage	139 $\mu\text{g}/100\text{ gfw}$
Englberger <i>et al.</i> (2006) <sup>41,42</sup>	Nine Pandanus cultivars of the Kiribati republic	154 to 3602 $\mu\text{g}/100\text{ gfw}$
	13 Pandanus cultivars of the Republic of the Marshall Island	260 to 3130 $\mu\text{g}/100\text{ gfw}$
van Jaarsveld <i>et al.</i> (2005) <sup>43</sup>	Orange-fleshed sweet potato	8329–10 699 $\mu\text{g}/100\text{ gfw}$
Welsch <i>et al.</i> (2010) <sup>44</sup>	White-fleshed cassava	60 to 150 $\mu\text{g}/100\text{ gfw}$
Tan (1989) <sup>45</sup>	Unrefined/crude palm oil	RAE 15 times higher than carrots, and 300 times that of tomatoes

and promotion of vitamin A-rich cultivars is considered a feasible approach to help alleviate VAD. The widespread promotion of vitamin A-rich banana (including plantain) cultivars has, for example, been reported to lead potentially to a reduction of 9.6–17.1% in the burden of illnesses due to VAD in three African countries: Ghana, Rwanda, and Uganda.<sup>30</sup>

In eastern DR Congo, plantain (AAB genome) and green cooking banana (AAA genome, East African highland banana – EAHB) have been reported as the second most important source of calories, after cassava.<sup>39</sup> Recent studies have reported plantains and EAHB to be potential sources of pVACs.<sup>32,38,39,46,47</sup> However, the above studies on pVACs in banana have focused on a limited number of EAHB and plantain cultivars. Screening of a wider range of plantain cultivars was deemed necessary to identify those cultivars that could meet or exceed the DRIs of vitamin A for susceptible / vulnerable groups. This study therefore assessed, through C<sub>30</sub>-RP-HPLC analysis, the pVACs content at ripening stages 1 and 5 for 48 plantain cultivars grown in different parts of the North Kivu and Ituri provinces of eastern DR Congo, which is also part of a secondary center of banana diversity.

## MATERIALS AND METHODS

### Study region and plantain cultivars

The eastern part of the DR Congo covers a wide variety of agro-ecologies that support a wide diversity of bananas, including plantains.<sup>48</sup> It is characterized by two rainy seasons (mid-August to mid-January and mid-February to mid-July) and two short dry seasons (mid-January to mid-February and mid-July to mid-August), and a mean precipitation varying between 1000 and 3170 mm. Altitude ranges from 700 to 5119 m above sea level (a.s.l.) (Mt Rwenzori top), and mean annual temperature varies from as low as 15 °C (high altitude) to as high as 31 °C (low altitude).<sup>28,49,50</sup> This region also has a high variability in its soils, including volcanic soils, alluvial soils, naturally weathered deep and humus-rich rock soils.<sup>28</sup>

This study was conducted on 48 different local plantain cultivars (AAB), previously collected through a *Musa* germplasm survey covering three districts in North Kivu province and five in Ituri province of eastern DR Congo (Table 2,<sup>51</sup>). It is estimated that the plantains used in this study represent about 30% of the known plantain diversity across DR Congo. Table 2 provides details of the cultivar types and their relative abundance at the sites of origin.

### Field experimental design and data collection

Field experiments were established in September 2010 at the Catholic University of Graben's (UCG) regional *Musa* germplasm collection at Butembo (1815 m a.s.l., 00.11786° N, 29.2587° E) in North Kivu province, for assessment of plantain cultivar yield and pro-vitamin A content. Butembo has sandy clay soils with pH 5.7, 5.1% organic carbon (OC), 0.26% nitrogen, 10.6 mg kg<sup>-1</sup> of available phosphorus and 1.14 cmol(+) kg<sup>-1</sup> of potassium.<sup>51</sup> The site had a mean annual rainfall of 1036 mm and a mean annual temperature of 19 °C.

For each of the 48 plantain cultivars, 15 plants were grown in three replications of five plants each, at a spacing of 2 m × 3 m. Banana production was entirely rain fed. No fertilizers and chemicals (for pest or disease control) were applied, and the fields were regularly mulched with dry grass.

The 48 plantain cultivars were observed during the period 2010–2015, over two cropping cycles, i.e. the plant and first ratoon

crop. Data collected for this study included bunch weight (kg) and the time to harvest (days). Time to harvest for the plant and first ratoon crop is, respectively, time from planting to harvest and time from harvest of the plant crop to harvest of the first ratoon crop.

Average annual yield (tons/ha/year) was based upon the two crop cycles and was calculated from bunch weight and the time to harvest using the formula described by Gaidashova *et al.* (2008)<sup>52</sup> as below: Average annual yield = [(bunch weight (kg)/number of days to harvest] × 365 × 1666.7/1000; where 365 = number of days in a year; 1666.7 = plant density/ha, and 1000 = conversion factor from kg to tons.

### Sample collection and preparation

The samples for pVACs analysis were collected from the Butembo field collection. Here, three physiologically mature disease-free plant crop bunches (when fruits were deep green, full, and rounded; i.e. ripening stage 1<sup>53</sup>) were selected per cultivar and harvested over a period spread out between 2012 and 2013. Two middle hands (second and third hand from the top of the bunch) per bunch were subsequently sampled (giving a total of six hands per cultivar) and used for carotenoid analysis. The fresh fruit samples were packed in perforated cardboard boxes and transported under ambient temperature (20 °C) to the National Agricultural Research Laboratory, Kawanda, Kampala, Uganda, within 24 h of harvest for further processing prior to analysis. Fruit ripeness was assessed based on peel color as described by Dadzie and Orchard (1997)<sup>53</sup> and Stover and Simmonds (1987):<sup>54</sup> 1 = green; 2 = green with trace of yellow; 3 = more green than yellow; 4 = more yellow than green; 5 = only green tips remaining; 6 = all yellow; 7 = yellow flecked with brown. In this study, fruits were sampled for pVACs assessment at ripening stages 1 (unripe) and 5 (ripe) as they reflect the extreme range of plantain pulp consumption. Plantains are mainly consumed (fried or boiled) at ripening stage 5. Nevertheless, unripe plantain is also often fried or boiled. At ripening stage 1, three fingers were randomly detached from each of the six banana hands per cultivar, cleaned, hand peeled, and cut into pieces. Fruit pulp pieces from the two hands collected from the same bunch were combined into a sub-sample, resulting in three subsamples per cultivar. Each sub-sample was placed in a labeled, zip-locked bag from which air was manually removed, and frozen at –80 °C. The remaining fingers on the six selected hands per cultivar were left to ripen naturally in a well aerated room. Sub-sampling of fruit pulp at ripening stage 5 was done using the same procedure applied during stage 1. Once both stages were sampled, samples were weighed and lyophilized for 72 h, after which they were re-weighed, re-packaged in labeled zip-locked bags, and kept frozen at –80 °C until transport to Belgium for nutrient analyses. Half of each sub-sample was transported on dry ice, using a courier service, to the Laboratory of Fruit Breeding and Biotechnology, Department of Bio-systems at the Catholic University of Leuven (KULEuven), Belgium for pVACs analysis.

### Extraction and analysis of carotenoids

Carotenoid extraction and analysis were carried out in triplicate according to procedures developed specifically for the analysis of banana tissues as described in Ekesa *et al.* (2013b).<sup>39</sup> One hundred milligram aliquots of powdered lyophilized fruit pulp were homogenized for 30 s at maximum speed in a 'Fast Prep' reciprocal shaker in 400 mL of ice-cooled extraction solvent (Thomas Scientific Inc., Swedesboro, New Jersey, USA). The extraction solvents consisted of tetrahydrofuran : methanol (THF : MeOH), 1:1 (v/v), containing 0.25% butylated hydroxytoluene (BHT) and 2% insoluble

**Table 2.** Plantain cultivars assessed for agronomic performance and pro-vitamin A carotenoid content in eastern Democratic Republic of Congo

Cultivar name	Type <sup>a</sup>	Site of origin	Relative abundance <sup>b</sup>	Citation <sup>c</sup>
Adili I	F	Ituri	*	A
Adili II	F	Ituri	*	A
Agbindolo	F	Ituri	**	A
Akange	F	Ituri	*	A
Akobanzi	FH	Ituri	*	A
Akoto membo	FH	Ituri	*	A
Akoto monama	FH	Ituri	*	A
Alongo	FH	Ituri	*	A
Apakumo	FH	Ituri	***	A
Ayaya	F	Ituri	*	A
Bakpulu	FH	Ituri	*	A
Bubu	FH	Ituri	*	A
Buembe	F	North Kivu	*	A, B
Kingulungulu	FH	North Kivu	*	A, B
Kirisirya plantain	F	North Kivu	*	A, B
Kothina	FH	North Kivu	**	A, B
Kothina I	FH	North Kivu	**	A
Kothina II	FH	North Kivu	**	A
Kothina III	FH	North Kivu	*	A
Mabilanga	FH	Ituri	*	A
Makaka	H	Ituri	*	A
Makpelekese	FH	Ituri	*	A
Mangondi	FH	Ituri	***	A
Mangondi I	FH	Ituri	*	A
Mangondi II	FH	Ituri	*	A
Manzenzele	F	Ituri	*	A
Mayayi	F	Ituri	*	A
Musilongo	FH	North Kivu	**	A, B
Ndonge	F	Ituri	*	A
Ngobia II-1	F	Ituri	*	A
Ngobia makelekele	FH	Ituri	*	A, B
Nguma	F	North Kivu	***	A, B
Nguma II	F	North Kivu	**	A
Nguma III	F	North Kivu	***	A
Nzirabahima	F	North Kivu	*	A, B
Plantain masunga	F	North Kivu	*	A
Plantain grand format I	FH	North Kivu	*	A
Plantain grand format II	FH	North Kivu	*	A
Sanza moya	H	North Kivu/Ituri	*	A, B
Sanza tatu	H	North Kivu	*	A, B
UCG II	FH	Ituri	*	A
UCG III	FH	North Kivu	*	A
UCG IV	F	North Kivu	*	A
UCG VIII	F	Ituri	*	A
Vuhembe	F	North Kivu	*	A, B
Vuhetera	F	North Kivu	*	A, B
Vuhindi	F	North Kivu/Ituri	**	A, B
Vulambya	F	North Kivu	*	A, B

<sup>a</sup> French (F), False Horn (FH) and Horn (H).

<sup>b</sup> '\*' less abundant and '\*\*\*' = highly abundant at site of origin.

<sup>c</sup> A and B, respectively, denote Sivirihauma, *et al.* (2017)<sup>51</sup> and Ocimati *et al.* (2016).<sup>48</sup>

polyvinylpyrrolidone (PVPP) to complex flavonoids and other secondary metabolites that can interfere with analyses.<sup>31,55</sup> Following centrifugation (16,873 xg for 20 min at 48 °C), the supernatant was transferred to a fresh micro-centrifuge tube (Heathrow Scientific LLC, Vernon Hills, Illinois, USA) and the

pellet was re-extracted twice with 400 mL of THF : MeOH, 1:1 (v/v), containing 0.25% BHT without PVPP. Supernatants were collected and combined, and 8-apo  $\beta$ -carotenol at a final concentration of 0.002 mg/mL was added as an internal standard. The combined supernatants were analyzed directly by reversed phase – high

performance liquid chromatography (RP-HPLC) using a Waters Alliance, 2690 Separations System fitted with an auto-sampler, thermostat at 88 °C, a pulse dampener and a 996 UV-visible photodiode array detector (Waters, Milford, MA, USA). The entire system was controlled and the data were collected and integrated using the Millennium 4.0 software package. A 150 mm × 4.6 mm, YMC C<sub>30</sub> 3 µm particle size HPLC column (Achrom, Zulte, Belgium) was employed, using a 24 min linear gradient from 2 to 50% *tert*-butyl methyl ether (t-BME) in MeOH at 1.0 mL/min, followed by a 1 min linear gradient to 95% t-BME in MeOH, which was maintained for 3 min to elute the remaining compounds. The column was then re-equilibrated for 5 min under starting conditions of 2% t-BME in MeOH, before the next injection. Peaks were quantified at 450 nm using a freshly prepared standard curve of all-*trans*-β-carotene and 8-*apo*-carotenal in extraction solvent, and identified on the basis of their characteristic absorption spectra and retention times relative to known standards.<sup>39,55</sup>

### Pro-vitamin A carotenoids data conversions

Results from the pVACs analysis were obtained as nmol/g dry weight (gdw) and converted to nmol/gfw [fresh matter weight = dry matter weight/ (100/ (100-moisture %))] and finally to µg/100 gfw using the conversion factor of 0.01863.<sup>56</sup> Fresh matter weight was measured after peeling the fruit, and dry matter weight was measured after lyophilization. To determine the relative vitamin A nutritional content of samples, total pVACs content were first converted to all-*trans*-β-carotene equivalents (t-BCEs) by adding up all the specific carotenoids, which included all-*trans*-β-carotene (t-BC), all-*trans*-α-carotene (t-AC) and their *cis*-isomers (c-BC, c-AC). As t-AC has half of the retinol activity of t-BC, and c-BC and c-AC each have 0.53 times the retinol activity of t-BC, the following formula [t-BCE = 0.5 t-AC + t-BC + 0.53 (c-BC + c-AC)] was used.<sup>55,57,58</sup>

On average, the consumption of 12 µg of t-BC from fruits and vegetables yields 1 µg of retinol (Institute of Medicine, 2001), therefore, t-BCE values were converted into retinol activity equivalents (RAE) assuming that 1/12th of the total t-BCEs ingested are taken up by the body.<sup>59</sup>

The DRIs of vitamin A for children below 5 years old and of women of reproductive age (15–49 years) equal 400 µg RAE and 700 µg RAE, respectively.<sup>60</sup> The RAE values (in µg/100 gfw) of the studied plantain fruits were compared to the above daily vitamin A DRIs when varying amounts of fruit pulp were consumed. Consumption of 250 g (approximately 1–2 fingers) of cooked plantain fruit by a child below 5 years and 500 g (approximately 3–4 fingers) by a woman of reproductive age has been reported to be within normal consumption levels in banana growing and consuming regions of Africa.<sup>61</sup> However, the above amounts may not always be attained across the various banana-based production systems in Africa. Therefore, DRI values were also calculated for smaller fruit pulp quantities, varying between 100–250 g for children below 5 years and 100–500 g for women of reproductive age. Taking into account the exceptionally high fruit pulp consumption levels of adults in the east and central African highland regions, higher DRI values can also be derived from values calculated for 100, 250, and 500 g of fruit pulp for women of reproductive age. The percentage DRI for the various cultivars and fruit ripening stages and for different fruit pulp quantities consumed was then computed as: (RAE of cultivar [µg/X gfw] × 100)/DRI, with DRI = 400 µg RAE for children below 5 years and 700 µg RAE for women of reproductive age.

### Statistical analysis

The analysis of variance (ANOVA) for yield (tons/ha/year), individual pVACs, total pVACs content, total t-BCEs, RAE, and DRIs for children below 5 years and women of reproductive age across the 48 plantain cultivars and the two ripening stages were determined using GenStat v. 12 statistical software.<sup>62</sup> The Least Significant Difference (LSD) function in GenStat v.12 was used to compare means at 5% significance level. In addition, GenStat v. 12 was also used to carry out regression analysis between on one hand plant growth cycle duration and bunch weights with pVACs content on the other hand.

## RESULTS AND DISCUSSION

### Provitamin A carotenoids content and RAE of plantains

The pVACs identified in the plantain cultivars under study were t-BC, t-AC, *cis*-AC and *cis*-BC (Table 3, Figs. 1 and 2). Trace amounts of all-*trans*-lutein were also recorded although this compound has no vitamin A activity. Similar pVACs profiles were reported for different banana cultivars by Davey *et al.* (2009)<sup>30,31,38</sup> and Ekesa *et al.* (2013, 2015).<sup>33,39,47</sup> At ripening stage 1, the plantain cultivars had a similar pVACs profile containing mainly t-BC with lower levels of t-AC, except 'Akange', which contained only t-BC, 'Kirisirya Plantain' (primarily lutein) and 'Plantain Grand Format', which consisted primarily of t-AC (Fig. 1). At ripening stage 5, the plantain cultivars had a similar pVACs profile with higher proportions of t-BC (47–70%) followed by t-AC (23–43%) and only trace amounts of *cis*-BC, *cis*-AC, and lutein (Fig. 2). This is similar to results from Davey *et al.* (2009)<sup>30,38</sup> and Ekesa *et al.* (2015)<sup>33</sup> who reported a larger proportion of t-BC (61 to 69%) compared to t-AC (30–38%) in some plantain cultivars; t-AC has only 50% of the RAE of t-BC and thus the relative proportions of t-AC and t-BC affect the overall vitamin A value in foods.<sup>57,58</sup> The proportion of t-BC relative to other pVACs is therefore a good indicator of the quality of vitamin A supplied by a given food. Studies on other crops such as maize and wheat have reported a much lower t-BC proportion of 10% to 20% of the total carotenoid content.<sup>38</sup> Thus the quality of the pVACs in the 48 plantains is good compared to some other plant pVACs sources.

A high variability ( $P < 0.001$ ) in the total pVACs content was observed amongst the 48 plantain cultivars and between the two ripening stages. The mean total pVACs content varied from 4 µg/100 gfw in 'Akange' to 548 µg/100 gfw in 'UCG II' at ripening stage 1, and from 175 µg/100 gfw in 'Akobanzi' to 1756 µg/100 gfw in 'Adili II' at stage 5 (Table 3). Wide variations in pVACs content were also reported for Asian cultivars (300 to 6360 µg/100 gfw) within and across banana genomic groups and for African cultivars<sup>30,34,35,38</sup> (Table 1).

Significant differences ( $P < 0.001$ ) were observed between the various cultivars and the two ripening stages (Table 3). Except for 'UCG II' in which the total pVACs decreased from 548 to 263 µg/100 gfw with ripening, a (predominantly significant) increase in the t-BC, t-AC, total pVACs, and t-BCE levels from ripening stage 1 to 5 was observed for all the cultivars (Table 3). For example, the largest increments in fruit pulp mean total pVACs of 118, 75, 72, 47, 45 and 45-fold were observed for 'Mabilanga', 'Akoto Membo', 'Ngobia Mukelekele', 'Adili II', 'Mangondi' and 'Mangondi I', and 'Akange', respectively (Table 3). Similar increases in pVACs from ripening stage 1 to 5 in different banana cultivars were observed by Ekesa *et al.* (2013, 2015)<sup>33,39,47</sup> and Newilah *et al.* (2008),<sup>63</sup> which are attributed to the enhanced carotenogenesis

**Table 3.** Mean content of individual and total pro-vitamin A carotenoids (pVACs) and all-*trans*- $\beta$ -carotene equivalents (t-BCE) in  $\mu\text{g}/100$  gfw in fruit pulp of 48 plantain (AAB) cultivars at ripening stages 1 and 5. Values are means of three individual samples per ripening stage

Cultivar name	Ripening stage	t-AC	t-BC	<i>Cis</i> -AC	<i>Cis</i> -BC	Lutein	Total-pVACs	t-BCE
Adili I	1	109.2	183.6	7.3	0.0	7.7	300.1	242.1
	5	283.1	311.4	15.9	0.0	48.9	610.4	461.4
Adili II	1	16.5	20.9	0.0	0.0	0.0	37.4	29.2
	5	740.5	958.3	9.9	47.4	152.1	1756.1	1358.9
Agbindolo	1	71.4	162.7	1.2	5.7	2.6	241.0	202.0
	5	162.9	336.3	26.7	17.8	23.4	543.7	441.4
Akange	1	0.0	4.0	0.0	0.0	0.0	4.0	4.0
	5	46.9	127.3	7.4	0.0	8.7	181.6	154.7
Akobanzi	1	4.8	7.6	0.0	0.0	0.0	12.4	10.0
	5	47.2	118.1	9.2	0.0	11.6	174.5	146.5
Akoto membo	1	1.5	10.2	0.0	0.0	0.0	11.7	10.2
	5	334.8	502.7	42.2	0.0	24.9	879.7	692.5
Akoto monama	1	24.9	30.3	0.0	0.0	2.3	55.2	42.8
	5	272.5	389.7	14.7	0.0	10.9	676.9	533.8
Alongo	1	8.9	15.4	0.0	0.0	0.0	24.3	19.9
	5	154.3	360.9	17.1	0.0	14.0	532.3	447.1
Apakumo	1	41.6	108.2	0.0	0.0	0.0	149.8	129.0
	5	124.0	289.7	0.0	12.4	0.0	426.1	358.2
Ayaya	1	8.3	20.1	0.0	0.0	0.0	28.4	24.3
	5	163.5	266.1	12.2	0.0	21.0	441.8	354.3
Bakpulu	1	10.4	26.3	0.0	0.0	0.0	36.7	31.5
	5	93.0	238.2	20.5	0.0	12.9	351.7	295.6
Bubu	1	17.5	47.1	0.0	0.0	0.0	64.6	55.8
	5	238.5	509.3	0.0	37.7	24.7	785.5	648.5
Buembe	1	31.2	80.4	0.0	2.6	0.0	114.2	97.4
	5	311.7	617.5	0.0	38.0	30.0	967.2	793.5
Kingulungulu	1	123.8	223.1	0.0	6.5	0.0	353.4	288.4
	5	261.8	468.6	11.8	7.2	0.0	749.4	609.6
Kirisirya plantain	1	1.2	2.2	0.0	0.0	59.7	3.4	2.7
	5	133.2	242.7	20.0	0.0	32.1	395.9	319.9
Kothina	1	19.2	42.7	0.0	0.0	0.0	61.9	52.3
	5	226.3	368.8	24.7	0.0	23.4	619.8	495.1
Kothina I	1	13.9	29.1	0.0	0.0	0.0	43.0	36.1
	5	204.1	453.1	22.9	0.0	9.7	680.1	567.3
Kothina II	1	3.4	9.5	0.0	0.0	0.0	12.9	11.2
	5	102.9	255.6	22.7	0.0	15.3	381.2	319.1
Kothina III	1	4.2	14.3	0.0	0.0	0.0	18.5	16.4
	5	195.4	436.8	33.1	0.0	33.5	665.3	552.0
Mabilanga	1	2.4	4.7	0.0	0.0	0.0	7.1	5.4
	5	236.6	448.2	23.0	0.0	37.2	707.8	578.7
Makaka	1	25.5	64.8	3.2	0.0	12.1	93.5	79.2
	5	67.1	176.8	15.4	0.0	20.3	259.3	218.5
Makpelekese	1	6.1	13.7	0.0	0.0	0.0	19.8	16.8
	5	141.6	307.0	18.5	0.0	41.3	467.1	387.6
Mangondi	1	4.5	7.8	0.0	0.0	0.0	12.3	10.0
	5	200.3	336.7	18.8	0.0	43.6	555.8	446.8
Mangondi I	1	4.5	7.8	0.0	0.0	0.0	12.3	10.0
	5	200.3	336.7	18.8	0.0	43.6	555.8	446.8
Mangondi II	1	11.6	28.3	0.0	0.0	0.0	39.9	34.1
	5	229.2	353.2	15.9	0.0	39.8	598.3	476.2
Manzenzele	1	6.9	11.0	0.0	0.0	3.7	17.9	14.5
	5	103.1	187.7	13.3	0.0	22.8	304.1	246.3
Mayayi	1	11.3	23.6	0.0	0.0	0.0	34.9	29.3
	5	328.8	631.0	43.7	0.0	44.7	1003.5	818.5
Musilongo	1	4.1	11.1	0.0	0.0	0.0	15.2	12.4
	5	145.6	308.2	18.3	0.0	23.9	472.1	390.7

**Table 3.** Continued

Cultivar name	Ripening stage	t-AC	t-BC	<i>Cis</i> -AC	<i>Cis</i> -BC	Lutein	Total-pVACs	t-BCE
Ndonge	1	4.6	8.3	0.0	0.0	0.0	12.9	10.5
	5	163.7	336.8	21.4	0.0	21.9	521.9	430.0
Ngobia II-1	1	47.2	122.6	0.0	23.0	3.2	192.8	147.4
	5	98.9	235.5	18.0	28.6	19.3	381.0	309.7
Ngobia mukelekele	1	4.0	5.0	0.0	0.0	0.0	9.0	7.0
	5	249.5	376.3	18.5	0.0	47.5	644.3	510.8
Nguma	1	34.4	88.4	0.0	2.8	0.0	125.6	107.1
	5	267.5	529.4	0.0	32.4	26.0	829.3	680.3
Nguma II	1	26.9	59.3	2.7	0.0	16.0	88.9	74.2
	5	164.1	344.3	22.1	0.0	40.3	530.5	438.1
Nguma III	1	12.6	31.4	0.0	0.0	0.0	44.0	37.7
	5	184.0	338.9	22.1	0.0	29.3	545.0	442.6
Nzirabahima	1	23.4	30.3	1.0	0.0	1.9	54.7	42.5
	5	521.6	645.3	33.2	0.0	21.5	1200.1	923.7
Plantain masunga	1	25.6	51.0	0.0	0.0	0.0	76.6	63.9
	5	234.6	494.1	10.5	13.0	13.0	752.2	623.9
Plantain grand format I	1	71.9	2.9	0.0	0.0	0.0	74.8	38.8
	5	94.9	165.1	10.6	0.0	28.0	270.6	218.0
Plantain grand format II	1	33.1	72.9	4.9	0.0	3.6	110.9	92.1
	5	103.0	232.0	20.8	0.0	20.2	355.8	294.5
Sanza moya	1	10.9	37.8	0.0	0.0	0.0	48.7	28.1
	5	113.0	331.6	8.1	23.9	3.9	476.6	405.1
Sanza tatu	1	24.8	34.1	0.0	0.0	0.0	58.9	46.5
	5	363.2	552.2	13.0	0.0	10.1	928.4	740.7
UCG II	1	183.2	351.4	13.6	0.0	18.2	548.2	450.2
	5	91.1	162.6	8.8	0.0	17.3	262.5	212.8
UCG III	1	8.1	9.8	0.0	0.0	0.0	17.9	213.8
	5	253.4	367.4	29.6	0.0	42.4	650.4	509.8
UCG IV	1	13.6	20.8	0.0	0.0	0.0	34.4	27.6
	5	207.4	407.3	0.0	0.0	9.4	614.7	511.0
UCG VIII	1	11.9	26.5	0.0	0.0	0.0	38.4	32.4
	5	99.2	213.3	19.9	0.0	20.7	332.4	273.4
Vuhembe	1	43.5	82.6	0.0	0.0	4.4	126.1	104.4
	5	147.8	285.0	16.2	0.0	34.2	449.0	367.5
Vuhetera	1	17.3	46.5	0.0	0.0	0.0	63.8	55.1
	5	234.1	499.8	0.0	37.0	24.3	770.9	636.4
Vuhindi	1	2.7	4.0	0.0	0.0	0.0	6.7	5.3
	5	91.1	163.2	12.6	0.0	17.6	266.9	215.4
Vulambya	1	40.9	106.7	0.0	0.0	0.0	147.6	127.2
	5	174.0	418.3	0.0	20.3	0.0	612.6	516.1
LSD (5%)		64.8	112.5	7.7	6.5	27.4	187.9	147.4
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

t-AC, all-*trans*-a-carotene; t-BC, all-*trans*-b-carotene; *cis*-AC, *cis*-a-carotene; *cis*-BC, *cis*-b-carotene.

that commonly occurs during maturation or ripening of fruits and vegetables.<sup>12,61,63</sup>

At ripening stage 5, 29 plantain cultivars (i.e. 60% of the studied cultivars) had a mean total pVACs content higher than 500 µg/100 gfw (i.e. RAE of >32 µg/100 gfw) while three cultivars had a mean total pVACs value above 1000 µg/100 gfw, with the highest value of 1756 µg/100 gfw recorded in the cultivar 'Adili II' (Tables 3 and 4). The quantities of pVACs measured in these DR Congolese plantains (ranging from 175 to 1756 µg/100 gfw, with BCE values between 147 and 1359 µg/100 gfw) are higher than those reported for East African highland and dessert banana cultivars,<sup>36,40</sup> while they are comparable to most Pacific

(*Musa* Iholena and Maoli-Pöpō'ulu subgroups) and other African plantains<sup>34–39,46,64</sup>; cf. Tables 1 and 3). These values are, however, significantly lower than, for example, the highest measured pVACs content in carrots (11 427 to 14 693 µg/100 gfw), tomatoes (3454 µg/100 gfw), spinach (9890 µg/100 gfw), orange-fleshed sweet potato (8329–10 699 µg/100 gfw), the Micronesian Fe'i *Musa* spp. cultivar 'Utimwas' (9000 µg/100 gfw), and the Pacific plantain (AAB) cultivar 'Mangat en Seipahn' (8207 µg/100 gfw) (Table 1).

The RAE values of banana cultivars studied at ripening stage 1 and 5 ranged from 0.3 to 37.5 µg/100 gfw and 12 to 113 µg/100 gfw, respectively (Table 4). Englberger *et al.*

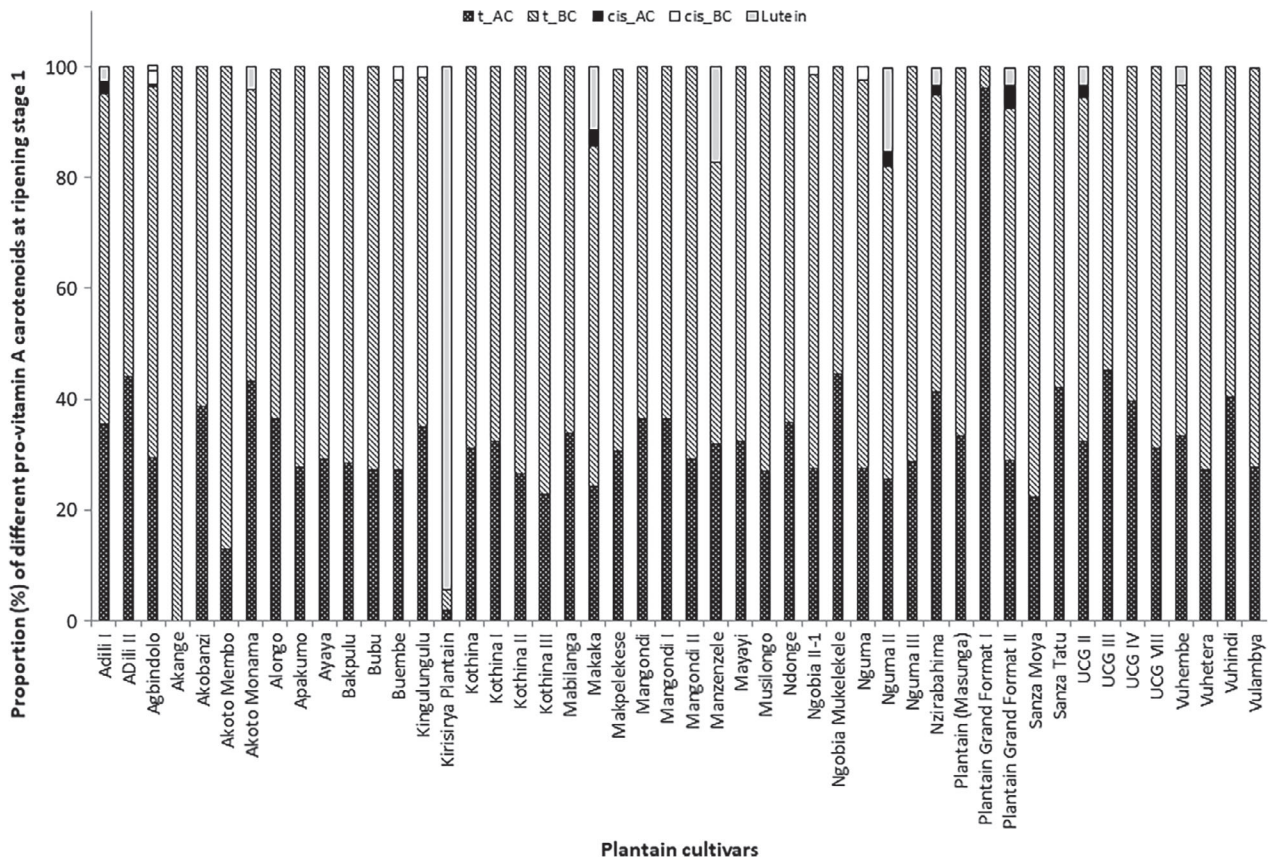


Figure 1. Proportion (%) of different pro-vitamin A carotenoids at ripening stage 1 for the assessed plantain landraces.

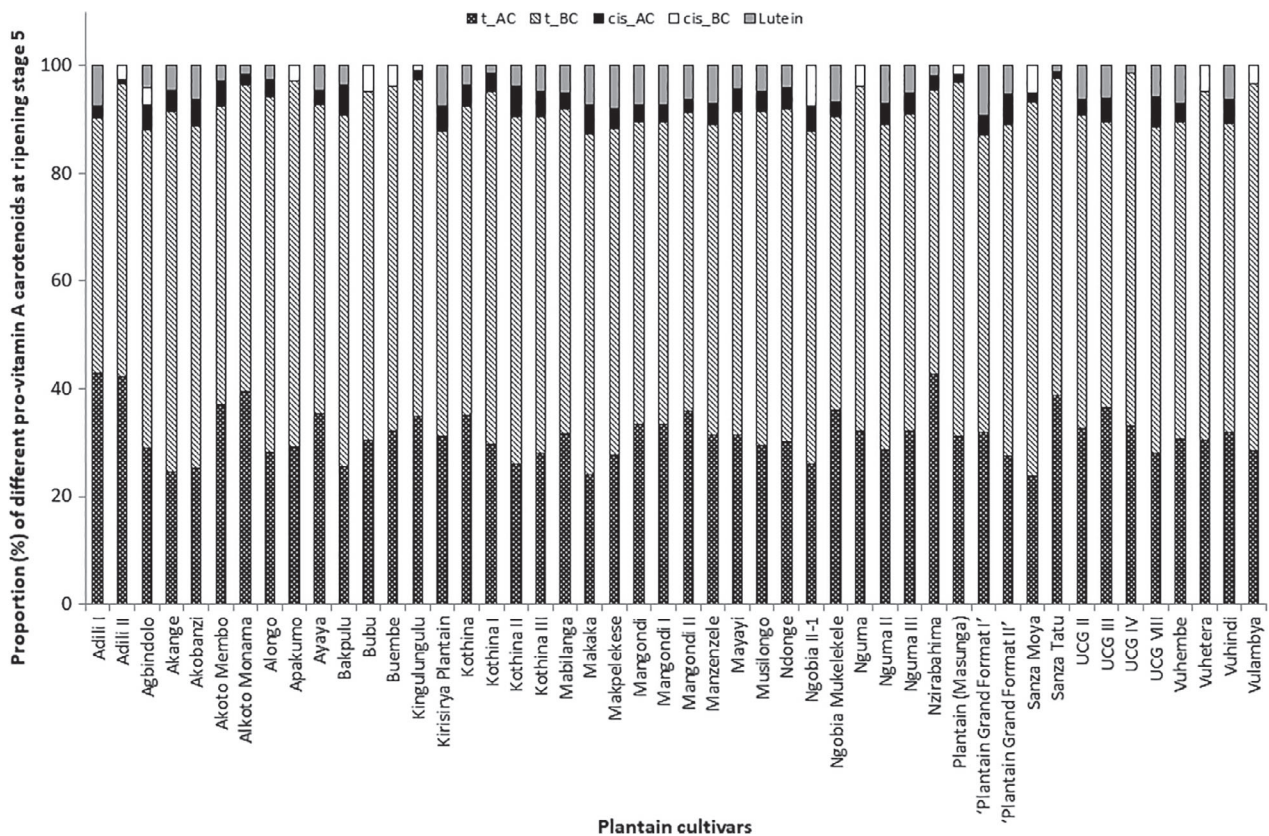


Figure 2. Proportion (%) of different pro-vitamin A carotenoids at ripening stage 5 for the assessed plantain landraces.

**Table 4.** Mean fruit pulp retinol activity equivalent (RAE) and the percentage contribution of the pVACs in 48 plantain (AAB) cultivars at ripening stage 1 and 5 toward the dietary reference intakes (DRIs) for children (1–5 years) and women of reproductive age

Cultivar name	Ripening stage	RAE $\mu\text{g}/100\text{gfw}$	% DRI Child (<5 years)		%DRI woman		
			Mean in 100 g of fruit pulp	Mean in 250 g of fruit pulp	Mean in 100 g of fruit pulp	Mean in 250 g of fruit pulp	Mean in 500 g of fruit pulp
Adili I	1	20.2	5.0	12.6	2.9	7.2	14.4
	5	38.5	9.6	24.0	5.5	13.7	27.5
Adili II	1	2.4	0.6	1.5	0.4	0.9	1.7
	5	113.2	28.3	70.8	16.2	40.4	80.9
Agbindolo	1	16.8	4.2	10.5	2.4	6.0	12.0
	5	36.8	9.2	23.0	5.3	13.1	26.3
Akange	1	0.3	0.1	0.2	0.1	0.1	0.2
	5	12.9	3.2	8.1	1.8	4.6	9.2
Akobanzi	1	0.8	0.2	0.5	0.1	0.3	0.6
	5	12.2	3.1	7.6	1.7	4.4	8.7
Akoto membo	1	0.9	0.2	0.5	0.1	0.3	0.6
	5	57.7	14.4	36.1	8.2	20.6	41.2
Akoto monama	1	3.6	0.9	2.2	0.5	1.3	2.6
	5	44.5	11.1	27.8	6.4	15.9	31.8
Alongo	1	1.7	0.4	1.0	0.2	0.6	1.2
	5	37.3	9.3	23.3	5.3	13.3	26.6
Apakumo	1	10.8	2.7	6.7	1.5	3.8	7.7
	5	29.9	7.5	18.7	4.3	10.7	21.3
Ayaya	1	2.0	0.5	1.3	0.3	0.7	1.5
	5	29.5	7.4	18.5	4.2	10.5	21.1
Bakpulu	1	2.6	0.7	1.6	0.4	0.9	1.9
	5	24.6	6.2	15.4	3.5	8.8	17.6
Bubu	1	4.7	1.2	2.9	0.7	1.7	3.3
	5	54.0	13.5	33.8	7.7	19.3	38.6
Buembe	1	8.1	2.0	5.1	1.2	2.9	5.8
	5	66.1	16.5	41.3	9.5	23.6	47.2
Kingulungulu	1	24.0	6.0	15.0	3.4	8.6	17.2
	5	50.8	12.7	31.8	7.3	18.1	36.3
Kirisiriyi plantain	1	2.3	0.1	0.1	0.0	0.1	0.2
	5	26.7	6.7	16.7	3.8	9.5	19.0
Kothina	1	4.4	1.1	2.7	0.6	1.6	3.1
	5	41.3	10.3	25.8	5.9	14.7	29.5
Kothina I	1	3.0	0.8	1.9	0.4	1.1	2.2
	5	47.3	11.8	29.5	6.8	16.9	33.8
Kothina II	1	0.9	0.2	0.6	0.1	0.3	0.7
	5	26.6	6.7	16.6	3.8	9.5	19.0
Kothina III	1	1.4	0.3	0.9	0.2	0.5	1.0
	5	46.0	11.5	28.8	6.6	16.4	32.9
Mabilanga	1	0.5	0.1	0.3	0.1	0.2	0.3
	5	48.2	12.1	30.1	6.9	17.2	34.5
Makaka	1	6.6	1.7	4.1	0.9	2.4	4.7
	5	18.2	4.6	11.4	0.3	6.5	13.0
Makpelekese	1	1.4	0.4	0.9	0.2	0.5	1.0
	5	32.3	8.1	20.2	4.6	11.5	23.1
Mangondi	1	0.8	0.2	0.5	0.1	0.3	0.6
	5	37.2	9.3	23.3	5.3	13.3	26.6
Mangondi I	1	0.8	0.2	0.5	0.1	0.3	0.6
	5	37.2	9.3	23.3	5.3	13.3	26.6
Mangondi II	1	2.8	0.7	1.8	0.4	1.0	2.0
	5	39.7	9.9	24.8	5.7	14.2	28.4
Manzenzele	1	1.2	0.3	0.8	0.2	0.4	0.9
	5	20.5	5.1	12.8	2.9	7.3	14.7
Mayayi	1	2.4	0.6	1.5	0.4	0.9	1.7
	5	68.2	17.1	42.6	9.7	24.4	48.7

**Table 4.** Continued

Cultivar name	Ripening stage	RAE µg/100gfw	% DRI Child (<5 years)		%DRI woman		
			Mean in 100 g of fruit pulp	Mean in 250 g of fruit pulp	Mean in 100 g of fruit pulp	Mean in 250 g of fruit pulp	Mean in 500 g of fruit pulp
Musilongo	1	1.0	0.3	0.7	0.2	0.4	0.7
	5	32.6	8.1	20.4	4.7	11.6	23.3
Ndonge	1	0.9	0.2	0.6	0.1	0.3	0.6
	5	35.8	9.0	22.4	5.1	12.8	25.6
Ngobia II-1	1	12.3	3.1	7.7	1.8	4.4	8.8
	5	25.8	6.5	16.1	3.7	9.2	18.4
Ngobia mukelekele	1	0.6	0.2	0.4	0.1	0.2	0.4
	5	42.6	10.6	26.6	6.1	15.2	30.4
Nguma	1	8.9	2.2	5.6	1.3	3.2	6.4
	5	56.7	14.2	35.4	8.1	20.2	40.5
Nguma II	1	6.2	1.6	3.9	0.9	2.2	4.4
	5	36.5	9.1	22.8	5.2	13.0	26.1
Nguma III	1	3.1	0.8	2.0	0.5	1.1	2.2
	5	36.9	9.2	23.1	5.3	13.2	26.4
Nzirabahima	1	3.5	0.9	2.2	0.5	1.3	2.5
	5	77.0	19.2	48.1	11.0	27.5	55.0
Plantain masunga	1	5.3	1.3	3.3	0.8	1.9	3.8
	5	52.0	13.0	32.5	7.4	18.6	37.1
Plantain grand format I	1	3.2	0.8	2.0	0.5	1.2	2.3
	5	18.2	4.5	11.4	2.6	6.5	13.0
Plantain grand format II	1	7.7	1.9	4.8	11.0	2.7	5.5
	5	24.5	6.1	15.3	3.5	8.8	17.5
Sanza moya	1	2.3	0.6	1.5	0.3	0.8	1.7
	5	33.8	8.4	21.1	4.8	12.1	24.1
Sanza tatu	1	3.9	1.0	2.4	0.5	1.4	2.8
	5	61.7	15.4	38.6	8.8	22.0	44.1
UCG II	1	37.5	9.4	23.5	5.4	13.4	26.8
	5	17.7	4.4	11.1	2.5	6.3	12.7
UCG III	1	1.2	0.3	0.7	0.2	0.4	0.8
	5	42.5	10.6	26.6	6.1	15.2	30.4
UCG IV	1	2.3	0.6	1.4	0.3	0.8	1.6
	5	42.6	10.7	26.6	6.1	15.2	30.4
UCG VIII	1	2.7	0.7	1.7	0.4	1.0	1.9
	5	22.8	5.7	14.2	3.3	8.1	16.3
Vuhembe	1	8.7	2.2	5.4	1.2	3.1	6.2
	5	30.6	7.7	19.1	4.4	10.9	21.9
Vuhetera	1	4.6	1.2	2.9	0.7	1.6	3.3
	5	53.0	13.3	33.2	7.6	18.9	37.9
Vuhindi	1	0.4	0.1	0.3	0.1	0.2	0.3
	5	18.0	4.5	11.2	2.6	6.4	12.8
Vulambya	1	10.6	2.7	6.6	1.5	3.8	7.6
	5	43.0	10.8	26.9	6.1	15.4	30.7
Lsd (5%)		12.3	3.1	7.7	1.8	4.4	8.8
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are means of three individual samples per ripening stage per cultivar.

The Dietary Reference Intakes (DRIs) of vitamin A for children 1–5 years old and women of reproductive age is 400 µg RAE and 700 µg RAE, respectively (FAO/WHO, 2002).<sup>60</sup>

(2006)<sup>37</sup> reported RAE values of 130 µg/100 gfw for the Fe'i cultivar 'Asupina', 79 µg/100 gfw for a horn plantain, and 49 µg/100 gfw for a Pacific plantain. Englberger *et al.* (2006)<sup>64</sup> reported that *Musa* cultivars with RAE values higher than 44 µg/100 gfw could be considered as excellent sources of pVACS. The RAE values recorded in 'Adili II' (113 µg/100 gfw)

and 'Nzirabahima' (77 µg/100 gfw) exceeded 70 at stage 5, whereas three additional cultivars, 'Mayayi', 'Buembe' and 'Sanza Tatu' had RAE values between 60 and 70 at stage 5, and can thus be considered as highly promising cultivars. An additional ten cultivars had RAE values of 44 µg/100 gfw and above and could also be considered as promising (Table 4).

**Table 5.** The ranking of 48 plantain cultivars for fruit mean total pro-vitamin A carotenoid content (pVACs) and their respective mean time to harvest, bunch weight and yields

Cultivar name	Rank for total pVACs	Mean time to harvest (days)	Bunch weight (kg)	Yield (t/ha/y)
Adili II	1	500.8 <sup>ghi</sup>	24.1 <sup>nopq</sup>	24.5 <sup>ijklm</sup>
Nzirabahima	2	396.0 <sup>b</sup>	22.0 <sup>ijkl</sup>	30.7 <sup>rs</sup>
Mayayi	3	595.0 <sup>p</sup>	22.3 <sup>ijklm</sup>	22.2 <sup>fgh</sup>
Buembe	4	566.2 <sup>no</sup>	20.1 <sup>efgh</sup>	19.6 <sup>de</sup>
Sanza tatu	5	504.3 <sup>hij</sup>	16.7 <sup>b</sup>	17.3 <sup>cd</sup>
Akoto membo	6	532.5 <sup>klm</sup>	26.6 <sup>s</sup>	26.4 <sup>mnp</sup>
Nguma	7	509.9 <sup>ijkl</sup>	28.3 <sup>t</sup>	27.4 <sup>nopq</sup>
Bubu	8	554.5 <sup>mn</sup>	25.5 <sup>qrs</sup>	24.4 <sup>ijklm</sup>
Vuhetera	9	501.3 <sup>ghi</sup>	21.0 <sup>fghij</sup>	22.2 <sup>fgh</sup>
Plantain masunga	10	588.7 <sup>op</sup>	22.3 <sup>ijklm</sup>	20.0 <sup>ef</sup>
Kingulungulu	11	—	—	—
Mabilanga	12	466.3 <sup>def</sup>	25.9 <sup>rs</sup>	29.5 <sup>qrs</sup>
Kothina III	13	465.7 <sup>def</sup>	21.9 <sup>ijkl</sup>	23.1 <sup>ghijk</sup>
UCG III	14	432.3 <sup>c</sup>	21.4 <sup>ghijk</sup>	26.5 <sup>mnp</sup>
Ngobia mukelekele	15	541.8 <sup>mn</sup>	19.9 <sup>efg</sup>	20.2 <sup>ef</sup>
Kothina I	16	478.5 <sup>efgh</sup>	21.9 <sup>ijkl</sup>	23.3 <sup>ghijk</sup>
Akoto monama	17	528.2 <sup>ijklm</sup>	12.8 <sup>a</sup>	13.5 <sup>ab</sup>
Adili I	18	505.7 <sup>hijk</sup>	22.5 <sup>klmn</sup>	22.2 <sup>fgh</sup>
Kothina	19	451.0 <sup>cde</sup>	18.2 <sup>bcd</sup>	21.0 <sup>efg</sup>
Mangondi II	20	—	—	—
UCG IV	21	349.3 <sup>a</sup>	22.8 <sup>klmn</sup>	35.4 <sup>t</sup>
Vulambya	22	387.2 <sup>b</sup>	20.0 <sup>efg</sup>	29.6 <sup>qrs</sup>
Mangondi	23	533.1 <sup>klm</sup>	24.8 <sup>opqr</sup>	24.2 <sup>ijklm</sup>
Mangondi I	24	—	—	—
Nguma III	25	539.2 <sup>mn</sup>	26.5 <sup>s</sup>	25.0 <sup>ijklm</sup>
Nguma II	26	474.8 <sup>efg</sup>	26.5 <sup>s</sup>	28.4 <sup>pqr</sup>
Agbindolo	27	600.5 <sup>p</sup>	17.4 <sup>bc</sup>	16.2 <sup>c</sup>
Alongo	28	593.9 <sup>op</sup>	23.8 <sup>mnp</sup>	21.2 <sup>efgh</sup>
Ndonge	29	548.8 <sup>mn</sup>	18.8 <sup>cde</sup>	16.5 <sup>c</sup>
Makpelekele	30	593.5 <sup>op</sup>	20.9 <sup>fghij</sup>	20.0 <sup>ef</sup>
Musilongo	31	478.2 <sup>efgh</sup>	24.1 <sup>nopq</sup>	27.9 <sup>opq</sup>
Vuhembe	32	397.4 <sup>b</sup>	23.2 <sup>lmno</sup>	31.2 <sup>s</sup>
Sanza moya	33	549.8 <sup>mn</sup>	13.0 <sup>a</sup>	11.8 <sup>a</sup>
Ayaya	34	435.8 <sup>c</sup>	21.2 <sup>ghijk</sup>	25.1 <sup>klmn</sup>
Kirisirya plantain	35	—	—	—
Apakumo	36	—	—	—
Kothina II	37	472.2 <sup>ef</sup>	19.4 <sup>def</sup>	21.1 <sup>efg</sup>
Ngobia II-1	38	—	—	—
Plantain grand format II	39	—	—	—
Bakpulu	40	—	—	—
UCG VIII	41	—	—	—
Manzenzele	42	531.1 <sup>ijklm</sup>	21.7 <sup>hijkl</sup>	23.5 <sup>hijkl</sup>
Plantain grand format I	43	643.1 <sup>q</sup>	32.7 <sup>u</sup>	25.8 <sup>lmno</sup>
Vuhindi	44	440.3 <sup>cd</sup>	20.0 <sup>efg</sup>	22.7 <sup>ghij</sup>
UCG II	45	541.5 <sup>mn</sup>	25.1 <sup>pqrs</sup>	27.9 <sup>opq</sup>
Makaka	46	530.4 <sup>klm</sup>	16.4 <sup>b</sup>	16.2 <sup>c</sup>
Akange	47	687.7 <sup>r</sup>	17.2 <sup>bc</sup>	14.2 <sup>bc</sup>
Akobanzi	48	534.8 <sup>lm</sup>	20.6 <sup>fghi</sup>	22.3 <sup>fghi</sup>
<b>Lsd (5%)</b>		27.9	1.6	2.3
<b>P value</b>		<0.001	<0.001	<0.001
<b>Cv %</b>		6.0	8.3	12.0

\*\*\*\* denote a significant difference between the cultivars within a yield attribute at  $P < 0.001$ .

Means in the column followed by the same letter are not significantly different at 5% least significant difference (Lsd).

Dash (—) denotes that yield data was not collected for the particular cultivar.

Daily consumption of 250 g of unprocessed fruit pulp of the cultivars under study at ripening stage 5 meets between 7.6% and 71.8% of the DRI for vitamin A of children below 5 years old (Table 4). Consumption of 500 g of fruit pulp meets between 8.7% and 81% of the DRI for vitamin A of women of reproductive age. The current results show that none of the plantain cultivars can meet 100% of the DRI for vitamin A of the target group when normal amounts of pulp are consumed (Table 4). However, in the east and central African highland region, daily consumption levels of above 500 g have also been reported.<sup>27,29</sup> Slight increments in consumption of some of the plantain cultivars above the normal consumption levels (i.e. 250 g in children below 5 years and 500 g in women of reproductive age) would profoundly increase the percentage of the DRI of vitamin A met. For example, for 'Adili II', a daily consumption of 618 g translates to 100% of the DRI for vitamin A in women of reproductive age.

In this study, the pVACs values used to estimate contribution to the DRIs are of raw plantains. Plantains in North Kivu, however, are mainly consumed boiled or fried / roasted.<sup>47</sup> It has been established that preparation methods affect the amount and bio-accessibility of pVACs.<sup>32,43,65,66</sup> For example, Ekesa *et al.* (2013)<sup>39,47</sup> observed total pVACs retention levels of 40–90% and >95% in boiled EAHB and plantains, respectively, and 100% retention when plantains were deep-fried in fully refined palm oil or crude red palm oil for 2 min. Deep frying with fully refined palm oil and crude red palm oil (with an extremely high pVACs content; see also Table 1) enhanced the BCE and RAE of plantain-based dishes by twofold and sixfold, respectively.<sup>33</sup> Promoting appropriate and healthy fruit processing methods could increase the RAE values of plantain-based dishes to meet the DRI requirements under normal consumption levels for both children below 5 years and women of reproductive age. Ekesa *et al.* (2015)<sup>33</sup> also observed the bio-accessibility of pVACs in boiled bananas and plantains to vary with the cultivar and the type of pVACs. These authors noted a significantly more efficient micellarization of t-BC in the boiled EAHB 'Vulumbya' (28.9%) than in boiled plantain 'Musilongo' (16.6%) and similar bio-accessibility levels for t-BC (17–29%) and t-AC (16–31%). The actual quantity of carotenoids available in these plantains after preparation and consumption therefore still needs to be determined.

#### Yield of the plantain cultivars and their relative abundance

Significant differences ( $P < 0.001$ ) were observed in the mean time to harvest (days), bunch weight (kg) and annual yield (t/ha/year) of the plantain cultivars computed over two crop cycles (i.e. the plant crop and first ratoon crop) (Table 5). Mean time to harvest for the plantain cultivars varied between 349 and 688 days. It is important to note that the production / cultivation site (Butembo) is at a high altitude of 1815 m a.s.l. and has a low mean annual temperature (19 °C). Flowering and harvesting of banana (including plantains) is strongly associated with altitude and temperature.<sup>67,68</sup> However, altitude effects on pVACs content have not yet been studied.

Englberger *et al.* (2006)<sup>37</sup> reported the Fe'i cultivar 'Utin lap', which has the highest pVACs content measured to date, grows very slowly, and as such, a high pVACs content has been postulated to be associated with slow plant growth. Similarly, Swennen and Wilson (1983)<sup>69</sup> reported that most plantains characterized by orange fruit pulp, and thus likely higher pVACs content, have long intervals between crop cycles. The long cycle duration is attributed to a shortage of gibberellins, which is associated with a high apical dominance and corresponding slow sucker growth. However, in this study, regression analysis did not find a strong association

between mean plant growth cycle duration and pVACs content ( $R^2 = 0.05$ ).

Bunch weights varied between 12.8 kg in 'Akoto monama' and 33 kg in 'Plantain Grand Format I'; 82% of the cultivars had acceptable bunch weights of 20 kg and above (Table 5). However, no strong association ( $R^2 = 0.05$ ) was observed between the bunch weights and the total pVACs content across all the cultivars. The mean annual yield of the 48 plantain cultivars varied between 12 t/ha/year in 'Sanza Moya' and 35 t/ha/year in 'UCG IV', with 82% having yields above 20 t/ha/year (Table 5). The best three cultivars in terms of pVACs concentration, namely 'Adili II', 'Nzirabahima' and 'Mayayi', recorded annual yields of 25, 31, and 22 t/ha/year, respectively. 'Nzirabahima', the second best cultivar in terms of pVACs content, ranked the third in terms of bunch yield. 'Nguma I' and 'Akoto Nembo', which had moderately high pVACs content, also rated among the cultivars with the best yields. In contrast, the top two performers in terms of bunch yield, 'UCG IV' and 'Vuhembe', respectively, ranked only 21st and 32nd in terms of pVACs (Table 5).

It is important to note that the three cultivars with the highest pVACs content are not widely distributed or grown in the study region (i.e. eastern DR Congo) (Table 2). This could partially explain the fact that this region still experiences high levels of VAD, especially among children below 5 years and women of reproductive age, despite the presence of such plantain cultivars. There is a need to understand the reasons for the low adoption or growth of these cultivars, and to promote their cultivation and consumption within households deliberately. The most important selection criteria influencing farmers' choices of plantain cultivars to adopt and or retain on farm in the central African region (Burundi, eastern DR Congo and Rwanda) include the taste and flavor of the pulp, size of the bunch, crop-cycle duration, and market demand.<sup>48,70</sup> Plantain cultivars with small bunch sizes have a lower market value and are more likely to be phased out / rejected by farmers irrespective of the nutritional value, whereas cultivars with average to large bunches are favored by farmers for their high marketability. The observed bunch weights and annual yields, especially for the cultivars with the highest levels of pVACs, are moderately high and would thus positively contribute to scaling-out efforts. For example, 'Nzirabahima' and 'Nguma', which respectively ranked second and seventh in vitamin A content, are expected to be highly marketable due to their large bunch sizes and taste. The orientation towards taking such cultivars with large bunches to markets could, however, potentially be an undoing in the fight against VAD, for the poor rural households. Sensitization will also be necessary to change such attitudes and to enhance household consumption of vitamin A-rich plantains.

## CONCLUSION

In this study, 15 plantain cultivars, including 'Adili II', 'Nzirabahima', 'Mayayi', 'Buembe', and 'Sanza Tatu' (associated with RAE values above 44 µg/100 gfw) can be considered as good sources of pVACs. Modest consumption (250 or 500 gfw) of the fruit pulp of the five best plantain cultivars at ripening stage 5 meets between 39–71% and 44–81% of vitamin A DRI respectively, for children below 5 years old and women of reproductive age. The 15 best plantain cultivars (especially the top five) could potentially be introduced / promoted as alternative sources of pro-vitamin A in banana-dependent communities. Given the cultural importance of plantains in diets in the region studied, targeting them in the fight against VAD offers an additional sustainable alternative to

complement existing pVACs-rich crops such as carrots and sweet potato. It is important to note that the best plantain cultivars for pVACs content are currently not widely cultivated and thus need to be actively promoted. Despite the fact that the amount of pVACs in fruit pulp did not have a strong association with crop-cycle duration and yield (in tons/ha/year), most of the plantain cultivars including the best five in term of pVACs content had moderate to large bunch sizes and yields (tons/ha/year). Bunch size and yield are critical criteria used by farmers in selecting *Musa* cultivars to grow and retain on farms, and could thus be helpful in promoting the scaling out of these cultivars. Additional studies are needed to further understand the factors responsible for the current often limited geographical spread and potential issues / drivers linked to their introduction / promotion, e.g. pest and disease resistance and culinary / organoleptic characteristics.

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