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Validation of Rapid (Colour-Based) Prescreening Techniques for Analysis of Fruit Provitamin A Contents in Banana (*Musa* spp.)

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Abstract

Banana and plantain (*Musa* spp.) fruits have been shown to be a potentially rich source of provitamin A carotenoids (pVACs) and can thus play a key role in reducing vitamin A deficiency in developing countries. Recently, the screening of over 170 *Musa* genotypes indicated that there is substantial genetic diversity in the pVACs contents of banana and plantain fruit pulp. Additional screening of the more than 6000 accessions maintained in over 60 field genebanks worldwide is highly desirable, but detailed pVACs analysis by spectrophotometry and reversed phase-high performance liquid chromatography (RP-HPLC) is both time consuming and expensive, due to the need for specialised equipment and technical expertise. The aim of this work was to validate alternative colour-based prescreening techniques for measuring fruit pVACs contents. The prescreening techniques used showed that pulps with white-cream coloration contained low levels of carotenoids, while those with more orange colour had levels of carotenoids ranging from low to high. The regression models tested indicated a positive correlation between *Musa* pulp colour and carotenoids content (significant at $P < 0.001$), especially for the lower colour scores. The correlation was stronger for the yellowness index measured by colorimetry than for the DSM colour chart scores. It is expected that these results will allow researchers to reduce the costs and time required for pVACs analysis by prescreening field germplasm collections by colour-based methods and selecting only the most interesting accessions for precision analysis.

INTRODUCTION

Vitamin A deficiency (VAD) is a global public health problem and is a major cause of blindness and mortality among children and women, particularly in Africa and Southeast Asia (Sommer et al., 1996; World Health Organization, 1992). Efforts to decrease VAD have focused on the production and promotion of vitamin A-rich foods, including eggs and orange-yellow fruits and vegetables such as papaya, pumpkin, cassava, carrot and sweet potato (Englberger et al., 2003; McLaren et al., 2001). Recently, the screening of over 170 *Musa* genotypes has indicated that bananas and plantains are another potentially rich source of provitamin A carotenoids (pVACs) which are converted into vitamin A in the human body (Davey et al., 2009a). While the fruit pVACs content of export banana cultivars of the

Cavendish type is low, traditional orange-fleshed cultivars seem in general to have higher carotenoids levels (Amorim et al., 2009; Davey et al., 2009a; Englberger, 2003). Further screening of more than 6000 *Musa* accessions maintained in over 60 field genebanks worldwide is highly desirable, but detailed pVACs analysis by spectrophotometry and high performance liquid chromatography (HPLC) is both time consuming and expensive, since it requires specialised equipment and technical expertise. The development of a rapid prescreening method based on fruit colour would facilitate the large-scale screening of germplasm collections as it uses relatively inexpensive tools and does not require a high level of technical expertise. It could therefore be used to prescreen entire field germplasm collections and select only the most interesting accessions for full pVACs analysis. The aim here was thus to assess the suitability of using fruit pulp colour evaluation as a prescreening method for pVACs contents in a wide variety of *Musa* cultivars.

MATERIALS AND METHODS

Sampling Procedure, Colour Coding and Carotenoids Analysis

A total of 439 fruit samples from 353 different cultivars were used in this study. All fruit samples were obtained from the germplasm collection maintained by the *Centre Africain de Recherches sur Bananiers et Plantains* (CARBAP) in Njombé, Cameroon (4º35'N, 9º39'E, 80 meters above sea level). Fruits were collected from the centre of hands situated at the top (proximal), middle and bottom (distal) end of each bunch, and where possible from 2-3 bunches harvested at optimal physiological maturity (appearance of a ripe fruit on the proximal hand).

After harvest, fruits were weighed and sliced lengthwise. Pulp colour was then assessed using the DSM Colour Chart for Egg Yolk Colour, which is a linear colour chart that ranges from 1 (light yellow) to 15 (dark orange). Fruit pulp colour was also analysed by colorimetry. The HunterLab colorimeter (Minolta CR-14) used measures three colour coordinates named 'L' (white-black), 'a' (green-red) and 'b' (blue-yellow). However, in this study, only the 'b' parameter was evaluated which is a quantitative measure of blue to yellow colour, with a negative value for blue and a positive value for yellow (yellowness index). Subsequently, individual carotenoids species were analysed by reversed phase-high performance liquid chromatography (RP-HPLC) as described by Davey et al. (2006, 2009a).

Statistical Analysis

Linear and multiple regressions, using the Curve Estimation procedure ($P < 0.05$) of the SPSS software package version 16.0, were carried out to identify correlations between pulp colour (measured by colour chart and colorimetry) and the content of total and individual carotenoids as measured by RP-HPLC. SPSS Curve Estimation automatically excludes from the analysis all cases with missing values for one or more of the variables (SPSS User's Guide, 2007). While the individual carotenoids contribute to the degree of orange colour of the fruit, the objective of this study was to determine the predictive value of the pulp colour for pVACs content. Colour codes and colorimetric measurements were therefore treated as independent variables and total and individual carotenoid contents as dependent variables. The coefficient of determination (R^2) was used to determine the best-fit correlation between observed values and model predictions. R^2 ranges from 0 to 1, where a value of 0 indicates the model has no predictive capability and a value of 1 means

the model has perfect predictive capability, i.e. that the regression line perfectly fits the data.

RESULTS AND DISCUSSION

Colour Assessment and Total Carotenoids Content

Regression models indicated a positive relation between DSM value and total carotenoids content (TCC), and between yellowness index and TCC (Fig. 1A, 1B), indicating as expected that TCC increases with increasing orange colour of the fruit pulp. For the DSM value, coefficients of determination of 0.313 for the linear regression and 0.428 for the power regression indicate that from 31% to 43% of the variation within the data could be explained by the regression models (significant at $P < 0.001$) (Table 1). For the yellowness index, coefficients of determination for the linear regression and the power regression were 0.391 and 0.415, respectively (significant at $P < 0.001$) (Table 1).

From Fig. 1A and 1B, it can further be seen that genotypes with a whiter pulp colour (lower DSM value or yellowness index) all had relatively low TCC values. However, the more orange genotypes (higher DSM value or yellowness index) had TCC values ranging from almost 0 to >100 nmol/gdw.

These findings broadly agree with data of Chávez et al. (2006), who determined a strong and positive association ($R^2 = 0.769$, $P < 0.01$) between colour intensity (visual colour) and TCC in cassava roots, but also found a better correlation for the lower colour ranges.

Colour Assessment and Individual Carotenoids Content

In a next step, the correlation between pulp colour and individual carotenoids content as determined by RP-HPLC was investigated. Fig. 2A shows the correlations between the DSM value and the mean all-*trans* α-carotene (t-AC), all-*trans* β-carotene, (t-BC), 13-*cis* β-carotene (c-BC) and lutein contents. Fig. 2B shows the same correlations with the yellowness index, determined colorimetrically. As for TCC, there seems to be a general increase in individual carotenoids content with increasing orange colour of the fruit pulp, except for lutein which tends to first increase and then decrease with increasing orange colour.

For the DSM value, the linear regression model had a coefficient of determination of 0.285 for t-AC content, 0.318 for t-BC content and 0.130 for c-BC content (significant at $P < 0.001$). No significant correlation was found for lutein. For the yellowness index, the linear regression model had a coefficient of determination of 0.374 for t-AC content and 0.409 for t-BC content (significant at $P < 0.001$). No significant correlation was found for c-BC and lutein contents.

These results indicate that the orange colour intensity of pulps appears to be primarily determined by t-AC and t-BC rather than by c-BC or lutein. This contrasts with the findings of Burgos et al. (2009), who reported a significant and negative correlation between yellow flesh colour and β-carotene concentration in tubers of potato accessions (*Solanum phureja*) ($R = -0.77$, $P < 0.001$). They found, nevertheless, a significant positive correlation between yellow pulp colour and the total carotenoids content and with two other carotenoid species, antheraxanthin and zeaxanthin, which are not found in *Musa* fruit pulp in any significant concentration ($R = 0.93$, 0.81 and 0.82, respectively, $P < 0.001$).

Colour Assessment and Vitamin A Activity

Ultimately, the nutritional value of a cultivar is determined by its net vitamin A activity, and not all pVACs have the same vitamin A potential. The relative vitamin A activity of c-BC has been estimated to be 53% of t-BC (Schieber and Carle, 2005), while that of t-AC is only 50% of the activity of t-BC (Fraser and Bramley, 2004; Trumbo et al., 2003). Lutein has no vitamin A activity. The vitamin A activity of ingested t-BC has been determined to be approximately 50% of that of retinol itself, meaning that 1 µg retinol is equivalent to 2 µg of t-BC. More recent data suggest that 6 µg of dietary t-BC is required to have the same effectiveness as 1 µg of purified t-BC. Therefore, an overall conversion factor of 12:1 is used to calculate the vitamin A activity of ingested t-BC (Yeum and Russell, 2002). The net vitamin A nutritional value or retinol activity equivalent (RAE) of banana fruit was thus calculated as: RAE = 1/12 of the total t-BC equivalents, with t-BC equivalent = 0.5 t-AC + t-BC + 0.53 c-BC.

For the correlation between DSM value and t-BC equivalents, coefficients of determination ranged from 0.318 (linear regression) to 0.423 (power regression) at significance levels of $P < 0.001$ (Fig. 3A). For the yellowness index, these coefficients varied from 0.400 (linear regression) to 0.432 (power regression), again at a significance level of $P < 0.001$ (Fig. 3B).

Englberger et al. (2006), while studying Fe'i bananas, also found a significant positive correlation ($R^2 = 0.43$, $P < 0.05$) between fruit RAE values and the colorimetric yellowness index (HunterLab colour coordinates). While in our study only the yellowness colorimetric parameter was analysed, Englberger et al. (2006) further assessed the redness index of pulps and reported a high significant linear relationship $Y = 0.056X + 0.66$ (where Y = colour and X = RAE) with a R^2 of 0.84 and $P < 0.001$. The authors concluded that in Fe'i bananas, an increase in RAE is highly correlated with increased redness of the fruit pulp.

Application of Colour-Based PreScreening in a Vitamin A Screening Programme

According to the HarvestPlus Challenge Program, target dietary micronutrient levels are expressed based on the 'Population Weighted Estimated Average Requirements' (EAR). The EAR is an approximation of the median of the distribution of nutrient requirements for individuals of the target population, and as such provides the most valid, single-point comparison for the estimates of the probable contribution of a food to the overall nutrient needs of a population (Tarasuk, 2006).

The EAR for vitamin A has been set at 250 µg RAE/day for children (Davey et al., 2009a). Assuming an average daily banana consumption of 250 gfw/day and assuming an average water content of 70%, banana accessions with an RAE of at least 0.501 µg/gfw (0.93 nmol/gfw) or 1.67 µg/gdw (3.10 nmol/gdw) can provide 50% of the daily vitamin A requirements (i.e. 125 µg RAE/day) for children. For processed banana products, potential losses during processing also have to be taken into account. From Fig. 4A and 4B, it can be seen that all accessions with an RAE of at least 1.67 µg/gdw have a DSM value of 7 or more, and a yellowness index of 62 or more.

CONCLUSION

Detailed analysis of individual pVACs by RP-HPLC provides accurate information on the concentration of individual carotenoids profile in fruit of *Musa* cultivars. However,

RP-HPLC is both time-consuming and expensive, requiring both specialised equipment and technical expertise.

In this study, pulp colour (measured by visual scoring and by colorimetry) and carotenoids content in banana fruit were positively correlated. As expected, this correlation was stronger for the yellowness index measured by colorimetry (objective colour measurement) than for the DSM score (subjective colour assessment). The correlation was also found to be stronger for fruit with lower colour scores (more white pulp) than for those with higher scores (more orange pulp). *Musa* genotypes with a white pulp colour all had relatively low carotenoids levels while the more orange genotypes displayed much more variation in carotenoids contents ranging from low to high.

Genotypes with a t-BC equivalent high enough to provide 50% of the daily EAR for children at realistic consumption levels were shown to have a DSM value of 7 or more and a yellowness index of more than 62. These results demonstrate that simple colour-based methods can be used for the visual inspection and rapid prescreening of field germplasm collections for carotenoids content to exclude low-carotenoid cultivars. Precision screening by spectrophotometry or RP-HPLC will still be needed however for the analysis of more orange genotypes. As such colour-based prescreening can help reduce time and costs in *Musa* carotenoids screening programmes.

Recently, it has been demonstrated that visible/near-infrared reflectance spectroscopy (vis/NIRS) can be used for the rapid, accurate and non-destructive analysis of carotenoids in lyophilised *Musa* fruit pulp and in *Zea mays* (Davey et al., 2009b; Brenna and Berardo, 2004). The high speed and low operational costs of this technique holds out much promise for implementation in screening and breeding programmes, but it remains to be seen whether the method is also suitable for the field evaluation of fresh fruit.

It is hoped that this study will contribute to a better use of *Musa* genetic resources in breeding and biofortification strategies to increase the content of vitamin A of this important staple food.

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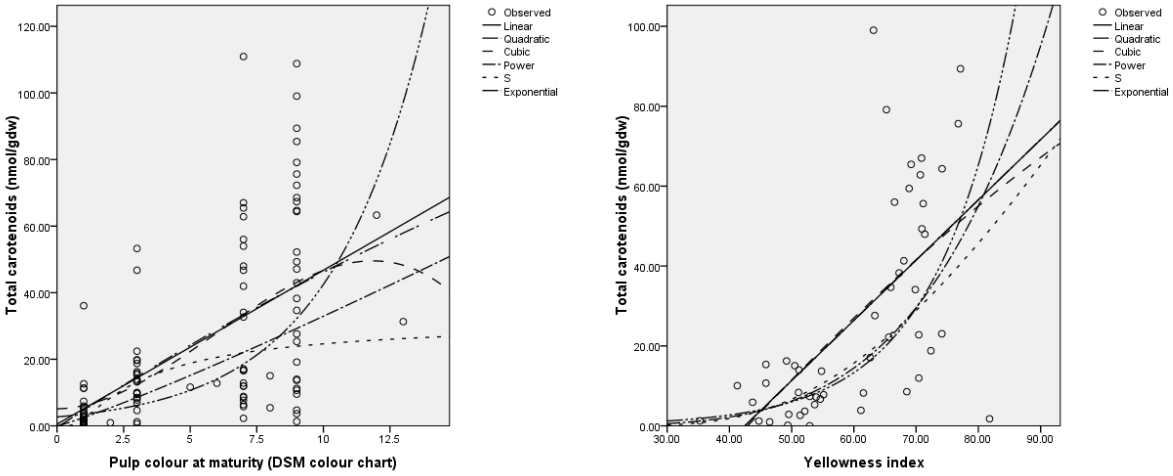
Tables

Table 1. Linear and best-fit regression models for the correlation between pulp colour at maturity, measured by DSM colour chart and by colorimetry, and total carotenoids content of *Musa* fruit pulp.

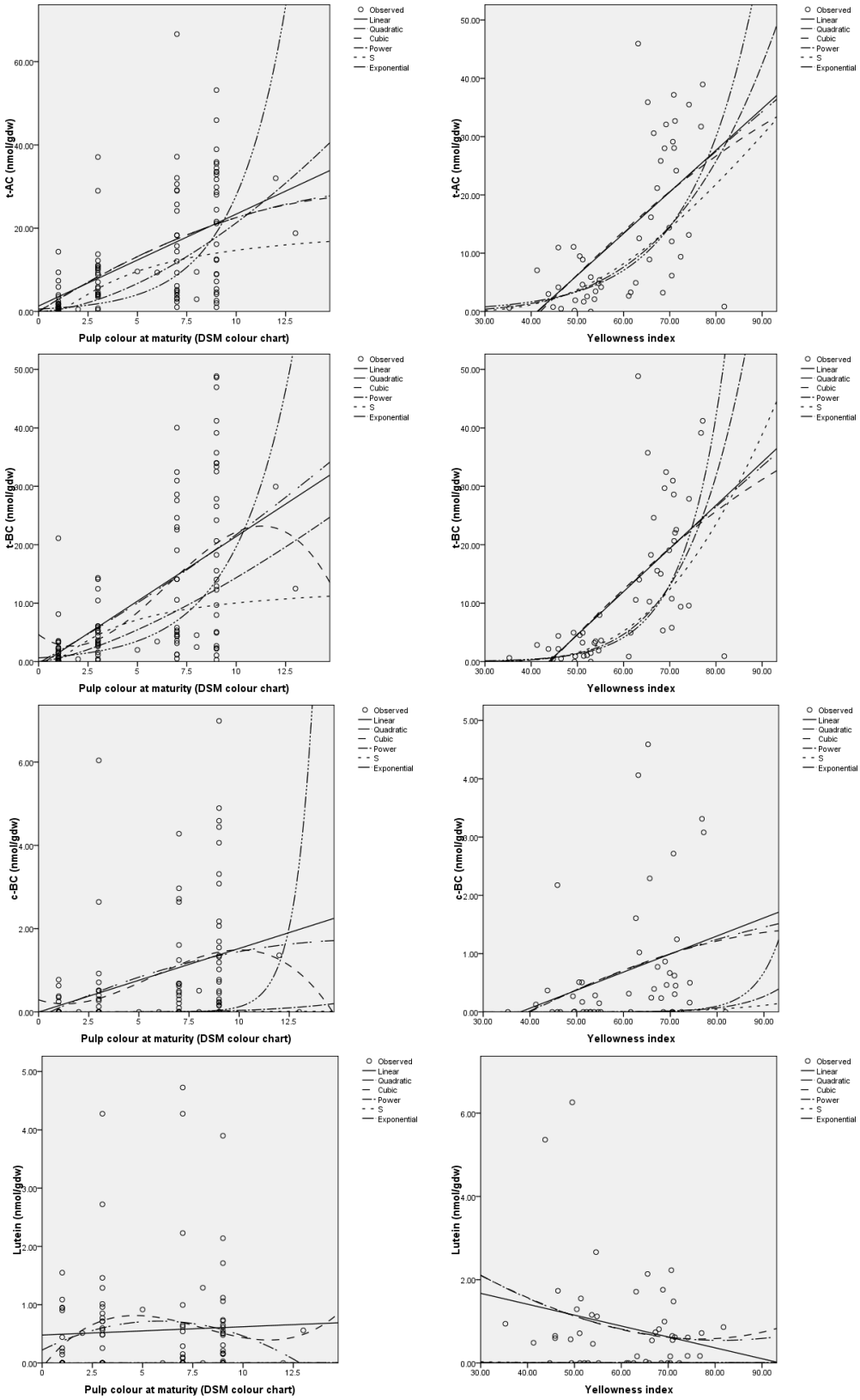
Regression Models		Pearson Correlation Coefficient	R Square	*P* value
Y = Total carotenoids content X = DSM colour chart value				
Linear	$Y = 0.513 + 4.623X$	0.559	0.313	< 0.001
Power	$Y = 2.494X^{1.121}$	0.654	0.428	< 0.001
S- curve	$Y = e^{[3.474 + (-2.699/X)]}$	0.642	0.412	< 0.001
Exponential	$Y = 2.684e^{(0.276X)}$	0.620	0.384	< 0.001
Y = Total carotenoids content X = Yellowness index (colorimetry)				
Linear	$Y = -64.039 + 1.507X$	0.625	0.391	< 0.001
Power	$Y = 8.027E\text{-}8X^{4.643}$	0.644	0.415	< 0.001
S- curve	$Y = e^{[7.029 + (-256.520/X)]}$	0.642	0.412	< 0.001
Exponential	$Y = 0.113e^{(0.080X)}$	0.636	0.404	< 0.001

Figures



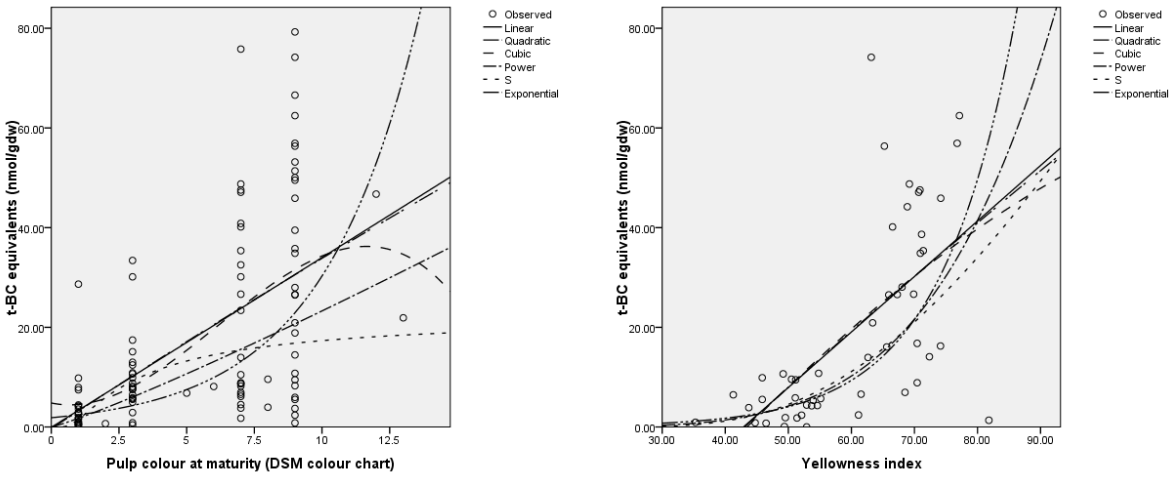
A B

Fig. 1. Correlation between pulp colour at maturity, measured by DSM colour chart (A) and by colorimetry (B), and total carotenoids content of *Musa* fruit pulp.



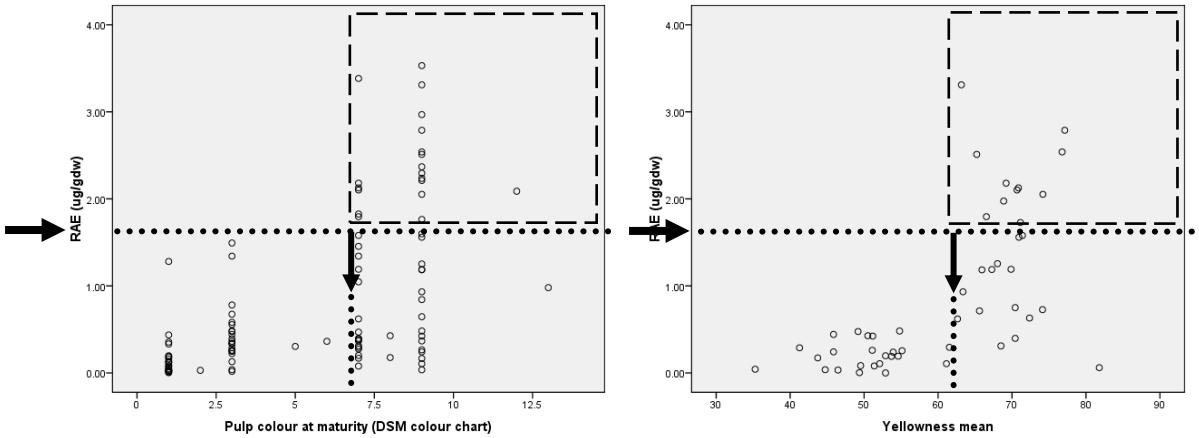
A B

Fig. 2. Correlation between pulp colour at maturity, measured by DSM colour chart (A) and by colorimetry (B), and individual carotenoids content of *Musa* fruit pulp.



A B

Fig. 3. Correlation between pulp colour at maturity, measured by DSM colour chart (A) and by colorimetry (B), and t-BC equivalents content of *Musa* fruit pulp.



A B

Fig. 4. Target groups of high-vitamin A cultivars: accessions with an retinol activity equivalent (RAE) of at least 1.67 µg/gdw (→) have a DSM value of 7 or more (A), and a yellowness index of 62 or more (B).