Biofortification of taro (*Colocasia esculenta*) through breeding for increased contents in carotenoids and anthocyanins

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Abstract:
Biofortification of taro (*Colocasia esculenta*) has never been studied. The aim of the present study is to compare the chemical compositions and individual constituent contents for major compounds (starch, sugars, cellulose, proteins, minerals), carotenoids and anthocyanins between parents and hybrids selected first for their agronomic performance and second for their corm characteristics (flesh colour, quality and taste). For major compounds, 45 selected hybrids were compared to 66 cultivars and for carotenoids and anthocyanins, 34 selected hybrids were compared to 79 cultivars. All plants were planted the same day within the same plot and harvested together to avoid environmental factors effects. Total sugars, cellulose and minerals contents presented moderate increases in hybrids. Carotenoids and anthocyanins contents were correlated with corm flesh colours. Anthocyanins contents could not be increased in the selected hybrids. However, total carotenoids contents were increased by more than four folds in the hybrids. The results of this study indicate that carotenoids contents can be rapidly improved by selecting plants of good agronomic performance and corm shape with increased density of yellow and orange colours. Potential applications to taro breeding programmes are discussed.
Keywords: anthocyanins, biofortification, carotenoids, primary compounds, taro

Introduction

Taro (*Colocasia esculenta*) is an important staple in many developing countries, particularly in the Pacific and in Melanesia where the annual consumption *per* inhabitant is among the highest in the world. Many traditional varieties exist and there is now a need to improve production and corm quality. In spite of its importance, taro is under-researched with insufficient knowledge on its micronutrients, especially carotenoids and anthocyanins.

A deficiency in vitamin A can lead to blindness and is a serious problem in developing countries. In the Pacific, vitamin A deficiency has been diagnosed since lifestyle changed and there is now a need to identify sources of provitamin A among locally-grown staple foods (Englberger *et al*., 2008). Human health benefits have also been shown to be related to non-provitamin A carotenoids. Lutein and zeaxanthin, for example, are found in the macula area of the human retina and are associated with a reduced risk of age-related retina degeneration (Mares *et al.*, 2006). More generally, natural carotenoids protect the skin from damaging solar radiations and reduce the risk of developing certain cancers (Stahl and Sies, 2005). Carotenoids also exhibit antioxidant properties and their regular consumption from fresh product has been proven to bring numerous health benefits (Miller *et al*., 1996; Sies and Stahl, 1995; Stanner *et al*., 2004). Since carotenoids have such a beneficial impact on human health, biofortification of taro, and other aroids, has the potential to play a role in health improvement programmes.

Phenolic compounds including flavonoids (*e.g*., anthocyanins, flavonols or flavanols) and phenylpropanoids (*e.g*., cinnamic acids, or cinnamic acid esters) are metabolites considered as valuable natural products. They exhibit different functional properties. The human health benefits of

anthocyanins intake have been thoroughly investigated. Evidence suggests that phenolic compounds consumption may protect from some disorders including cancers (Ding *et al.*, 2006; Yang, *et al*., 2001), cardiovascular diseases (Bell and Gochenaur, 2006; Ross and Kasum, 2002) and age-related neurodegenerative declines (Lau *et al*., 2005).

Carotenoids and phenolic compounds composition and content of taro have been studied and remarkable variation was observed among varieties (Champagne *et al*., 2010, 2011). Taro genetic improvement is based on the selection of genotypes on their *per se* value. The identification of parents with complementary traits, their recombination and the subsequent screening of large full-sib families are required to identify improved hybrids. Breeders need well-designed technologies and reliable data to select the most suitable parents and progenies. The limited knowledge available on taro secondary metabolites is a serious constraint to its biofortification through breeding. Major compounds (starch, sugars, cellulose, proteins, minerals) and secondary metabolites are involved in the palatability of traditional varieties. Colours are linked to the presence of secondary metabolites such as carotenoids and flavonoids and are essential traits of different cultivars. Moreover, the traditional selection system practiced by local farmers for attractive and palatable chemotypes has generated tremendous diversity which deserves to be studied. Because of socio-cultural requirements and traditional exchanges of germplasm, smallholders pay a particular attention to morphological variation, such as corm flesh colours which are directly determined by their physico-chemical composition.

Plant biofortification is the nutritional enhancement of crops through breeding. Various breeding programmes are now improving major compounds such as proteins and sugars, but also secondary metabolites such as carotenoids and anthocyanins. These programmes start with the evaluation of germplasm followed by crossing the selected accessions. Crosses can be done in polycross plots where natural open pollination is used to produce half-sib progenies and, when bulked together, populations for recurrent selection. Once seedlings are obtained, they must be clonally propagated and multiplied through clonal generations before accurate assessment of their performance can be conducted. Depending on their scope, programmes may differ slightly but their rationale remains the same. Heavy selection pressure is applied at the seedling stage for vigour and resistance to diseases. This selection process is visual without any data recording in order to minimize the costs and maximize the number of genotypes assessed. The selected clones are then released as new varieties. A new selection cycle can begin, in which the new selected varieties are used as parents. Unfortunately, chemotypes with interesting properties can be eliminated because of high selection pressure on other traits. The selection process is equivalent to mass recurrent selection since great numbers have to be screened to achieve some progress. Each individual offspring can be selected to yield a new cultivar. Most current biofortification programs use this method (Bradshaw, 2010).

Previous studies have unveiled opportunities for improvement as a result of high variation and particular relationships between compounds (Champagne *et al.*, 2009). Starch content in taro is negatively correlated to other major compounds (Lebot *et al*., 2011). Local consumers are also favouring certain chemical compositions. A chemical composition suitable for preparing a good traditional dish *laplap* in Vanuatu (a pudding-like dish) is more complex to fulfill than for daily consumption through simple boiling. The same is observed with the West African *fufu* prepared from taro corms. Varietal characteristics are of utmost importance. The objectives of the present study were to *i)* assess progresses achieved within current breeding lines in order to confirm biofortification potentials, *ii)* evaluate conventional breeding method for biofortification in major compounds (starch, sugars, proteins, minerals and cellulose) and secondary metabolites (carotenoids and anthocyanins). The selection process and its perspectives are then discussed.

Materials and Methods

Origin of genotypes

Most cultivars used as parents were from Vanuatu and were selected for corm quality (Bourrieau, 2000). Others were from South-East Asia (Lebot *et al*., 2005). Taros from both genetic pools were

cross-pollinated via controlled crosses to produce highly variable progenies. Seeds were mixed before planting to generate a highly variable population (Lebot *et al*., 2004). Cultivars and hybrids were grown together on the same plot at Téouma (17°45'S/168°18'E, on Efate island in Vanuatu) to prevent variation due to environmental factors. Accessions were planted at the same time and harvested at optimum maturity, to avoid ontogenic variation. Taro hybrids were selected for major agronomical traits through four consecutive generations (the seminal, F_1 and three clonal ones, C_1, C_2 and C_3):

- F_1 or seminal generation (originally from seeds): hybrids were mostly selected for agronomic performance (vigour), the major taro pathogen (*Phytophthora colocasiae*) being absent from Vanuatu.
- C_1 or first clonal generation: hybrids were selected again for the same trait and for yield which was estimated from individual weights. Low yielding hybrids (< one kilo per plant) were discarded.
- C_2 or second clonal generation: 5 to 10 plants per hybrid were evaluated for the same previous traits with an additional care to quality characteristics. Shape of the corms should be smooth and oblong. White flesh was not favoured because local consumers prefer coloured flesh. However, all white fleshed genotypes were not eliminated because some produced nice corm shapes and high yields. Corms hardness (ease of hand-cutting the central section) was also evaluated because it is related to dry matter content. Watery corms were rejected.
- C_3 or third clonal generation: 10 to 20 plants per hybrid were evaluated for all of the previous traits plus for tasting quality through consumption preference by local staff.

These assessments were mainly qualitative and sensorial and were conducted on thousands of hybrids. About 90% of the hybrids were eliminated during these three successive selection steps. Selected hybrids were compared to the parent cultivars used for breeding. Quantitative evaluation is still in progress to reduce hybrids number.

Samples preparation

Accessions analysed for major compounds involved 66 cultivars (parents) and 45 C_3 hybrids. Accessions analysed for carotenoids and anthocyanins included 79 cultivars (parents) and 34 C_3 hybrids. For major compounds, corms were peeled, washed, dried with a towel and cut into 2 mm thick transversal slices. Slices were dried in a ventilated oven at 60°C until constant weight. The dried flours were enclosed in paper bags and kept in a dry place until analysis. For carotenoids and anthocyanins, the preparation procedure was very same that previously described (Champagne *et al.*, 2010, 2011)

Primary compounds analysis

Analyses of major compounds were performed as previously described (Champagne *et al.*, 2009). Briefly, about 150 g of dry matter prepared in Efate (Vanuatu) were sent to France, where residual moisture, starch, sugars, proteins, minerals and cellulose were quantified. For dry matter determination, samples of flours were dried again in an air oven to remove residual moisture and then analyzed on a dry matter basis according to NF (Norme Française) V 18-109. Mineral contents were estimated from ash produced at 550°C (NF V 18-101). Crude cellulose was measured using Weende's method (NF V 03-040). Protein content was measured using the Kjeldahl method (NF V 18-100) for quantification of total nitrogen (N x 6.25). After starch extraction, reducing sugars were estimated using the standard iodometric method of Luff Schoorl (CEE 98\54\CE). Starch was quantified using the Ewers protocol (NF ISO10-520). All measurements are expressed in percentages of dry matter (DM).

Reagents and standards

Acetone, methanol and chlorhydric acid were purchased from VWR Int. (Fontenay-sous-Bois, France). *Tert*-butyl-methyl-ether, ammonium acetate and ethyl acetate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Acetic acid was purchased from J.T. Baker (Phillipsburg, USA) and ethylic

ether from Cooper (Melun, France). All-*trans*-*β*-carotene and all-*trans*-lutein were pure standards purchased from Carotenature GmbH (Lupsingen, Switzerland) and lycopene from Sigma-Aldrich Co. (St. Louis, USA). Phytoene and zeaxanthin were obtained from *Escherichia coli* harbouring the plasmids pAC-PHYT and pAC-ZEAX kindly provided by Dr F.X. Cunningham Jr. (University of Maryland, USA). Carotenoids were extracted from bacteria cultures using ethyl ether. Cyanidin-3-glucoside was purchased from Extrasynthese (Genay, France).

Carotenoids extraction and analysis

The extraction method was performed as previously described (Champagne *et al.*, 2010). Briefly, a 2-4 g freeze-dried powder sample was homogenized in 10 mL acetone using a polytron Biotrona 6403 (Küssnacht, Switzerland). To ensure full recovery of analytes, knifes were rinsed with 5 mL of acetone and the 5 mL was then pooled with the first 10 mL. The sedimentation of the powder was achieved by centrifugation at 4°C at 3000 g for 10 min. The supernatant was recovered with a Pasteur pipette and the extraction process was repeated on the pellets. In order to guarantee optimal extraction conditions, the process optimization was followed using High-Performance Liquid Chromatography - Diode Array Detector (HPLC-DAD). The DAD was set at 460 and 290 nm for coloured and non-coloured carotenoids. Generally two to four extractions were needed for optimal results. Each extract was evaporated to dryness under a nitrogen stream. The extracts were then dissolved in an adequate volume of ethyl acetate and filtered on a 0.45 μm PTFE filter (C.I.L., Sainte-Foy-La-Grande, France) prior to injection.

Carotenoids were analyzed by HPLC-DAD using the method described by Fraser *et al.* (2000) with modifications. Extracts were separated on a Spectra system (Thermo Finnigan) equipped with a reverse-phase C30 column (YMC Inc. Europe GmbH, Germany), 5 µm, 4.6 x 250 mm. The mobile phases were methanol as eluent A, methanol/ammonium acetate 1% in water (5:1, v:v) as eluent B, and *tert*-butyl-methyl ether as eluent C. The injection volume was 50 μL, the flow rate was set at 1 $mL.min^{-1}$ and the column temperature was set at 25°C. The gradient program was performed as follows: initial conditions 0-12 min, 95%A /5%B; 12-12.1 min, to 80%A /5%B/15%C; 12.1-40 min, to 30%A /5%B /65%C; 40-43 min, to 5%B /95%C; 43-46 min, 5%B /95%C; 46-49 min, to 95%A /5%B; and back to the initial conditions for re-equilibration. Carotenoids were monitored between 200 and 800 nm with an UV-visible DAD (UV-6000, Thermo Finnigan). Data were collected and processed using Chromeleon software v.6.60 (Dionex Co., Sunnyvale, USA). When sample were shown to be over-concentrated via HPLC run, it was re-analyze with higher dilution. An external astaxanthin standard was injected daily to monitor repeatability of the HPLC analytical separation through changes in retention time, and repeatability of the detection through peak area. All compounds eluted in 45 min.

Anthocyanins extraction and analysis

The phenolic compounds were extracted from the corm using a method previously described (Champagne *et al.*, 2011). Briefly, the procedure was optimized using HPLC-DAD monitoring. A 2 g freeze-dried powder was homogenized in 15 mL of 7% acetic acid in milliQ water (v/v) using a polytron Biotrona 6403 (Küssnacht, Switzerland), knifes were rinsed with 5 mL of 7% acetic acid in milliQ water then the 5 mL were pooled with the first 15 mL. Extraction took place in a capped opaque tube at room temperature overnight and under stirring. As water used as an extraction solvent yields an extract with a high content of impurities, solid phase extraction (SPE) was used to eliminate non-phenolic compounds including organic acids, sugars, and soluble proteins, among others. Samples were purified and concentrated with a SPE system from Grace-Alltech, GracePure SPE C18-Max, 500 mg/3 mL (Columbia, USA). For each step of the purification procedure, samples were kept away from light and when possible, were maintained at 4°C. SPE columns were pre-treated with 3 mL of 0.1% HCl in methanol (v/v) and were balanced with 6 mL of 7% acetic acid in milliQ water (v/v). Residues contained in the extraction solution separated by centrifugation at 4°C, 4800 g, for 10 min. Supernatants were applied to the SPE column, and then bounded phenolics were washed with 6 mL of 7% acetic acid in milliQ water. In order to keep anthocyanins in the red

flavylium form which confers higher stability, they were eluted with 1 mL of 0.1% HCl (v/v) in methanol, prior to injection. The recovery rate of the SPE procedure was calculated as the percent ratio between the quantity found after the purification step and the amount added as standard. The rate was 95.2% for cyanidin-3-glucoside (expressed as the mean of triplicates). This rate was used to calculate anthocyanin contents.

A volume of 20 μL of the anthocyanins extract solution was injected by an automated sample injector ASI-100 and gradient was performed with a HPLC pump P680, both from Dionex Co. (Sunnyvale, USA). Separation was performed on a reversed-phase Ultrasphere ODS column 250 x 4.6 mm, 5 μm particle sizes fitted with an Ultrasphere C18 guard column 45 x 4.6 mm purchased from Beckman Coulter Inc. (Fullerton, USA). The column and guard column temperature was maintained at 25°C with an external oven Igloo-CIL Peltier C.I.L. (Sainte-Foy-La-Grande, France). Solvent A was composed of water and formic acid (90/10, v/v), and solvent B of methanol and formic acid (90/10, v/v). A binary gradient at 0.6 mL.min^{-1} flow rate was used. The initial conditions were 90%A/10%B; 0-40 min, to 60%A/40%B; 40-44 min, to 100%B; 44-48 min, back to the initial conditions, and finally 48-70 min for re-equilibration. Anthocyanins were monitored at 520 nm with an UV - Visible DAD 340U Dionex Co. (Sunnyvale, USA). Data were collected and processed using Chromeleon software v.6.60 (Dionex Co., Sunnyvale, USA). For anthocyanins seen on HPLC chromatograms of extracts, detection and quantification were based on their maximum absorption wavelengths (λmax), therefore at 520 nm. All compounds eluted in 42 min.

Determination of peak purity and identification

Carotenoids quantification was achieved by external calibration curves made of five carotenoids standard concentrations injected in triplicate. Concentrations were calculated using molar extinction coefficients. Correlation coefficients were linear ($r \geq 0.98$). When available, contents were calculated with HPLC-quality standards. Unidentified compounds were labelled as follows: a figure representing characteristic retention time (in minutes), preceded by C for carotenoids and A for anthocyanins (*i.e.* C24.9: a carotenoid exhibiting a retention time of 24.9 min).

The purity of carotenoids standards was determined by HPLC-DAD and checked to be over 90% for each standard (Rodriguez-Amaya and Kimura, 2004). Each peak was checked to make sure it displayed the same characteristic spectrum on the ascending and descending slopes and at maximum. When standards were not commercially available, contents were expressed as all-*trans*-*β*-carotene equivalent (*β*Ceq). For instance, *cis*-*β*-carotene isomers were quantified as all-*trans*-*β*-carotene. For anthocyanins, standard and peak purity were checked as previously mentioned for carotenoids. However, all contents were expressed as cyanidin-3-glucoside equivalent (CGeq). Total contents were estimated on basis of total peaks area at 460 and 520 nm and therefore expressed in *β*Ceq and CGeq, respectively for carotenoids and anthocyanins. For carotenoids, phytoene contents determined at 290 nm were added.

Colours assessment

Colour codes ranging from 1 to 7 were attributed to each storage organ at harvest. Colours were assessed visually. The corm flesh colour code was attributed as follow: 1 = white, 2 = yellow, 3 = orange, 4 = pink, 5 = red, 6 = light-purple, 7 = dark-purple, several numbers imply several colours, *e.g.*: 3-6 = orange-light purple.

Statistical analyses

Coefficients of variation of the mean were calculated to provide an estimate of the dispersion of a probability distribution of specific datasets and are reported as percentages (CV%). Linear correlation analysis was conducted to establish the relationships between corm flesh colour and carotenoids and anthocyanins contents with the free open source software environment for data analysis and graphics, R version v2.9.0 (9) and the "RcmdrPlugin. FactoMineR" packages with mean-centred data scaled to unit variance. Relationships between major compounds, visually determined corm flesh colour codes, and quality were estimated by calculating Pearson's product-moment correlations.

Significance was determined using Student's *t* test. Analyses of variance were carried out using transformed data if required. Significance of differences among least square means was determined by Turkey's test ($P \leq 0.05$).

Results and discussion

Impact of selection method on major compounds

Taro is a staple across the Pacific and local farmers practice traditional selection by mainly focusing on starch contents. This could explain the very low CV% for starch in the 66 cultivars (Table 1). Variability of other major compounds is large and congruent with previous results. The average starch content (and maximum and minimum values) was higher in the group of cultivars than in hybrids in opposite to average total sugar contents and in agreement with previous reports on the inverse relationship between the accumulations of these two substances in taro (Champagne *et al.*, 2009, Lebot *et al.*, 2011). The hybrids exhibited lower CV%. Not intentionally, our selection method may have, therefore, favoured hybrids with high total sugars content and this could reduce starch levels. Variability in total proteins contents was significantly enhanced in hybrids compared to cultivars, with respective CVs of 48% and 25% respectively (Table 1).

Our selection method decreased the variability in minerals content (lower CV%). Though the mean value was virtually unchanged, maximum values for mineral content were severely curbed. Taste quality of hybrids was frequently evaluated by the local technical staff of the VARTC research station. It appeared that minerals content plays a crucial role in consumers' acceptance. These results confirmed preliminary results reporting that a "good" variety must exhibit high dry matter and starch contents coupled with low total minerals content (Bourrieau, 2000, Lebot *et al.*, 2004, Lebot *et al.*, 2011).

It has previously been also shown that mid-range starch content is preferred for varieties to be boiled and roasted rather than transformed into *laplap* (Champagne *et al.*, 2009). Biofortification of sugars, proteins and minerals therefore seems achievable for "table" type of varieties. However, as these are negatively correlated with starch, it is expected that the starch content will be reduced. Genetic improvement for higher starch content has to be done within an independent set of hybrids where starch and dry matter contents can be jointly improved as the two are positively correlated (Lebot *et al.*, 2011).

Impact of selection method on carotenoids

Contents of total and major carotenoids varied greatly among cultivars and hybrids (Table 2). Coupled with previous results based on comparative evaluation of different clones of the same genotype to double check the robustness of the quantification, this indicates again that individual secondary metabolites contents are genetically controlled (Champagne *et al.*, 2010). A previous study has shown that satisfaction of local consumers' preferences is not compromised by an enhancement of flesh colour (*i.e.* by increasing carotenoids content) (Champagne *et al.*, 2009). Maximum content of each individual carotenoid was higher in hybrids compared to cultivars, except for lutein (Table 2). More particularly, an increase from 0.17 to 2.04 $\mu g\beta$Ceq/gDM of P25.5 content was observed (unidentified compound, putative *cis*-isomer of *β*-carotene). All-*trans*-*β*-carotene maximum value increased by a factor of 3.5. The average content of all carotenoids increased in the hybrid group. For instance, all-*trans*-*β*-carotene and zeaxanthin were respectively increased by a factor of 7 and 36. These results were in opposition to CV% variations that exhibited remarkable decreases in hybrids, most likely due to lesser sample size. Surprisingly, phytoene was detected in small amount in 4 hybrids, while it was not detectable in the parent cultivars. The same was observed with lycopene which was found in only one hybrid. Since phytoene and lycopene are non-cyclic carotenoids found in earlier steps of the carotenoid biosynthetic pathway, their detection may result from either a disturbance of downstream enzymatic steps or from a global enhancement of the whole biosynthetic pathway in the hybrids.

Impact of selection method on anthocyanins

The largest peak found in *C. esculenta* purple-fleshed genotypes corresponded to compound A22.1 and represented 57-74.5% of the total peak area. Only two previous studies identified anthocyanins in *C. esculenta*, the first as pelargonidin-3-glucoside, cyanidin-3-rahmnoside and cyanidin-3-glucoside (Chan and Kao-Jao, 1977), and the second as cyanidine-3-rutinoside (Terasawa *et al.*, 2007). However, our analyses could not reveal the presence of pelargonidin-3-glucoside in *C. esculenta*. Cultivars exhibited mid-range anthocyanins contents. No anthocyanins were found in the widely consumed local Fijian cultivars of taro (Lako *et al.,* 2007), indicating that Vanuatu cultivars are richer in anthocyanins. As expected, the white-fleshed cultivars displayed no detectable anthocyanins.

A significantly lower content was observed for most anthocyanins in hybrids than in cultivars (Table 3). This was particularly clear for maxima of both individual and total anthocyanins contents. The only compound which maximum and CV% were almost unaffected was A28.5. Nevertheless, the major anthocyanin (A22.1) content correlated to total anthocyanins content and showed higher CV% and lower means. A broader distribution of anthocyanins contents was thus revealed in the hybrid group, coupled with decreased maxima. The maximum hybrid value was clearly lower than for cultivars which maximum value of 243.3 μgCGeq/gDM was close to a previously reported value of 265.6 μgCGeq/gDM despite our wider sampling of 113 taros against 33 (Champagne *et al.*, 2011).

After grouping accessions into classes corresponding to flesh colour, classes were sorted by ascending order of total carotenoids (Table 4) or total anthocyanins means (Table 5). This revealed that yellow flesh taros exhibited the smallest anthocyanins contents, while dark-purple taros exhibited the highest. In addition, higher total anthocyanins contents were found in orange and white flesh taros than in yellow ones though they were very small compared to the values observed in the pink-purple varieties. Orange cultivars had the highest total carotenoids (mostly made of isomers of β-carotene) contents. The red varieties contained surprisingly high levels of carotenoids. Because red carotenoids were not detected and these varieties contained high anthocyanins content, it is very likely that the anthocyanins masked the carotenoids to the human eye. Tough not apparent from these tables, a colour gradient was obvious when observing sliced-open corms of light-purple fleshed taro (Fig. 1). The younger part (the proximal, upper end) was more coloured (darker). This colour gradient was not observed in yellow fleshed corms but existed in orange fleshed ones where it went from orange at the proximal end to yellow at the distal end.

Chemotypes promoted by the selection method

Variations in major compounds have previously been studied within a larger sample size and are therefore not discussed here (Champagne *et al.*, 2009, Lebot *et al*., 2011). However, taro carotenoids and anthocyanins compositions and contents have never been studied on such a broad sample scale. The extent of variation unveiled by the present study indicates that biofortification opportunities exist.

Selection for corm characteristics was applied during the second and third clonal generations only, not in F_1 and C_1 (Fig. 2). In addition, selection based on corm shape was also combined with a selection for hybrids with coloured corm fleshes. Therefore, the white fleshed corms were usually discarded because they were not attractive to local consumers in Vanuatu. Also, the export markets to New Zealand and Australia are looking for pink flesh taros. Our selection also discarded accessions with low dry matter content. The present study indeed revealed that our selection process promoted high soluble sugars and carotenoids, and low minerals and anthocyanins, contents simultaneously. In addition, lower CV% among hybrids compared to cultivars suggested that the selection pressure reduced the range of variation of secondary metabolites content. Taken together, these findings reveal the impact of the selection method and indicate that potential maxima are not reached yet. Consequently, biofortification opportunities still exist for improving taro corm quality.

In the Pacific, taro is an ancient crop. It is a diploid species and genetic analyses have revealed that a narrow genetic base was introduced in Vanuatu (Kreike *et al*., 2004; Caillon *et al.*, 2006; Quero-García *et al.*, 2004, 2006). Among the 79 parents (cultivars) used to produce hybrids, only eight had a non-Vanuatu origin. Since this crop is traditionally very important in Vanuatu,

morphotypes corresponding to local preferences were prioritised by the method. The principal use of taro is evidently related to starch and its content appeared to be relatively stable (Table 1).

Conclusions

This study represents a first attempt to assess the biofortification potential of taro through breeding for increased carotenoids and anthocyanins contents. Previous studies have shown that variation in major compounds is genetically controlled and that there is tremendous potential for genetic improvement by crossing parents with suitable chemotypes. Dry matter and starch contents are positively correlated to each other but negatively correlated to other compounds (sugars, cellulose, proteins and minerals). As quality of taro corm has been shown to be closely related to starch and dry matter contents and negatively related to proteins, cellulose, sugars and minerals content, the focus on starch is likely to improve quality if it does not go beyond a certain point (approx. 78-82%DM) to avoid dryness in the mouth.

The present study has shown that it is fairly easy to improve total carotenoids content through this simple breeding process. The situation for anthocyanins is more complex. Though orange taros had the highest carotenoids contents and deep-purple taros the highest anthocyanins contents, the simple screening of a germplasm by eye or with a chromameter may not be the best option. Chromameter measurements may be biased by the existence of orange and red pigments gradients within tubers. Red corms were also shown to contain significant quantities of both anthocyanins and carotenoids. Though this demonstrates that simultaneous biofortification for both classes of compounds is possible, it reveals that the human eye may be made blind to some pigments due to colour co-suppression effects.

Acknowledgements

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Table 1. Variation of major compounds for 66 parents (*cvs*) and 45 hybrids (*hyb*) of taro (expressed as percentage of DM).

	Starch		**Sugars**		**Proteins**		**Minerals**		**Cellulose**	
	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*
Min	64.60	*60.77*	0.90	*3.23*	2.30	*2.42*	1.47	*2.68*	1.40	*2.16*
Max	88.20	*87.05*	17.30	*18.58*	9.20	*14.79*	8.13	*5.86*	7.30	*6.64*
Mean	79.34	*76.58*	4.07	*6.58*	5.40	*5.18*	3.93	*4.15*	3.26	*3.51*
CV%	6.5	*7.8*	66.0	*40.8*	25.4	*48.0*	30.3	*16.6*	32.2	*29.3*

Table 2. Variation in major and total carotenoid content for 79 *cvs* and 34 *hyb* of taro from polycross (mg/gDM).

	lutein		**zeaxanthin**		**phytoene**		**C25,5***		**13-*cis*-β-carotene***		**all-*trans*-β-carotene**		**9-*cis*-β-carotene***		**Total**	
	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*
Min.	n.d.	*n.d.*	n.d.	*n.d.*	n.d.	*n.d.*	n.d.	*n.d.*	n.d.	*n.d.*	n.d.	*n.d.*	n.d.	*n.d.*	0.09	*n.d.*
Max.	1.21	*0.82*	0.14	*1.36*	n.d.	*0.19*	0.17	*2.04*	2.72	*4.77*	4.03	*15.83*	1.79	*2.30*	9.77	*25.58*
Mean	0.16	*0.28*	0.00	*0.13*	n.d.	*0.01*	0.03	*0.50*	0.37	*1.44*	0.53	*3.53*	0.28	*0.80*	1.47	*6.79*
CV%	164	*95*	613	*241*	0	*316*	188	*139*	184	*119*	180	*137*	163	*107*	155	*121*
Median	0.06	*0.12*	0.00	*0.00*	0.00	*0.00*	0.00	*0.16*	0.16	*0.24*	0.21	*0.61*	0.16	*0.19*	0.74	*1.64*

* *in all*-trans-β-*carotene equivalent*

Table 3. Variation in major and total anthocyanins content for 79 *cvs* and 34 *hyb* of taro from polycross (mg/gDM).

	A21.0*		**A22.1***		**A24.2***		**A26.6***		**A28.5***		**Total***	
	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*	cvs	*hyb*
Min.	n.d.	*n.d.*	n.d.	*n.d.*	n.d.	*n.d.*	n.d.	*n.d.*	n.d.	*n.d.*	n.d.	*n.d.*
Max.	5.58	*1.68*	243.26	*104.23*	12.74	*0.28*	32.86	*6.60*	22.13	*22.86*	306.79	*118.87*
Mean	0.12	*0.10*	10.06	*6.88*	0.29	*0.02*	0.72	*0.70*	1.15	*1.41*	12.33	*9.11*
CV%	546.5	*328.3*	400.1	*305.7*	571.5	*343.6*	528.3	*221.7*	313.1	*300.2*	386.7	*267.7*
Median	0.00	*0.00*	0.07	*40.92*	0.00	*0.00*	0.00	*3.36*	0.00	*9.14*	3.03	*55.64*

* *in cyanidine-3-glucoside equivalent*

Table 4. Variation in major and total carotenoids content for 113 accessions of taro grouped by flesh colour.

Flesh color	Nb. Acc.	Means expressed in mg/gDM							
		Lutein	Zeaxanthine	Phytoene	C25,5*	13-*cis*-β-carotene*	all-*trans*-β-carotene	9-*cis*-β-carotene*	Total*
4	16	0.05^{a}	0.01^{a}	0.01^{a}	0.06^{a}	0.15^{a}	0.20^{a}	0.13^{a}	0.65^{a}
1	14	0.10^{b}	0.03^{b}	0.00^{a}	0.06^{a}	0.16^{ab}	0.22^{b}	0.15^{b}	0.77^{bc}
7	3	0.14^{b}	0.04^{b}	0.00^{a}	0.05^{a}	0.16^{b}	0.26^{c}	0.11^{ab}	0.82^{c}
6	15	0.10^{b}	0.00^{a}	0.00^{a}	0.05^{a}	0.18^{c}	0.35^{d}	0.17^{bc}	0.90^{cd}
2	31	0.12^{b}	0.01^{a}	0.00^{a}	0.04^{b}	0.24^{d}	0.40^{e}	0.18^{c}	1.04^{e}
5	12	0.19^{c}	0.09^{c}	0.03^{b}	0.23^{c}	0.39^{e}	0.86^{f}	0.28^{d}	2.11^{f}
3	21	0.53^{d}	0.13^{d}	0.00^{a}	0.56^{d}	2.53^{f}	5.31^{g}	1.45^{e}	10.56^{g}
3-6	1	0.81^{e}	0.00^{a}	0.00^{a}	1.51^{e}	4.77^{g}	15.83^{h}	2.30^{f}	25.27^{h}
113 acc	**min.**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.087
	max.	1.21	1.36	0.19	2.04	4.77	15.83	2.30	25.57
	mean	0.20	0.04	0.00	0.18	0.69	1.44	0.43	3.074
	CV%	136.4	423.5	589.6	247.7	173.0	214.2	148.4	176.9
	Median	0.08	0.00	0.00	0.00	0.16	0.22	0.16	0.89

* *in all*-trans-β-*carotene equivalent*

Mean values with the same letter in a column are not significantly different at P ≤ 0.05 (Turkey's test).

Table 5. Variation in major and total anthocyanins content for 113 accessions of taro grouped by flesh colour.

Flesh color	Nb. acc.	Means expressed in µg/gDM*					
		A21.0	A22.1	A24.2	A26.6	A28.5	Total
2	31	0.06[a]	0.45[a]	0.08[a]	0.10[a]	0.18[a]	0.86[a]
3	21	0.03[b]	1.24[b]	0.01[b]	0.13[a]	0.09[b]	1.49[b]
1	14	0.00[c]	1.45[c]	0.00[b]	0.04[b]	0.15[a]	1.64[b]
4	16	0.03[b]	2.58[d]	0.01[b]	0.20[c]	0.53[c]	3.35[c]
3-6	1	0.00[c]	4.08[e]	0.00[b]	2.16[d]	0.37[d]	6.61[d]
5	12	0.22[d]	17.76[f]	0.98[c]	1.61[e]	3.98[e]	24.55[e]
6	3	0.48[e]	20.41[f]	0.51[d]	2.62[f]	1.97[f]	25.98[e]
7	15	0.00[c]	129.45[g]	0.03[ab]	2.95[g]	13.38[g]	145.81[f]
113 acc	**min.**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	max.	5.58	243.26	12.74	32.86	22.86	306.79
	mean	0.11	9.10	0.21	0.71	1.23	11.36
	CV%	505.4	390.0	668.2	459.1	308.1	369.4
	Median	0.00	3.29	0.00	0.76	0.00	7.00

* *in cyanidine-3-glucoside equivalent*

Mean values with the same letter in a column are not significantly different at P ≤ 0.05 (Turkey's test).

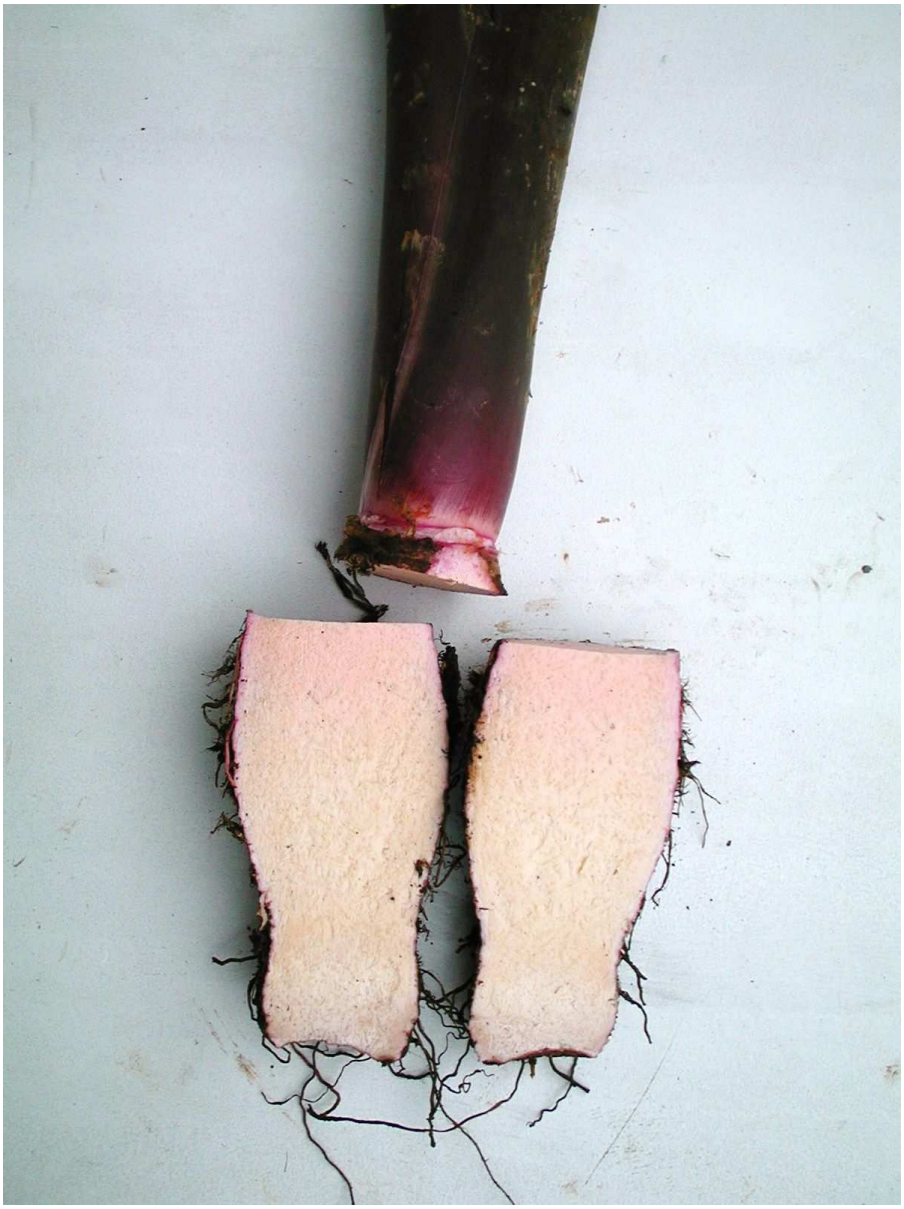

Figure 1: Color gradient along longitudinal section of orange-fleshed corm of taro

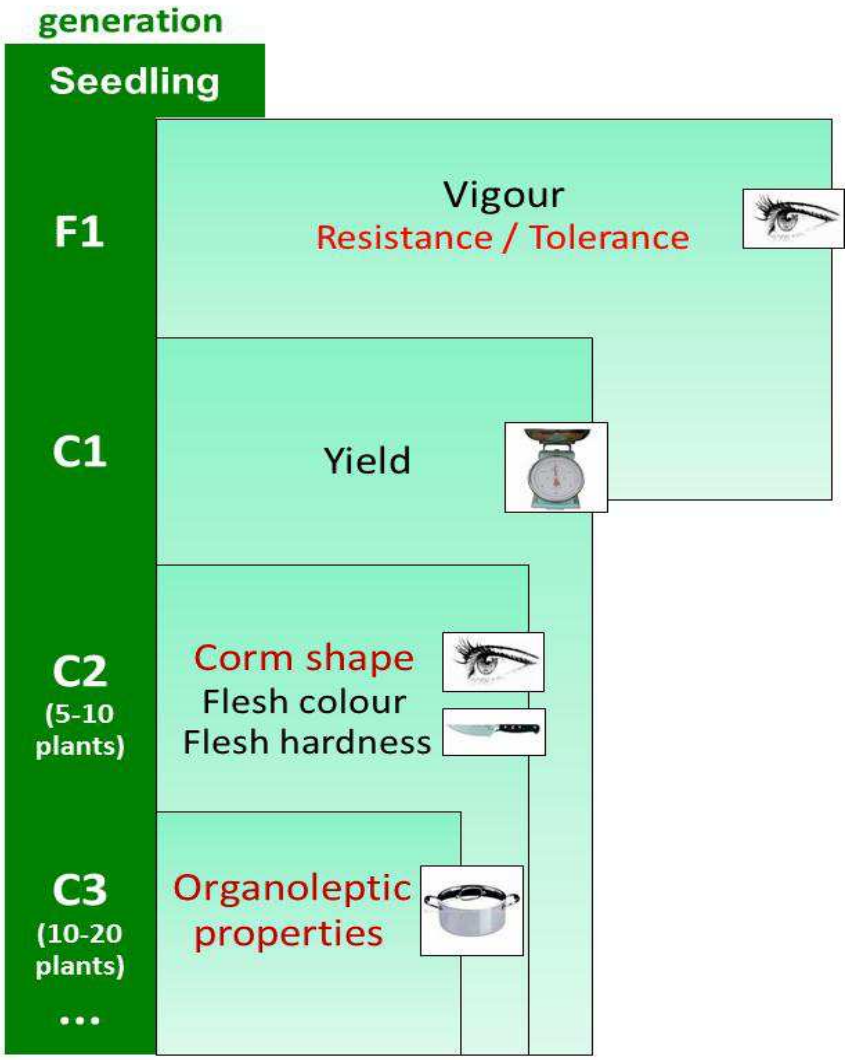

Figure 2: Flow chart diagram of the selection process