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Characterization of taro [*Colocasia esculenta* (L.) Schott] germplasm for improved flavonoid composition and content

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Abstract

The starchy corms of taro (*Colocasia esculenta*) are consumed throughout the tropics and are essential for food security in many developing countries. Taro corms are increasingly processed into fries, chips, flours or flakes in urban areas, and varieties with attractive corm flesh colours are now needed. The identification of flavonoids in taro corms would add value to this crop. The present study developed a high-performance thin layer chromatography (HPTLC) protocol for the high-throughput screening of flavonoids in taro germplasm. Overall, 350 different accessions were analysed including 259 varieties from Vanuatu, one from Vietnam, eight from Thailand, eight from the Philippines, six from Malaysia, two from Japan and 18 from Indonesia. Forty-eight breeding lines (hybrids) including 21 from Vanuatu, 21 from Samoa, four from Hawaii and two from Papua New Guinea were also analysed. Ten flavones, namely luteolin-6-*C*-hexoside-8-*C*-pentoside, schaftoside, luteolin-3′,7-di-*O*-glucoside, homoorientin, isovitexin, orientin, luteolin-4′-*O*-glucoside, luteolin-7-*O*-glucoside, vitexin and apigenin-7-*O*-glucoside, were successfully detected in the corm and are responsible for the attractive yellow colour of the flesh and fibres. Quantitatively, luteolin-6-*C*-hexoside-8-*C*-pentoside and schaftoside were the most important of all the detected flavonoids. However, only 18% of the varieties analysed presented these two compounds and 80% presented poor flavonoid composition. No geographical structure of the variation was detected and the most flavone-rich varieties originated from Vanuatu, Thailand, the Philippines, Malaysia and Indonesia. The compounds detected in the present study were significantly and positively correlated, suggesting that there is potential for fast improvement through controlled crosses, subsequent evaluation of full-sib progenies and cloning of elite individuals.

Keywords: *Colocasia esculenta*; flavonoid glycosides; HPTLC

Introduction

Taro [*Colocasia esculenta* (L.) Schott], a tropical species cultivated for its starchy corm, plays an essential role in food security in many developing countries (Kaushal *et al.*, 2013). Germplasm collections are maintained *ex situ* in the field or *in vitro*, and close to 6000 accessions have been morphologically described (Jackson, 1994). These collections are far from being complete, and it is thought that there are probably more than 15,000 varieties of taro (Rao, 1996). These varieties present remarkable morphological variation for vegetative traits (lamina shape, petiole colours, height of plants, number

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of suckers and stolons) with, however, a narrow genetic base in many countries as they are always introduced and propagated asexually. National germplasm collections are, therefore, assembling limited allelic diversity (Kreike *et al.*, 2004); thus it is essential to characterize, evaluate and exchange germplasm on an international scale to secure breeding efforts. This is crucial in countries where pests (e.g. *Papuana* spp.) and leaf blight (*Phytophthora colocasiae*) are threats to production (Singh *et al.*, 2012).

Breeding programmes in India, Samoa, Hawaii and Papua New Guinea are gradually building resistance to *P. colocasiae*. In Vanuatu, the breeding programme is attempting to improve the corm quality aspects, focusing on flesh colour and its attractiveness (Champagne *et al.*, 2013). Compared with the common whitish/greyish aspect of the corm flesh of many varieties, the yellow and pink colours are favoured by consumers. A HPLC screening of different roots and tubers has revealed that taro is the species with the widest range of flavonoids, with very high concentrations (up to 326 mg/100 g dry weight), but their individual identification was not conducted (Champagne *et al.*, 2011). Taro shoots and leaves are also consumed, and the study of their phenolic composition has identified several flavones and hydroxycinnamic acids (Ferreres *et al.*, 2012). In Japan, HPLC analysis has identified shaftoside, isoschaftoside, orientin, isovitexin, isoorientin, orientin and luteolin-7-*O*-glucoside as the major flavonols, and suggested that taro leaves are a good source of dietary antioxidant (Leong *et al.*, 2010). In Portugal, many flavones have also been identified in taro leaves using HPLC, with luteoline-6-*C*-hexoside present in higher amounts (Ferreres *et al.*, 2012). Qualitative and quantitative differences were observed, but only three varieties were analysed (Gonçalves *et al.*, 2013).

High-performance thin layer chromatography (HPTLC) is an eco-friendly technique that necessitates low volumes of solvents and is increasingly used to screen plant samples for phenolic compounds (Boudesocque *et al.*, 2013; Sagi *et al.*, 2014). Compared with HPLC, HPTLC offers interesting perspectives to screen the numerous varieties of taro. Since there is limited knowledge on the flavonoids present in the corm, the most important part of the plant for human consumption, the objectives of the present study were (1) to analyse with HPTLC 350 different taro varieties originating from distant geographical growing centres, (2) to identify the main flavonoids present in the fresh corms and (3) to develop a method to characterize taro germplasm and identify varieties rich in flavonoids. Implications for germplasm conservation and breeding programmes are discussed.

Materials and methods

Germplasm

All varieties were harvested when plants were 8–10 months old from the VARTC (Vanuatu Agricultural Research and Technical Centre) germplasm collection on the Island of Espiritu Santo, Northern Vanuatu (166°15′ E, 15°23′ S). Accessions were selected for their good eating quality and agronomic performance. All were scored for their corm flesh colour (flesh and fibres) as white, yellow, orange, pink, red, red purple and purple. Overall, 350 different accessions were analysed including 259 local varieties from Vanuatu, one from Vietnam, eight from Thailand, eight from the Philippines, six from Malaysia, two from Japan and 18 from Indonesia. All were *dasheen*-type genotypes (cultivated for their central corm) except the two Japanese varieties that were *eddoes* (cultivated for their side cormels). Forty-eight breeding lines (hybrids) were also analysed including 21 from the Vanuatu taro improvement programme, 21 from Samoa, two from Papua New Guinea and four from Hawaii.

Sample preparation

After harvest, the fresh corms were hand-washed, peeled and a slice of approximately 200 g was cut from the central part of the corm. This slice was hand-grated with a cheese grater, and the resulting purée was wrapped in aluminium foil and labelled. This procedure was standardized for taking a constant preparation time of less than 5 min. Samples were then frozen and kept in a freezer at −10°C until extraction. For each sample, 10 g of frozen corm were ground in a coffee grinder. Two grams of the resulting paste were placed in a 50 ml plastic centrifuge tube with 10 ml methanol–H_2O (7:3, v/v) and hand-shaken. Samples were sonicated for 30 min and centrifuged at 4500 rpm during 10 min. The supernatant was then transferred to 5 ml vials and stored in a fridge at 4°C in the dark until analysis.

Standards and reagents

Apigenin, catechin, epicatechin, isoharmnetin, kaempferol, luteolin and quercetin were purchased from LKT Laboratories Inc. (St Paul, MN, USA). Apigenin-7-*O*-glucoside, luteolin-4′-*O*-glucoside, luteolin-7-*O*-glucoside, luteolin-3′,7-di-*O*-glucoside, schaftoside, vitexin, isovitexin, homoorientin (isoorientin) and orientin were purchased from Extrasynthese (Genay, France). Standard stock solutions were prepared by dissolving the appropriate

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amount of each compound in methanol (1.0 mg/ml). Stock solutions were stored at 4°C in the dark and were stable for several weeks. All solvents were of analytical grade. Ethyl acetate, methanol, acetic acid, formic acid and natural product (NP, 2-aminoethyl diphenyl borinate) were purchased from Sigma-Aldrich (St Quentin, France).

High-performance thin layer chromatography

Analyses were performed on Merck (Darmstadt, Germany) silica gel 60 F_{254} plates (20 × 10 cm), using a Camag (Muttenz, Switzerland) HPTLC system equipped with an automatic thin layer chromatography (TLC) sampler (ATS 4), an automatic developing chamber (ADC 2), a visualizer and a TLC scanner 4 controlled with winCATS software. Standards and sample solutions were applied band-wise (band length 8 mm, delivery speed 250 nl/s, track distance 8.0 mm and distance from the edge 15 mm) at a concentration rate of 10 µl per band. Twenty taro extracts were applied on a single plate. After 30 s of pre-drying, plates were developed at room temperature with ethyl acetate, methanol, acetic acid, formic acid and water (30:1:2:1:3, by vol.) as the mobile phase (10 ml) for a maximum migration distance of 80 mm. The tank interior was vapour-saturated with developing solvent (25 ml) prior to migration. After development, plates were heated at 100°C for 3 min and then immersed in a NP solution (1 g/200 ml of ethyl acetate) for 1 s at speed 5 with an automatic immersion device. After derivation, visual inspection and documentation of the chromatograms were carried out at 366 nm. Bands revealed on the plate were identified by their retention factor (R_f) values, and each individual variety or hybrid (track) was scored for the presence or absence of bands. Plates were then scanned under the following conditions: reflectance mode 366 nm; D2 and W lamp slit dimension 8.00 mm × 0.20 mm; scanning speed 20 mm/s; data resolution 100 µm/step. Peak area measurements (area units; a.u.) were also used to compare the values of individual varieties.

High-performance liquid chromatography

HPLC–DAD–MS/MS analysis was conducted to identify a particular marker (R_f 0.5) because of its abundance and the lack of available commercial standard to verify its identity. Fresh corm samples were frozen and freeze-dried in Santo, Vanuatu, before being shipped to Lyon, France. A 100 mg sample of freeze-dried powder was extracted with 1 ml methanol–water (1:1), sonicated for 15 min and centrifuged at 20,238 ***g*** for 10 min. A second extraction was conducted with 1 ml methanol. Supernatants were pooled and dried using a rotational vacuum concentrator (Labconco, MO, USA) and dissolved in methanol–water (80:20) + 0.05% TFA (trifluoroacetic acid) at a final concentration of 10 mg/ml. Samples were analysed by reverse-phase HPLC using an Agilent 1100 series HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a gradient pump, a cooled autosampler and a UV–vis photodiode-array detector (210 and 600 nm). Samples were injected through a 5 µm column (250 × 4.6 mm, Kromasil C18) coupled to a guard column (10 × 4.6 mm, Kromasil C18) at 30°C. The mobile phase was aqueous acetic acid (0.4%, v/v, eluent A) and acetonitrile (eluent B) at 1 ml/min. After photometric analysis, chromatographed material was subjected to mass analysis by ESI-QTOF (electrospray ionization quadrupole time of flight, Agilent 6530) in positive and negative modes. The ESI source was optimized as follows: scan spectra from *m/z* 100 to 2000; capillary voltage 3.5 kV; fragmentor 120 V; fixed collision-induced dissociation energy 20 eV. Nitrogen was used as the nebulizing gas with a flow rate of 11 l/min and a temperature of 310°C at 40 psi. Compounds were identified by the analysis of their UV, HRMS and MS/MS characteristics using MassHunter Quantitative Analysis software (Agilent Technologies).

Statistical analyses

Data resulting from scored absence (0) or presence (1) of individual bands, as well as from the scanning of peak areas at 366 nm, were entered into Excel spreadsheet format. Statistical analyses were performed using XLStat software (Microsoft). Cluster analysis (unweighted pair group method with arithmetic mean; UPGMA) was conducted using the DARwin software (Perrier and Jacquemoud-Collet, 2006).

Results

No less than 25 HPTLC bands were detected at 366 nm and scored for their presence or absence in the extracts of 350 varieties. Based on previous studies, 16 commercial standards were applied on the plates next to the taro varieties. Only nine molecules had R_f and post-NP-staining colour that were similar to taro substances: schaftoside, luteolin-3′,7-di-*O*-glucoside, homoorientin, isovitexin, orientin, luteolin-4′-*O*-glucoside, luteolin-7-*O*-glucoside, vitexin and apigenin-7-*O*-glucoside (Fig. 1). A scan of individual standards was conducted to obtain their UV spectra. These were very similar as far as their λ_{max} were concerned (Fig. 2) and matched the UV spectra of taro substances with similar R_f, therefore confirming

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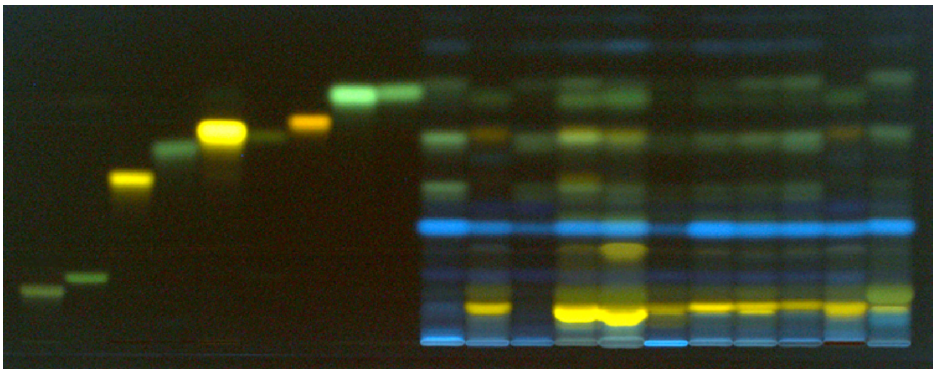

Fig. 1. HPTLC standards (left) and chromatograms of different taro varieties (right). Schaftoside, luteolin-3′,7-di-*O*-glucoside, homoorientin, isovitexin, orientin, luteolin-4′-*O*-glucoside, luteolin-7-*O*-glucoside, vitexin and apigenin-7-*O*-glucoside. The lower yellow band (R_f 0.5) was identified by HPLC–DAD–MS/MS as luteolin-6-*C*-hexoside-8-*C*-pentoside.

their identity. A bright yellow band located at R_f 0.5 was also detected but with no corresponding standard available. The HPLC–DAD–MS/MS analysis identified this compound as luteolin-6-*C*-hexoside-8-*C*-pentoside (L). Altogether, ten molecules were, therefore, successfully identified and scored.

After band scoring, the 350 taro varieties were grouped into 16 distinct chemotypes based on their HPTLC profiles (Table 1). It appeared that there was no geographical structure of the variation, with chemotypes 4, 6, 10, 13 and 14 being widely distributed and the large sampling from Vanuatu exhibiting 13 chemotypes out of a total of 16. Tremendous variation was observed between varieties for the total number of bands detected. It ranged from only three bands in one hybrid from Samoa (chemotype 9) to 21 bands in 22 varieties from Vanuatu, three varieties from Indonesia, one hybrid from Papua New Guinea and one from Vanuatu (chemotypes 12 and 13).

Cluster analysis conducted on the data matrix composed of the 16 chemotypes (Table 1) revealed four major clusters (Fig. 3). Cluster 1 was composed of chemotypes 12 to 16, which presented all ten detected flavonoids, except chemotype 16 which lacked luteolin-3′,7-di-*O*-glucoside, apigenin-7-*O*-glucoside and vitexin. These chemotypes were rich in various flavonoids and represented 41 varieties and 11 hybrids with different geographical origins (Table 1). All the varieties

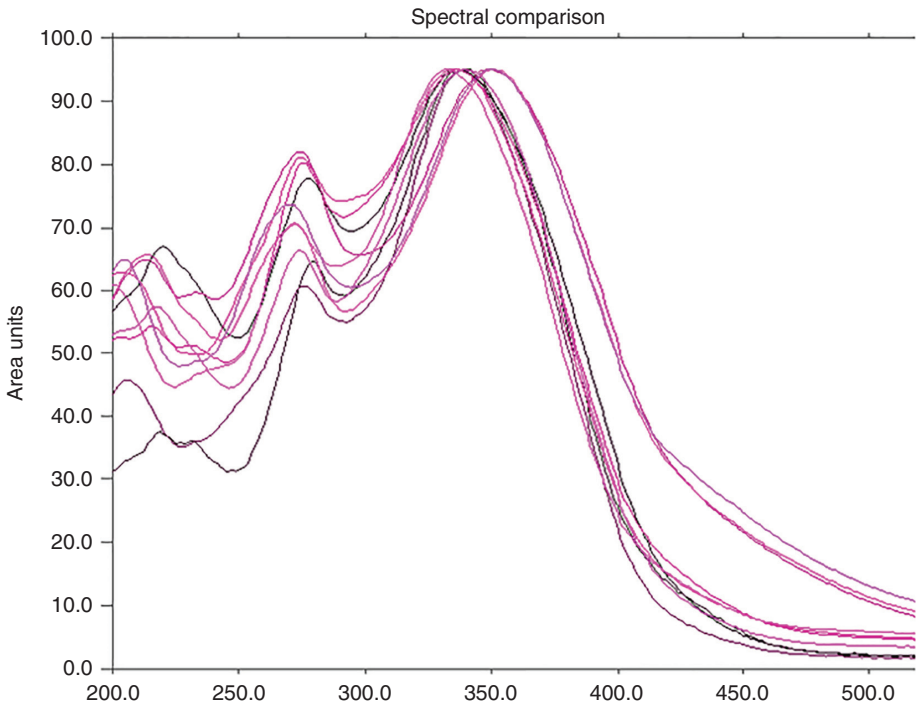

Fig. 2. UV spectra of schaftoside, luteolin-3′,7-di-*O*-glucoside, homoorientin, isovitexin, luteolin-4′-*O*-glucoside, orientin, luteolin-7-*O*-glucoside, vitexin and apigenin-7-*O*-glucoside.

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Table 1. Number of varieties and hybrids per country presenting HPTLC bands (R_f values recorded at 366 nm)

	No. of varieties							No. of hybrids			
Chemotype	VU	VN	TH	PH	MY	JP	ID	SM	PG	HI	VU
1	22						2				
2	4			1							
3	2										
4	111		6	2	2	1	5	7		2	5
5	20			4							1
6	37						6	5		2	7
7	18										1
8	6										
9								1			
10	10		1			1		1			1
11								3			
12	3										
13	19						3		1		1
14	4			1	1		2	4	1		3
15	3										
16		1	1		3						2

	R_f values of scored bands																								
		L	S	L3					H	I					O	L		L7			A7	V			
Chemotype	0.01	0.05	0.06	0.07	0.08	0.09	0.16	0.22	0.24	0.27	0.33	0.35	0.39	0.46	0.48	0.50	0.51	0.54	0.57	0.61	0.65	0.71	0.76	0.78	0.80
1	1	0	0	0	1	1	1	0	0	1	0	0	0	0	1	1	0	1	1	0	0	1	1	1	1
2	1	0	0	0	1	0	1	0	0	1	0	0	0	0	1	1	0	1	1	0	0	0	1	1	1
3	1	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	1	1	0	0	0	1	1	1
4	0	0	0	0	1	1	1	0	0	1	1	0	0	0	1	1	0	1	1	0	0	1	1	1	1
5	0	0	0	0	1	0	1	0	0	1	0	0	0	0	1	1	0	1	1	0	0	0	1	1	1
6	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	1	1	0	0	0	1	1	1
7	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	1
8	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	1
9	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
10	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
11	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
12	1	1	1	1	0	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
13	0	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
14	0	1	1	1	0	0	1	0	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1
15	0	1	1	1	0	0	1	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
16	0	1	1	0	0	0	1	0	1	1	0	0	0	1	1	1	0	1	1	0	0	0	1	1	1

VU, Vanuatu; VN, Vietnam; TH, Thailand; PH, Philippines; MY, Malaysia; JP, Japan; ID, Indonesia; SM, Samoa; PG, Papua New Guinea; HI, Hawaii; L, luteolin-6-*C*-hexoside-8-*C*-pentoside; S, schaftoside; L3, luteolin-3′,7-di-*O*-glucoside; H, homoorientin; I, isovitexin; L4, luteolin-4′-*O*-glucoside; O, orientin; L7, luteolin-7-*O*-glucoside; A7, apigenin-7-*O*-glucoside; V, vitexin.

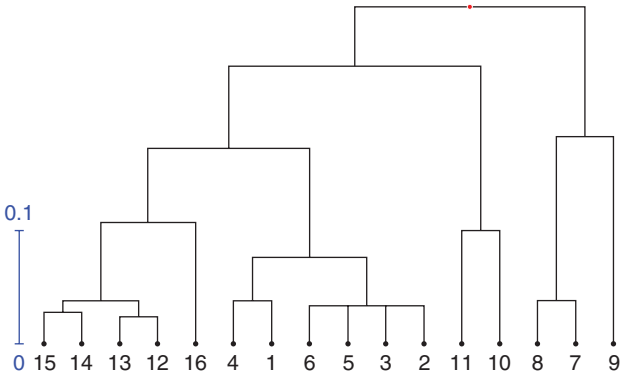

Fig. 3. UPGMA (Dice) of the data matrix composed of 16 chemotypes × 25 scored HPTLC bands reveals four major clusters. There is no geographical structure of the variation. From left to right, cluster 1 (chemotypes 15, 14, 13, 12 and 16) assembles coloured fleshed varieties presenting up to 21 bands and ten identified compounds, but making only 15% of the total number of the varieties analysed. Cluster 2 assembles varieties with white corm flesh and 80% of the varieties analysed. Cluster 3 (chemotypes 11 and 10) and cluster 4 (chemotypes 8, 7 and 9) are composed of varieties poor in flavonoids with very few detected compounds.

and hybrids present in this cluster displayed yellow to purple corm flesh and fibre colours. They, however, represented only 15% of the total number of accessions analysed (350).

Cluster 2 was composed of chemotypes 1 to 6 and was characterized by the presence of isovitexin, luteolin-4′-*O*-glucoside, luteolin-7-*O*-glucoside and orientin, and by the absence of the six other identified flavonoids. This cluster composed of the majority (80%) of the analysed taro varieties (249 accessions) and hybrids (30 accessions) and was geographically widely distributed. Most of the varieties and hybrids (198 accessions) from this cluster displayed white corm flesh and fibres and 81 had light yellow corm flesh and fibres. A purple band at R_f 0.1 (appearing in white light and at 366 nm just above the loading zone) was obviously not correlated with the corm and fibre flesh colours.

Cluster 3 was represented by only 12 varieties from Vanuatu (10), Thailand (1) and Japan (1) and five hybrids with very few flavonoids. All these varieties displayed white corm flesh and fibres. Of the ten identified substances, only isovitexin could be detected and only five unidentified bands were present. These accessions appeared to be very poor in flavonoids. Cluster 4 was composed of 24 varieties and two hybrids from Vanuatu and Samoa. Only seven bands out of 25 could be detected with luteolin-4′-*O*-glucoside and orientin as identified substances. These varieties were also poor in flavonoids and presented white and pink corm flesh and fibre colours.

Principal component analysis (PCA) conducted on the data matrix containing 350 varieties × peak areas (in a.u.) is shown in Fig. 4. Chromatograms of the extracts scanned at 366 nm indicated that schaftoside and luteolin-3′,7-di-*O*-glucoside were not sufficiently separated to obtain individual peak areas for these two compounds. Their peak values corresponded most probably to a combination of both. It was also possible that a third compound was also present in this green band located at R_f 0.06 as the HPLC–DAD–MS/MS analysis also detected isoschaftoside. However, in the absence of a commercial standard for this compound, it was not possible to confirm its identity on the plates. The PCA was, therefore, conducted on nine rather than on the ten identified compounds. The majority of the varieties were located on the left-hand side of axis 1 and corresponded to clusters 2, 3 and 4 of the dendrogram (Fig. 4). Varieties rich in flavonoids (cluster 1) were clearly separated from others on axis 1 with very variable contents of the nine individual compounds (statistical elements). Coefficients of correlation (Pearson) among the quantitative values (peak areas in a.u.) of the nine compounds are presented in Table 2. It appeared that accumulations of most compounds (measured by densitometry) were significantly and positively correlated with each other.

Discussion

HPLC analysis is the most commonly used technique to study flavonoids and colours of plant parts (Zhu *et al.*, 2012). However, the analytical protocols are rather time consuming, and in most studies, a limited sample of

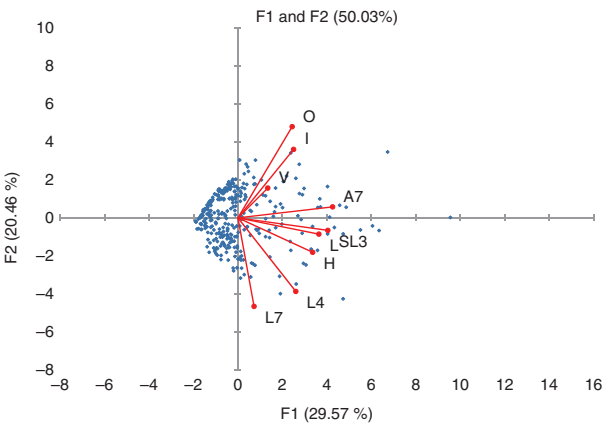

Fig. 4. PCA conducted on the data matrix composed of 350 varieties × 9 identified compounds (peak areas in a.u.). Varieties rich in flavonoids (cluster 1 of the UPGMA) are located on the right of the first axis and those poor in flavonoids (clusters 2, 3 and 4) are on the left. The nine compounds (variables) were positively correlated. O, orientin; I, isovitexin; V, vitexin; A7, apigenin-7-*O*-glucoside; S, schaftoside; L3, luteolin-3′,7-di-*O*-glucoside; L, luteolin-6-*C*-hexoside-8-*C*-pentoside; H, homoorientin; L4, luteolin-4′-*O*-glucoside; L7, luteolin-7-*O*-glucoside.

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Table 2. Coefficients of correlation (Pearson, *n*) between flavonoids (peak areas measured at 366 nm, in a.u.)

Variables	L	S + L3	H	I	O	L4	L7	V
S + L3	0.583**							
H	0.251**	0.366**						
I	0.146	0.189**	0.089					
O	0.209**	0.253**	0.328**	−0.017				
L4	0.105	0.128	0.096	0.521**	−0.126			
L7	0.074	−0.001	0.221**	−0.103	0.422**	−0.298		
V	0.378**	0.447**	0.356**	0.286**	0.323**	0.406**	0.028	
A7	0.014	0.017	0.153	0.149	0.081	0.255**	−0.025	0.096

L, luteolin-6-*C*-hexoside-8-*C*-pentoside; S, schaftoside; L3, luteolin-3′,7-di-*O*-glucoside; H, homoorientin; I, isovitexin; O, orientin; L4, luteolin-4′-*O*-glucoside; L7, luteolin-7-*O*-glucoside; V, vitexin; A7, apigenin-7-*O*-glucoside.
**Significant at $P < 0.01$ and $n > 250$, r value at 1% = 0.162.

varieties, often less than 50, is analysed, although germplasm collections host several hundreds of accessions (Morris *et al.*, 2014). It is consequently difficult to use HPLC for germplasm characterization of taro genetic resources that are composed of thousands of varieties distributed in many different countries. For taro leaves, HPLC studies of two varieties revealed similar qualitative profiles with minor quantitative differences (Leong *et al.*, 2010; Ferreres *et al.*, 2012; Gonçalves *et al.*, 2013). Luteolin-6-*C*-hexoside is the most important compound, and several luteolin and apigenin glycosides have also been identified. Our study detected these glycosides in the taro corm, which represents the most consumed part of the plant, and the use of HPTLC allowed the screening of a very large sampling of varieties (350) from different geographical origins. In the case of taro, our results indicate that conclusions based on the analysis of a few varieties could be misleading as there is tremendous qualitative and quantitative variation. Whether this pattern also applies to other root and tuber crop or plant species remains to be studied; however, it suggests that flavonoid analysis conducted on a few genotypes cannot be conclusive to characterize a species. A broad sample composed of distant genotypes is needed to assess the extent of the variation.

HPTLC is not as resolutive as HPLC for the accurate separation of individual compounds. It is, therefore, possible that a single HPTLC band corresponds to two or three different molecules insufficiently separated on the plate. In our case, schaftoside and luteolin-3′,7-di-*O*-glucoside were not well separated and were probably hiding other molecules (e.g. isoschaftoside) behind their respective bands. Also, HPLC allows for the detection of a greater number of molecules per run, while HPTLC has a comparatively about three to four more limited output. The HPLC analysis of taro leaves, for example, detected 41 phenolic metabolites (Ferreres *et al.*, 2012). In the present study of taro corms, we detected 25 distinct bands (metabolites), and this can be considered as a great number of HPTLC bands for a single plate (Sagi *et al.*, 2014). The attractiveness of the HPTLC technique comes from its cost-efficiency and high-throughput performance as 20 varieties can be analysed in a single plate in less than 1 h with limited volumes of solvents (35 ml per plate in our case). As there are several thousands of varieties of taro, HPTLC appears as a promising tool for germplasm characterization. There is, however, a major technical constraint due to the limited access to fresh corm samples. The present protocol necessitates for proper flavonoid analysis, either to have access to frozen purées or to freeze-dried samples. This part of our protocol needs to be further improved before HPTLC can be used on a larger geographical scale.

Also, our sample is not representative of taro diversity as 259 varieties out of a total of 350 originated from different islands of Vanuatu. The genetic base of taro in Vanuatu is known to be genetically narrow compared with the allelic diversity found in Southeast Asia (Kreike *et al.*, 2004). The fact that significant chemotype variation exists in Vanuatu indicates that these molecules are most probably controlled by sets or genes that are not linked to the markers used in previous DNA studies. Molecular markers have been widely used to characterize taro germplasm collections in an attempt to rationalize their preservation and to facilitate breeders' access to genetically distant parents. Isozymes and ribosomal DNA have been used to study the variation in taro from China, Taiwan and Japan (Matsuda, 2002). RAPD (random amplified polymorphic DNA) analysis results generally agree with isozyme groupings (Irwin *et al.*, 1998), and AFLP (amplified fragment length polymorphism) markers have revealed greater diversity in Southeast Asia than in the Pacific (Kreike *et al.*, 2004). Stratification of collections using AFLP analysis did not correlate with morphotypes but was found useful to detect duplicates (Quero-García *et al.*, 2004). AFLP and DArT (diversity array technology) markers also confirmed traditional knowledge and the three sources of genetic diversity known to farmers: clonal introductions; selection of

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somatic mutations; volunteers resulting from sexual recombination (Caillon *et al.*, 2006; VandenBroucke *et al.*, 2015). SSRs have confirmed the conclusion revealed by isozymes, RAPDs, AFLPs and DArTs (Sardos *et al.*, 2012) so that all markers revealed a clear geographical structure of the allelic diversity, with different countries hosting unique alleles, resulting in patterns of isolation by distance. Surprisingly, there are no correlations between molecular markers and the two botanical varieties (var. *esculenta* and var. *antiquorum*), and markers could not differentiate the two morphological types of varieties (i.e. *dasheen* cultivated for its main corm and *eddoe* cultivated for its side cormels). They did not differentiate either particular morphotypes with or without stolons, for example (Noyer *et al.*, 2004).

For breeding purposes, DNA markers have shown that different taro genepools have to be combined to broaden the base of national programmes. In Oceania, local varieties are the result of very ancient selection focusing on *dasheen*-type corm quality and yield, but these varieties are highly susceptible to taro leaf blight (Singh *et al.*, 2012). In Asia, co-evolution with *P. colocasiae* has produced resistant genotypes, but local varieties present numerous suckers and stolons and many varieties are *eddoe* type cultivated for their cormels. It is now accepted that taro breeding programmes should use varieties from diverse and distant geographical origins. Crosses between varieties from the same country do not produce a very diverse offspring, slowing down the selection process (Quero-García *et al.*, 2009). As taro breeding is based on parents selected on their *per se* values, accurate characterization of the germplasm is of utmost importance. Taro genetic maps have been produced and revealed putative QTLs for corm yield and corm dimensions, whereas a major dominant gene, responsible for the yellow colour of the corm flesh, has also been identified (Quero-García *et al.*, 2006). It is unknown, however, whether this gene is related to the biosynthesis of flavonoid glycosides detected in the present study that are responsible for the bright yellow colour of the corm flesh. Although a significant investment has been put into studies of DNA diversity, the impact has not been as great as might have been hoped.

Many studies have shown that some flavonoids present antioxidant properties as well as anti-inflammatory and neuroprotective effects. It is, therefore, interesting to attempt to increase their content and composition via conventional breeding to improve the nutritional value of taro. Apigenin and luteolin, and their naturally occurring glycosides formed by their combination with sugars, have well-documented health benefits (Verbeek *et al.*, 2004; Lamy *et al.*, 2008; Soto-Vaca *et al.*, 2012; Bumke-Vogt *et al.*, 2014; Wu *et al.*, 2015). The improvement of polyphenol composition is also attractive for managing pest pressure (Treutter, 2010). For example, schaftoside and isoschaftoside (apigenin glycosides) have nematicidal properties against root-knot nematodes (Du *et al.*, 2011) and these properties could be useful in taro breeding.

Our results indicate the absence of a single geographical origin for flavonoid-rich varieties. Chemotypes 12 to 16, for example, are present in Vanuatu, Malaysia, the Philippines, Vietnam and Indonesia. It is, therefore, possible to select parents from distant origins with high and diverse flavonoid content and to compose a sub-population rich in apigenin and luteolin glycosides for a recurrent selection strategy. The strategy being based on accumulation of desired alleles through successive reproduction cycles and the fact that the compounds detected in the present study are significantly and positively correlated suggest that there could be a potential for fast improvement through controlled genetic recombination, subsequent evaluation of the resulting progenies and cloning of elite individuals to be used as parents in future cycles. Breeding for flavonoid concentration and composition in taro could lead to more attractive corm flesh colours for the urban consumers, but also to health benefits for populations who use this plant as a staple.

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