Genet Resour Crop Evol (2018) 65:1699–1709
https://doi.org/10.1007/s10722-018-0647-0

RESEARCH ARTICLE

Secondary metabolites content may clarify the traditional selection process of the greater yam cultivars (*Dioscorea alata* L.)

V. Lebot · R. Malapa · K. Abraham · T. Molisalé · N. Van Kien · B. Gueye · J. Waki

Received: 6 December 2017 / Accepted: 2 May 2018 / Published online: 15 May 2018

Abstract *Dioscorea alata* L. is one of the most widely grown and economically important yam species. Hundreds of accessions are maintained ex situ in germplasm collections and have been characterized with descriptors but new tools are still needed to assess tuber chemical composition. The objectives of the present study were to analyze saponins and catechins profiles in 388 *D. alata* cultivars (landraces) from distant geographical sources (Nigeria, Vietnam, Papua New Guinea and Vanuatu) and to compare them with those of 162 selected hybrids. The relationships between these compounds and tuber flesh oxidation and browning were also studied in order to understand their possible role in the ancient cultivars selection process. Dioscin and gracillin, the most documented *Dioscorea* saponins, were absent among the 550 *D. alata* cultivars and hybrids analyzed using HP-TLC. Two saponins and four catechins were quantitated, including epicatechin. Mean total catechins and saponins values were very low for most cultivars and higher mean values were found in hybrids. Correlation coefficients revealed possible relationships between total saponins and catechins contents with speed of oxidation, presence of mucilage and flour colour. Distribution of cultivars values within each country indicate that these were mostly selected for their low saponins and catechins contents through simple visual assessment. Metabolite profiles can be used to improve the phenotyping efficiency of *D. alata* hybrids generated through conventional breeding.

R. Malapa—Deceased

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10722-018-0647-0) contains supplementary material, which is available to authorized users.

V. Lebot (✉)
CIRAD, UMR AGAP, P.O. Box 946, Port-Vila, Vanuatu
e-mail: lebot@vanuatu.com.vu

R. Malapa · T. Molisalé
VARTC, P.O. Box 231, Luganville, Santo, Vanuatu

K. Abraham
ICAR-CTCRI, Thiruvananthapuram, Kerala 695017, India

N. Van Kien
PRC, An Khanh, Hoai Duc, Hanoi, Vietnam

B. Gueye
IITA, PMB 5320, Oyo Road, Ibadan 200001, Oyo State, Nigeria

J. Waki
NARI, P.O. Box 4415, Lae, Morobe Province, Papua New Guinea

Keywords Anti-nutritional compounds · Catechins · *Dioscorea alata* · Domestication · Saponins · Tuber quality

Introduction

The greater yam (*Dioscorea alata* L., section Enantiophyllum) is the most economically important yam species (Degras 1993). It is also the most widely grown because of its good organoleptic characteristics (Dufie et al. 2013), ease of cultivation and remarkable agronomic performances with high yield potential. Its exact geographical area of origin is unknown. A comprehensive analysis of morpho-agronomic and intra-specific variation conducted on 235 *D. alata* cultivars (landraces) concluded that the most bizarre and least improved types were found in New Guinea along with other types found elsewhere. It was therefore concluded that the centre of variation of *D. alata* is first New Guinea and secondly Indonesia (Martin and Rhodes 1977). It is possible that thousands of ancient cultivars of *D. alata* exist around the tropics. The most interesting ones were selected by the USDA in Mayaguez, Puerto Rico, and were widely distributed in the 1970s (Martin 1976). Nowadays, diverse germplasm collections are found in India (CTCRI, Trivandrum, Kerala), Nigeria (IITA, Ibadan), Vanuatu (VARTC, Santo), Vietnam (PRC, Hanoi), and in the West Indies (CIRAD/INRA, Guadeloupe). Hundreds of accessions are maintained ex situ and have been characterized with morpho-agronomic descriptors. Many local cultivars present attractive tuber shape and flesh colours and excellent taste after cooking but are often very susceptible to anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. et Sacc.), the major threat for *D. alata* (Asiedu and Sartie 2010).

Breeding programmes have been initiated in Guadeloupe, India, Nigeria and Vanuatu, and hundreds of hybrids are now being evaluated, mostly for tolerance to anthracnose (Abraham et al. 2013). In practice, as *D. alata* is dioceous, seeds obtained from controlled crosses or in polycross blocks result in hybrids which are evaluated through successive clonal generations. Tremendous variation occurs in hybrids especially for tuber shape, flesh color and oxidation. Tuber flesh oxidation is often associated with off-flavors, including bitterness, and accounts for undesirable colors after cooking. Cultivars with high anthocyanin content are often prone to oxidation (Martin 1976). The tuber flesh of the most appreciated cultivars is always very white and free of oxidation. Different phenols may contribute to the extent of flesh whiteness and thus the tuber flesh should be low in pigments (Egesi et al. 2003).

Polyphenols have been detected in *D. alata* (Ozo et al. 1984). In some cultivars they are found in significant proportions and are considered as anti-nutritional factors (Ezeocha and Ojimelukwe 2012). Several studies, however, reported the antioxidant properties of *D. alata* with significant differences between cultivars (Dilworth et al. 2012). They reveal that *D. alata* represent a potential source of natural antioxidants and may be a good candidate for pharmaceutical plant based products (Narkhede et al. 2013; Dey et al. 2016). It has been hypothesized that its antidiabetic and hypoglycemic activities are most probably linked to its flavonoids content (Maithili et al. 2011; Olubobokun et al. 2013). Catechin has been identified as the main flavonoid but has been suspected to contribute to tuber flesh browning as it is a good substrate for polyphenol oxidase and can undergo oxidative polymerization to form tannins (Akissoe et al. 2005). So, the presence of catechins could be interesting for a biofunctional food but they also represent a constraint for commercial purposes because they are precursors of the brown compounds formed when processing in puree or flour.

Saponins have been isolated from *D. alata* and they are considered as antinutritional compounds (Ezeocha and Ojimelukwe 2012). They are associated with bitter taste and are suspected to contribute to poor yam tuber quality (Coursey 1967). Dioscin is an interesting bioactive saponin found in wild *Dioscorea* spp. (Zhang et al. 2015) and in *D. cayenensis* Lam, an African species of the Enantiophyllum section (Sautour et al. 2007). It has been detected in *D. alata* cultivars from China (Wu et al. 2016). Diosgenin, an aglycon of dioscin obtained after acid hydrolysis of the raw tuber pulp, is a precursor for steroids with high commercial value (Jesus et al. 2016) and has also been identified in a *D. alata* cultivar from India (Shah and Lele 2012). Other studies, however, concluded that dioscin was absent in *D. alata* cultivars from the IITA collection in Nigeria (Kwon et al. 2015). It is therefore unclear if the presence of dioscin (and diosgenin) is

genetically controlled, with some cultivars presenting this saponin while it is absent from others, or if these discrepancies are due to analytical artifacts and taxonomic misidentifications.

The objectives of the present study were: (i) to screen 388 *D. alata* landraces cultivars from distant geographical sources (Nigeria, Vietnam, Papua New Guinea and Vanuatu) and to analyze their variation in catechins and saponins profiles, (ii) to compare the same 388 cultivars with 162 selected hybrids profiles, (iii) to study the possible relationships between these compounds and tuber flesh oxidation and browning, and (iv) to understand their possible role in the ancient cultivars selection process to see how these metabolite profiles could be used to improve the phenotyping efficiency of hybrids generated through conventional breeding.

Materials and methods

Plant materials

The geographical origins of the tuber samples analysed are presented in Table 1. In Vanuatu, local cultivars of *D. alata* have been collected from throughout the archipelago over the last two decades and are maintained, characterized and field evaluated annually by the Vanuatu Agricultural Research and Technical Centre (VARTC) on the island of Santo (15°23′S and 166°51′E, ~ 80 m above sea level). Locally selected hybrids from India (India OP) originated from half-sib progenies introduced to Vanuatu in 2002 as true botanical seeds. Several hundred seeds were germinated at Tagabé experimental station, Efaté, and were field evaluated. Interesting genotypes were selected on (i) sex (female), (ii) tolerance to anthracnose, (iii) compact tuber shape, (iv) no tuber flesh oxidation and no excessive mucilage. Selected hybrids, obtained after four successive clonal generations were propagated with minisets in VARTC, Santo for field evaluation. Finally, 29 genotypes were selected in 2011 and are used as female plants in the VARTC breeding programme. Hybrids of *D. alata* were produced in VARTC through controlled pollinations between these 29 Indian female hybrids and Vanuatu male genotypes (local cultivars). Several hundred hybrids have been produced in 2013–2015 but the majority were eliminated based on their susceptibility to anthracnose, poor tuber shape, and fast oxidizing tuber flesh. Overall, 132 hybrids were selected and are still being evaluated (Table 1). In August 2016, all Vanuatu accessions (cultivars and hybrids) were grown in a common field, on tropical brown soil, to minimise variation due to environmental factors. Accessions were planted with seed tubers of 300–500 g per plant (1 × 1 m spacing) and staked on poles of two meters high. Vanuatu accessions and hybrids were scored with IPGRI morpho-agronomic descriptors no 7.6.34 (oxidation after cutting: 1 = < 1 min, 2 = 1–2 min, 3 = > 2 min, 4 = none) and no 7.6.36 (amount of mucilage released by cut tuber: 3 = low, 5 = intermediate, 7 = high) (IPGRI/IITA 1997). In Nigeria, Vietnam and Papua New Guinea, diverse local cultivars were selected from the field germplasm collections maintained ex situ in Ibadan (IITA), Hanoi (PRC) and Lae (NARI) (Table 1) and dry matter samples were prepared. The vernacular names of the cultivars analysed and their detailed geographical origins were recorded in the excel spread sheet (supplementary material).

Table 1 Geographical origin of the accessions and samples analyzed

Origin	Germplasm collection	Accessions	Geographical coverage	Type
Vanuatu	VARTC, Santo	216	13 islands	Cultivars
Papua New Guinea	NARI, Lae	12	1 province	Cultivars
Vietnam	PRC, Hanoi	131	32 provinces	Cultivars
Nigeria	IITA, Ibadan	29	7 countries	Cultivars
India OP	VARTC, Santo	30	–	Hybrids
Hybrids INxVU	VARTC, Santo	132	–	Hybrids
Total		550		

Preparation of methanolic extracts

Samples from Vanuatu, Papua New Guinea and Vietnam were prepared following the same protocol. After harvest, a tuber sample representative of the accession was washed under cool running water and peeled with a knife. A slice of approximately 200 g was cut through the central section of the tuber and processed into "French-fries" shape of 1 × 1 cm section. For each accession, the fresh weight was recorded and the sample was oven-dried at 60 °C till constant weight. Dry matter samples were kept in zip-lock bags and sent to the Department of Agriculture Laboratory in Port-Vila, Efaté, Vanuatu. Samples were then processed in fine flour using a coffee grinder (SEB, Prep'Line 850, Dijon, France) and were then preserved into plastic bags at room temperature. In IITA (Ibadan, Nigeria), two samples per accession, originating from two different tubers of the same plant, were prepared following the same procedure but were freeze dried using a freeze drier (Christ Beta: 1–8 LD Plus, Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany) before shipment to Vanuatu for further analysis. After grinding, the flour colour of all samples was assessed visually following a gradient from light to dark (1 = white, 2 = cream, 3 = beige, 4 = yellow, 5 = brown, 6 = dark brown). For each sample, 10 g of flour were transferred to a 50-mL polypropylene centrifuge tube (CellStar Tubes, Greiner Bio-One GmBH, Frickenhausen, Germany), and 20 mL of methanol (95%) were added. The tubes were hand shaken during 1 min and then sonicated in a water bath for 10 min and centrifuged at 4500 rpm for 10 min in a Universal 32 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). The sample preparation took less than one hour for eight samples corresponding to the maximum number of samples accepted by the centrifuge. After centrifugation, the supernatant was transferred to 9-mm-wide opening screw thread vial of 2 mL in glass (Chromacol™, Thermo Fisher™, USA). Vials were stored in a refrigerator at 4 °C until analysis.

Standards and reagents

Dioscin standard was purchased from Chromadex (Irvine, Ca, USA). Gracillin standard was purchased from CarboSynth (Compton, Berkshire, UK). Catechin and Epicatechin standards were purchased from ExtraSynthèse (Genay, France). Standard stock solutions were prepared by dissolving 1.0 mg/mL in methanol. Stock solutions were stored at 4 °C in the dark and were stable for several months. All solvents were of analytical grade. Acetone, chloroform, ethyl acetate, methanol, toluene, formic and acetic acids, sulphuric acid and anisaldehyde, were purchased from Sigma-Aldrich (St Quentin, France). The anisaldehyde sulphuric reagent was prepared by dissolving 1 mL of anisaldehyde in 170 mL methanol, 10 mL sulphuric acid and 20 mL acetic acid. It was kept in the fridge and prepared again as soon as it was not colourless.

High-performance thin layer chromatography

Analyses were performed on Merck (Darmstardt, Germany) silica gel 60 F_{254} plates (glass plates 20 × 10 cm, reference 1.05642.0001), using a Camag (Muttenz, Switzerland) system equipped with an automatic TLC sampler (ATS4), an automatic developing chamber (ADC2), a visualiser and a TLC scanner 4. These machines were controlled on-line with winCATS™ software (Camag, Switzerland). Standards and sample solutions were applied band wise on the plates with a band length of 8 mm at 250 nL/s delivery speed, at a concentration rate of 5 µL per band for phenolics, catechins and saponins. Twenty extracts were applied on a single plate. As *D. cayenensis* Lam. (an African Enantiophyllum species) has already been documented for its saponins (Sautour et al. 2007), eight cultivars of *D. cayenensis* were compared on the same plate with *D. alata* and *D. nummularia* Lam. cultivars (both belonging to the Enantiophyllum section) to identify saponins bands. After 30 s of pre-drying, plates were developed at room temperature. The mobile phase was toluene: ethyl acetate: formic acid (3:6:1, v/v, 10 mL) for a maximum distance of 80 mm. For post-chromatographic derivatization, the plate was automatically immersed into the anisaldehyde reagent with the TLC Immersion Device III (Camag) using a vertical speed of 5 cm/s and 1 s immersion time and heated on the TLC Plate Heater III (Camag) at 110 °C for 5 min. Digital images of the plates were documented in white light by the TLC Visualizer documentation system equipped with a high-resolution 12 bit CCD digital camera (Camag). For all plates, images were captured with an exposure time of 35 ms. Two saponins and

four catechins were scanned after derivatization at 440 nm. All scans were done with D2 and W lamp, slit dimension 8.00 mm × 0.20 mm, scanning speed 20 mm/s, data resolution 100 µm/step.

Statistical analyses

Peak purity tests were done by comparing UV spectra and λmax absorption (nm) of the individual compounds (dioscin, gracillin, catechin and epicatechin) in standard and sample tracks. For determination of the linearity curve, different amounts of stock solutions (0.1, 0.2, 0.3, 0.4, 0.5 µL) of each standard were applied on HP-TLC plates that were developed with the same mobile phase as described above and scanned at 440 nm after deritivization. Linear ranges were computed using the least squares method. Repeatability was confirmed by applying five repetitions of each standard at five different concentration levels (0.1, 0.2, 0.3, 0.4, 0.5 µL) and the variance among repetitions was expressed as the repeatability standard deviation (%RSD). Peak areas measurements (in area units, AU) were used to compare sample values. Raw data from peak areas were recorded using Excel™ (Microsoft Corporation) spread sheet format. Statistical analyses (mean standard deviation and coefficients of correlation) and ANOVA Fisher's test of Least Significant Difference (LSD at $p < 0.05$) were performed using XLStat™ software (Microsoft Corporation).

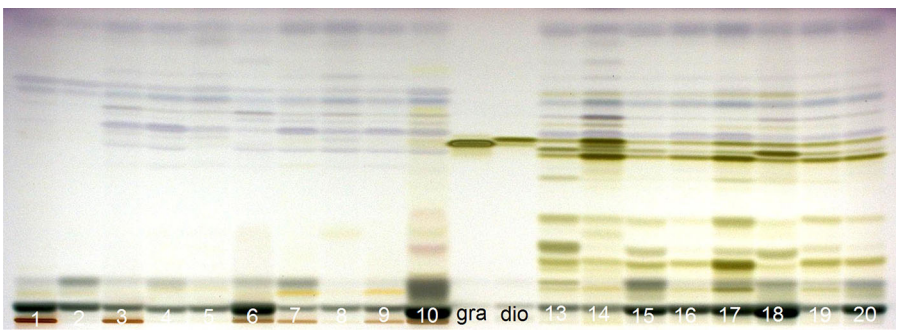


Fig. 1 Gracillin (gra) and dioscin (dio) are absent from chromatograms of *D. alata* cultivars. Three cultivars from Vietnam are in tracks 1–3, three cultivars from Vanuatu are in track 4, 5 and 8, one cultivar from Nigeria is in track 6 and three cultivars of *D. nummularia* from Vanuatu are in tracks 7, 9 and 10. In tracks 13–20 are eight cultivars of *D. cayenensis* from Vanuatu, used as control genotypes presenting dioscin and gracillin and other saponins

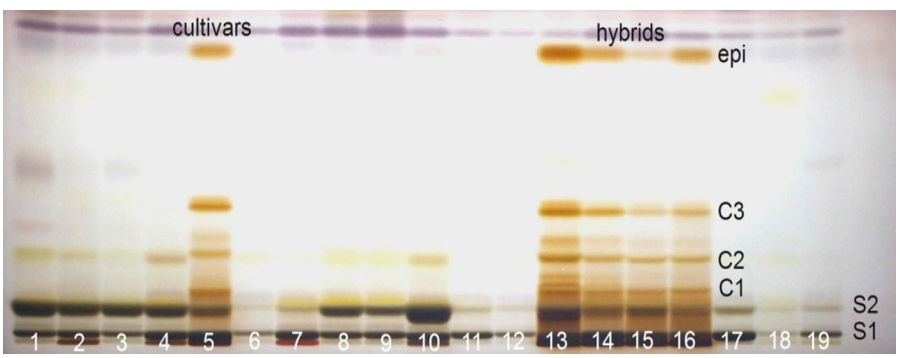


Fig. 2 HP-TLC plate of *D. alata* cultivars and hybrids. The two dark bands at the bottom of the plate are saponins (S1 and S2) and the orange bands in the upper part are catechins (C1, C2, C3 and epicatechin). Scanning at 440 nm allows quantification in peak area units (AU) and comparison between tracks. Cultivars with very low saponins and no catechins are in tracks 6, 11 and 12. Hybrids with very low values are found in tracks 18 and 19. Cultivar *Da 1539* from Vanuatu (track 5) is rich in catechins and hybrids (in tracks 13–16) also present high catechins content

Results

HP-TLC chromatograms are presented in Figs. 1, 2. Saponins occur in dark bands located on the bottom part of the HP-TLC plate (Fig. 1). Pure standards of gracillin (gra) and dioscin (dio) were applied on the same plate to allow identification of bands. In the present study, dioscin and gracillin were absent from the 550 *D. alata* cultivars and hybrids analyzed. Only two individual saponins were detected in *D. alata* but could not be identified. They were confirmed as saponins using their UV spectra and were also detected in *D. nummularia* and *D. cayenensis*. It is possible that these two bands correspond in fact to two groups of closely related compounds overlapping on the silica plate. However, as the two standards (gra & dio) and *D. cayenensis* saponins are well separated on the same plate, we will assume that they most likely correspond to only two saponins (S1, S2).

Catechins are clearly identified as orange bands using the pure standard epicatechin. Catechin was not detected. Four catechins were successfully scanned, including epicatechin (epi), the most important one located at the top of the plate. Most cultivars from Vanuatu, Vietnam and Papua New Guinea (PNG) present chromatograms with very limited amounts of saponins and catechins. All accessions from IITA, Nigeria and a few *D. alata* cultivars (i.e., Da 1539 from Vanuatu) and hybrids (India OP and INxVU) are rich in saponins (S1, S2) and exhibit characteristic chromatograms with orange catechins bands (tracks no 5 and 13–16 in Fig. 1).

No obvious differences were observed between freeze dried samples prepared in Nigeria and oven dried samples prepared in other countries. Saponins and catechins were detected in comparable contents and HP-TLC chromatograms were identical.

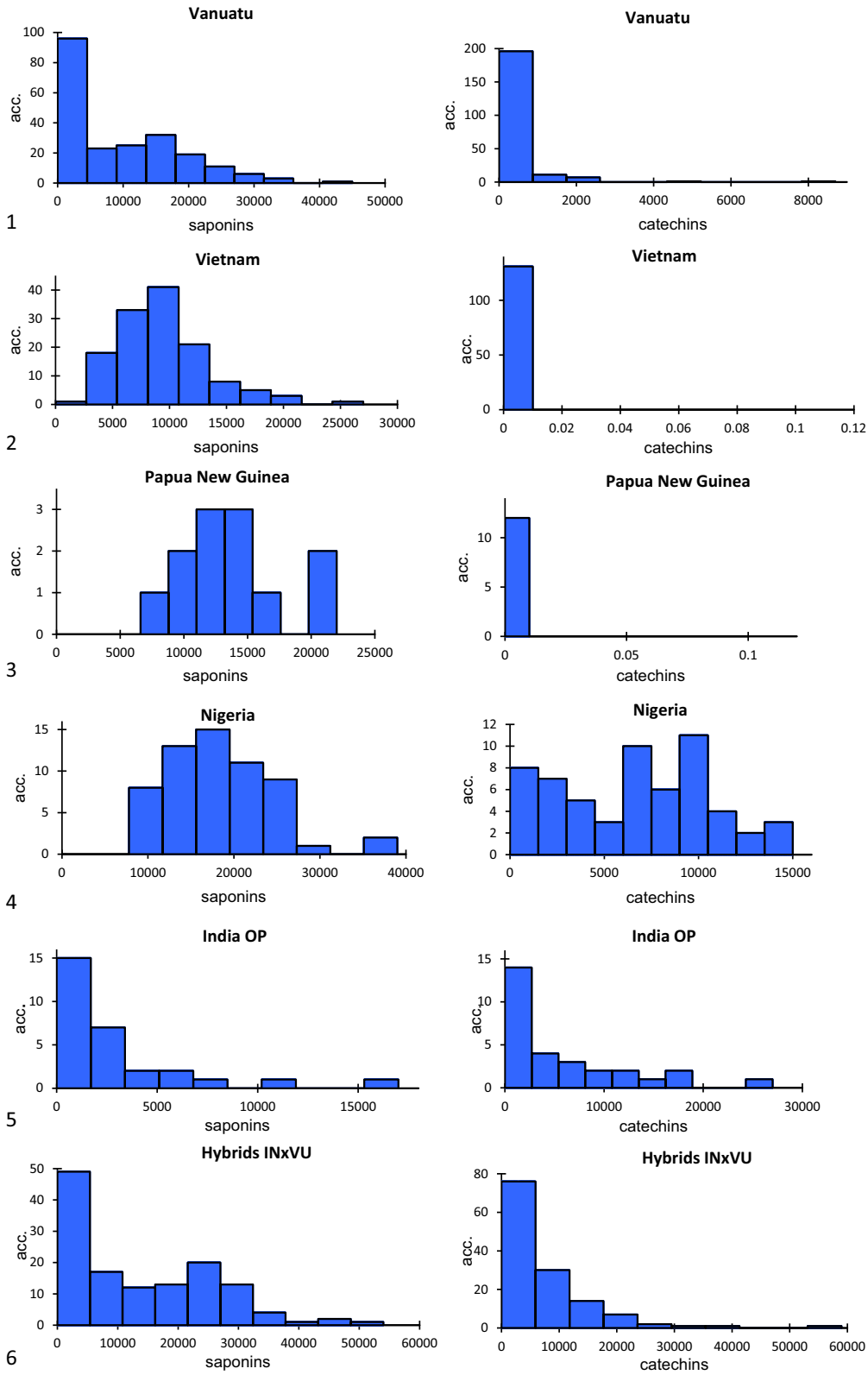
For each group of cultivars and hybrids, the mean values for two saponins (S1, S2) and four catechins

Fig. 3 Distribution of classes of saponins and catechins contents for cultivars from Vanuatu, Vietnam, Papua New Guinea and Nigeria and comparison with classes for selected hybrids from India (OP) and hybrids resulting from crosses between Indian female plants and Vanuatu males (INxVU). For saponins and catechins, the numbers on the horizontal line refer to the classes in peak area units. Numbers of accessions analyzed for each class are on the left vertical axis

Table 2 Saponins and catechins contents in cultivars and hybrids of *Dioscorea alata* (values are in AU, HP-TLC peak area units)

Origin		S1	S2	Total saponins	C1	C2	C3	Epicatechin	Total catechins
Vanuatu	Mean	5571	4152	9723[d]	21	41	49	106	218[d]
$n = 216$	Min	126	136	372	0	0	0	0	0
	Max	25,721	21,885	44,901	2819	1586	3290	3704	8627
Papua New Guinea	Mean	6099	7835	13933[c]	–	–	–	–	–
$n = 12$	Min	3698	3916	8636					
	Max	9978	14,306	21,362					
Vietnam	Mean	5498	3905	9404[d]	–	–	–	–	–
$n = 131$	Min	1804	794	2598					
	Max	12,046	15,664	26,896					
Nigeria	Mean	14,556	4043	18599[a]	3086	2598	1000	668	7352[b]
$n = 29$	Min	7353	515	8273	273	0	0	0	964
	Max	26,280	12,974	38,913	11,185	11,181	7687	9338	14,363
India OP	Mean	1507	1588	3095[e]	1309	628	1758	2276	5972[c]
$n = 30$	Min	232	211	629	0	0	0	0	707
	Max	4893	12,440	16,566	14,075	6924	11,792	7352	26,810
Hybrids INxVU	Mean	8073	7683	15695[b]	4295	1727	1382	1141	8521[a]
$n = 132$	Min	111	220	511	0	0	0	0	605
	Max	28,333	30,554	53,841	22,735	11,746	9499	16,616	58,044

Means with different letters within each column are significantly different at $p < 0.05$


Vanuatu
acc.
saponins
Vanuatu
acc.
catechins
1
Vietnam
acc.
saponins
Vietnam
acc.
catechins
2
Papua New Guinea
acc.
saponins
Papua New Guinea
acc.
catechins
3
Nigeria
acc.
saponins
Nigeria
acc.
catechins
4
India OP
acc.
saponins
India OP
acc.
catechins
5
Hybrids INxVU
acc.
saponins
Hybrids INxVU
acc.
catechins
6

(C1, C2, C3, epi) are presented in Table 2. The highest total mean value for saponins was found in cultivars from Nigeria (18599 AU) and in INxVU hybrids (15695 AU). Cultivars from Vanuatu and Vietnam presented comparable mean values (9723 and 9404), while cultivars from PNG had an intermediate mean value (13933). However, within each group, remarkable variation (min–max) was observed, especially within Vanuatu cultivars (372-44901) and within INxVU hybrids (511-53841).

Catechins were not found in cultivars from Vietnam and Papua New Guinea and only 25 cultivars from Vanuatu presented catechins in low values. Surprisingly, most cultivars from Nigeria presented catechins but the highest mean values were found in hybrids (8521) with selected genotypes from India presenting an intermediate mean value (5972). Distributions of saponins and catechins classes for the four groups of cultivars (Vanuatu, Vietnam, PNG, Nigeria), are presented in Fig. 3. Except for the case of Nigeria, it appears that in Vanuatu, Vietnam and PNG, local cultivars are characterized by the absence, or the extremely low levels, of catechins. Interestingly, OP hybrids introduced from India as true seeds and selected in Vanuatu after several clonal generations, also present low levels of saponins and catechins. Likewise, the 132 INxVU hybrids which are presently field evaluated tend to present low saponins and catechins. However, for each group (India and local hybrids) some accessions are still presenting very high values for both saponins and catechins.

The hybrid selection process includes the qualitative assessment of the speed of oxidation after cutting and the amount of mucilage exuded after cutting (descriptors 7.6.34 and no 7.6.36). Correlation coefficients were computed to detect possible relationships between total saponins and catechins with oxidation, mucilage and flour colour. For saponins these coefficients were respectively: -0.2422^{**}, $+0.5122^{**}$ and $+0.1469$. For catechins, these were -0.5888^{**}, $+0.2841^{**}$ and $+0.4096^{**}$ (**significant at $P < 0.01$ and $n > 250$, r value at 1% = 0.162). Although these coefficients are not high, it appears that this visual selection has an impact on the total saponins and catechins contents of selected genotypes.

Discussion

Anti-nutritional compounds are found in all root and tuber crop species. In most cases they act as repellents and protect the plant against predators (Mierziak et al. 2014). They are usually found in greater proportion in the wild forms and are considerably reduced in the most improved cultivars. Domestication is often seen as a selection of suitable chemotypes aiming at reducing undesirable compounds. This is the case for glycoalcaloids in potato, cyanogens in cassava, latex in sweet potato, oxalates in aroids and in *Dioscorea* spp., saponins and flavonoids are considered as such (Ezeocha and Ojimelukwe 2012). The greater yam presents low levels of anti-nutritional compounds (Udensi et al. 2010; Didier et al. 2014; Viji et al. 2016). Several antinutrients have been identified such as alkaloids, saponins, flavonoids, and tannins and their contents were significantly reduced in the boiled tubers (Ezeocha and Ojimelukwe 2012). A first attempt to detect dioscin in 48 *D. alata* cultivars from the IITA collection using HPLC, concluded that it was absent from all cultivars (Kwon et al. 2015). The present study conducted on a much wider sample, confirms this result and, therefore, previous investigations revealing the presence of dioscin in *D. alata* have to be taken with caution.

It is, however, shown that saponins are present in *D. alata* and their content is very variable between cultivars but more research is needed to identify these individual saponins. Previous reports identifying diosgenin from *D. alata* (Shah and Lele 2012) indicate that this aglycon could be produced from other saponins after hydrolysis of the raw tubers. When looking at the overall picture (Fig. 3), it appears that saponins were down selected during the domestication and selection processes and a good cultivar is low in saponins. It is possible that these secondary metabolites, although present in very small amounts might play a role in the perception of taste. It has already been shown that secondary metabolites profiling conducted with gas chromatography (GC–MS) could be used for a better identification of genotypes in breeding programmes but only five *D. alata* accessions were characterized and saponins were not studied (Price et al. 2017). HP-TLC is consequently an efficient tool for screening yam germplasm for saponins.

Catechins have been suspected to contribute to tuber flesh oxidation and browning (Akissoe et al. 2005). Like other flavonoids, they are known to protect the plants against insects. They are reported as feeding deterrents but when in significant amount, their presence can alter the palatability of the plants, reduce their nutritive value, decrease digestibility or even act as toxins (Simmonds 2003; Mierziak et al. 2014). The presence of catechins in significant proportions in some genotypes of *D. alata* can be considered as a wild trait. In our study, most cultivars presented low or no catechins, while hybrids between parents originating from distant genepools, presented high catechins. These high catechins content, along with other deleterious traits such as hairy tubers, poor shape and spines at the base of the stems, are part of the genetic load observed in hybrid plants resulting from controlled pollinations and true seeds. The fact that some accessions from Nigeria present high levels of catechins, might indicate that the browning of the flour or puree is not considered as a constraint compared to other agronomic advantages that these cultivars might have. This could also indicate that these accessions were clonally introduced a long time ago and that *D. alata* does not produce seeds in farmers' fields in Africa to allow further selection. More research is needed to characterize a broader sample of African cultivars of *D. alata*. Incorporation of plants resulting from sexual reproduction exists in Africa for other *Dioscorea* spp. (Scarcelli et al. 2006) but natural crosses are rare for *D. alata*. Sexual reproduction has been involved in the diversification of this species (Arnau et al. 2017), but polyploidy and dioecy reduce the opportunities for selection of seedlings with low catechins contents.

Molecular markers have recently shed some light on the genetic diversity existing within this species. In China, ISSR and SPRAP markers have shown that *D. alata* and *D. persimilis* Prain et Burkill, its closest wild relatives, are well differentiated with low genetic diversity within each species (Wu et al. 2014). However, a global study conducted with SSRs on 384 *D. alata* cultivars (landraces) from Asia, Africa, the Caribbean and the Pacific, revealed wide genetic diversity and structuring associated with geographic origin, ploidy levels (2×, 3×, 4×) and morpho-agronomic characteristics. No center of origin was identified but two diversification genepools (Vanuatu and India) were differentiated and high diversity levels were found in international germplasm collections (Arnau et al. 2017). In Vanuatu, SSR and DArT markers were used to elucidate the origin of 80 *D. alata* cultivars and the study concluded that the local diversification seems to be dominated by the contribution of somaclonal variation, while the selection of seedlings played a minor role in this archipelago. However, the greater yam being dioceous, when male and female plants of equivalent ploidy flower, fruit set occurs in Vanuatu and produce several hundred fertile seeds per plant (VandenBroucke et al. 2015). The same phenomenon probably occurred elsewhere and volunteer seedlings were clonally propagated, evaluated and selected, giving raise to the cultivars known today. Those with rapidly oxidizing flesh were not retained.

For taxonomists, *D. alata* is unknown in the wild state anywhere in the world and it has therefore been considered as a cultigen (Coursey 1967). It has been postulated that the greater yam originated in cultivation in Assam or Burma where related species, *D. hamiltonii* Hook f. and *D. persimilis,* are found growing wild (Burkill 1951). This theory has, however, been considered as rather speculative (Martin 1976) and these two binomials are now classified as synonyms (Wilkin et al. 2007). Phylogenetic relationships of *Dioscorea* spp. from SE Asia have been reconstructed with chloroplast sequence data and have shown that *D. alata* clusters together with *D. hamiltonii-persimilis, D. fordii* Prain et Burkill, *D. nummularia*, *D. lepcharum* Prain et Burkill and other unidentified *Dioscorea* sp. (Hsu et al. 2013). Relationships were also investigated using plastid sequences and nuclear microsatellite (SSR) markers and indicated that *D. alata* was related to the Asian Enantiophyllum species (*D. oryzetorum* Prain et Burkill and *D. inopinata* Prain et Burkill) but also to *D. nummularia* (Viruel et al. 2016) and this is supported by the occurrence of natural hybrids between the two species (Chaïr et al. 2016). Asian Enantiophyllum species appear closely related and the situation remains complex as *D. hamiltonii-persimilis* is found only in mainland Asia while *D. nummularia* is a Sahul species found east of the Wallace line, in New Guinea and Melanesia (Chaïr et al. 2016), so the geographical origin of *D. alata* remains obscure.

However, archaeological data from New Guinea demonstrate that the exploitation of yams (*D. alata*, *D. bulbifera* L., *D. esculenta* (Lour.) Burkill and *D.*

nummularia) started more than 40,000 years ago on the Sahul plate, composed of New Guinea and Australia. The analysis of abundant starch grains found on stone artefacts confirmed that *D. alata* was used for subsistence when the first humans arrived in Ivane valley, a very isolated region in the eastern highlands (Summerhayes et al. 2010). This would indicate that *D. alata* was growing wild in New Guinea well before the introduction of its most dangerous predator, the pig (*Sus scrofa* L.), introduced only 12,000–6000 years ago in New Guinea (O'Connor et al. 2011). It is therefore necessary, considering the economic importance of *D. alata* worldwide, to encourage and strengthen activities aiming at collecting and characterising genetic resources on a broad scale as these resources are, in the absence of wild forms, essential for future breeding programmes.

Conclusion

Dioscin and gracillin were absent from the 550 accessions analyzed and as the samples were representative of a wide geographical diversity, this suggests that they are not present in *D. alata*. Secondary metabolites profiling appears as an attractive taxonomic tool to differentiate species of the Enantiophyllum section. The domestication process which has led to the selection of present cultivars has favored genotypes with low saponins and catechins contents. This slow process was most likely based on a visual selection of attractive tuber flesh but it is also possible that these secondary metabolites impact the tuber taste. HP-TLC is a cost efficient technique that allows the high throughput screening of numerous yam accessions and could be used in breeding programs to detect hybrids with low levels of saponins and catechins.

Acknowledgements This work was financially supported by the 'Agropolis Fondation' under reference ID 1403-023 through the 'Investissements d'avenir' programme (Labex Agro: ANR-10-LABX-0001-01). Special thanks are due to Rosanna Molisalé and Madeleine Shem for assistance in preparing samples and methanolic extracts.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

Abraham K, Nemorin A, Lebot V, Arnau G (2013) Meiosis and sexual fertility of autotetraploid clones of greater yam *Dioscorea alata* L. Genet Resour Crop Evol 60:819–823

Akissoe N, Mestres C, Hounhouigan J, Nago M (2005) Biochemical origin of browning during the processing of fresh yam (*Dioscorea* spp.) into dried product. J Agric Food Chem 53:2552–2557

Arnau G, Bhattacharjee R, Sheela MN, Chair H, Malapa R, Lebot V, Abraham K, Perrier X, Petro D, Penet L, Pavis C (2017) Understanding the genetic diversity and population structure of yam (*Dioscorea alata* L.) using microsatellite markers. PLoS ONE 12(3):e0174150

Asiedu R, Sartie A (2010) Crops that feed the world: yams for income and food security. Food Sec 2:205–315

Burkill IH (1951) Dioscoreaceae: flora Malesiana. In: Hansen B (ed) Dioscoreaceae: studies in the flora of Thailand. Dansk Botanisk Arkiv, Copenhagen, pp 461–463

Chaïr H, Sardos J, Supply A, Mournet P, Malapa R, Lebot V (2016) Plastid phylogenetics of Oceania yams (*Dioscorea* spp., Dioscoreaceae) reveals natural interspecific hybridization of the greater yam (*D. alata*). Bot J Linn Soc 180(3):319–333

Coursey DG (1967) Yams: an account of the nature, origins, cultivation and utilisation of the useful members of the *Dioscoreaceae*. Tropical Agriculture Series, Longmans

Degras LM (1993) The yam: a tropical root crop. MacMillan Press Ltd, London

Dey P, Chowdhuri SR, Sarkar MP, Chaudhur TL (2016) Evaluation of anti-inflammatory activity and standardisation of hydro-methanol extract of underground tuber of *Dioscorea alata*. Pharm Biol 54(8):1474–1482

Didier AC, Hubert KK, Djè KM, Koné FM, Yapi AYD, Kouadio JPN, Kouamé LP (2014) Assessment of some antinutritional compounds and some organic acids of "bètè-bètè" yam (*Dioscorea alata*) tubers as influenced by boiling times. Asian J Appl Sci 2(1):494–504

Dilworth L, Brown K, Wright R, Oliver M, Hall S, Asemota H (2012) Antioxidants, minerals and bioactive compounds in tropical staples. Afr J Food Sci Technol 3(4):90–98

Dufie WMF, Oduro I, Ellis WO, Asiedu R, Maziya-Dixon B (2013) Potential health benefits of water yam (*Dioscorea alata*). Funct Food 4:1496–1501

Egesi CN, Asiedu R, Egunjobi JK, Bokanga M (2003) Genetic diversity of organoleptic properties in water yam (*Dioscorea alata* L.). J Sci Food Agric 83:858–865

Ezeocha VC, Ojimelukwe PC (2012) The impact of cooking on the proximate composition and anti-nutritional factors of water yam (*Dioscorea alata*). J Stored Prod Postharv Res 3(13):172–176

Hsu KM, Tsai JL, Chen MY, Ku HM, Liu SC (2013) Molecular phylogeny of *Dioscorea* (*Dioscoreaceae*) in East and Southeast Asia. Blumea 58:21–27

IPGRI/IITA (1997) Descriptors for Yam (*Dioscorea* spp.). International Institute of Tropical Agriculture, Ibadan, Nigeria/International Plant Genetic Resources Institute, Rome, Italy

Jesus M, Martins APJ, Gallardo E, Silvestre S (2016) Diosgenin: recent highlights on pharmacology and analytical

methodology. J Anal Met Chem. https://doi.org/10.1155/2016/4156293
Kwon YK, Jie EY, Sartie A, Kim DJ, Liu JR, Min BW, Kim SW (2015) Rapid metabolomics discrimination and prediction of dioscin content from African yam tubers using Fourier transform-infrared spectroscopy combined with multivariate analysis. Food Chem 166:389–396
Maithili V, Dhanabal SP, Manhendran S, Vavivelan R (2011) Antidiabetic activity of ethanolic extract of tubers of *Dioscorea alata* L. in alloxan induced diabetic rats. Indian J Pharm 43(4):455–459
Martin FW (1976) Tropical yams and their potential. Part 3. *Dioscorea alata*. Agriculture handbook No. 46, USDA-USAID, Washington DC
Martin FW, Rhodes AM (1977) Intra-specific classification of *Dioscorea alata*. Trop Agric (Trinidad) 54(1):1–13
Mierziak J, Kostyn K, Kulma A (2014) Flavonoids as important molecules of plant interactions with the environment. Molecules 19:16240–16265. https://doi.org/10.3390/molecules191016240
Narkhede A, Gill J, Thakur K, Singh D, Singh E, Kulkarni O, Harsulkar A, Jagtap S (2013) Total polyphenolic content and free radical quenching potential of *Dioscorea alata* L. tubers. Int J Pharm Pharmaceut Sci 5(3):866–869
O'Connor S, Barham A, Aplin K, Dobney K, Fairbairn A, Richards M (2011) The power of paradigms: examining the evidential basis for early to mid-holocene pigs and pottery in melanesia. J Pac Archaeo 2(2):1–25
Olubobokun TH, Aluko EO, Iyare E, Anyaehie UB (2013) *Dioscorea alata* L. reduces body weight by reducing food intake and fasting blood glucose level. Br J Med Med Res 3(4):1871–1880
Ozo ON, Caygill JC, Coursey DG (1984) Phenolics of five yam (*Dioscorea*) species. Phytochemistry 23(2):329–411
Price EJ, Bhattacharjee R, Lopez-Monte A, Fraser PD (2017) Metabolite profiling of yam (*Dioscorea* spp.) accessions for use in crop improvement programmes. Metabolomics 13:144. https://doi.org/10.1007/s11306-017-1279-7
Sautour M, Mittaine-Offer AC, Lacaille-Dubois MA (2007) The *Dioscorea* genus: a review of bioactive steroid saponins. J Nat Med 61:91–101
Scarcelli N, Tostain S, Vigouroux Y, Agbangla C, Dainou O, Pham JL (2006) Farmers' use of wild relative and sexual reproduction in a vegetatively propagated crop: the case of yam in Benin. Mol Ecol 15(9):2421–2431
Shah HJ, Lele SS (2012) Extraction of diosgenin, a bioactive compound from natural source *Dioscorea alata* var. *purpurea*. J Anal Bioanal Tech 3:141. https://doi.org/10.4172/2155-9872.1000141
Simmonds MSJ (2003) Flavonoid–insect interactions: recent advances in our knowledge. Phytochemistry 64:21–30
Summerhayes GR, Leavesley M, Fairbairn A, Mandui H, Field J, Ford A, Fullagar R (2010) Human adaptation and plant use in highland New Guinea 49,000 to 44,000 years ago. Science 330:78–81
Udensi EA, Oselebe HO, Onuoha AU (2010) Antinutritional assessment of *D. alata* varieties Pakistan. J Nut 9(2):179–181
Vanden Broucke H, Mournet P, Vignes H, Chair H, Malapa R, Duval MF, Lebot V (2015) Somaclonal variants of taro (*Colocasia esculenta* Schott) and yam (*Dioscorea alata* L.) are incorporated into farmers' varietal portfolios in Vanuatu. Genet Res Crop Evol 63(3):495–511
Viji MO, Neeba W, Veena VV (2016) Phytochemical profiling of tuber extracts of *Dioscorea alata*. VISTAS 5(1):133–137
Viruel J, Segarra-Moragues JG, Raz L, Forest F, Wilkin P, Sanmartin I, Catalan P (2016) Late cretaceous–early eocene origin of yams (Dioscorea, Dioscoreaceae) in the Laurasian Palaearctic and their subsequent Oligocene-Miocene diversification. J Biogeogr 43:750–762
Wilkin P, Thapyai C, Chayamarit K (2007) Lectotypification of *Dioscorea* L. (*Dioscoreaceae*) names from Thailand. Kew Bull 62:251–258
Wu ZG, Li XX, Lin XC, Jiang W, Tao ZM, Nitin M, Fan CY, Bao XQ (2014) Genetic diversity analysis of yams (*Dioscorea* spp.) cultivated in China using ISSR and SRAP markers. Genet Res Crop Evol 61:639–650
Wu ZG, Jiang W, Nitin M, Bao XQ, Chen SL, Tao ZM (2016) Characterizing diversity based on nutritional and bioactive compositions of yam germplasm (*Dioscorea* spp.) commonly cultivated in China. J Food Drug Anal 24:367–375
Zhang X, Han X, Yin L, Xu Qi Y, Xu Y, Sun H, Lin Y, Liu K, Peng J (2015) Potent effects of dioscin against liver Fibrosis. Nat Sci Rep 5:9713. https://doi.org/10.1038/srep09713