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RESEARCH ARTICLE

Propagation of lusala (*Dioscorea hirtiflora*), a wild yam, for *in situ* and *ex situ* conservation and potential domestication

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Summary

Lusala (*Dioscorea hirtiflora* Benth. subsp. *pedicellata* Milne-Redh) is an important wild edible tuber foraged widely from natural forests in Southern Zambia, but at risk from overharvesting and deforestation. Its propagation was investigated in glasshouse studies to explore potential domestication and future *in situ* and *ex situ* genetic resources conservation. Almost all tubers planted with visible shoot buds produced vines, with no effect of tuber size on vine emergence or tuber yield. Few tubers without visible shoot buds at planting produced vines, but those that did not re-tuberized. The progeny provided good vine emergence and similar tuber yield, with vines from tubers produced by re-tuberization being more vigorous. Re-tuberization in the absence of vine emergence also occurred in other experiments. Minisetts cut from the proximal end of tubers provided better vine emergence (with more from 20-mm than 10-mm-long sections) and greater tuber yield than mid- or distal minisetts. Nodal stem cuttings rooted well, vined, and provided small tubers. This study shows that lusala can be propagated successfully from tubers, minisetts, nodal vine cuttings, or mini-tubers from nodal vine cuttings, for genetic resources conservation and/or domestication. Domestication is likely to be hampered by the long period required for vines to emerge and establish. More sustainable foraging, including re-planting in natural forests, is recommended to balance consumption of lusala in the region and promote its long-term conservation.

Keywords: *Dioscorea hirtiflora* subsp. *pedicellata*; Emergence; Minisetts; Propagation; Re-tuberization; Tubers; Vines; Wild yam

Introduction

The genus *Dioscorea* (Dioscoreaceae, yam family) comprises about 450 species distributed in tropical and subtropical regions (Purseglove, 1972; Wilkin *et al.*, 2005). Yams are climbing vines exploited for their starchy tubers for food from cultivated crops or wild plants (Magwe-Tido *et al.*, 2016). Global farmed yam production was estimated at about 68 million tons in 2014 with 96% of tubers produced in West Africa (FAOSTAT, 2016). Yams are an important staple food with production systems dominated by *Dioscorea rotundata* Poir. (white yam), *Dioscorea cayenensis* Poir. (yellow yam), and *Dioscorea alata* L. (water yam) (Orkwor and Ekanayake, 1998). Wild yams may also be exploited for food (Ackermann, 2004; Andriamparany, 2015; Devineau *et al.*, 2008; Laly *et al.*, 2019). The wild edible yam *Dioscorea hirtiflora* Benth. is an example, and three subspecies have been identified: *hirtiflora* across West Africa, Angola, and the Democratic Republic of Congo (DRC); *orientalis* in Kenya, Malawi, Mozambique, and Tanzania; and *pedicellata* in

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Uganda, DRC, the Caprivi strip in Namibia, Tanzania, Mozambique, Malawi, Zimbabwe, and Zambia (RBG Kew, 2018; Wilkin, 2001).

D. hirtiflora Benth. subsp. *pedicellata* Milne-Redh is known locally as lusala, busala, or lwiidi within the Tonga ethnic group of Southern Zambia and is an important wild edible tuber there (Wilkin, 2001; Forestry Department, 2016; Zulu *et al.*, 2019). Lusala is an annual of open woodland and grassland with scattered trees growing at altitudes of 200–1700 m with vines that can reach 3 to 8 m, often twining around shrubs or trees, producing 1–6 cylindrical tubers of up to 5 cm in diameter (Wilkin, 2001). The tubers are initially dormant (for about 6–7 months), with new vines often emerging at the onset of the rainy season. Yield per plant has not been estimated, and foragers harvest tubers from clumps of plants periodically. In southern Zambia, most rural households forage the tubers from forests to consume and/or trade seasonally (March–September), and it is in high demand from urban populations (Zulu *et al.*, 2019).

Some wild yams are toxic to humans and require treatment to eliminate toxicity (Poornima and Ravishanker, 2009). The West African *D. hirtiflora* is one such example and is consumed in times of food shortage after preparation to reduce or eliminate the toxicity (Fern, 2019). In contrast, lusala from Central Africa is edible (Wilkin, 2001) and does not appear to be toxic: foragers chew it raw, while it is widely consumed once cooked (Zulu *et al.*, 2019). Lusala's nutritional value is similar to cultivated yams in most respects, but protein and iron contents are superior being some 2 and 10 times greater, respectively (Nyirenda *et al.*, 2007). It is described as tasty and blends well with groundnuts, eggs, fish, and meat in cooked meals (Zulu *et al.*, 2019). Despite its local importance, lusala is largely undomesticated, with limited research attention, while populations in the wild are declining due to overharvesting and deforestation (Forestry Department, 2016; Zulu *et al.*, 2019). Domestication may be one route to the sustainable utilization of lusala to relieve the pressure from foraging on this species in the wild.

Suitable propagation methods are necessary in the domestication of any plant (Aighewi *et al.*, 2015) and for plant genetic resources conservation and utilization. In cultivated yams, propagation often involves the selection of small whole tubers (seed yams) from the ware crop or minisetts cut from large tubers to provide planting material (Morse, 2018; Morse and McNamara, 2018), while nodal vine cuttings are grown to produce mini-tubers for use as seed yams in research stations (Aighewi *et al.*, 2015; Balogun and Gueye, 2014). The propagation of cultivated yams from small whole tubers diverts as much as 30% of the annual harvest from consumption to future crop production (Ekanayake and Asiedu, 2003). Hence, planting material accounts for about 50% of cultivated yam production costs (Balogun and Gueye, 2014). This cost is reduced by propagation from minisetts (Ekanayake and Asiedu, 2003). However, there is a limit to reducing sett size for optimal propagation: the smaller the setts the smaller the tubers produced (Morse, 2018; Morse and McNamara, 2018).

Lusala is a wild edible yam in high demand (Zulu *et al.*, 2019) with potential for domestication. We report a series of studies conducted in glasshouses on plant propagation and establishment in order to better understand lusala's suitability for domestication. Tuber yield was also recorded to compare among treatments and across experiments in order to identify if any propagation treatments were deleterious to subsequent growth and yield. A further objective was to develop advice on propagation for its future genetic resources conservation and utilization.

Materials and Methods

Seventy-five tubers of lusala (*D. hirtiflora* subsp. *pedicellata*; *D. hirtiflora* henceforth here) or large tuber sections (the long thin tubers of lusala often break during harvest), varying in weight and totalling 1 kg (average weight 13.3 g), were harvested from a single site in the southern Miombo forest in Chongwe district (15°15'S, 28°29'E, 1106 m a.s.l.), Lusaka Province, Zambia on November 2, 2016. The tubers were washed and treated with a mixture of the insecticide and

nematicide Phorate 10G and the fungicide Mancozeb, inspected and certified, then couriered to the UK and received on November 21, 2016. These tubers and their vegetative progeny were used in all the investigations reported here. Tubers were classified into those in which there was visible evidence of the end of the dormant phase, namely shoot bud development with the sprout locus just breaking through the skin of the tuber, or not (i.e., those that lacked visible buds). Using the classification derived from the physiological status of yam tubers in previous studies (Craufurd *et al.*, 2001; Hamadina *et al.*, 2010; Ile *et al.*, 2006; Lang *et al.*, 1987), these were designated as non-dormant (ND) or dormant (D), respectively.

Five experiments were conducted between 2016 and 2019 in heated glasshouses at the Crop Environment Laboratory, Whiteknights, University of Reading, UK (51°26'N, 00°56'W, 65 m a.s.l.). Environmental control was limited to heating, to avoid cold damage, with supplementary lighting (SON-T sodium lights, Experiments 1-4; mercury vapour lights, Experiment 5; Phillips, Guildford, UK) in winter; day length was not altered. Plants were each grown in separate 4-liter (except in Experiment 3) pots in a mixture of potting and bedding compost (Clover Peat Ltd, Dungannon, UK) and perlite (2.0–5.0 mm; Sinclair Pro Ltd, Cheshire, UK) in the ratio 4:1. This medium was used for all experiments (including with vine cuttings). The tops of tubers were covered with 20 mm of growing medium. Tubers were planted either vertically or horizontally in Experiment 1, but as no difference was detected all tubers were planted horizontally in the subsequent experiments. Tap water was applied using a fine rose watering can once a day. There was no supplemental fertilization. All vines were tied to a stake as they grew.

At the end of each of the five experiments, the contents of every pot were examined closely. This was because in some cases where vines did not emerge small new tubers were, nonetheless, produced: this phenomenon is described here as re-tuberization.

Experiment 1: Effect of tuber weight and shoot bud development on emergence, growth, and yield

The original lusala tubers from Zambia provided the planting material. Each tuber was weighed and grouped into one of three categories: 1 to 4.9 g ($n = 35$), 5 to 9.9 g ($n = 22$), or >10 g ($n = 18$) with mean weights (\pm S.E.M.) of 3.13 ± 0.17 g, 7.39 ± 0.32 g, and 16.65 ± 1.39 g, respectively. Tubers were further classified into ND and D based on visual assessment (see above). The tubers were planted on November 29, 2016 using a randomized complete block design with four blocks and six treatments. The minimum and maximum air temperatures (i.e., night and day) in the glasshouse averaged throughout the investigation were 19.1 and 33.0 °C, respectively. The emergence of vines above the growing medium was recorded every 7 days until 77 days after planting (DAP) (February 15, 2017). The experiment was terminated on May 6, 2017 (158 DAP), when vine length, number of leaves per plant, presence of tubers, and tuber fresh weight yield were determined.

Experiment 2: Emergence, growth, and yield from planting tubers from emerged or re-tuberized plants

Experiment 1 generated tubers of two contrasting types: tubers from emerged plants, that is, vines emerged and thereafter tuberization occurred (T_V , mean weight 9.70 ± 1.50 g); or tubers from re-tuberization with no emergence (T_{NV} , mean weight 4.26 ± 0.72 g). These two types of tubers provided the planting material for Experiment 2, with an additional treatment of 20-mm-long minisetts (M_V , mean weight 1.74 ± 0.13 g) cut from the middle of tubers harvested from plants with vines. The tubers had been stored for 143 days at room temperature in loose-folded paper bags from harvest until planting on September 26, 2017. Leaf primordia development was not apparent in any tuber at planting. A randomized complete block design was used comprising four blocks and three treatments (six replicates each, one per pot, per block). The experiment was

conducted in the glasshouse with minimum and maximum air temperatures averaged throughout the investigation of 17.3 and 33.4 °C, respectively. Vine emergence was recorded every 7 days until 133 DAP (February 6, 2018). The number of leaves and vine length were recorded at 72 (December 7, 2017) and 90 DAP (December 25, 2017). Plants were harvested at 220 DAP (May 4, 2018) and the presence of tubers and tuber fresh weight recorded.

Experiment 3: Establishment, growth and yield from single-node vine cuttings

Lusala vines from Experiment 1 provided the planting material. The middle portion of each vine selected (all of which appeared healthy) was cut at an angle of 45°, midway between nodes, to provide single-node cuttings. Four separate propagation environments were provided within a heated glasshouse with minimum and maximum air temperatures averaged throughout the investigation of 19.1 and 33.0 °C, respectively: (i) open; (ii) open with misting; (iii) under polythene; and (iv) under polythene with misting. Misting was provided by a MacPenny Mist Control Unit (Wright Rain, Southampton, UK) in which an electronic leaf moisture sensor placed among the vine cuttings triggered misting automatically when dry. Mist bursts lasted 10 seconds. Each environment comprised a randomized complete block design with four blocks and two treatments comprising either 0.25% w/w 1 naphthylacetic acid (NAA; Bayer CropScience Ltd, Cambridge, UK) applied to the base of cuttings or control (untreated) vine cuttings. The cuttings were planted in the standard media (i.e., potting and bedding compost) in 0.9-liter pots on February 1, 2017. Within each environment, each treatment combination comprised 16 cuttings.

At 30 DAP (March 3, 2017), eight plants from each treatment combination were selected at random for destructive leaf and root sampling. The number of leaves and leaf area were assessed using the WinDIAS 3 Image Analysis System 2012 (Delta-T Devices Ltd, Cambridge, UK). The numbers of roots per vine cutting, the presence of tubers (i.e., tuberization), and below ground (i.e., roots and tubers) biomass dry weight were also recorded for these plants. The remaining eight plants from each treatment combination were harvested 150 DAP (July 1, 2017) to determine the presence of tubers and yield at senescence.

Experiment 4: Emergence, growth, and yield from different miniset lengths and origin (position on the tuber, i.e., proximal, middle, or distal)

Tubers (referred to as mini-tubers below as small in size) produced by single-node vine cuttings (Experiment 3) were cut to provide 10- and 20-mm-long minisets from three sections: proximal (PM, near the vine); middle (MM); and distal (DM, furthest from vine). Their mean weights were PM10, 0.47 ± 0.04 g; PM20, 1.51 ± 0.14 g; DM10, 0.15 ± 0.02 g; DM20, 0.46 ± 0.07 g; MM10, 0.22 ± 0.02 g; MM20, 0.74 ± 0.08 g). A control (intact mini-tuber, mean weight 1.52 ± 0.19 g) provided the seventh treatment. None of the whole or cut mini-tubers planted showed evidence of shoot bud development. The mini-tubers and minisets were planted on September 25, 2017 and grown in a heated glasshouse with average minimum and maximum air temperatures averaged throughout the investigation of 17.3 and 33.4 °C, respectively, in three blocks with 15 replicates per treatment. Vine emergence was recorded every 7 days until 210 DAP (April 25, 2018). The number of leaves and vine length were recorded at 161 (March 5, 2018) and 228 DAP (May 11, 2018). Plants were harvested 228 DAP, and the presence of tubers and tuber fresh weight recorded.

Experiment 5: Emergence, growth, and yield from tubers and minisets with and without shoot buds visible

Whole tubers harvested from Experiment 2 were stored for 185 days at laboratory temperature in loose-folded paper bags from harvest until planting. Tubers were planted on November 5, 2018. The minimum and maximum air temperatures inside the glasshouse averaged throughout the

investigation were 15.9 and 32.0 °C, respectively. Eight treatments were provided: whole tubers with visible leaf primordia (T_{ND} , mean weight 23.14 ± 2.93 g), or without (T_D , 15.70 ± 2.64 g); 20-mm-long minisets from the proximal end of tubers with visible shoot buds ($PM20_{ND}$, 1.78 ± 0.21 g), or without ($PM20_D$, 1.44 ± 0.11 g); 20-mm-long mid-tuber minisets with visible shoot buds ($MM20_{ND}$, 2.51 ± 0.29 g), or without ($MM20_D$, 2.10 ± 0.21 g); and 20-mm-long minisets from the distal end of tubers with visible shoot buds ($DM20_{ND}$, 1.38 ± 0.16 g), or without ($DM20_D$, 1.66 ± 1.16 g). There were four blocks with each treatment represented by 12 replicates. Vine emergence was recorded every 7 days until 175 DAP (April 29, 2019). The number of leaves and vine length were recorded at 189 DAP (May 13, 2019). Plants were harvested 207 DAP (May 31, 2019), and the presence of tubers and tuber fresh weight recorded.

Statistical analyses

Genstat 17 (VSN International Ltd, Hemel Hempstead, UK) was used for statistical analyses. The Kruskal–Wallis H test was applied to leaf and vine length data (due to non-homogeneity of variance and non-normal distribution) with *post hoc* pairwise comparisons between groups using the Mann–Whitney *U*-test with Bonferroni correction. Analysis of variance, and *post hoc* analysis with Dunnett's T3, was applied to tuber yield data (data log-transformed where necessary to satisfy normality of variance assumptions). In Experiment 3, *t* tests were performed on number of leaves, leaf area, number of roots, and tuber weight data.

Results

The duration from planting tubers to vine emergence, and final emergence percentage, varied considerably within treatments, among treatments, and among experiments (Figure 1, Table 1). Among Experiments 1, 2, 4, and 5, emergence was earliest in Experiments 1 and 5, at c. 20 DAP, and latest in Experiment 4, with some vines not emerging until around 200 DAP. The within-treatment variation in duration to emergence among extreme individual vines was sometimes considerable, as much as 100 days (Figure 1b–d). In contrast, mid-weight ND tubers (shoot bud visible) planted in Experiment 1 showed little variation and all vines emerged between 21 and 35 DAP (Figure 1a).

Effect of shoot bud development and tuber weight on emergence

ND tubers planted in Experiment 1 had greater ability to produce vines than dormant tubers (Figure 1a, Table 1). Among tubers without shoot buds visible at planting, only a few of the lightest category produced vines during the study with 18% emerged at 21 DAP but none thereafter. Almost all tubers of all weights with shoot buds visible at planting had produced vines by 56 DAP (96% for 1 to 4.9 g, 100% for heavier tubers), but this took longer on average and with greater variation in duration to emerge for the lightest tubers.

Effect of planting tubers from emerged or re-tuberized plants on emergence

Both types of tubers planted in Experiment 2 ultimately provided high (T_V) or full (T_{NV}) vine emergence but required >100 days to achieve this (Figure 1b, Table 1), whereas vines emerged from only a third of the minisets (M_V) over a similar period.

Effect of minisett length and source (proximal, middle, and distal) on emergence

Vines emerged from just over half of the mini-tubers (harvested from plants propagated by cuttings) in Experiment 4, but this required 182 days (Figure 1c, Table 1). Similarly, all the 20-mm-

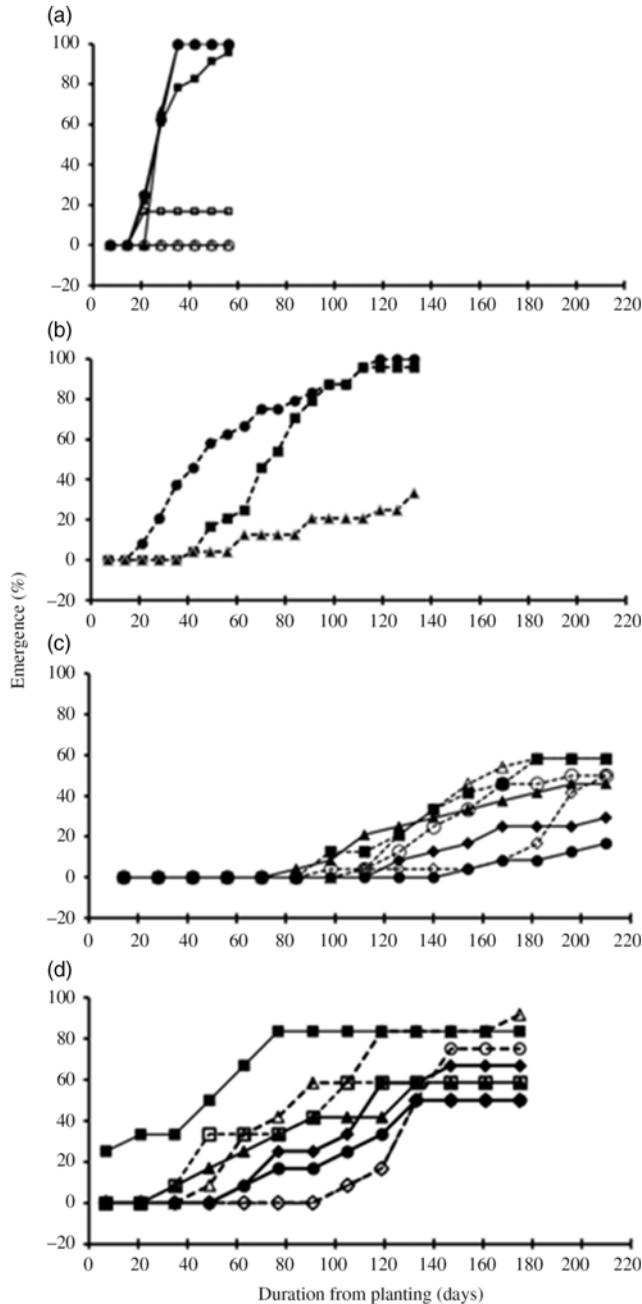


Figure 1. The cumulative progress of emergence from planting lusala (*Dioscorea hirtiflora* subsp. *pedicellata*) tubers, mini-tubers, and minisett in four experiments. (a) Emergence from three weight classes of tubers (1–4.9 g, ■, □; 5–9.9 g, ●, ○; >10 g, ▲, △) with (solid symbols) or without (open symbols) visible shoot buds at planting (Experiment 1). (b) Emergence from tubers harvested from Experiment 1 from plants (■) or from re-tuberization in the absence of vine emergence (●), or from 20-mm-long minisett (▲) (Experiment 2). (c) Emergence from mini-tubers (■), or of 10- (solid lines) or 20-mm-long (broken lines) minisett cut from proximal (▲), middle (●), or distal ends of mini-tubers (◆) produced from single-node cuttings (Experiment 4). (d) Emergence from planting tubers (■, □) or 20-mm-long minisett cut from proximal (▲, △), middle (●, ○), or distal end (◆, ◇) of tubers with (solid symbols and lines) or without (open symbols and dotted lines) visible shoot buds at planting (Experiment 5).

Table 1. Comparison among four experiments of maximum sprouting (Max, %), duration from planting to maximum sprouting (Max, days), and duration from tuber harvest to maximum sprouting (DTH-Max, days) in Iusala (*Dioscorea hirtiflora* subsp. *pedicellata*)

Experiment	Treatment	Max (%)	Max (days)	DTH-Max (9 days)
1	1–4.9 g ND*	96	56	83
	5–9.9 g ND*	100	35	62
	>10 g ND*	100	35	62
	1–4.9 g D [†]	18	21	48
	5–9.9 g D [†]	0	–	–
	>10 g D [†]	0	–	–
2	T _{NV} [†]	100	119	262
	T _V [†]	96	112	255
	M _V [†]	33	133	276
4	Mini-tuber	58	182	269
	PM10 [‡]	46	196	283
	PM20 [‡]	58	182	269
	MM10 [‡]	17	210	297
	MM20 [‡]	50	196	283
	DM10 [‡]	29	210	297
	DM20 [‡]	50	210	297
5	T _{ND} [*]	58	105	290
	T _D [*]	83	77	262
	PM20 _{ND} ^{* §}	92	175	360
	PM20 _D [§]	58	133	318
	MM20 _{ND} ^{* §}	75	147	332
	MM20 _D [§]	50	133	318
	DM20 _{ND} ^{* §}	50	133	318
	DM20 _D ^{* §}	67	147	332

*ND = not dormant (shoot bud visible); D = dormant.

[†]T_{NV} = tuber re-tuberized without producing vine (from Experiment 1); T_V = tuber from plants with vines; M_V = minisett cut from tuber from plants with vines.

[‡]10- or 20-mm-long minisett cut from proximal (PM), middle (MM), or distal (DM) end of minituber.

[§]20-mm-long minisett cut from proximal (PM), middle (MM), or distal (DM) end of tubers.

long minisett treatments showed 50–58% emergence by 182–210 days. The 10-mm-long minisett treatments also emerged over a similar period, but more so from 10-mm-long minisett cut from near the vine (PM) than distal ones (DM), with lowest emergence from mid-section minisett (MM).

Effect of tuber, minisett position, and shoot bud development on emergence

Whole tubers with shoot buds visible at planting provided the most rapid emergence of vines and distal minisett without visible shoot buds the slowest (Figure 1d). The presence of visible shoot buds at planting resulted in earlier emergence, but this difference was greater among tuber treatments than minisett. Among the latter, emergence occurred soonest with proximal minisett. Whole tubers with visible shoot buds when planted showed >80% vine emergence by 77 DAP, with 92% emergence by 175 DAP for proximal minisett without visible shoot buds.

Effect of shoot bud development and tuber weight on number of leaves and vine length

The lightest tuber weight class among those with visible shoot buds provided the most leaves per plant and the longest vines in Experiment 1, with the largest weight class almost as productive and indistinguishable given the high variability (Table 2). Tubers with visible shoot buds at planting provided more leaves (Mann–Whitney U = 186, p < 0.05) and longer vines (U = 305, p < 0.05)

Table 2. Average number of leaves and length of vine (\pm S.E.M.) for *lusala* (*Dioscorea hirtiflora* subsp. *pedicellata*): at 158 days after planting (DAP) grown from tubers with (ND) or without visible shoot buds (D) (Experiment 1); at 72 and 90 DAP grown from tubers or minisettes (Experiment 2); at 161 and 228 DAP grown from mini-tubers or minisettes (Experiment 4); at 189 DAP grown from tubers (T) or 20-mm-long minisettes cut from the proximal (PM), middle (MM), or distal (DM) end of tubers with visible shoot buds (ND) or without (D) (Experiment 5). Mean values calculated for all tubers, mini-tubers, or minisettes planted

Experiment and treatment	Leaves (no.)	Vine length (mm)
Experiment 1		
Weight class (ND)		
1–4.9 g	15.3 \pm 3.7	614 \pm 167
5–9.9 g	7.0 \pm 3.4	331 \pm 231
>10 g	10.7 \pm 10.7	563 \pm 563
Weight class (D)		
1–4.9 g	2.2 \pm 2.2	97 \pm 97
5–9.9 g	0*	0*
>10 g	0*	0*
Experiment 2†		
72 DAP		
T_{NV}	7.0 \pm 1.6	490 \pm 118
T_V	1.5 \pm 0.7	153 \pm 69
M_V	0.1 \pm 0.1	11 \pm 11
90 DAP		
T_{NV}	5.5 \pm 3.5	695 \pm 136
T_V	5.5 \pm 1.8	562 \pm 136
M_V	0.3 \pm 0.2	27 \pm 21
Experiment 4‡		
161 DAP		
Mini-tuber	3.9 \pm 1.3	100 \pm 41
PM10	2.7 \pm 0.7	59 \pm 17
PM20	3.9 \pm 1.2	153 \pm 70
MM10	0.1 \pm 0.1	3 \pm 3
MM20	2.3 \pm 0.9	97 \pm 54
DM10	0.3 \pm 0.2	9 \pm 5
DM20	0.3 \pm 0.3	9 \pm 6
228 DAP		
Mini-tuber	21.0 \pm 4.4	488 \pm 147
PM10	7.6 \pm 2.2	123 \pm 52
PM20	21.9 \pm 2.7	835 \pm 180
MM10	2.1 \pm 1.0	29 \pm 13
MM20	12.1 \pm 3.4	367 \pm 136
DM10	3.5 \pm 1.3	45 \pm 26
DM20	4.0 \pm 1.3	136 \pm 72
Experiment 5		
T_D	70.9 \pm 16.8	1496 \pm 284
T_{ND}	67.3 \pm 15.2	1577 \pm 299
PM20 _D	25.7 \pm 5.3	540 \pm 137
PM20 _{ND}	13.4 \pm 4.9	370 \pm 147
MM20 _D	15.4 \pm 4.1	331 \pm 85
MM20 _{ND}	6.8 \pm 3.2	294 \pm 161
DM20 _D	12.0 \pm 6.7	257 \pm 109
DM20 _{ND}	11.3 \pm 3.5	296 \pm 117

*No sprouting and so nil.

†Tubers harvested from Experiment 1 either from plants which produced vines (T_V) or did not (T_{NV}), or minisettes cut from tubers harvested from the former (M_V).

‡10- or 20-mm-long minisett cut from proximal (PM), middle (MM), or distal (DM) end of mini-tuber.

Table 3. Rooting, tuberization, and fresh tuber yield of *lusala* (*Dioscorea hirtiflora* subsp. *pedicellata*) propagated from vine cuttings treated with 0.25% w/w 1 naphthylacetic acid (NAA) applied to the base of cuttings, or not (control), and grown in four environments (Experiment 3)

Propagation environment	Treatment	Cuttings rooted at 30 DAP (%)	Cuttings tuberized at 30 DAP (%)	Cuttings tuberized at 150 DAP (%)	Fresh tuber yield/vine at 150 DAP (g)
Open					
	NAA	63	50	75	5.6 ± 1.4
	Control	63	38	88	5.6 ± 1.0
Mist					
	NAA	88	63	75	2.7 ± 0.9
	Control	88	50	100	4.5 ± 0.9
Polythene					
	NAA	100	63	75	3.8 ± 1.1
	Control	100	88	100	4.1 ± 0.7
Polythene and mist					
	NAA	100	25	100	2.9 ± 0.5
	Control	100	25	88	3.9 ± 0.8

DAP, days after planting.

than those without. Among the latter, only the lightest tuber weight class produced vines and leaves during the study.

Effect of planting tubers from emerged or re-tuberized plants on number of leaves and vine length

In Experiment 2, the re-tuberized tubers provided the most leaves per plant and the longest vines, at both observation times, with comparatively little vegetative growth detected from minisets (Table 2). At 72 DAP, the Kruskal–Wallis H test showed significant differences among treatments for the number of leaves ($H(2) = 22.87, p < 0.001$) ranking $T_{NV} > T_V > M_V$ and vine length ($H(2) = 21.76, p < 0.001$) also ranking $T_{NV} > T_V > M_V$. *Post hoc* comparisons (Mann–Whitney *U*-tests) found significant differences ($p < 0.05$) between T_{NV} and each of T_V and M_V for both numbers of leaves and vine length. At 90 DAP, differences among treatments were also significant for the number of leaves ($H(2) = 25.98, p < 0.001$) and vine length ($H(2) = 23.54, p < 0.001$) both ranking $T_{NV} > T_V > M_V$. Mann–Whitney *U*-tests found significant differences ($p < 0.05$) between M_V and each of T_{NV} and T_V for number of leaves and for vine length.

Establishment and growth from single-node vine cuttings

Cuttings under polythene showed 100% rooting at 30 DAP regardless of misting or rooting hormone treatments (Table 3). Those under mist only showed 88% rooting at this time, and without misting in the open only 63%, again regardless of whether rooting hormone was applied (Table 3). Tuberization started early and was evident 30 DAP, with no consistent effect of NAA. It was greatest under polythene without misting (88% control; 63% for NAA) and lowest under polythene with misting (25% with or without NAA). Tuberization was high in all treatments by 150 DAP. The NAA treatment reduced leaf area and the number of roots in the polythene-cover environment at 30 DAP but had no effect in any other combination of dependent variable and environment (Table 4). The polythene-cover environment promoted the best vegetative growth, particularly when assessed by leaf area (Table 4), but this did not result in greater tuber yield (Table 3).

Table 4. Average number of leaves, leaf area (LA), number of roots and below ground dry biomass (BGDB) (\pm S.E.M.) at 30 days after planting (DAP) for lusala (*Dioscorea hirtiflora* subsp. *pedicellata*) grown from single-node vine cuttings propagated from vine cuttings treated with 0.25% w/w 1 naphthylacetic acid (NAA) applied to the base of cuttings, or not (control), in four environments and error probability (p) for an effect of NAA from a t -test (Experiment 3)

Propagation environment	Variable	Treatment	Mean	p
Open	No. of leaves	NAA	0.4 \pm 0.3	0.27
		Control	0.9 \pm 0.4	
	LA (cm ²)	NAA	1.3 \pm 1.0	0.31
		Control	3.4 \pm 1.8	
	No. of roots	NAA	6.9 \pm 2.2	0.83
		Control	6.1 \pm 2.6	
	BGDB (g)	NAA	0.1 \pm 0.0	0.72
		Control	0.1 \pm 0.0	
Mist	No. of leaves	NAA	1.1 \pm 0.4	0.43
		Control	1.6 \pm 0.5	
	LA (cm ²)	NAA	4.1 \pm 2.2	0.78
		Control	4.9 \pm 1.9	
	No. of roots	NAA	8.1 \pm 2.3	0.75
		Control	7.0 \pm 2.5	
	BGDB (g)	NAA	0.1 \pm 0.0	0.84
		Control	0.1 \pm 0.0	
Polythene	No. of leaves	NAA	2.0 \pm 0.3	0.30
		Control	2.5 \pm 0.3	
	LA (cm ²)	NAA	10.5 \pm 2.6	0.01
		Control	25.3 \pm 4.1	
	No. of roots	NAA	10.5 \pm 1.1	0.03
		Control	16.5 \pm 2.2	
	BGDB (g)	NAA	0.1 \pm 0.1	0.52
		Control	0.1 \pm 0.1	
Polythene and mist	No. of leaves	NAA	2.3 \pm 0.5	0.16
		Control	1.4 \pm 0.4	
	LA (cm ²)	NAA	3.2 \pm 0.9	0.60
		Control	2.5 \pm 0.9	
	No. of roots	NAA	5.4 \pm 1.3	0.94
		Control	5.3 \pm 1.0	
	BGDB (g)	NAA	0.1 \pm 0.0	0.99
		Control	0.1 \pm 0.0	

Effect of miniset length and source (proximal, middle, and distal) on number of leaves and vine length

The number of leaves varied up to 10-fold among the treatments and vine length yet more at both 161 and 228 DAP in Experiment 4 (Table 2). This was significant in all four cases: the treatments affected the number of leaves at 161 DAP ($H(6) = 36.34$, $p < 0.001$), being ranked PM20 > PM10 > mini-tuber > MM20 > DM10 > DM20 > MM10; and vine length ($H(2) = 39.43$, $p < 0.001$) with ranking PM20 > mini-tuber > PM10 > MM20 > DM10 > DM20 > MM10. The six same treatment pairs differed ($p < 0.05$) for both variables at 161 DAP for *post hoc* comparisons using the Mann–Whitney U -test: between PM20 and DM10; between PM20 and DM20; between PM20 and MM10; between PM10 and MM10; between MM10 and MM20; and between mini-tuber and MM10.

The treatments also affected the number of leaves at 228 DAP ($H(6) = 45.40$, $p < 0.001$) ranking PM20 > mini-tuber > MM20 > PM10 > DM20 > DM10 > MM10; and vine length ($H(6) = 31.0$, $p < 0.001$) with ranking of PM20 > mini-tuber > MM20 > PM10 > DM20 >

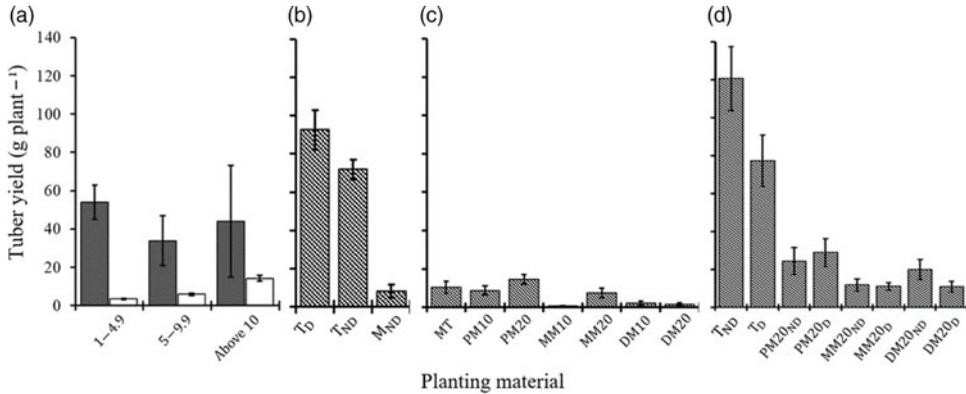


Figure 2. Lusala (*Dioscorea hirtiflora* subsp. *pedicellata*) tuber fresh weight yield per plant \pm S.E.M. (vertical bars about the means) in four experiments. (a) Yield from planting three weight classes of tubers (g) with (solid columns) or without (open columns) visible shoot buds at planting (Experiment 1). (b) Yield from planting tubers harvested from planted tubers that had re-tuberized without producing vines (T_{NV}), from plants with vines (T_V), or from planting 20-mm-long minisetts (M_V) (Experiment 2). (c) Yield from planting mini-tubers (MT, control) or 10- or 20-mm-long minisetts cut from proximal (PM), middle (MM), or distal (DM) ends of MT produced from single-node cuttings (Experiment 4). (d) Yield from planting tubers with (T_{ND}) or without visible shoot buds (T_D), or 20-mm-long proximal minisetts with ($PM20_{ND}$) or without visible shoot buds ($PM20_D$), 20-mm-long middle minisetts with ($MM20_{ND}$) or without visible shoot buds ($MM20_D$), or 20-mm-long distal minisetts with ($DM20_{ND}$) or without visible shoot buds ($DM20_D$) (Experiment 5).

MM10 > DM10. *Post hoc* comparisons with the Mann–Whitney *U*-test found differences ($p < 0.05$) in the number of leaves between each of PM20 and mini-tubers with DM10, DM20, and MM10; and in vine length between each of PM20 and mini-tubers with MM10 and DM10.

Effect of planting tubers and minisetts with and without visible shoot bud on number of leaves and vine length

The numbers of leaves and vine length were affected considerably by the treatments imposed in Experiment 5, with the best treatments providing more leaves and longer vines than in other experiments (Table 2). The treatments in Experiment 5 affected both numbers of leaves ($H(7) = 23.41$, $p < 0.001$) and vine length ($H(7) = 22.16$, $p < 0.001$) at 189 DAP. The presence of visible shoot buds in the planting material had no effect on either variable. Plants from tubers appeared to outperform those from minisetts considerably. However, only the comparisons of minisetts cut from the middle of tubers with visible shoot buds with each of the two whole tuber treatments provided significant differences ($p < 0.05$) for both number of leaves and vine length.

Effect of shoot bud development and tuber weight on tuber yield

A high proportion of the tubers planted without visible shoot buds (i.e., dormant tubers) did not sprout during Experiment 1, and so no vines emerged, but somewhat surprisingly they re-tuberized: 73% of those weighing 1 to 4.9 g, and 100% of the two heavier weight classes. Two-way analysis of variance (on log-transformed data) showed no interaction between weight class and shoot bud development on yield ($F(2,66) = 1.2$, $p > 0.05$). The effect of tuber weight class was also non-significant ($F(2,66) = 2.8$, $p > 0.05$), but the large effect of visible leaf primordia (Figure 2a) was ($F(1,66) = 30.8$, $p < 0.001$). On average, mean yield was 48.3 ± 7.1 g plant⁻¹ from tubers planted with shoot buds visible and only 8.5 ± 0.9 g plant⁻¹ without (Figure 2a).

Effect of planting tubers from plants or from re-tuberization on tuber yield

Planting tubers from re-tuberization (T_{NV}) or from plants with vines (T_V) provided similar yields in Experiment 2 (Figure 2b), while the yield from minisetts (M_V) was considerably lower ($F(2,69) = 39.71$, $p < 0.001$). A *post hoc* Dunnett test showed $M_V < T_{NV}$ and $< T_V$ ($p < 0.05$), but the latter pair did not differ ($p > 0.05$). The 4% of tubers from plants with vines (T_V) which did not produce vines (Figure 1b) all re-tuberized, while 12% of the minisetts (M_V) failed to produce vines but did re-tuberize.

Tuber yield from single-node vine cuttings

Treatment of cuttings with NAA did not affect tuber yield at 150 DAP within any environment ($p > 0.05$) in Experiment 3. Across the four environments, the highest and lowest yields recorded were in the open (5.6 g plant^{-1}) and mist treatments (2.7 g plant^{-1}), respectively (Table 3). All tuber yields were comparatively low, however, being similar to the low yield from planting dormant tubers in Experiment 1 (Figure 2a).

Effect of miniset size and source (proximal, middle, and distal) on yield of tubers

Three of the miniset treatments (PM10, PM20, and MM20) provided similar yield to the mini-tuber control, with much lower yield in the remaining treatments in Experiment 4 (Figure 2c). This was significant ($F(6,96) = 12.22$, $p < 0.001$). *Post hoc* comparisons using Bonferroni tests confirmed the differences among the above two groups of treatments. Tubers were only produced by plants with vines and no re-tuberization occurred. Planting material that neither produced vines nor re-tuberized by the end of the experiment had rotted.

Yield of tubers grown from tubers and minisetts with and without visible shoot bud

The treatments in Experiment 5 affected fresh tuber yield at 207 DAP considerably ($F(7,87) = 10.80$, $p < 0.001$). Plants from whole tubers yielded several-fold (c. 400%) more than those from minisetts (Figure 2d). The latter were all comparatively similar in yield, but with proximal minisetts yielding more than those cut from other tuber sections. Whole tubers with visible shoot buds at planting provided about 50% greater yield ($120.6 \pm 16.8 \text{ g}$) than those without ($77.2 \pm 13.5 \text{ g}$). Whereas no treatment provided 100% emergence (Figure 1d), all but one treatment provided 100% tuberization (the exception was $PM20_{ND}$ where it was 92%). Thus, re-tuberization ranged from 8% ($PM20_D$) to 58% ($MM20_{ND}$).

Discussion

Lusala was propagated successfully from tubers, mini-tubers, minisetts, and vine cuttings. Many of the treatments had similar effects on emergence, growth, and yield of lusala as those reported for cultivated yam species, but emergence and establishment were often delayed considerably in lusala. Emergence began as early as 21 DAP and as late as 180 DAP among the five experiments, with some minisetts providing even later emergence (Figure 1). Lusala tubers tended to provide greater and more rapid emergence than did minisetts, with larger minisetts and/or those cut from the proximal end of tubers performing better than smaller and/or mid- and distal minisetts (Table 1, Figure 1). Emergence occurred sooner if the tubers had lost dormancy (i.e., shoot bud visible) when planted. This was most evident in Experiment 1 (Figure 1a) and from a comparison with the much delayed emergence in Experiments 2 and 4 (Figure 1b, c), where no tubers had visible shoot buds at time of planting.

In cultivated yams, tuber dormancy is similarly characterized by a prolonged period before the tubers eventually sprout (Craufurd *et al.*, 2001). Visible shoot buds on tubers denote the end of the

Table 5. Comparison of maximum sprouting (Max, %) and durations from planting to earliest sprouting (P-ES) and maximum sprouting (P-Max) for lusala (*Dioscorea hirtiflora* subsp. *pedicellata*) in the best-performing treatments in Experiments 1, 2, 4, and 5 with published results for four cultivated yams (*Dioscorea* spp)

Species	Max (%)	P-ES (days)	P-Max (days)
<i>D. hirtiflora</i> [*]	94.3	21	56
<i>D. hirtiflora</i> [†]	97.9	21 – 42	119
<i>D. hirtiflora</i> [‡]	58.3	98	182
<i>D. hirtiflora</i> [§]	58.3	112	182
<i>D. hirtiflora</i> [¶]	58.0	35	105
<i>D. hirtiflora</i> ^{**}	83.0	7	77
<i>D. hirtiflora</i> ^{††}	92.0	49	175
<i>D. hirtiflora</i> ^{†††}	58.0	35	133
<i>Dioscorea alata</i> ^{§§}	83.6	8 – 14	57
<i>Dioscorea rotundata</i> ^{§§}	69.6	23 – 35	57
<i>Dioscorea cayenensis</i> ^{§§}	67.1	23 – 35	64
<i>Dioscorea dumetorum</i> ^{§§}	37.8	25 – 50	92

^{*}Mean of three weight classes of tubers with visible shoot buds (Experiment 1).

[†]Mean for both whole tuber treatments (Experiment 2).

[‡]Intact mini-tuber (control, Experiment 4).

[§]20-mm-long minisetts from proximal end of tubers (PM20, Experiment 4).

[¶]Whole tubers without visible shoot buds (Experiment 5).

^{**}Whole tubers with visible shoot buds (Experiment 5).

^{††}20-mm-long minisetts from proximal end of tubers without visible shoot buds (PM20_{ND}, Experiment 5).

^{†††}20-mm-long minisetts from proximal end of tubers with visible shoot buds (PM20_D, Experiment 5).

^{§§}Minisetts (means from three seasons); data from Igwilo and Okoli (1988).

dormancy period (Awologbi and Hamadina, 2016). Larger whole tubers or larger minisetts of cultivated yams show greater emergence, and the resultant plants survive better than those from smaller ones (Aighewi *et al.*, 2015; Enyi, 1972; Law-Ogbomo and Remison, 2009; Morse and McNamara, 2018). Proximal minisetts emerge better than those cut from distal and middle regions (Orkwor and Ekanayake, 1998; Passam, 1977). Durations to emergence vary widely in cultivated yams—from as early as 14 to as many as 90 DAP (Ekanayake and Asiedu, 2003). Some treatments of Experiments 1, 2, 4, and 5 provided emergence percentages comparable with those for cultivated yams (Table 5). Durations to maximum emergence in lusala tended to be greater, except in Experiment 1 for tubers with visible shoot buds.

In addition to different types and sources of planting material, the month of lusala planting varied among our investigations. While durations from planting to emergence varied considerably among experiments, variation in the months of emergence was much less. In the majority of treatments, emergence occurred between November and January, except for mini-tubers harvested from vine cuttings (Experiment 4) or for mid- and distal minisetts (Experiment 5) where it was delayed further. Hence, lusala emergence from planting tubers coincided with the rainy season in Zambia which starts in November and December and ends in April (Speybroeck *et al.*, 2002). These results also correspond with the long dormancy periods in cultivated yams and failure to sprout and emerge, even if planted earlier, until dormancy is lost (Orkwor and Ekanayake, 1998).

The considerable re-tuberization without vine emergence in Experiment 1 was predominantly from tubers planted, while still dormant. Clearly, this dormancy prevented shoot and leaf development by these tubers throughout the experiment but did not prevent tuber development, with the original tuber weight planted largely conserved in the progeny (Figure 2a). Interestingly, all of the resultant small tubers produced vines (and tubers) with earlier emergence than their peers (tubers from emerged plants) after planting in the subsequent Experiment 2 (Figure 1b), suggesting that they were the less dormant of this treatment pair. Considerable re-tuberization without vine production was also detected in Experiments 2 and 5 (but not Experiment 4). Rainfall is

unpredictable in Zambia (Hachigonta *et al.*, 2008). Hence, re-tuberization without emergence could aid lusala's survival in natural forests and help it cope with future climate change. This is because the trait delays vine emergence to the second rainy season, thereby providing dissemination over time and so promoting resilience.

Growth and yield from glasshouse studies cannot be extrapolated simply to yield in the wild or in cultivation. These studies were designed to investigate plant establishment by propagation, not potential lusala yields in the field or forest. Plants were close together, not provided with additional fertilizer, to which cultivated yams respond well (Ekanayake and Asiedu, 2003); UK winter light levels are low; and they were grown as monocrops. Nonetheless, comparisons among treatments and the five experiments show that propagation from vine cuttings, mini-tubers, and minisetts produced much lower tuber yields than from whole tubers; hence they are more useful for plant multiplication than food production. These results are also of value in comparing treatment effects with those reported for cultivated yams.

The length of vines and number of leaves (Table 2) were greater from: tubers with visible shoot buds and small or large tubers (Experiment 1); re-tuberized tubers (Experiment 2); larger minisetts and/or those cut from the proximal end of tubers (Experiments 4 and 5); and minisetts from tubers with visible shoot buds (Experiment 5). Similarly in cultivated yams, bigger minisetts or tubers show better growth (Aighewi *et al.*, 2015; Nyi, 1972; Law-Ogbomo and Remison, 2009; Morse and McNamara, 2018), and proximal minisetts provide greater growth than minisetts from middle and distal regions (Orkwor and Ekanayake, 1998; Passam, 1977). Lusala tuber yield from minisetts was lower than that from tubers (Figure 2). This tallies with cultivated yam studies where the size of planting material is correlated positively with yield (Kayode, 1984; Law-Ogbomo and Remison, 2009). Minisetts cut from the proximal end of tubers provided greater yield than those from other sections. The low yield from planting 10-mm-long mid-tuber minisetts (Figure 2c) matches reports in cultivated yams (Coursey, 1967; Orkwor and Ekanayake, 1998).

The best-yielding treatments in Experiments 1, 2, 4, and 5 provided 38–47, 70–90, 10–14, and 120 g plant⁻¹, respectively (Figure 2). The yields in Experiment 4 (Figure 2c) were low because of low-planting weights (minisetts or mini-tubers). The higher yield in Experiments 2 and 5 is compatible with longer growing periods (199 and 203 days from emergence to harvest) compared with Experiments 1 and 4 (137 and 116 days) and so greater radiation capture and conversion to assimilate (Monteith, 1977). These estimates of yield per plant cannot be compared with those from foraging in Zambia because foragers collect tubers from multiple plants (lusala 'hotspots'). Foragers take 20–44 minutes to collect 1 kg of tubers (Zulu *et al.*, 2019), and so we suggest that the range of yields when whole tubers were planted here, 3–120 g per plant (Figure 2), is not incompatible with those reports. In contrast, cultivated yams can produce as much as 33 kg of tubers per plant (Ekanayake and Asiedu, 2003). Hence, lusala yields are inferior, as expected for crop and wild relative comparisons.

The very limited cultivation of lusala to date in Southern Zambia replicates wild foraging close to the homestead in order to reduce tuber collection duration and competition for tubers from other foragers (Zulu *et al.*, 2019). Each rural household in the region cultivates a small area of land, on average, and foraging lusala from common forest lands provides additional, dry season, resources from beyond the village (Zulu *et al.*, 2019). The combination of long durations to emergence and then from emergence to harvest combined with lower yield than cultivated yams mitigate against rapid domestication of lusala, particularly in comparison with successive short-duration leaf vegetable crops in a smallholding for example. The report from a wild yam domestication study in Madagascar of reduced tuber yield in experiments compared with regeneration in forests (Andriamparany, 2015) further discourages investment in lusala's rapid domestication.

The current results are, however, valuable to the conservation of this wild edible plant, threatened by deforestation and overcollection (Zulu *et al.*, 2019), and to more sustainable foraging practices. The plant can be propagated successfully from tubers, minisetts, or cuttings. All three approaches can be used for *ex situ* conservation in living collections and can support *in situ*

conservation by improving foraging practices. A sustainably managed wild harvest might be better for the environment, and for rural households, than the large-scale agricultural production of lusala, particularly if it is supported by *in situ* conservation. Lusala grows well in scrubland and forest, the trees and shrubs being live stakes, and there is no need for weeding. It has the potential to contribute to a managed semi-natural environment that would offer other ecosystem services too. In the past, foragers re-buried small tubers to produce plants for the following year, but this practice appears to have been lost due to intense competition among foragers (Zulu *et al.*, 2019). This all points to the need for more sustainable foraging; lusala propagation from tubers, minisets, or cuttings can all be applied to support this. To propagate from tubers requires little beyond considerable patience. Re-planting small tubers with visible shoot buds would aid sustainable foraging at comparatively low cost. Planting 20-mm-long minisets cut from the proximal end of tubers would limit the fraction of foraged tubers diverted from the food chain, because foragers' families could consume the remainder of each tuber (the majority of the original tuber). Moreover, one vine has the potential to provide many cuttings. Plant multiplication by propagating from vines early in the rainy season, well before tubers mature, would reduce competition with foraging. Such an intervention could succeed with little equipment and expertise required (Table 3). Future studies should investigate lusala regeneration *in situ* using participatory research approaches in order to encourage farmer, forager, and community innovation to sustain the plant. This study is a key resource to support the early involvement of local communities in research to explore whether lusala would merit semi-domestication, rather than full domestication given long durations to emergence and harvest as well as low tuber yields. It would also aid the acceptance of more sustainable foraging of this diminishing resource. Such actions would support lusala conservation *in situ* and habitat conservation over the long term. Our results are also of value to the *ex situ* conservation of lusala and the wider utilization of this germplasm.

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