LEGUME SOCIETY

LEGUME PERSPECTIVES

Add a tuber to the pod:

on edible tuberous legumes

The journal of the International Legume Society

Issue 19 • November 2020

ISSN
2340-1559 (electronic issue)

Quarterly publication
January, April, July and October
(additional issues possible)

Published by
International Legume Society (ILS)

Co-published by
CSIC, Institute for Sustainable Agriculture, Córdoba, Spain
Instituto de Tecnologia Química e Biológica António Xavier
(Universidade Nova de Lisboa), Oeiras, Portugal

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Front cover:
Ahipa (*Pachyrhizus ahipa*) plant at harvest,
showing pods and tubers. Photo courtesy E.O. Leidi.

EDITORIAL

Although seeds are the major focus of global agriculture, there are also other storage organs, such as tubers and other underground storage organs. The Legume Society is delighted to present this issue of Legumes Perspectives, devoted to edible tuberous legumes that have been largely neglected relative to grain legume crops. We hope to increase the attention paid to these interesting species that produce edible tubers. Tuberous legumes have been domesticated and used as crops mostly in tropical regions of South America, Africa, and Southeastern Asia. None of these is extensively cultivated nowadays, however all harbor a great potential, especially in context of climate change and food security.

On behalf of the Legume Society, we wish to thank the authors of the articles in this issue for their thoughtful and well-prepared contributions.

Petr Smýkal,
Steven B. Cannon
Eric B. von Wettberg
Managing editors of the issue

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Carte blanche to…

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The Fabaceae is the third largest family of flowering plants, with over 800 genera and 20,000 species. It is an extremely diverse family of worldwide distribution, from arctic-alpine herbs, to annual xerophytes and forest trees. Cultivated legumes also fulfill many human needs besides their uses for direct human consumption: providing valuable timber, dyes, forage or fodder, medicine, soil fertility, etc. A little-known use of leguminous species is to provide edible underground storage organs. This trait of forming tubers and other underground storage organs seems to be either ecologically-related to highly seasonal habitats, or developmentally-related to forest canopy density.

Agricultural research has traditionally focused on staples, while relatively little attention has been given to minor (or underutilized or neglected) crops. Moreover, unlike most staples, many of these neglected species are adapted to various marginal growing conditions such as those of arid areas, salt-affected soils, etc. Furthermore, many crops considered neglected at a global level are staples at a national or regional level. The limited information available on many important and frequently basic aspects of neglected and underutilized crops hinders their development and use. But many of these species have important adaptations and long histories of cultural significance, highlighting their potential for sustainable agricultural intensification and climate resilience.

This issue begins with a taxonomic overview of genera that produce underground storage organs. The remaining articles are organized following the phylogeny presented in this taxonomic overview. Some of the species are quite well-known, such as Pachyrhizus (P. erosus, "jicama" being the most widely-grown in this genus), while others have seen no domestication and are only used regionally, through wild-harvesting (e.g. North American Pediomelum esculentum, "prairie turnip" or Tylosema esculentum, "marama bean"). We also include a review of Vigna subterranea, "bambara ground nut", which though it doesn't produce a tuber, it does produce underground seeds, and is of interest both for its local importance as a crop in sub-Saharan Africa, and for its taxonomic proximity to several other legume crop species in the same genus (including Vigna vexillata, "zombi pea," which is also profiled in this issue).

We hope that the information provided in this issue on tuberous legume species will contribute to their further utilization and understanding.

Left to right: Eric B. von Wettberg, Petr Smýkal and Steven Cannon.

Which came first: the tuber or the vine? A taxonomic overview of underground storage in the legumes

Jacob S. Stai[1], Eric B. von Wettberg[2], Petr Smýkal[3], Steven B. Cannon[4]

Abstract: Tuberous legumes are found in approximately 25 of the ~765 legume genera, and across four of the six subfamilies. Tubers (underground storage organs) have evolved repeatedly within the legumes, apparently in response to particular environmental conditions. Tuberous legumes are most often associated either with viny or prostrate/crawling aboveground growth. The tuberous+viny habit is found in liminal habitats, where the tuber apparently enables rapid vine growth into the canopy of nearby shrubs or trees. The tuberous+prostrate (or shrubby) habit is associated with droughty conditions, where the tuber evidently serves to store water and energy during harsh environmental periods. Edible tuberous species can be utilized for their conveniently stored nutrient reserves.

Key words: tuberous legumes, underground storage organs, tuber, vining habit, prostrate habit

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Agriculture, or the science and art of cultivating plants and livestock, might be colloquially thought of as the appropriation of nutritional reserves from plants and animals to serve the needs of humans. Plants pack away nutrients in many forms: in fruits, seeds, carbohydrate-rich sap, and in various other storage organs. Although seeds have been a natural focus for agriculture, tubers and other underground storage organs (USO) have been as well - in crops such as potato (*Solanum* and *Ipomea*), taro, manioc, African yams, carrots, parsnips, etc. In this issue of Legume Perspectives, we focus on these specialized organs as they occur in various legume species.

Underground storage can be accomplished in several ways anatomically: in rhizomes (underground stems, common in many grasses); in root tubers (thickened primary roots, such as in carrots); in specialized storage roots (e.g. *Dahlia* or yacón [*Smallanthus sonchifolius*]); or in proper tubers (thickenings along underground stems, as in *Solanum* potato). All of these forms seem to have some anatomical advantages over seeds for the purpose of nutrient storage. Seeds need to include complete propagules, with embryos and programs for dormancy and germination, and mechanisms of dispersal. In contrast, the primary requirements of underground storage are programs to manage the control of sink and source metabolism, and anatomy that permits accumulation of storage materials (typically starch, water, and sugars).

Why wouldn't USO therefore be more common across a taxonomy? Storage is, by definition, energetically costly. The benefit to the plant must outweigh the costs. By our count, USO are observed in 25 of the ~765 legume genera, and across four of the six subfamilies (Table 1, Figure 1, derived from data from the Legume Phylogenetic Working Group, 2017 (1)). Clues to the environmental conditions for evolution of such USO are suggested by the two main ecological-developmental syndromes that are typical for these genera.

The first syndrome occurs in grassland or desert environments that are highly seasonal, with a cold or dry season unsuitable for plant growth. Most tuberous legume taxa in these environments are small herbs or shrubs, but a few are perennial sprawling vines. In such seasonal settings a USO is of clear advantage because it can facilitate regrowth in a suitable season. Likewise, upon development of a tuber to store energy, a vining habit would be of clear advantage even in desert or grassland conditions without shrubs to climb onto, if the increased growth allows the plant to both survive lean times more effectively. One possible risk of overgrowth would be overuse of available water resources for cooling, but tubers may assist with this as

Table 1. Legume genera with underground storage organs. Only one species is indicated per genus; some genera have multiple species with such organs. Environment key: S scrubland; D desert; M montane; F forests; DG dry grassland; G grassland; R riparian.

Genus	Species (e.g.)	Region	Common names	Habitat	Habit
Phaseolus	*coccineus*	Central America	scarlet runner bean	S	vine
Vigna	*vexillata*	pantropical	tuber cowpea	S	vine
Lablab	*purpureus*	N Africa	hyacinth bean	S	vine
Vatovaea	*pseudolablab*	E Africa	olkalei	DG	vining shrub
Sphenostylis	*stenocarpa*	C&S Africa	African yam bean	DG,F	vine
Neorautanenia	*brachypus*	S Africa	zhombwe	D	prostrate herb
Pachyrhizus	*erosus*	C&S-America	jicama	DG,F	vine
Pueraria	*montana*	pantropical	kudzu	M,F	vine
Pediomelum	*esculenta*	N-America	Indian breadroot	DG	low herb
Pseudeminia	*comosa*	eastern Africa		S	vine
Psophocarpus	*tetragonolobus*	New Guinea	winged bean	G	vine
Flemingia	*vestita*	Australia, India	sohphlang	M,F	vine
Apios	*americana*	N-America, SE Asia	potato bean	R	vine
Lathyrus	*tuberosus*	Europe, W Asia	tuberous pea	G	climbing herb
Trifolium	*somalense*	E Africa		D	low herb
Coursetia	*caribaea*	C&S-America	anil falso	F	low herb
Peteria	*scoparia*	S N-America	camote del monte	D	shrub
Weberbauerella	*chilensis*	S-America		D	shrub
Weberbauerella	*tuberosum*	S Africa		D	low herb
Luetzelburgia	*andina*	S-America		D	shrub
Elephantorrhiza	*burkei*	S Africa	elephant root, sumach bean	D	shrub
Hoffmannseggia	*glauca*	S N-America	Indian rushpea	D	shrub
Neoapaloxylon	*tuberosum*	Madagascar	tala	D	shrub
Tylosema	*esculentum*	S Africa	marama bean	D	prostrate vine

well.

The marama bean (*Tylosema esculenta*) of the Kalahari is a clear demonstration of this model. *Tylosema* tubers have a variety of local uses, one of which is as an emergency water supply. They can reach prodigious sizes; according to Lost Crops of Africa: Volume II, a marama tuber weighing 277 kg was once dug up in Botswana. That publication estimated that perhaps 250 kg of this would have been water, earning the plant the moniker of a "living cistern".

The second syndrome takes place in environments where the canopy is not closed, but where the ground is sheltered and shaded by shrubs and smaller trees. This includes both broad scrubland landscapes and liminal habitats such as forest edges or riparian zones. In these environments, every one of the tuberous legumes of which we are aware has a vining habitat. In these scrubland habitats or riparian zones, vining plants compete in a rapid scramble to the tops of canopies; and vines growing from larger tubers would be at a clear advantage over vines growing from smaller seeds, because they start this construction race with far more raw materials at their disposal. Kudzu (*Pueraria lobata*) demonstrates this famously in the southeastern United States, and in its native range across east Asia and the south Pacific.

In our examination of the distribution of USOs across the legumes (Figure 1), we note a high frequency in the Phaseoleae. This suggests USO as a more likely ancestral state for this group than for many others. But we also note that every single tuberous member of the Phaseoleae of which we are aware has either a vining or prostrate habit, leading us to the question of which came first: the tuber or the vine?

Simple parsimony within the Phaseoleae would recommend the vine as more likely than the tuber, since there are more vining members of the Phaseoleae than tuberous; but most tuberous Phaseoleae are vines specifically after the model of our second syndrome, which takes a vining habit as a context that a tuber could augment. This is important because it is the first syndrome, not the second, which has arisen in the most diverse set of Fabaceae lineages, the Phaseoleae included (e.g. *Neorautanenia brachypus*).

Acknowledgements

This research was supported in part by the US Department of Agriculture, Agricultural Research Service, project 5030-21000-062-00D. USDA is an equal opportunity provider and employer. E.J.B.v.W is further supported by the USDA Hatch program through the Vermont State Agricultural Experimental Station

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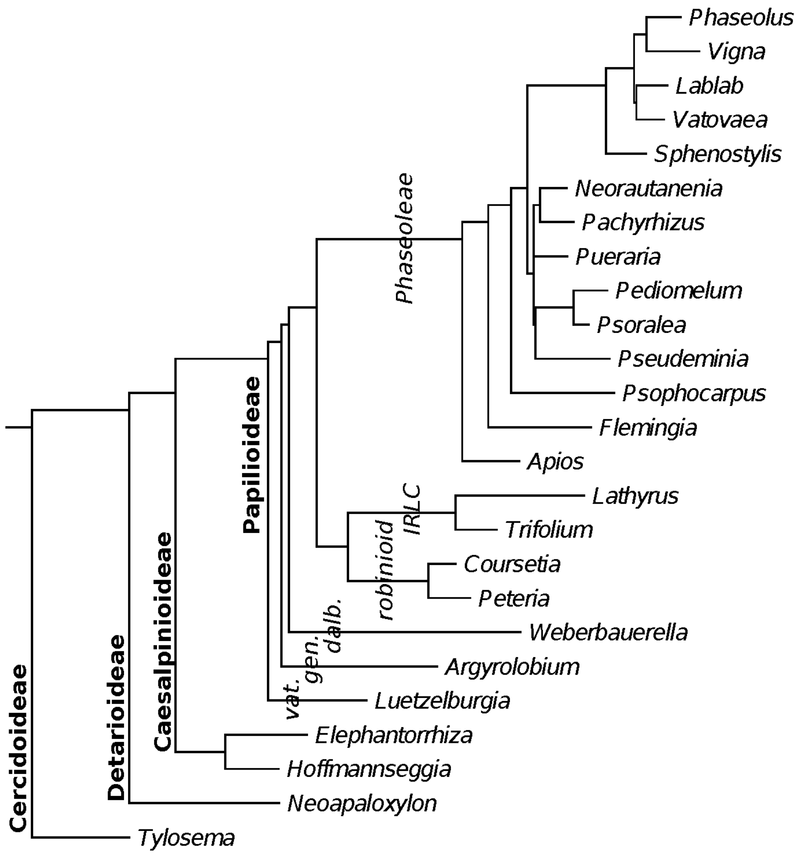

Figure 1. Legume genera with underground storage organs. See table 1 for species names and habitats. Phylogeny was calculated using matK sequences selected genus representatives from LPWG, 2017 (1). Clade abbreviations: "vat" = vataireoid; "gen" = genistoid; "dalb" = dalbergioid; IRLC = inverted-repeat-lacking clade.

The unusual biogeography of zombi pea, *Vigna vexillata*

Eric B. von Wettberg[1], Ken Naito[2], Andi Kur[1], Ndiko Ludidi[3]

Abstract: *Vigna vexillata*, also called "zombi pea" and "tuber cowpea," has sufficient genetic similarity with domesticated cowpea (*V. unguiculata*) to be partially reproductively compatible. *V. vexillata* is of interest for several reasons. It has been used in both northeast Africa and in Indonesia for its edible seeds and tubers. Phylogenetic and biogeographic evidence suggests independent domestication in both Africa and Indonesia. As an underutilized crop, research priorities include: germplasm collection and characterization, evaluation of agronomic characteristics (such as competitive partitioning between seed and tuber, and photoperiod interactions), biological and nutritional properties of seeds and tubers, and responses to biotic and abiotic stresses.

Key words: *Vigna vexillata*, zombi pea, tuber cowpea, underutilized crops

The zombi pea, *Vigna vexillata* (L.) A. Rich, is an underutilized legume species with a pantropical distribution and with a large amount of genetic and phenotypic variability. While its wild distribution includes all five continents (1-3), cultivated varieties have been reported only in Sudan and Indonesia (4, 5). These two cultivated forms are not cross compatible, suggesting that the wild form has substantial genetic variation within it and is likely a species complex. The Sudanese cultivated form, which is moderately tolerant to drought, salt and some biotic stresses, is generally cultivated for seeds. However, in Bali, where varieties are more susceptible to many kinds of stresses, it is generally cultivated for its tubers. Similarly, *V. vexillata* var. *lobatifolia*, which grows naturally in the Namibu desert, is widely eaten by indigenous people. It forms fleshy and crispy tubers that are edible even raw. The tubers of *V. vexillata*, as well as seeds, contains 15% N and can be a rich source of protein. Across all the regions where it is consumed, it often serves an important food security role, particularly in times of famine.

Besides being partially reproductively compatible with cowpea (*V. ungiculata*) and serving as a potential source of tolerance to biotic and abiotic stresses (e.g. 6, 7), *V. vexillata* has attracted some scientific attention because of its curious domestication history. Within the last two decades, several lines of data have been produced to suggest two independent domestication events for the Sudanese and Indonesian cultivars. This hypothesis has been substantiated by isozyme marker analysis of herbarium specimens (8); RAPD analysis of *V. angustifloria*, *V. macrosperma*, and *V. vexillata* (9); morphological and agronomic evaluation and test crosses between Bali and Sudan accessions (10-12);

Figure 1. Left: *V. vexillata* var. *macrosperma*; 165 g. Right: *V. vexillata* var. *lobatifolia*; 218 g. Both plants were harvested at 112 days. Photos courtesy Norihiko Tomooka.

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analysis of nuclear DNA-ITS and chloroplast *atpB-rbcL* spacer regions of *Vigna* (13) and most recently, by SSR marker analysis of 422 different wild and cultivated accessions of *V. vexillata* (14) (Figure 1). In the most comprehensive of the aforementioned studies, Dachapak *et al.* (14) showed that cultivated African and Asian accessions form separate clusters in both neighbor-joining and principle coordinate analysis; however, these results were obtained from only 14 accessions of cultivated *V. vexillata,* of which 8 were of African origin and 3 of Asian origin. Consequently, resolution of these domestications has been precluded by a lack of accessible germplasm from Indonesian accessions, a condition that was noted in the remaining studies as well. Collection of germplasm of *V. vexillata* from Bali will lend resolution to future research by increasing material available for genetic analysis.

Collectively, these studies suggest that the domestication of the zombi pea occurred as two independent events; however, currently little conclusion can be drawn regarding the domestication of *V. vexillata* specific to the Indonesian accessions, primarily due to a lack of collected germplasm and subsequently limited sample sizes in the aforementioned studies. While the details of these domestications are relatively unrefined, their independent occurrence provides a unique opportunity to explore domestication of tuber forming legumes.

As an underutilized legume, there are several aspects of the biology of Zombi pea warranting further investigation. Germplasm collections of wild and cultivated are needed, with both access to researchers and appropriate benefit sharing following international agreements. Furthermore, information on variation in its capacity for tuber formation, and trade-offs between tuber and seed production are needed. As tuber formation is likely a means of surviving dry seasons, tuber formation may vary geographically across the enormous *V. vexillata* range. The biological properties of seeds and tubers warrant more investigation, as some promising compounds for medicinal uses have been found previously (e.g. 15, 16). More information on cultural uses, beyond its use as food source during famines, is needed to understand how it is used, and how human movement may have contributed to its global distribution. Finally, as a reservoir of potentially adaptive traits for cowpea, information is needed on its stress tolerance and disease resistance.

RESEARCH TIMELINE

1954 – Ferguson (4) → "*V. vexillata* is a unique crop of Sudan where it is grown on a small scale in one locality for edible root & seeds"
1998 – Garba & Pasquet (8) → suggest independent domestication of Bali & Sudan *vexillata* based on herbarium accessions & isozyme variation
1998 – Spinosa *et al.* (9) → RAPD to analyze var. *angustifloria, macrosperma,* & *vexillata,* found no relationship between botanical varieties and geographical origins
2006 – Karuniawan *et al.* (5) → confirmed reports that *V. vexillata* is grown on Bali & East Timor as a cultivated root crop
2010 – Damayanti *et al.* (a,b,c) (10-12) → "studied agronomic and morphological traits of *vexillata* germplasm and found that accessions from Bali & Africa are different, but used a small number of accessions." Bali cultivar is not cross compatible with the African
2016 – Takahashi *et al.* (13) → "determined phylogenetic relationship of many *Vigna* accessions (including 9 *vexillata*) using DNA-ITC and chloroplast *atpB-rbcL* and found that Bali accessions are genetically different from other accessions
2017 – Dachapak *et al.* (14) → "report a genetic diversity analysis of 422 zombi pea accessions from various origins using SSR markers from azuki bean, cowpea, and mungbean"
- all of the above suggests that Bali was a separate cultivation event
- suggest that Bali cultivar was domesticated from wild Indian
- cultivated zombi pea may also be grown in Sri Lanka?

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In vitro culture of Bambara groundnut, *Vigna subterranea*: State of the Art

Napkalo Silue[1], Yaya Toure[2], Sergio Ochatt[3], Mongomaké Kone[2,*]

Abstract: Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an indigenous grain legume with a prominent place in the strategies to ensure food security in sub-Saharan Africa. Developing an efficient *in vitro* regeneration system is a prerequisite for application of genetic transformation in Bambara groundnut breeding. Therefore, several studies have been undertaken with various explants derived from *in vitro* seed germination and mature seeds. The morphogenetic responses induced by treatments including basal salt media, combinations and concentrations of growth regulators, concentrations and sources of carbohydrates have been recorded. This review highlights the main findings on *in vitro* breeding approaches in Bambara groundnut.

Key words: *in vitro* biotechnology, *Vigna subterranea* (L.) Verdc, morphogenetic responses

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Introduction

Bambara groundnut [*Vigna subterranea* (L.) Verdc.] is an indigenous crop cultivated mainly by women in arid and semiarid regions of sub-Saharan Africa. Seeds can be eaten fresh (semi-ripe), as a pulse (dry and mature) or ground into flour (1). (Editor's note: though not a tuber-producing legume, we include *V. subterranea* in this issue for its importance in local agroecosystems, and for potential in other arid environments, and in-keeping with the theme of underground storage organs. It is also interesting for its taxonomic proximity to other *Vigna* species, including *V. vexillata*, discussed in the preceding article). Bambara plays an important role in food security, particularly in terms of protein requirements for low-income farmers. The seeds are used also as feed for pigs and poultry and the haulm as fodder (2). However, as for most indigenous African plants (3), few research efforts have been devoted to the plant. Pod yields remain low and unpredictable (650 - 850 kg. ha^{-1}), because of the variability observed in growth and development within each landrace (4). The seeds contain anti-nutritional factors like tannins, oxalates and trypsin inhibitors that often lower product quality and protein availability (5).

Genetic selection, induced mutagenesis and, more recently, biotechnological techniques can result in a larger and more efficient cultivation of Bambara groundnut. Thus, approaches such as somatic hybridization and gene transfer may be used to improve this species and to transfer its favourable stress-resistant traits to other legumes but, for this, establishment of a regeneration system is the main prerequisite. Biotechnological tools have been used for various minor legumes including *Vigna mungo, Lathyrus sativus, Vigna angularis* and *Vigna sesquipedalis* (3), but literature in this domain for *Vigna subterranea* (L.) is scanty, with just a few formal reports so far. The present review summarizes the major results obtained since the first reports signalling *in vitro* culture techniques in Bambara groundnut (6,7).

In vitro seed germination and shortening of generations cycles in Bambara groundnut

In vitro seed germination in Bambara groundnut provided clean plant material (Figure 1j-l) for subsequent *in vitro* propagation, but was also used in the first report on the acceleration of generation cycles in Bambara, with up to 4 generations

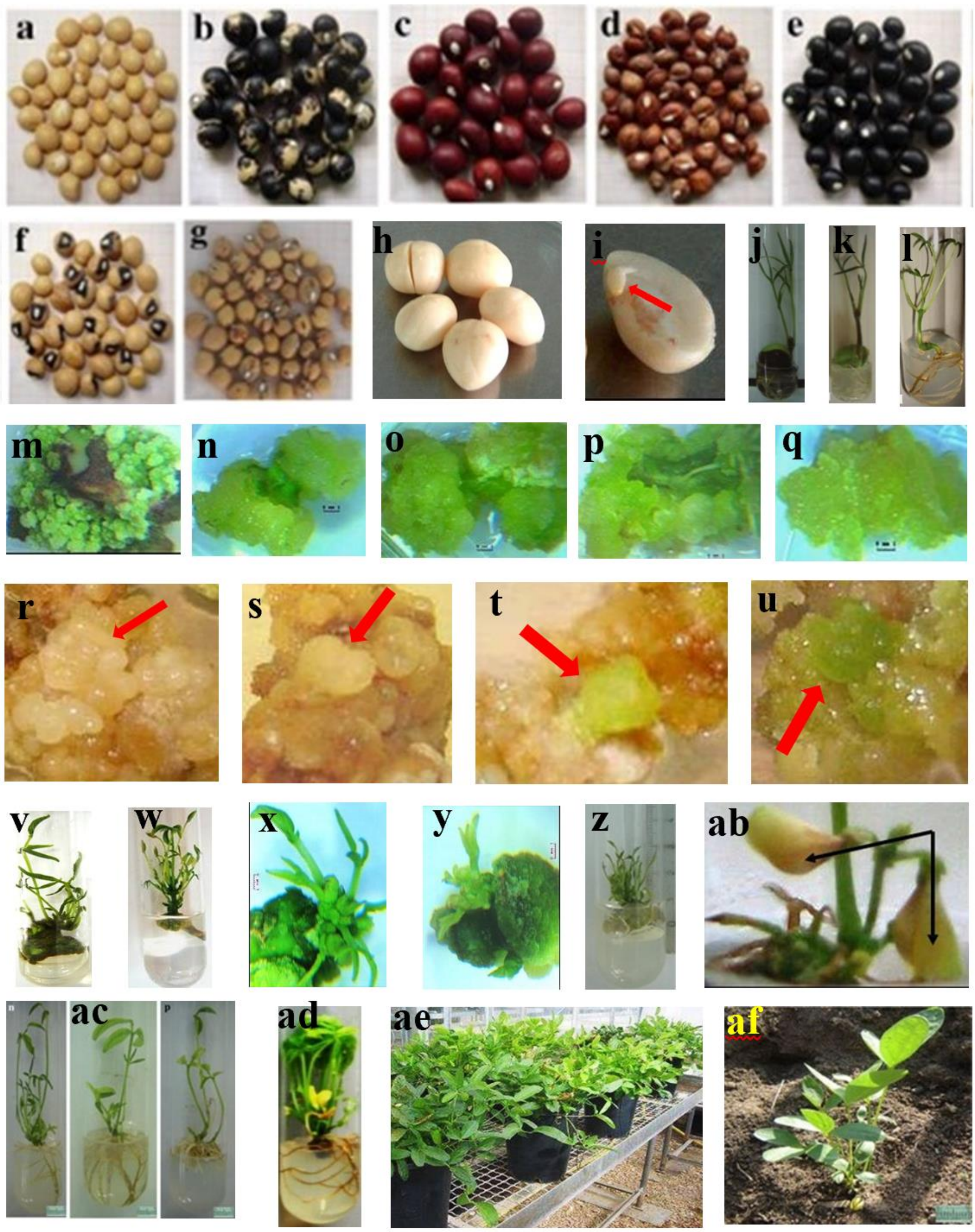

Figure 1. Morphogenetic responses from various explants excised from two-week-old *in vitro* germinated seedlings from mature Bambara groundnut seeds. (a-g) variability in colour and size of mature seeds of Bambara groundnut landraces; (h) seed removed from the shell after surface disinfection; (i) excised seed to remove the embryo axis (red arrow); (j-l) two week-old- plantlets from seed germination with shell, without shell and from the embryo axis; (m-q) callus growth from cotyledon, leaf, petiole, epicotyl and root explants; (r-u) induction of globular somatic embryos and development at the heart, torpedo and cotyledonary stages (red arrows); (v-z) induction of buds and development of shoot buds from cotyledon, embryo axis, hypocotyl, epicotyl and apex explants; (ab) direct induction of flowers (black arrows) from the cotyledon explant; (ac) elongation and rooting of regenerated plants on MS medium lacking growth regulator or supplemented with IBA and NAA; (ad) *in vitro* flowering of regenerated plant; (ae) regenerated plant acclimatized in greenhouse; (af) regenerated plant acclimatized and successfully transferred to the field.

per year combining *in vitro* and *in vivo* culture (8). Then, modifying this strategy, gamma irradiation was applied to *in vitro* germinated seeds to improve the productivity in Bambara (7).

These investigations revealed that although peeled seeds germinated within 7 days while the unpeeled control needed 14 days, by 21 days germination percentage and plantlet growth were comparable among treatments. Embryo axis explants gave 96.2% germination by 21 days of culture and 0.5-1 mg/l NAA enhanced root growth compared to auxin-free medium, but size of the embryo axis-derived plantlets was about one quarter that of the peeled or unpeeled seeds.

More recently, in addition to the use of seeds with (Figure 1a-g) or without seed coats (Figure 1h) or the embryo axis (Figure 1i), Koné *et al.* (9) evaluated: (i) full strength (MS), half (1/2 MS), and quarter-strength Murashige and Skoog (10) salts (1/4 MS); (ii) 3 % of sucrose, glucose, and fructose and (iii) 1, 2, 3, 4, 5, and 6% of the best carbon source defined in ii) for their effectiveness in promoting germination and subsequent seedling growth. Thus, explant type was the only factor with a major influence on germination capacity and embryo axis followed by seeds without coat gave the best rate of germination, while the best seedling growth was observed with the seeds without coat followed by the embryo axis, on half MS medium containing 3 % sucrose.

Callus induction and differentiation in Bambara groundnut

An efficient callusing protocol in Bambara would be useful for hybrid embryo rescue, *in vitro* mutagenesis, cell line screening, isolation and fusion of protoplasts, plant regeneration and gene transfer. Moreover, cell suspension cultures initiated from callus are of great importance in secondary metabolites production with industrial and pharmaceutical applications.

Callus induction and differentiation was first reported using cotyledon (Figure 1m) and epicotyl (Figure 1p) explants (11). For all Bambara landraces tested: Ci1, Ci3, Ci4, GB2 and MB, best callusing was on a medium with 3 mg/l BAP + 0.5 mg/l NAA, coupled with direct regeneration of roots from cotyledon and epicotyl explants or after a callusing phase. Koné *et al.* (12) extended the explants to leaves (Figure 1n), petioles (Figure 1o) and roots (Figure 1q) of seedlings from embryo axes of landraces Ci1, Ci2, Ci3, Ci4, GB1, GB2 and MB. Growth regulators were critical for the frequency of callus formation and differentiation potential, and the petioles gave the best responses on MS medium with 3-5 mg/l BAP and 0.5 mg/l NAA. After 3 months, callus produced both adventitious buds (11-27%) and somatic embryos (50-68%) (Figure 1r-u), with a strong genotypic effect. On the other hand, Konaté *et al.* (13) reported callus induction and proliferation from embryo axes on MS medium with vitamins B5 and various growth regulators. After four weeks of culture, best results were obtained from the basal part of embryo axes on a medium with 2,4-D (0.5 mg/L) and 84 mM sucrose, and with landraces Ci2, Ci3, Ci4, Ci5, Ci6, Ci7, Ci10 and Ci21. Soon after, they also reported callus induction and proliferation from cotyledon explants, which was best with the distal segment of landrace Ci7, on a medium with 2,4-D or Picloram, while combining auxins with various cytokinins resulted in reduction of both callus formation and proliferation (14).

Whole plant regeneration in Bambara groundnut

Lacroix *et al.* (6) were the first to report regeneration of fertile plants through direct shoot organogenesis from embryo axes from the mature seeds of two landraces. They used a medium with BAP and NAA for six days, then cut axes transversely, transferred them onto a medium with BAP alone where 100% gave 5-8 shoots each. Shoots rooted on NAA and plants were transferred to the greenhouse, where all regenerants were morphologically normal, fertile and diploid as shown by flow cytometric analyses and chloroplast counts of guard cells. Koné *et al.* (11) established the first protocol for *de novo* plant regeneration from cotyledon (Figure 1v) and epicotyl (Figure 1y) explants of several landraces from Côte d'Ivoire, with successful regeneration of normal and fertile plants from both explants and all landraces. Of interest in this work was the formation of flowers (Figure 1a,b), without any intervening callus phase, and the distinction between landraces according to their relative nuclear DNA content, determined by flow cytometry (Figure 2). In another study, multiple shoots were obtained from both epicotyls and hypocotyls but regeneration efficiency was higher with the former and using BAP, while equimolar concentrations of kinetin or TDZ or adding NAA for shoot bud induction did not improve results. Shoots were rooted on auxin-free half-strength MS medium, 62% of plantlets were weaned and 73% of these survived in soil (15).

Owunibi *et al.* (16) reported direct organogenesis from nodal segments with pre-existing meristems of 28-days-old plantlets from embryo axes of two accessions of Bambara groundnut, TVSU1834 (Nigeria) and TVSU255 (Niger), of which the former responded best.

Koné *et al.* (17) regenerated buds from embryo-free cotyledon explants of ten Bambara landraces excised transversally or

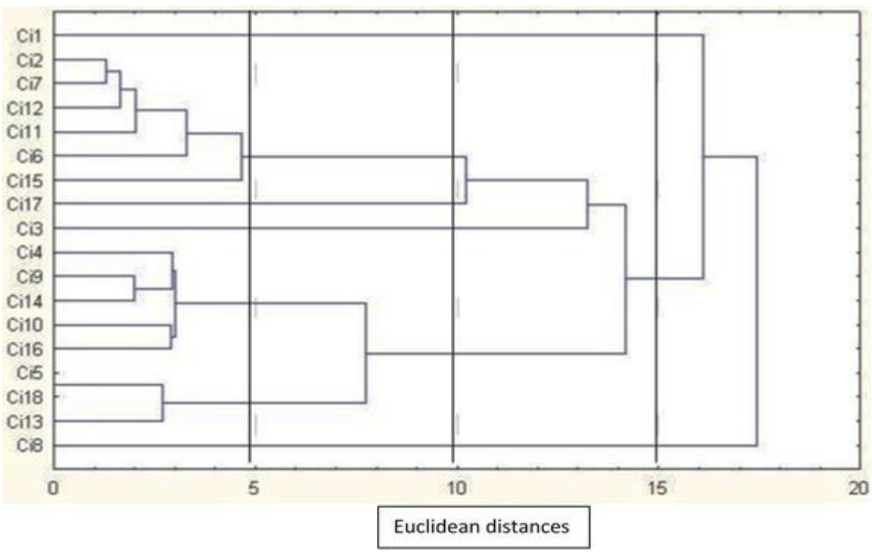

Figure 2. Dendrogram of the variability in genome size between 18 *Vigna subterranea* (L.) landraces.

longitudinally (referred to as proximal, middle and distal) and placed onto the medium with the adaxial or abaxial side down. Multiple shoot formation occurred on MS medium with BAP alone or combined with NAA, but was restricted to the proximal segment of cotyledons, adaxial side down on the medium. Caulogenesis was best with landraces Ci6, Ci2, Ci4 and Ci5, regenerated shoots were rooted on hormone-free MS medium and all rooted plantlets survived transfer to a sand soil mixture and morphologically normal plants were hardened and transferred to greenhouse for further growth to maturity and seed set.

Finally, Napkalo *et al.* (18) described a rapid, simple and efficient micropropagation protocol from shoot tips (Figure 1z) of Bambara groundnut using MS medium with B5 vitamins, sucrose and with different concentrations of various cytokinins, of which Thidiazuron at 0.45 µM was best. The individual shoots rooted on MS medium (Figure 1ac), were hardened in the greenhouse (Figure 1ae) and established in the field (Figure 1af), where 70-80 % survived, morphologically normal and fertile.

Conclusion

The literature review showed that different morphogenetic responses can be observed in Bambara groundnut with different explants. Among these, the embryo axes and cotyledon fragments were obtained from mature seeds, while the epicotyl, hypocotyl, leaf, root, nodal segment and apex explants were taken from two-weeks-old plantlets. Seedlings were issued from the germination of mature seeds with or without a shell and the development of the embryo axis. The main morphogenetic responses observed were the induction of calli, the formation of roots before or after a callogenesis phase, the induction of multiple buds and their subsequent development into shoot buds and the induction of somatic embryos on media supplemented with different concentrations and combinations of 2,4-D, BAP, and NAA. Any shoot buds obtained were rooted on MS medium without growth regulators, regenerated plants were identical to the mother plants and about 70% survived upon field transfer.

Perspectives

Callogenesis protocols established will be useful for *in vitro* hybrid embryo rescue, *in vitro* mutagenesis, cell line screening and plant regeneration and will be applicable for genetic transformation in Bambara groundnut. Likewise, the efficient regeneration systems established by direct organogenesis will help genetic improvement of this grain legume crop relevant for low-income, food-deficit countries in Africa by rendering it more amenable to the exploitation of novel biotechnological breeding tools. Plant regeneration from shoot apex can be efficiently used for mass propagation, germplasm preservation and probably also for gene transfer. Future work will focus on developing somatic embryos and their conversion into whole plantlets. The regeneration of plants from calli will also be exploited for the early selection of Bambara groundnut lines tolerant and / or resistant to biotic and abiotic stresses.

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African yam bean, *Sphenostylis stenocarpa*

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Abstract: Many tuberous legumes are underutilized, with little or no research attention given to their genetic improvement, thereby limiting their potential to support food, nutritional, and economic security. Understanding and exploiting the potential of African yam bean, *Sphenostylis stenocarpa,* is important for increasing its role, particularly in sub-Saharan Africa under climate change. This crop is utilized as seeds and as tuberous roots, as both are of high nutritional value. The Genetic Resources Center (GRC) of the International Institute of Tropical Agriculture (IITA), based in Ibadan, Nigeria is focusing on understanding the genetic diversity in our collection and evaluating key traits to build a platform for pre-breeding and breeding.

Key words: Genetic resources, genetic improvement, underutilized, *Sphenostylis stenocarpa*

African yam bean, *Sphenostylis stenocarpa* (Hochst. ex A. Rich.), is an underutilized annual tuberous legume that serves as a food and nutritional security crop in sub-Saharan Africa and is the most economically important species in the genus *Sphenostylis* (1). It produces beans in pods with varying seed patterns and colors (2). The seed is rich in protein (19.5%), carbohydrates (62.6%), fat (2.5%), vitamins and minerals. The protein is made up of over 32% essential amino acids, with lysine and leucine being predominant (3). Omeire (4) reported higher levels of amino acids (lysine, histidine, arginine, valine) than previously recorded in other legumes.

It also produces edible tubers that contain approximately 19% crude protein (5). The tubers (Figure 1) resemble sweet potato, mature in five to eight months (6), and come in various shapes such as spindle, round, oval, and irregular. In West Africa only the seeds are currently consumed. These are rich in protein and minerals, particularly potassium, magnesium and iron. Accessions have been evaluated that could be used as parents in crop improvement strategies (7).

The crop has many sociocultural uses as a traditional crop in Africa for instance, during lean periods in the eastern parts of Nigeria the crop is utilised as substitute for cowpea (*Vigna unguiculata* (L.) Walp.) The lectin content in seeds have been reported to be effective in the control of some leguminous pests.

Genetic diversity assessment of the available landraces and continuous germplasm exploration and collection to fill gaps in the existing *ex-situ* collection will enhance the success of breeding. GRC has been carrying out morphological and molecular characterisation of our international collections.

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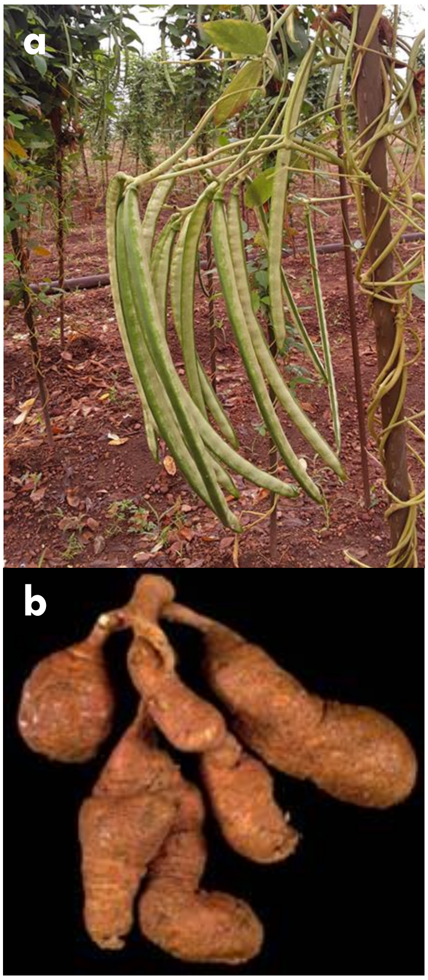

Figure 1. a) African yam bean pods and b) tuberous roots.

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Ahipa, *Pachyrhizus ahipa*: a legume with edible tuberous roots

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Abstract: Legumes are mostly known by their protein and oil rich seeds, but the main value of the old Andean legume crop "ahipa" (*Pachyrhizus ahipa*) resides in its nutritious tuberous roots. Traditionally, ahipa roots have been consumed fresh, but nowadays they are also a source of valuable starch and other sub-products. Ahipa can be cropped without the need of N fertilizers due to the capacity to fix atmospheric N_2, in symbiosis with appropriate rhizobial strains. In temperate regions, with the use of efficient rhizobia, economic yields may be obtained in rainfed or irrigated agriculture.

Key words: *Pachyrhizus ahipa,* tuberous-root, starch, sugars, proteins

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Introduction

Among the different species of root crops native to the Andean region of South America, there are some which are little-known and today at the risk of extinction (1, 2). Cultural changes, social factors, management difficulties together with the increased production of cash crops are pushing these species away from their traditionally areas of production (3, 4). Ahipa (*Pachyrhizus ahipa* [Wedd.] Parodi) is a tuberous-root producing legume cultivated by indigenous cultures in pre-Columbian South America (4). As with many other crops in the Andean region, loss of valuable cultivars is likely as the cultivation is being gradually abandoned by traditional producers (4-8). In Northern Argentina, the last record of ahipa production was dated in the 1990s (9, 10). However, ahipa cropping still remains in Bolivia although in very limited and isolated areas (7). Ahipa roots are traditionally consumed fresh, and according to traditional knowledge, they hold certain medicinal properties (2).

Here, we provide an update on some physiological, agronomic, and food properties of ahipa. Increasing popularity of this crop may lead to new uses and may reduce the loss of genetic biodiversity.

Roots

The tuberous roots of ahipa (Figure 1) have traditionally been consumed raw, as a vegetable, providing vitamin C and energy (starch and sugars) in the diet of Andean communities (5, 11); or processed into juice as traditionally consumed during the festival of *Todos Santos* (2, 6). Ahipa food properties include energy and dietary fiber, with low anti-nutrients contents (12). The roots also can be used for preparation of staple foods like West African *gari*, which is normally made with manioc root (*Manihot esculenta* Crantz) (13), or in the production of raw materials like biodegradable plastics for industrial applications (14, 15). Particular properties of the root starch make it an

Figure 1. Ahipa plant at harvest, showing pods and tuber. Photo courtesy E. O. Leidi.

alternative for gluten-free flour (16) or food thickener (15, 17), and it has also been tested for production of biodegradable starch films (14).

Two parts may be distinguished in the root system of ahipa: a cylindrical xylopodium, with sprouting capacity; and the proper tuberous root, which is predominantly fusiform or radish-shaped (7, 18), with a variable thickness depending on the accession (4) and the sowing time and density of plantation (19). The process of tuberization begins at around 60 days after germination by the increased production of root parenchyma cells by the vascular cambium (18). Interestingly, the beginning of tuberization and flowering are simultaneous processes in well characterized day-length-neutral accessions like AC521 (19), a period in which reproductive pruning, i.e. the removal of flowers, should start for increasing final root yield (4). Available accessions produce a single tuberous root per plant although some individuals produced secondary roots with storage capacity. Ahipa roots constitute a more nutritious energy source than other root crops, providing proteins, dietary fibres and minerals (11, 45). Traditional uses of roots in folk medicine (7) have been related to their curative properties (stomach and lung diseases) which have yet to be scientifically proved. The dry matter content in ahipa roots ranges from 16.3–21.1% and it contains up to 48–54% starch and 19–28% sugars (17, 20, 21).

The protein contents in the roots are higher than in other starch-accumulating species (manioc, sweet potato (*Ipomoea batatas* (L.) Lam.), yam (*Dioscorea* L. spp.)) and they may contribute significantly to its nutritional quality (22). No major storage protein in ahipa roots has been identified and are mostly salt-soluble (60% of total tuber nitrogen) (22).

Seeds

The seeds of ahipa (Figure 1) can potentially become a valuable source of protein and oil for feed and industrial uses once the rotenone content is removed, yielding similar values to soybean (20). The ahipa seeds contain high protein content (22). The high oil content of seeds provides similar fat yield to soybean (*Glycine max* (L.) Merr.) (21) although the main differential features are the high palmitic acid and total tocopherol contents (20). A main deterrent for the use of seeds for feed or food is – as mentioned above – the content of the toxic rotenone ($C_{23}H_{22}O_6$). Variation in rotenone content has been found in ahipa seeds (23) and breeding programmes for developing rotenone-free cultivars are in progress (24).

Agronomy

Most of the available agronomical data on yam bean involve the tropical Mesoamerican relative *P. erosus* (25), but there are main differences between ahipa and *P. erosus* in growth habit, ecophysiology and product quality (4). Cultivation of ahipa in the main production areas of Bolivia (Departments of Chuquisaca and Tarija) follows traditional knowledge and it requires intensive hand labour mostly for management (weeding, reproductive pruning, etc.) (26).

For harvesting roots of marketable size (0.3–0.5 kg), around 180 days of cultivation are required for growing conditions in SW Spain but available accessions are highly variable in final root size. Maximum root weight obtained in isolated plants in field conditions reached 1.5 kg. However, roots for raw materials extraction or for animal feeding should require shorter growth periods. Sowing date affects productivity when delayed as growth season is shortened (27).

Low temperature is a main limiting factor to be considered as it greatly reduced productivity in areas of cool nights (27). Low temperature in the autumn (air temperature just reaching -1ºC) may cause frost damage in shoots. Apparently, the species do not show acclimation induced by shortening days and cool nights (28).

Flower pruning and root yield

In order to obtain increased root yield reproductive organs (inflorescences or flowers only) are removed by hand to reduce sink size and avoid fruit-root competition for carbon assimilates and nutrients (29, 30). By following such management practice, a significant increase in root yield is obtained (31, 32) but with the need of intensive manual labour. Assays with growth regulators (gibberellins or ethylene-releasing chemicals) provided satisfactory results for reducing reproductive development and increasing root yields (29). There is an important variation in the number of flowers produced in available accessions (33). In field trials, yield of unpruned accessions ranged from 9 to 40 tonnes ha^{-1} (20). Seed inoculation with selected rhizobia strains significantly increased root yield soils of low N content (27, 34).

Figure 2. Seed inoculation with rhizobia results in initially spherical nodules which later develop in indeterminate nodules with many branches.

Climate and soil requirements

The limited cultivated areas with the species in South America have a semiarid climate with mean temperatures of 16–18 ºC and wide diurnal oscillations and 400–700 mm of annual precipitation (4). The species seems to be rather sensitive to low temperature at germination (optimum around 30ºC) or high temperature during vegetation (above 35ºC) (35).

Soils from the original habitat of the species in South America may differ widely to those in South Portugal and South West Spain where different ahipa landraces have been tested (27). However, sandy soils with reduced mechanical impedance and good aeration would facilitate root growth and reduce risks of anoxia in rainy seasons.

Nitrogen

A distinctive feature over other carbohydrate-producing crops relies on its N_2 fixation capacity with rhizobia (32, 34, 36). Nodules formed in ahipa roots are initially spherical and then develop as indeterminate nodules with many branches (Figure 2). Seed inoculation with effective rhizobia greatly increases root and seed production and the selection of strains for symbiotic effectiveness may provide greater shoot growth and N content (34). Inoculation with superior strains increased significantly ahipa seed and root yield and seed protein content under field conditions. The estimation of N provided by symbiotic N_2 fixation reached 160–260 kg N ha^{-1} while the amount of N left in crop residues was approximately 50 kg N ha^{-1}.

Conclusions

Ahipa, a tuberous-root legume, appears a promising alternative to other traditional crop sources of raw materials, like starch and proteins. Still to be studied are the nutraceutical properties traditionally associated to fresh root consumption. With a low environmental impact and low input requirement, ahipa provides an attractive alternative to traditional sources of carbohydrates for conserving non-renewable resources and maintaining farmer profitability.

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Economic and ethnobotanical uses of tubers in the genus *Pueraria* DC

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Abstract: Plants in the genus *Pueraria* DC., including the invasive kudzu, produce large, deep tubers that can weigh upwards of 400 pounds. Within its native Asia, tubers from various *Pueraria* species have been used for millennia for food and medicine, with more contemporary uses including soil stabilization and enrichment. Three species in particular provide unique ethnobotanical and economic uses: *Pueraria tuberosa* has been used as a food source and contraceptive for centuries in India; *Pueraria candollei* var. *mirifica* is purported to have strong rejuvenating properties, with laboratory research confirming numerous potential medical uses to treat symptoms of menopause in women; *Pueraria lobata* (kudzu) is a mainstay in traditional and contemporary Chinese medicine and a source of highly-prized starch used to produce fine delicacies in Japan. Here, a synopsis of the ethnobotanical and economical uses of tubers from three *Pueraria* species are reviewed.

Key words: *Pueraria*, kudzu, Fabaceae, ethnomedicine, Leguminosae

Introduction

Pueraria DC. is a genus of woody lianas or vines native to East and Southeast Asia. Recent molecular systematic work resulted in revision of the genus towards a more natural circumscription of ca. 16 species through the segregation of *Haymondia* A.N. Egan & B.Pan, *Toxicopueraria* A.N.Egan & B.Pan, and *Neustanthus* Benth., and placement of *P. stricta* Kurz to *Teyleria* Backer (1, 2). *Pueraria* species grow as large vines supported by deep root systems with large tubers that can grow to great sizes and depths. Kudzu (Ge Gen in China, Kuzu in Japan; *Pueraria lobata* subsp. *lobata*) is the most widely known and widely grown *Pueraria* species, having been introduced outside of Asia to the United States and elsewhere. The economic and ethnobotanical uses of *Pueraria* species have been documented for millennia, with kudzu mentioned as far back as 500 B.C. in China (3) and 600 A.D. in Japan (4). The earliest accounts mostly refer to kudzu (also called Ko or Ka) as a source of fibers for cloth making and weaving. Kudzu is also mentioned in China's first medicinal work, *Shen Nong Ben Cao Jing* (Divine Husbandman's Classic of Materia Medica, Anonymous, *ca.* 25-200 AD).

While kudzu is often the species most referenced in early, and even contemporary, literature, a number of *Pueraria* species may be or have been interchangeably used for certain purposes, particularly fiber usage. Three species, however, are well known for their economically and ethnobotanically important tubers: kudzu, *Pueraria tuberosa* (patal kumra) and kwao khruea (*Pueraria candollei* var. *mirifica*) (Figure 1). A synopsis of the economic and ethnobotanical use of tubers in each species is offered here.

Pueraria tuberosa

Pueraria tuberosa is likely native to the Himalayan regions, from Pakistan and the western Himalayas to Nepal and Sikkim in India. *Pueraria tuberosa* is also found throughout India, where it has likely become established by escaping cultivation through a long history of ethnobotanical use as food or more particularly in ayurvedic medicine (5). *Pueraria tuberosa*, also known by numerous local vernacular names, not limited to Indian Kudzu, patal kumra, Vidarikanda, or Vidari, has been used as a food source by tribes in the Himalayan regions where the tuber is eaten either raw or more commonly boiled (6).

In ethnomedicine, *P. tuberosa* has been traditionally used as a rejuvenating tonic towards sexual disorders, inflammation, cardiotonic, diuretic, and galactagogue, among others (7). Many of these ethnomedicinal uses have also been investigated in the laboratory setting. For example, a tuber mash is used to treat arthritis by tribal people of the Eastern Ghats in Andhra Pradesh (8). Several studies have shown the effectiveness of various *P. tuberosa* tuber extracts to relieve inflammation through different modes of action, likely through such isoflavonoids as daidzein or puerarin (7) or isoorientin (9). In recent decades, *P. tuberosa* has been investigated for its properties as a contraceptive, an ethnomedicinal use that has been documented among several Indian tribes for

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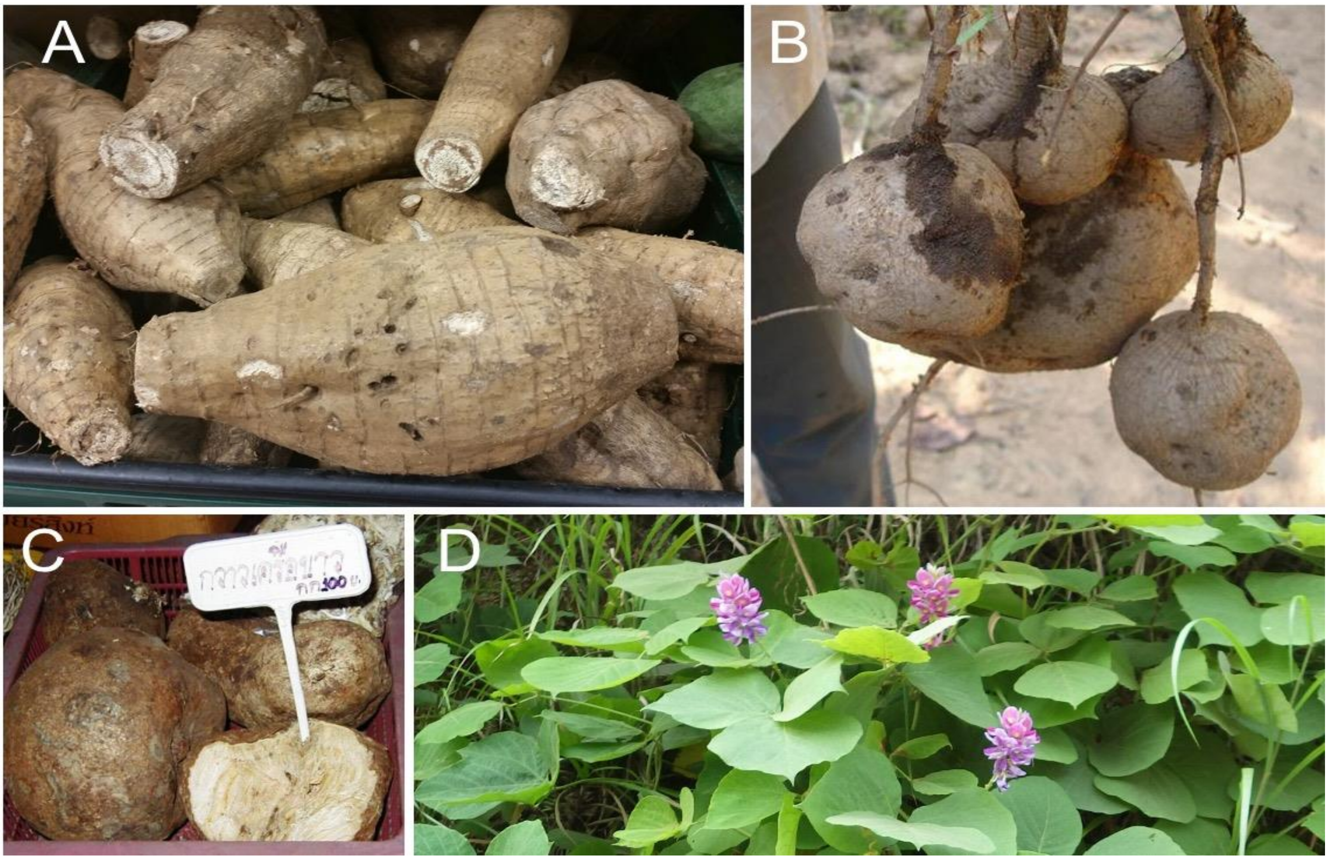

Figure 1. A) roots of Kudzu, photo by Emöke Dénes, used under creative commons 2.5 license, https://creativecommons.org/licenses/by-sa/2.5/legalcode; B) roots of *Pueraria tuberosa* from India, photo by Pankaj Oudhia, www.discoverlife.org; C) roots of *Pueraria candollei* var. *mirifica* in a market in Thailand, photo by VanLap Hoàng used under creative commons 2.0, https://creativecommons.org/licenses/by/2.0/legalcode; D) flowers and leaves of kudzu in China, photo by Ashley N. Egan.

many years and the effects of which are likely due to the presence of a number of isoflavonoids isolated from the tubers (10, 11).

Pueraria candollei var. *mirifica*

Pueraria candollei var. *mirifica*, hereafter *P.c. mirifica*, is endemic to Thailand where it has a renowned, centuries old history of use as a rejuvenating plant, particularly for its ability to restore vitality to menopausal women and andropausal men. Known as Kwao khruea (กวาวเครือ) in Chiangmai and northern provinces of Thailand, the large tuberous roots are dried and powdered, then mixed with a bit of honey to produce peppercorn-sized pills that are reputed to restore menstruation to post-menopausal women and hair to bald men (12, 13, 14). A number of other therapeutic effects claimed include improved sleep, promoting appetite and general vigor, reversal of greying hair, improved and more youthful complexion, and stimulation of breast development (12). Indeed, these 'miraculous' properties are the reason for its name (Latin, *mirificus* = amazing) (15).

Early laboratory research on ethanolic extractions from pulverized tubers of *P.c. mirifica* were shown to induce oestrus in mice (16). Since then, numerous studies have been conducted on *P.c. mirifica* root compounds, elucidating a distinct number of unique phytoesterols, isoflavonoids and coumestans present in the tubers (17). Key players in its efficacy are deoxymiroestrol and miroestrol, compounds from plants that mimic the biological activity of estrogen in humans and have been shown to be the most potent phytoestrogens in nature to date (18, 19).

Myriad laboratory research endeavors have investigated these phytoestrogens. However, the vast majority of these studies have been conducted on non-human mammals, with only a few clinical trials completed. That said, clinical trials show great promise for the relief of various climacteric symptoms in post-menopausal women (14). As an example, in a randomized, double-blind, placebo- controlled study, Manonai *et al.* (20) reported reduced genital tract dysfunction and vaginal dryness and reversal of atrophy of the vaginal wall, all symptoms of menopause in women. Manonai *et al.* (21) also showed that *P.c.mirifica* may help reduce osteoporosis in menopausal women.

Clearly, *P.c.mirifica* includes a number of important phytochemicals that have potential medicinal applications for treatment of menopausal symptoms or as dietary supplements to help ease this transition in women's lives. However, further research, particularly clinical trials, are needed to ascertain efficacy, safety and dosage limits for human use, with standardization of active elements included. The active phytoestrogens within a 1 gram sample of pulverized *P.c. mirifica* tuber can vary as much as 20-fold, depending on harvest time, geographical region, and extraction protocol (17). Use of *P.c.mirifica* root is not without its side effects, which, depending upon dosage, can be considerable. For example, study of *P.c. mirifica* supplementation in Donryu rats found significant induction of mammary adenocarcinomas (22). Several preparations have recently been marketed without regulatory oversight as dietary supplements in the United States, mostly directed towards breast augmentation in women. A simple Google search of "*Pueraria mirifica*" will call up numerous shopping opportunities for dietary supplements. While the benefits of *P.c.mirifica* are evident, standardization of chemical contents make its use challenging and warrant caution. Nevertheless, this tuber has numerous positive prospects for medicinal use.

Pueraria lobata

Kudzu has a long ethnobotanical history in Asia and includes a complex nomenclature. The kudzu species complex includes three taxa variously recognized at species, variety, or subspecies level. Most recently, kudzu has been recognized as a variety under the scientific name of *Pueraria montana* var. *lobata* (Willd.) Maesen & Almeida ex Sanjappa & Predeep. This taxon includes a complicated synonymy, including *Pueraria lobata* (Willd.) Ohwi var. *lobata*. Recent morphological, genomic, and phylogenetic research suggests that *Pueraria montana* (Lour.) Merr. comprises a distinct morphological and genetic species apart from *lobata* and *thomsonii* (A.N. Egan, unpublished data). As such, I champion recognition of *Pueraria montana* as its own species and adhere to the nomenclature suggested by Ohashi *et al.* (23), recognizing kudzu [*Pueraria lobata* (Willd.) Ohwi subsp. *lobata*] and one sister variety, *Pueraria lobata* subsp. *thomsonii* (Benth.) H. Ohashi & Tateishi, sometimes called Thomson's kudzu, but often called kudzu interchangeably.

Kudzu's native distribution includes China, Korea, and Japan, where it's history of ethnobotanical use may correlate with its phylogeography as assessed through microsatellites (24, A.N. Egan, unpublished data). Kudzu is highly prized in these countries where it has been wild-gathered and cultivated for millennia for use as food, fodder, fiber, paper, and medicine (4). Some of the oldest textile fragments unearthed in China date to ca. 4,000 BCE and are made of kudzu (25). With Indochina and southwestern China representing the center of diversity of *Pueraria*, some researchers believe that kudzu originated in China and was brought to Japan and Korea through human-mediated translocation for ethnobotanical use (4). The earliest archeological evidence of kudzu textiles in Japan dates to 300-538 AD from a kofun burial mound on Kyushu and use of kudzu textiles continued for millenia by Samurai and Japanese nobility as evidenced in writings and archeological material dating to the Edo period (1603-1868 AD) (26).

Table 1. Selected ethnobotanical and ethnomedicinal uses of *Pueraria* tubers.

Species (and vernacular)	Uses	Place	References
P. candollei var. *mirifica* (Airy Shaw & Suvat.) Niyomdham -- Kwao khruea (Thailand)			
	Rejuvenating tonic	Thailand	13, 15, 47
P. lobata subsp. *lobata* (Willd.) Ohwi -- Ge Gen, Ka (China); kudzu (United States, Europe); ko, kuzu (Japan)			
	Soil erosion control	United States	40, 48
	Soil enrichment	United States	48, 49
	Food	E & SE Asia, New Guinea, Polynesia, Philippines	50, 39, 51, 38, 52, 53
	Asthma, colds, fever, skin rashes, influenza, diabetes, flatulence, diarrhea, indigestion and other digestive disorders	E & SE Asia	54-58, 4, 47, 27
	Deafness	China	59
	Hypertension symptoms	China	58, 60
	Antidote to croton oil and other poisons	China	57, 47
	Topical treatment for dog bites	China	57
	Alcoholism and hangover	China, Japan	4
	Obesity	Japan	4
P. lobata subsp. *thomsonii* (Benth.) H. Ohashi & Tateishi			
	Ailments very similar to kudzu	Asia	see above
P. tuberosa (Willd.) DC. -- Patal kumra (India - Gondi)			
	Food	India	61, 5
	Tonic, emetic, galactagogue, cooling medicine, demulcent, and poultice to treat gravel, low fever, ulcers and menorrhagia	India	62
	Asthma	India	63

Ethnomedicine and Pharmacology

While kudzu's use as a fiber plays a key role in Asian ethnobotany, the use of its tubers for medicinal, food, and cultural uses continues from ancient times to today. Kudzu is considered one of the most common ingredients in traditional Chinese (27), Japanese, and Korean pharmacopoeias (28). Known as Radix Puerariae in China, root extracts from both *P.l.lobata* (called Puerariae lobatae radix or Ge Gen) and *P.l.thomsonii* (called Puerariae thomsonii radix or Fenge) are used as both food and medicine, sometimes interchangeably (28), although more often *P.l.lobata* is used medicinally whereas *P.l.thomsonii* is used as a food source. Recent research has suggested that *P.l.lobata* has higher concentrations of phytochemicals making it the better candidate for medicinal use. Indeed, a number of biochemical and molecular analyses suggest distinction between root extracts from the different taxa (e.g. 28 - 31). Ge Gen powder is formed from the dried tuber usually of *P.l.lobata* and is widely sold as a patent medicine in China known as Yufeng Ningxin used to treat spasms and promote blood circulation (28). Much of the contemporary Asian pharmacopoeia stems from traditional ethnomedicinal uses that span millennia and have been documented over the last century (Table 1; for a more extensive review see 32). In China, Ge Gen is also used to treat fever, headache and neck stiffness, to promote measles rash manifestation and resolution, promote production of body fluids to relieve thirst and treat diabetes, and to resolve diarrhea (27). In Japan the two varieties of *P. lobata* are known as kuzu and two traditional medicinal preparations are the use of the whole root dried and powdered used to make teas known as kuzu-ko and the purified, white starch from roots used to make kudzu cream preparations called kakkon (4). Kuzu is prized for its treatment of colds and influenza in Japan. During a kuzu collecting expedition, the author was herself treated with kudzu tea for cold-like symptoms while in Japan. Kudzu teas and preparations are also suggested for treatment of acid indigestion and upset stomach, largely due to its alkaline quality, acting much the same as milk of magnesia.

Many of these uses are attributed to the unique isoflavone and phytoestrogen compounds in the roots, such as Puerarin, Daidzin, Daidzein, and other phytochemicals. The pharmacological properties of Puerarin include a wide array of effects, including cardioprotection, vasodilation, antioxidant and anticancer activity, neuroprotection, anti-inflammation, osteogenesis, alcoholism inhibition, and insulin resistance attenuation (33). Modern scientific research has investigated a number of the traditional uses and discovered some remarkable results to treat specific conditions. For example, women lose estrogen during and after menopause, the loss of which can cause bone loss. This is problematic for women who need bone grafts or healing after bone trauma. Given the phytoestrogenic principles of several chemicals produced by *Pueraria* species (see discussion above under *Pueraria mirifica*), the use of *Pueraria* root extracts to bolster hormonal levels in white rabbits has been shown to aid in bone healing and bone formation (34). Kudzu extracts have also shown effective at helping to combat obesity and reducing serum glucose levels in diabetic patients as well as aiding to decrease alcoholism by reducing the amount of alcohol drunk during a single session (35). However, as discussed above for *P. mirifica*, much of the pharmacological research has been done on non-human mammals and few clinical trials exist (36).

Food Consumption

Kudzu tubers have long been used as a starch source in Asia, the Philippines, New Guinea, Fiji, and New Caledonia, where tubers were cooked by roasting, boiling, or processed for starch flour (18, 37, 38, 39). Perhaps the place where kudzu is most famous as a food source is in Japan, where it is a highly prized source of a fine, clear starch used as a thickening and jelling agent for fine cuisine. Refined kudzu root powder produces a strong starch that jells or sets with a clear, translucent appearance, making it a perfect ingredient to make kudzu-mochi and other fine desserts in Japan (4). Kudzu can be found regularly on restaurant menus in Japan, particularly for kudzu-mochi desserts, kudzu noodles, and other main and dessert courses. To this day, use of kudzu for starch dominates in Nara prefecture, Honshu, where the present-day Morino Yoshino-Kuzu Honpo shop was established over 450 years ago (4).

Given its historical use to treat metabolic syndrome and diabetes mellitus, use of kudzu root as a food source for diabetic and obese patients, and those struggling with digestive disorders, shows promise (4, 36). The author has used kudzu root powder in preparing gravies and puddings for friends and family that are gluten intolerant and for which wheat flour could not be used. In Japan, kudzu root creams and teas are routinely prescribed in hospitals as the major starch source and a main course meal in food preparations for patients with digestion issues (4).

Soil stabilization and enrichment

In its native range, kudzu is contemporarily used for medicine and food, however very different uses were ultimate reasons for kudzu's introduction into the United States nearly 150 years ago. Kudzu was first brought to the United States at the 1876 Philadelphia Centennial Expedition Fair where it was planted outside a Japanese pavilion. It was recognized as a favorable, fast-growing ornamental. Not long after, it was discovered that kudzu's fast growth, deep and large root system, and ability to root at the nodes of stems that contact the ground made it a panacea for soil erosion problems and its large leaves seemed a reasonable forage for cattle (40). Taking advantage of the deep and strong root systems, millions of seedlings were planted by the U.S. government in the early to mid 1900's to stabilize slopes, river and lake banks, and railroad cuts. Further, the U.S. government subsidized farmers to grow acres of kudzu as a cover crop for soil enrichment due to its nodulation and nitrogen enrichment of soils and for fodder (41). It wasn't until the 1950's that America realized the threat of "the plant that ate the south". Today in the U.S., kudzu is known as a notorious invasive species that creates monocultures across the landscape (Figure 2) and can tear down forests. In most states, it is now illegal to plant kudzu, however, personal discussions by the author with various farmers and growers across the southeastern United States found that some still prefer to plant kudzu, particularly along irrigation ditches for soil stabilization (A.N. Egan, personal observations). Currently, kudzu occupies between 4-6 million acres across the American landscape. Eradication of kudzu is a difficult, long, and arduous process due to its deep, penetrating root system and ability to root at the nodes.

Potential for Biofuels

Whilst giving a seminar on the introduction history and genetics of kudzu in Japan, the author was warmly greeted by several Japanese botanists who insisted that, if kudzu truly grew to the extent showcased in the U.S., its harvest and sale of root starch could easily fund her entire research program! (A.N. Egan, personal communication). Indeed, a major reason why kudzu is not considered a noxious weed in its native China, Korea, and Japan, is because it is widely used and wild-harvested in these areas (42). Several researchers and American naturalists have suggested that we do the same. One potential use for kudzu is as a biofuel. Kudzu has been shown to produce ethanol comparable to that of corn (43). Experiments have shown that acetone-butanol-ethanol fermentation by *Clostridium acetobutylicum* of kudzu alone, without the need for costly additional nutritional supplements to aid the bacterial fermentation process, was highly successful (44). While the entire plant can be used as a starch and glucose source for fermentation, the roots are the primary starch source. With belowground biomass of between 5 and 13 t ha^{-1} estimated across Maryland, Alabama, and Georgia, use of kudzu tubers for biofuel production has promise (45). The prospective gain from use of already established kudzu patches across the U.S. will have to be weighed against the upheaval of the soil and environment caused by digging up kudzu's large and deep root systems and the potential invasive spread of cultivation of biofuel species (46).

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Figure 2. Kudzu patch at Bowman's Island, Chattahoochee River National Recreation Area, Georgia. Photo by National Park Service, used with permission.

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Ethnography of prairie turnip, *Pediomelum esculentum*

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Abstract: Prairie turnip, *Pediomelum esculentum,* is a tuber-producing legume in the tribe Psoraleae, native to the Great Plains of North America. It is an herbaceous perennial (1), emerging in late spring to early summer and flowering in the mid-summer. It was wild-harvested throughout the year by Native Americans in the Great Plains region. *Pediomelum* is also of interest for evidence of a nitrogen-fixing endophyte, for which there is evidence of vertically-transmitted infection.

Key words: Ethnography, underutilized, prairie turnip, *Pediomelum*

Epigraph

"Other seeds were issued to us, of watermelons, big squashes, onions, turnips, and other vegetables. Some of these we tried to eat, but did not like them very well; even the turnips and big squashes, we thought not so good as our own squashes and our wild prairie turnips. Moreover, we did not know how to dry these new vegetables for winter; so we often did not trouble even to harvest them."– Buffalo Bird Woman, 1917 (2)

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Prairie turnip (*Pediomelum esculentum* (Pursh) Rydb.) is a temperate tuberous member of the family Fabaceae and tribe Psoraleae with chromosome number n = 11 (3). Prairie turnip is native to the Great Plains of North America, ranging from Alberta and Manitoba south to Texas, and from the Front Range to the Mississippi, with populations east of the Mississippi occurring primarily in Wisconsin (4). It prefers well-drained, often rocky soils, and in moderate climates does not require irrigation after seed germination, though irrigation can be beneficial in dry ones (5). It is an herbaceous perennial (1), emerging in late spring to early summer and flowering in the middle of July, with fruit maturation near July's end. In nature, an abscission layer forms 2-5cm belowground after fruiting, 2-5cm above the storage tuber, releasing the whole herbaceous part of the stem system to scatter seed across the prairie like a tumbleweed (1). It does not, however, resemble the famously-invasive Russian *Kali* tumbles.

Prairie turnip has a roughly egg-sized storage taproot (6) (Figure 1), with a thin inedible bark that must be peeled before consumption (5). Mature taproots are typically produced after two to four years'

Figure 1. *Pediomelum esculentum*. Image courtesy the University of Iowa Press, http://uipress.lib.uiowa.edu/ppi/display.php?record=Psoralea_esculenta

growth (7), and garden experiments suggested a four-year planting scheme as optimal (10). Reported estimates of protein content vary: Stahnke (1) reported 7.5% protein content by dry weight, whereas the USDA's FoodData Central nutrition database reports ~15% protein by dry weight when raw, half of which remains after boiling (8, 9). The roots have a firm texture even when cooked long (10). When observed under a microscope, the roots contain cells which specialize to store either starch or protein, but not both (1).

Virtually all Native Americans throughout the Great Plains region used prairie turnip as a primary dietary staple or as a significant component of their diet. This was so year round, but especially in the winter months (5). Although popular consciousness treats buffalo hunting as the primary nutritional source for indigenous people on the Great Plains, the importance of vegetable foods to local indigenous economies is plainly seen throughout the historical record. Reid (7) describes a report that the Arapaho and Cheyenne exchanged flour pounded from these roots to the Arikara for their cultivated maize, "at a profit of three or four measures for one." Likewise, Reid (7) mentions eyewitness historical accounts which reported that at the council preceding their summer bison hunt, the Omaha people would plan their route in a way that accounted for three important things: always wood and a plentiful supply of water foremost, but then also a place where they could gather prairie turnips in great quantities. Great quantities were useful because of the prairie turnip's excellent storage properties, as it keeps almost indefinitely when dried; and if the foliage was maintained, that portion could be braided into ropes for ease of both storage and transport, as the Lakota did (7). Prairie turnip continues to be an active part of the economies and cultures of indigenous communities today; prairie turnip flour is often the secret ingredient in American Indian frybread, as the traditional agent to make it fluffy (11).

One other point about the prairie turnip's anatomy and uses deserves mention: it appears to be the host for a constitutive, vertically-transmitted infection with a nitrogen-fixing endophyte (12). This symbiotic endophyte, described as being most closely related to *Bacillus nealsonii* or *Bacillus circulans*, could be isolated from sterilized seeds from three different sources, as well as from both the nodules and the tubers of prairie turnips grown under sterile conditions. As Deutscher writes (pg. 43) (12), "The presence of these *Bacillus* spp. in all of these tissue implies the endophyte is passed vertically from one generation to the next and, upon germination, begins a systemic colonization process where it spreads throughout the plant." This represents not just a novel symbiotic partner for a legume, but an entirely different modality by which symbiotic nitrogen-fixing relationships can occur not previously documented in legumes, known rather from sugarcane (13).

Lastly, it must be mentioned that determining which scientific name to use to refer to this species in this article was not entirely trivial. *Pediomelum esculentum* was settled on, but prairie turnip is commonly referred to by the synonymous name *Psoralea esculenta*, including in peer-reviewed literature (a few seconds of searching provided the author with examples as recent as 2019). Indeed, many species of the genera *Psoralea* and *Pediomelum* are routinely called by equivalent names in both genera, reflecting general confusion. Use of the *Pediomelum* name was chosen by reference to the 2016 thesis of Abubakar Bello (14), who reconstructed the phylogenetic history of the tribe Psoraleae using eight conserved molecular markers and found strong genetic support for the North American *Pediomelum* genus and African *Psoralea* genus that were proposed by Grimes (15).

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Winged bean, *Psophocarpus tetragonolobus*

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Abstract: Many tuberous legumes are underutilized, with little or no research attention given to their genetic improvement, thereby limiting their potential to support food, nutritional, and economic security. Understanding and exploiting the potential of winged bean will enhance its role in regions where it can be cultivated. This crop is utilized as seeds and as tuberous roots, as both have high nutritional value. The Genetic Resources Center (GRC) of the International Institute of Tropical Agriculture (IITA), based in Ibadan, Nigeria is focusing on understanding the genetic diversity in our collection and evaluating key traits to build a platform for pre-breeding and breeding.

Key words: Genetic resources, genetic improvement, underutilized, *Psophocarpus tetragonolobus*

Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is a tropical legume belonging to the family Fabaceae and tribe Phaseoleae, with chromosome number of 2n = 2x = 18. The likely origins of winged bean are in Papua New Guinea, Mauritius, Madagascar and India (1). The genus *Psophocarpus* contains nine species, eight of which are wild (1). Winged bean is a twining perennial plant that is cultivated as an annual and has highly nutritious tuberous roots and quadrangular pods, whose length can be up to 30cm long with longitudinal wings subtended on its vegetative part (Figure 1). The pods contain

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Figure 1. Winged bean plant, showing mature and immature pods.

5-20 seeds, which can vary in colour from white, through varying shades of yellow and brown to black or mottled (Figure 2). *Psophocarpus tetragonolobus* is commonly called "princess pea," "goa bean," "asparagus pea" (for their delicate asparagus-like flavour of young, immature pods), "four-angled bean," "Manila bean," "supermarket bean," or "winged bean." It combines many of the desirable characteristics of the green bean, garden pea, spinach, mushroom, soybean, bean sprout and potato (1). Apart from the stalk, almost all the entire plant is fit for human consumption. The tender pods are the most widely eaten part of the plant (and best eaten when immature, as the mature green pods are fibrous). The pods can be harvested within two to three months of planting. The tubers contain a high amount of protein in dry weight (20%) (2). The seeds contain a high percentage of crude protein content ranging between 29.8% and 42.5% Harding *et al.* (3) observed a higher capacity for nodulation and nitrogen fixation in winged bean than in any other tropical legumes such as cowpea, common bean, groundnut, soybean, etc. The exceptionally high protein level in the various plant parts could be attributed to the high nodulation and nitrogen fixing rates (2).

Winged bean is rich in tocopherol, an antioxidant that increases vitamin A use in the body (2). The tubers can be used as a root vegetable, similar to potato, and have a nutty flavour. They are also much richer in protein than potatoes. The dried seeds can be useful as flour and also to make a coffee-like drink. Winged bean can also be used to produce winged bean milk made from water, winged beans, and emulsifier. The milk has similar characteristics as soymilk. Winged bean provides many opportunities for economic benefit. Mature winged bean seeds can command high prices (2). In spite of this promotion it is only rarely found in home gardens or cultivated by farmers (2). GRC is exploring the potential of winged bean by understanding the genetic diversity within our collection and evaluating variation in key traits.

Figure 2. Winged tubers (left) and pods and seeds (right).

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Apios americana: natural history and ethnobotany

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Abstract: *Apios americana*, an herbaceous perennial legume with climbing, vinous habit, produces underground stem tubers along stolons. *A. americana* was wild-harvested by Native Americans throughout eastern North America. There is also some evidence of cultivation and transport of landraces. Early historical records of *Apios* in North America describe its important role in the diet of numerous tribes. Cooking methods included: boiling, frying, drying and grinding into flour. They were cooked, variously, with maple syrup, animal fat, roasted, or used in stews. *Apios* remains promising as a crop, particularly for its perenniality, its ability to tolerate wet soils, and its adaptation across much of eastern North America, and its nutritional value – with high levels of protein and complex starches.

Key words: *Apios americana*, ethnobotany, hopniss, potato bean

Apios americana is an herbaceous perennial legume with a climbing and vinous growth form. It is notable for the stem tubers that form as thickenings along stolons. It has many common names including Groundnut, Wild Bean, Hopniss, Potato Bean, and Indian Potato. The genus *Apios* is composed of three species native to Asia (*A. fortunei*, *A. carnea*, and *A. delavayi*) and two species found in North America (*A. americana*, *A. priceana*) (1, 2). DNA sequence analysis from *Apios* identifies it as a monophyletic lineage that diverged early from the other Phaseoloid legumes (1). The two North American *Apios*

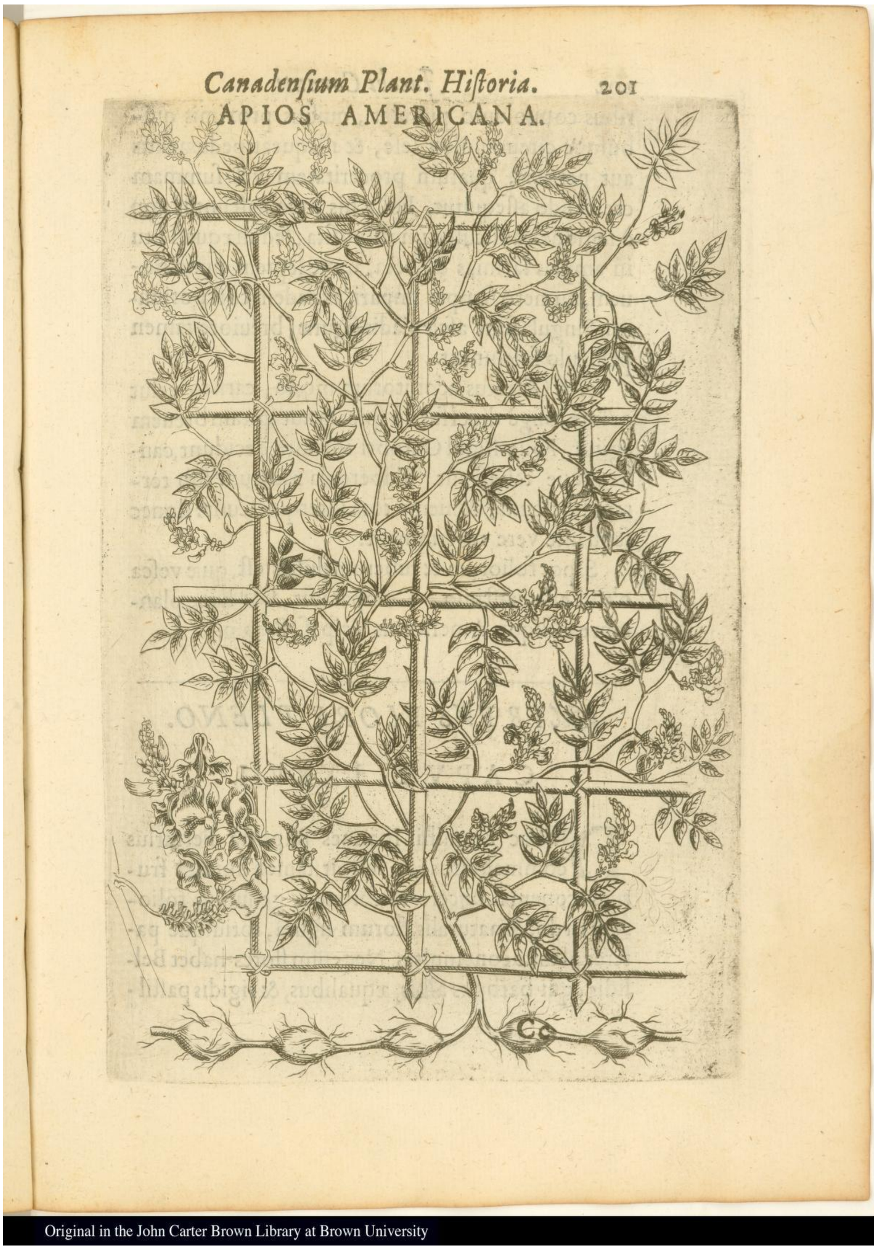

Figure 1. Engraving of Apios by Jacques Philippe Cornut, 1635. Cornut was a Parisian physician who obtained specimens for his garden from French traders and explorers. Image credit: John Carter Brown Library, Box 1894, Brown University, Providence, R.I. 02912. JCB call number: E635 C819c.

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Figure 2. Flowers of two accessions from the Blackmon breeding lines (showing differing flower color). Credit for the image: Steven Cannon.

Figure 3. Pods from an accession collected in Sturbridge, Massachusetts. Credit for the image: Steven Cannon.

species form a clade that evolved from Asian ancestors in the late Miocene or early Pliocene after crossing a land-bridge (1). *Apios priceana*, restricted mainly to rocky open woodlands and threatened by habitat loss, is known from 47 populations in 22 counties in Alabama, Kentucky, Mississippi and Tennessee (2). *Apios americana* (referred to henceforth as *Apios*) grows throughout eastern and central North America from southeastern Canada to the Gulf of Mexico (2).

Apios thrives in diverse ecosystems including temperate forests, thickets, prairies, sloughs, riverbanks, pondbanks, seeps, and wetlands (3). Typical locations possess moist soils close to standing or flowing bodies of water, although it can succeed in soils that are much drier (3, 4). Indeed, *Apios* was often found in upland meadows before fire suppression became commonplace (5), and high productivity and shorter rhizomes are encouraged by well-drained, neutral pH soils (4). *Apios* grows well in both full or intermittent sunlight and can flourish in loamy, sandy, or gravelly soils (3). Being adapted to many climatic and environmental regimes, *Apios* can survive both severe flooding and moderate drought (6). As with many of the other legumes, *Apios* can fix atmospheric nitrogen in the soil through symbiosis with rhizobial bacteria (7).

The *Apios* vine grows vigorously after initial establishment and can spread 10-20 feet in a growing season. Although the plant does well in open areas by trailing, the vines readily climb up vertical supports such as woody or robust herbaceous plants (Figure 1). The alternate compound leaves have a pinnate arrangement of 3 to 11 ovate leaflets along the rachis with a single terminal leaflet and varying pubescence (4). The fragrant pink, purple, and earth-toned flowers appear in the summer (July-October), clustered in axillary racemes (Figure 2) (3). These flowers produce relatively small pods with seeds roughly the size of mung bean (*Vigna radiata*) seeds (Figure 3).

The stolon comprises a series of nodes and internodes, axillary buds with scale leaves at the nodes, and a terminal hook (8). The stolon length can range between two to four feet under field conditions in a single growing season (6). Tubers form when the internodes swell at the beginning of the basal zone of each axillary bud. The swelling extends basipetally along the internode while the tubers also expand horizontally (8). The completed tuber usually occupies the region basal to the node where adventitious roots also appear (20-50% of internode), although sometimes the entire internode swells (8). The tuber-laden stolon appears like a beaded string or necklace (9). Tuber characteristics exhibit high variation among *Apios* populations, including shape, size, number per plant, density per length of stolon, and yield. The mother tuber (overwintering tuber), from which shoots emerge in the spring, also varies in size relative to the daughter tubers produced during the growing season. The smooth and thin skins of young tubers are covered in lenticels, whereas older tubers have a thick and gnarled surface. Tubers exude a creamy latex after cutting as a natural wound-sealing reaction. *Apios* vines are highly frost sensitive and do not survive the winter. The above-ground shoot system regenerates from the below-ground tubers each spring.

Native Americans used *Apios* as food throughout its historical range, from the Atlantic coast to as far west as the Missouri river basin (5, 10). The starch and protein rich tubers were of paramount interest, although the seeds were also sometimes cooked and used for food (10). Even though the shoots and flowers are also edible, there are fewer reports of these organs being used in significant amounts. Beardsley (10) reports on its consumption by varied ethnic groups including the Iroquois confederacy, Great Sioux Nation, Menomini, Potawatomi, Meskwaki, Lenape (Delaware), Ojibwe (Chippewa), and Muskogee (Creek). The French Jesuit missionary Le Jeune noted that Native Americans subsisted upon *Apios* in eastern Canada (10).

European colonists also consumed *Apios* tubers during the period of settlement (10). Early English visitors to the Atlantic seaboard described *Apios* as being plentiful, widely distributed, relatively easy to harvest, and tasty when cooked. The pilgrims of Plymouth relied on *Apios* for survival due to scarce grain supplies during the early years of the colony (10). They learned how to collect and cook the tubers from the Wampanoags (9). James Smith, an 18th century soldier and frontiersman, reported that *Apios* tasted similar to sweet potatoes after dressing with raccoon fat (10). The Swedish Botanist Peter Kalm, a visitor to eastern North America in 1749, reported that both Swedish and English colonists harvested the tubers and that Native Americans ate both tubers and seeds (10). Speaking from personal experience, Henry David Thoreau reported on *Apios* as a quality source of food when boiled or roasted (9).

Beardsley (10) mentions various cooking methods used by the Native Americans that include boiling in water, roasting in hot ash, and stewing with meat or corn. Dean (9) describes Native Americans frying the tubers or drying and grinding them into flour. The flavor is described as similar to a potato yet drier and nuttier (9), or as a cross between a potato and boiled peanuts with a mealy texture (4). Foragers report the tubers are sweeter or tastier if dug up following the first frost in late autumn (9). *Apios* tubers make quality chips as the low levels of reducing sugars prevent browning when they are fried (4).

Many Native Americans stored tubers for the winter by peeling and drying them in the sun on racks (10). The Meskwaki and Ojibwe would parboil and slice the peeled tubers before drying them (10). The Menomini made preserves of sun-dried tubers using maple syrup or maple sugar (10). Drying the tubers results in a higher relative protein content than the fresh ones (10). Many contemporary cultivators have found that the tubers store well when kept dormant in a cool and moist location such as a refrigerator, a root cellar, or in soil outside (3).

The tubers are an excellent source of both carbohydrates and protein. The majority of short-length carbohydrates in tubers (86-90%) are glucose and sucrose, with the remaining 10-14% composed of oligosaccharides such as raffinose and stachyose (11). Starch, deposited as granules, makes up almost 70% of the total carbohydrate of tubers (11). The cell walls of tubers comprise cellulose, hemicellulose, and pectins (11). The dry-weight protein content varies from 13-17%; this value is three times greater than found in potatoes or other common root vegetables (4).

We do not recommend eating *Apios* raw. Pancreatic hypertrophy in rats fed with raw *Apios* tubers suggested the presence of protease inhibitors that interfere with protein hydrolysis and act as an anti-nutrient (12). Cooking the tubers destroys these compounds and eliminates this problem (12). A few people experience gastrointestinal distress after eating *Apios* tubers, sometimes after having eaten the tuber previously without negative consequences (3).

Many authorities mentioned the deliberate cultivation of *Apios* by Native American groups, including the Iroquois and Muskogee (10). Beardsley (10) was unconvinced that *Apios* was ever intensively cultivated in an organized manner in cleared fields, and more accurately described it as a wild-gathered food. Nevertheless, wild *Apios* would have been protected from threats and may have been relocated closer to settlements for ease of access (10). Alternatively, Elfers (5) argued that Native Americans produced open savannas through controlled burning and *Apios* grew there in profusion among the grasses and widely-spaced trees. *Apios* populations, particularly in drier upland environments, may have been introduced purposefully for human consumption (5). It is also possible that tubers may have been transported over large-scale distances as populations moved to new territories or were exchanged as part of commercial relations among cultures.

Breeding work on *Apios* was carried out by Dr. Bill Blackmon and Berthal Reynolds in Louisiana in the 1980s and early '90s. Further breeding and evaluation work has been continued by researchers and enthusiasts in recent years, as reviewed in Belamkar *et al.* (2020; this issue).

Acknowledgements

We acknowledge the many independent botanists, gardeners, and foraging enthusiasts, who continue the research and exploration of *Apios* as a wild food and crop. This research was supported in part by the US Department of Agriculture, Agricultural Research Service, project 5030-21000-062-00D. USDA is an equal opportunity provider and employer.

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RESEARCH

Apios americana: crop improvement and genomic characterization

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Abstract: Over the last 40 years, several researchers have worked to develop improved cultivars of *Apios americana*. Notable improvements have been made in yield and tuber size. Research over this period has also led to better understanding of genetic and genomic limitations (e.g. sterile triploid populations) and characteristics of floral biology (an unusual tripping mechanism in the flower, which has made manual pollination difficult). Some genomic resources have also been developed, including transcriptomic assemblies and a genetic map.

Key words: *Apios americana*, genomic, crop improvement

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Wider adoption of *Apios americana* (hereafter just "*Apios*") by cultivators requires domestication and development of superior cultivars to overcome certain agricultural hurdles. Aggressive stolon and vine growth makes it difficult to prevent their spread to areas where *Apios* may be unwanted or difficult for mechanical harvesting. The *Apios* tubers can be somewhat small and laborious to dig from soil. Moreover, unharvested tubers sprout up in the spring without regard to the field layout and can be similar to volunteer weeds and also inhibit efficient harvesting.

Despite these challenges to industrial-scale *Apios* cultivation, it has been successfully produced commercially in both Japan and South Korea. Indeed, *Apios* has been grown and eaten in the Aomori Prefecture of Japan for over a century (1). *Apios* consumption in East Asia is popular mainly due to consumer confidence in its nutritional benefits and medical properties related to postpartum health, blood pressure, cancer, and osteoporosis (1, 2). In contrast, the inability of Europeans to adapt *Apios* to their known methods of agriculture may help explain its relative modern disuse (3). *Apios* was first brought to Europe in the late 16th century and investigated as a potential cultivated crop (4). References to its presence in gardens persist as late as the 19th century; e.g. there was interest in its cultivation in Ireland to provide an alternative to potatoes during the Great Famine of 1845-1852 (4). Nevertheless, these historical attempts at domestication and expanded acceptance were unsuccessful due to such factors as low tuber yields from slow-growing *Apios* plants (4). Further, the need to develop an alternative to potato was reduced with the development of potatoes resistant to late blight and other diseases (4).

Diploid populations of *Apios* are characteristic of the South and lower Midwest, but triploid populations that bear sterile flowers predominate in the northern part of the United States (5, 6). Diploid and triploid forms are morphologically identical and hard to differentiate in the field (5). Diploid populations spread both sexually by seed and asexually by stolon growth and tuber division, whereas triploid plants totally rely upon asexual means of reproduction (5,

Figure 1. ***Apios*** **accession LA-1849, selected by B.D. Reynolds and W. Blackmon in the late 1980s. Photo credit: Steven Cannon, USDA-ARS North Central Regional Plant Introduction Station, 2011, Ames, IA**

6). Fruit set is not common even in diploid *Apios*, perhaps due to partial self-incompatibility and lack of genetic diversity among vegetative clones that makes most cross-pollinations into effective self-pollinations (5). Moreover, a lack of suitable insect pollinators that can correctly trip the flowers could also impair sexual reproduction (5). It appeared that only 10% of the flowers were tripped in two Connecticut populations, out of which only 50% set fruits (5).

Dr. William Blackmon, Berthal D. Reynolds, and their colleagues led a breeding program to domesticate *Apios* from 1985 to 1994 at the Louisiana Agricultural Experiment Station in Baton Rouge (3, 4). Germplasm involving 210 wild accessions was collected from 19 states of the USA, although the majority came from Louisiana followed by Florida and North Carolina (4). A conventional breeding process of hybridization largely involving open pollinations, intermating of selected accessions, and screening for tuber yield was followed by the assessment of over 2,200 *Apios* breeding lines. They successfully identified advanced breeding lines that produced larger individual tubers, and with tubers more closely spaced on the stolon (4). *Apios* genotypes having significant yields after only one season in the ground were identified (4) (Figure 1). Subsequently, targeted breeding efforts were pursued to develop *Apios* germplasm producing higher tuber yields on un-trellised vines that had reduced tendency to climb and twine and were resistant to aerial web blight disease caused by *Rhizoctonia solani* (4). This *Apios* breeding program no longer exists in Louisiana, but Dr. Blackmon preserved about 40 advanced breeding lines of improved *Apios* at his property in Virginia (3). A set of 40 Blackmon and an additional 13 *Apios* genotypes are now maintained and cultivated by Dr. Steven Cannon and colleagues in the United States Department of Agriculture in Ames, Iowa.

These 53 genotypes were evaluated during 2010-2013 using 20 above- and below-ground trait descriptors at Ames, IA, and with 4 yield-related traits in 2013 at Mechanicsville, VA and Lititz, PA (7). A significant amount of diversity was revealed among the genotypes for 18 traits in field-grown plants and for 7 traits in potted and bag-grown plants (7). Fourteen of the 18 traits measured under field-conditions had broad-sense heritability greater than 61% indicating the effectiveness of these phenotypic descriptors for screening new *Apios* germplasm, and also the ability to breed and develop germplasm and cultivars using these traits (7). They classified 53 *Apios* genotypes into four phenotypically distinct clusters. One comprised early-emerging, high-yielding genotypes that produced large mother tubers, short stolons, and few child tubers, while another showed high values for all belowground traits except those associated with mother tubers (7). Also, there were late-emerging genotypes that were either intermediate performing or poorly performing (7). The genotypes comprising clusters 1 and 2 represented the two ideotypes of high-yielding genotypes in the collection. In addition, four above-ground measurements (internode length, plant vigor, stem diameter at 2 and 5 months) showed strong phenotypic correlations with tuber number and yield per plant (7). The comprehensive analysis led to identification of *Apios* breeding lines with consistently high yields up to ~1,500 g tubers per plant (similar to yields in conventional tuber crops) across geographic locations (7) (Figure 1). The detailed characteristics of each of these high-yielding lines along with 14 traits with high broad-sense heritability can facilitate identification of parental lines for crossing and developing cultivars, and provides a foundational framework for a cultivar development program.

Belamkar *et al.* (8) conducted next generation RNA sequencing of six tissues of a single *Apios* genotype to generate a high-quality *de novo* reference transcriptome assembly consisting of 48,615 contigs with average contig length of 1,173 bp, and a gene expression catalog with 28,738 contigs expressed in at least one of the tissues sampled. They also sequenced the leaf transcriptome from an additional 52 *Apios* genotypes, mapped the reads to the reference transcriptome assembly, and identified 58,154 high-quality single nucleotide polymorphism (SNPs) and 39,609 gene expression markers (GEMs) across the *Apios* germplasm. A population structure analysis using the SNPs identified 6 genotypic clusters across the *Apios* collection. These clusters largely matched the pedigree of the genotypes (8). Interestingly, using the GEMs also revealed similar clusters across the *Apios* collection. This is one of the early studies demonstrating the utility of GEMs equivalent to SNP markers for population structure and marker-trait analysis. Further, the reference transcripts were mapped onto the *Phaseolus vulgaris* genome to obtain provisional genetic locations for 46,852 SNPs and to investigate linkage disequilibrium (LD) in the collection (8). The LD dropped rapidly within 10-15 kb further indicating *Apios* largely as an outcrossing species. Finally, marker-trait analyses identified 21 SNP markers associated with 14 phenotypic traits (six aboveground and eight belowground), and 28 GEMs associated with nine phenotypic traits (four aboveground and five belowground) (8).

Cross-pollination by insects visiting many plants often results in multiple pollen parents fertilizing seeds within individual pods. Singh *et al.* (9) utilized the open-pollinated behavior of *Apios* to construct a high-density genetic linkage map by assessing the SNP marker segregation in an F_1 population using a pseudo-testcross strategy. The F_1 mapping population was derived from open-pollinations between a known maternal parent and 37 potential pollen sources (9). SNPs were identified and genotyped by aligning the expressed sequences of individuals in the mapping population against the maternal parent derived *de novo* reference transcriptome assembly (9). The sequenced transcriptomes of potential pollen donors further led to eliminate the possibilities of self-pollination in the population (9). This observation matches the rapid LD decay observed across the 52 genotypes and indication of outcrossing mating system of *Apios* highlighted in (8). The final *Apios* linkage map had 1,121 recombinationally distinct loci that were distributed over 11 linkage groups and covered a total 939 cM genetic distance (9). Genome sequence comparisons with other members of the *Phaseoleae* (warm-season legumes) showed extended syntenies for 9 out of 11 *Apios* linkage groups (9). The markers on linkage groups Lg-01 and Lg-06 displayed linear correspondences with two linkage groups in *Phaseolus vulgaris*, *Vigna radiata*, and *Vigna angularis* as well as four linkage groups in *Glycine max* (9). Additionally, Singh *et al.* (9) detected a translocation event that occurred in Lg-08 after the divergence of *P. vulgaris* and *G. max* from *A. americana*, *V. radiata*, and *V. angularis*.

In an era of drastic climatic changes, population explosion and food insecurity, alternative crops that are adapted to a range of environmental conditions and are highly nutritious are of great interest. We believe *Apios* is one such alternative crop with a huge potential to be a crop for the future. Moreover, genome sequencing efforts in combination with automated phenotyping (unmanned aerial vehicles and ground penetrating radars), advanced quantitative genomics (genomic selection and marker-assisted selection) and biometrical techniques, and gene-editing tools can help accelerate the process of domestication of orphan crops like *Apios*.

Acknowledgements

We thank Dr. Bill Blackmon for his pioneering work and for generously sharing germplasm, and Gautam Bhattacharya for encouragement over the course of our work with *Apios*. This research was supported in part by the US. Department of Agriculture, Agricultural Research Service, project 5030-21000-062-00D. USDA is an equal opportunity provider and employer.

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European tuberous *Lathyrus* species

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Abstract: There are several *Lathyrus* species, native to Europe, that have either thickened or tuberous roots. The two with tuberous roots are *L. linifolius* and *L. tuberosus*. Of these, *L. tuberosus* ("tuber vetchling") has been widely used in Europe for food. Its uses include flavorings, fodder (from leaves), oil (from seeds), and as a potato-like food (though much richer in protein than potato). The other tuberous *Lathyrus* species, *L. linifolius* ("bitter vetch") is of interest for possible medicinal or nutraceutical uses. In particular, it has been reported to serve as an appetite suppressant.

Key words: Lathyrus, tuber vetchling, bitter vetch, *Lathyrus linifolius, Lathyrus tuberosus*

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The European flora has very few native legume species which have tuberous or thickened root. *Lathyrus niger* (L.) Bernh, *L. vernus* (L.) Bernh., and *L. pannonicus* are listed as geophytes (1), owing to thickened roots. *L. niger* occurs throughout Europe, particularly in the Balkans, and it occurs up to Scandinavia. *L. vernus* grows also in Europe (with the exception of the UK, western France and the northern part of Scandinavia), eastwards to the Altai mountains in Asia. *Lathyrus pannonicus* is found in the central, eastern, and southern parts of Europe as well as in western and inner Asia (2). These species are perennial early spring plants with fast development and flowering, often before trees are covered with leaves.

However there are two European *Lathyrus* species which can be considered true tuberous legumes: bitter vetch (*L. linifolius* L. (Reichard) Bässler) and tuber vetchling (*L. tuberosus* L.).

Tuber vetchling (*Lathyrus tuberosus*) is a perennial sprawling plant with edible tubers 3 to 5 cm long attached to roots (Figure 1). The tubers are found at ~14 cm below the soil surface and unbranched roots can reach 70 cm of depth. The tubers form stolons and new roots during the development of the plant. Subsequently, tubers can form new stems and grow as a separate plant. Vegetative propagation of *L. tuberosus* is very successful and sexual reproduction via seeds occurs to provide genetic diversifications or to colonize new habitats. Typical habitat is grassy places, broad-leaved woodlands, forest margins, hedgerows, and banks up to 1600 m above sea level. It often occurs in disturbed habitats, along roads, in forest margins and weed communities (3). It is assumed that it spread simultaneously with cereal cultivation across Europe. Later on, it was introduced to North America and can be found in Northern Africa.

It is still occasionally grown for its flower

Figure 1. *Lathyrus tuberosus* plant. Photo courtesy László Erdős.

odour and edible tubers (4). However, this plant was much more widely used in the past. In the 16th century, flowers were distilled to produce perfume. In the 18th century in the Lower Rhine Valley of Germany and in the Netherlands, it was grown on a larger scale to produce tubers which were cooked or roasted for human nutrition (5, 6). Its Dutch name means earth-acorn, indicating its use. Similarly, it was commended for its "gentle nutty flavour" ("*macusson*") in French markets, and a variety of common names differing by regions indicates its widespread use (7). Interestingly, it was also used to flavour scotch whiskey. The production of fermented beverages or bread baking were occasional other uses of the tuber, while oil was pressed from the seeds (*7).* Borbás (8) noted that in Hungary *L. tuberosus* was grown (i.e., cultivated) like a crop (9). It gave double benefit: its leaves were used as fodder, while the tubers were eaten by pigs. The tubers were eaten in autumn mainly by children (10). Halász (11) reported than in Moldavia (Eastern Romania), children used to collect the tubers after the fields were ploughed. Sometimes pigs are used to root for the tubers. Also, when the fields are ploughed in the spring, crows are able to spot the tubers. Thus, the presence of many crows on the ploughfield may indicate that there are the tubers of *L. tuberosus* (12). Forgó (13) wrote that tubers can be eaten in three ways: i) raw (i.e., uncooked, unroasted), ii) after roasting, or iii) after cooking them in boiling water. It can be eaten as a delicacy, or as a garnish. Its taste is similar to that of chestnut. He adds that in the Netherlands, the tubers were roasted and eaten with tea. The tubers were eaten during famines, but its taste is so good that it was also eaten during times of plenty (13). Forgó adds that pigs also like the tubers, so if this weed is spreading on ploughlands, it can be eradicated by pigs. Recent studies show that *L. tuberosus* is still consumed by the rural population in Turkey and in Transylvania (14).

Lathyrus tuberosus succeeds on soils where other crops fail to grow, being adapted to a broad range of conditions including high soil salinity. Experiments with *L. tuberosus* as a forage crop were conducted in the beginning of the 20th century in Germany (15) but were abandoned thereafter. The tubers contain 16-20% starch, 5% sugar and 10-12% protein. Notably high is the vitamin C content of 160 mg/100 g (twice as high as the reference daily intake and three times higher than the vitamin C content of lemon) and calcium amount, which is almost twice as high as in cow milk (16). However, α-amino-8-oxaly-amino-butyric acid and other lathyrogenic substances are found especially in seeds (7) and care has to be taken when eating regularly or in higher quantities.

A similar tuberous species is bitter vetch (*Lathyrus linifolius* L. (Reichard) Bässler), also called cairmeal in Gaelic or heath pea. This is also a perennial species, however without climbing habit. It is native to most of Europe except for the eastern and cold climate northern parts and is reported extinct in north Africa (Algeria mountains). It is an endangered species in Switzerland and Austria. It is valued as a horticultural species, being cultivated for aesthetic reasons and aromatic flowers. *L. linifolius* is found in extensively grazed and ungrazed semi-natural, low altitude (20–350 m a.s.l.) grasslands, with soils of low nutrient status and low pH (17,18). Although it is a rather rare plant which is absent in agricultural environment, there are rich ethnobotanical record of uses, predominantly in 17th century historical records from the Scottish highlands (19 - 23). Its root tubers were used as food in times of famine, to offset symptoms of inebriation, to relieve flatulence, in chest ailments and as a flavouring, mainly of beverages. Raw tubers were also sliced and infused in hot water or alcohol, to produce beverages with a liquorice-like flavour. The tubers were also used as flour and were fermented to make beer, which might be distilled to produce spirits (24). Apparently, before potatoes bitter vetch tubers were harvested and dried to help against famine in the Scottish Highlands (19). The plant fell out of use in the 1800s with the cultivation of the potato, which was easier to farm and produced higher yields. Notably, it is generally reported to promote feelings of satiation. Sir Robert Sibbald, founding member of the Royal College of Physicians, mentions these tubers in his book from 1699, "*Provision for the Poor in time of Dearth and Scarcity*" (25). The plant was used not only by poor people but also in the court of Charles II in 1685, helping well living people to diet. This property draw attention of researchers today. Its use as an appetite suppressant was rediscovered by Brian Moffat during excavation of the monastery at Soutra Aisle, Scotland's largest medieval hospital (22). He proposed that the monks were using the tubers medicinally. It is also thought that these tubers provided a boost of energy, and were used by soldiers. The active component is suspected to be trans-anethole, a derivative of phenylpropene, which contributes to the odor and flavor of anise and fennel (Apiaceae), anise myrtle (Myrtaceae), liquorice (Fabaceae), although this remains to be confirmed. Recent research revealed that consumption of bitter vetch tubers by rats significantly altered the expression of hypothalamus genes involved in regulating metabolism (26). Therefore, this species has potential for development as a therapeutic and novel crop.

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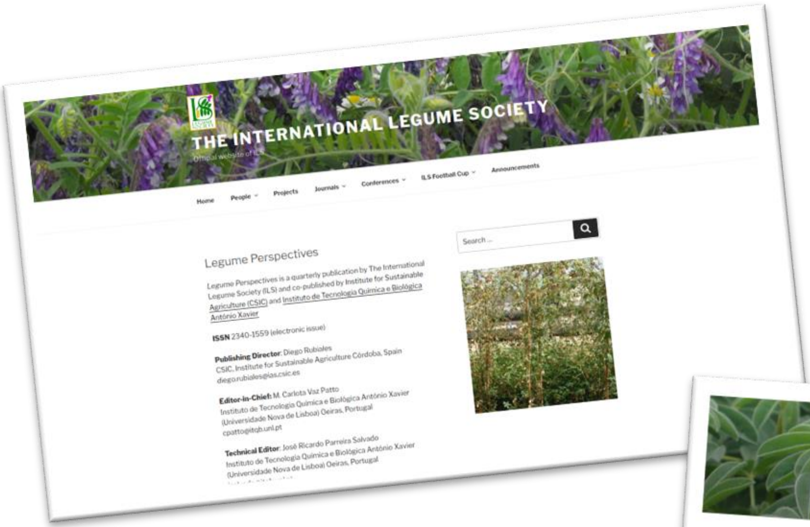

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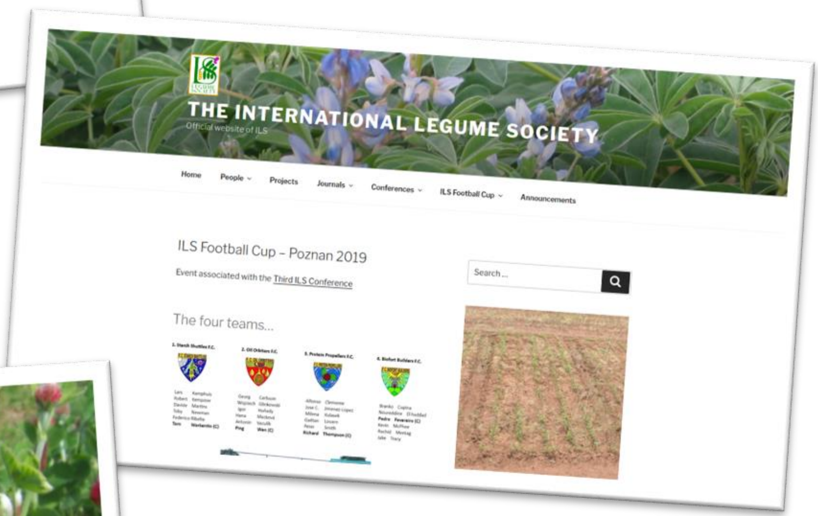

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