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REVIEW

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Development of marama bean, an orphan legume, as a crop

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Abstract

Advances have been recently made in the breeding and characterization of three major legume crops of the semiarid tropics, chickpea, pigeon pea, and groundnut. However, other wild-growing legumes, called "orphan legumes," with potential as crops, but are not yet cultivated would benefit from further attention. This review considers the domestication of one such plant, marama bean (*Tylosema esculentum*). Marama has long been recognized as a potential crop particularly for southern Africa, but coordinated, long-term research and development has been lacking. Marama is a perennial, outcrossing hexaploid, growing under extreme conditions in a restricted geographic range; only natural stands exist that are likely to be overexploited. Marama has two potential units of economic yield, seed and tuber. The seed is protein- and oil-rich, with potential in the pharmaceutical and cosmetic industries while the tuber is high in carbohydrates. In this review, the different aspects of bringing a wild plant into cultivation are considered, together with the additional socioeconomic benefits of developing a breeding program. An international collaboration is analyzing aspects of the plant structure and physiology, molecular biology, and its interactions with environment with a view to developing marama as a crop, with a role in food security, that could be harvested by local communities. Molecular topics considered include next-generation sequencing for developing molecular maps, genotyping by sequencing, identification of quantitative trait loci for important agronomic traits, protein quality, and incompatibility mechanisms. To achieve domestication requires seed gardens and fields, and the phenotyping of marama material from different locations. Community engagement will also ensure agronomic sustainability and farmer participation. This review describes an approach for a successful outcome of a breeding program to introduce improved marama and highlights the challenges in achieving this, which is a paradigm for the difficulties in the cultivation of "orphan species."

KEYWORDS

crop development, genome sequencing, marama bean, phenotyping, *Tylosema esculentum*

1 | INTRODUCTION

Global food security is heavily dependent on the major cereal crops maize, wheat, and rice which provide ~60% of the energy and ~56% of the protein that is directly consumed by people from plants (Sogbohossou et al., 2018). Yield of grain per unit area of land is very dependent on the application of fertilizers containing nitrogen, potassium, and phosphorus. Legumes, particularly soybean and groundnut (peanut), provide substantial energy and protein; on an average (unweighted by population), pulses contribute about 3% of total calories consumed in developing countries (Akibode & Maredia, 2012). They are less dependent on nitrogen fertilizers than cereals, although still requiring phosphate and potassium. A number of other cereals, such as millets (small-grained cereals including *Eleusine coracana, Panicum miliaceum,* and *Setraria italica*), sorghum (*Sorghum bicolor*), and teff (*Eragrostis tef*), as well as legumes, such as chickpea (*Cicer arietinum),* are widely and substantially exploited, depending on local conditions. Such crops provide a major fraction of human nutritional needs, especially in demanding climates, for communities dependent on subsistence agriculture, which is still practiced in much of Africa (Lawlor, 2010). These crops are regarded as having particular potential but are underdeveloped in terms of research and breeding. There are active efforts to improve them, by increasing yields, disease resistance, etc. Other species, for example, bambara ground nut, *Vigna subterranea,* are used widely in subsistence agriculture but have received less attention. A further group of essentially unimproved legume species, including the Tepary bean (*Phaseolus acutifolius*) (Mhlaba et al., 2018), Tarwi (*Lupinus mutabilis*) (Ad Hoc Panel of the Advisory Committee on Technology Innovation, Board on Science and Technology for International Development, & National Research Council, 1989), and marama bean (*Tylosema esculentum),* which is reviewed by Lawlor (2018), and Cullis and Kunert (2017), are not used as crops yet, although seed and other plant parts are collected from wild-growing populations and consumed. These underutilized species, often referred to as "orphan crops," have characteristics which strongly indicate that introducing them into agriculture could reduce the dependence on the main, staple cereals and legumes and preserve and foster food diversity. In addition, they have features which are adapted to particular climatic and soil conditions, suggesting that the species would provide additional, valuable opportunities for agriculture productivity and stability. An additional benefit would be to decrease pressures on wild populations of such species. The considerable challenges to improvement of "orphan crops" include the following: low production of economically desirable yield, lack of knowledge about basic husbandry, pest, diseases, nonexistent (or extremely limited) variety development, absence of a value chain for products if exploitation is envisaged beyond subsistence agriculture, low economic status and limited potential to provide profits, essential for commercial exploitation, from their improvement. Development also takes place against a background of competition from existing crops: Maize, for example, dominates in much of Africa but is of limited value in subsistence farming in challenging environmental conditions, especially where drought is frequent (Chimwamurombe & Munsanje, 2018; Lawlor, 2010).

There are a number of legumes which are considered "orphans" including marama bean, focus of this review. Such "orphan legumes" have considerable potential, not least because their seeds often have large protein and/or oil content, but are not currently exploited in agriculture. How to increase the contribution that they can make, particularly in subsistence agriculture in climatically marginal areas for plant growth and production, has been the subject of much speculation and increasing research activity over the past decades. There has been only limited research and development, and selection and breeding has (by definition) been neglected. Field trials have been established for some species but are too short-lived, particularly for perennial species, to enable effective selection of potential breeding material and for breeding. Phenotypic characterization of plant material has generally been limited, as it is time- and labor-consuming and thus expensive, plus requiring sound management and complex data analysis. Conventional plant breeding methods, especially for outbreeding perennial plants, have thus been regarded as of limited value. However, more recent progress in applying new methods in plant breeding to orphan crops, particularly legumes, has changed perceptions and the possibility of rapid domestication (Nadeem et al., 2018; Moose & Mumm, 2008).

Conventional plant breeding is being greatly improved by the application of DNA marker-assisted selection (MAS), with development of molecular marker systems for crop plants, including cereals and legumes (Baloch et al., 2017; Nadeem et al., 2018) and the use of high-throughput phenotyping also promises to increase the rate of genetic gain (Araus & Cairns, 2014). The most widely used markers and their applications, covering all aspects of MAS and association mapping as applied to plant breeding, have been recently reviewed (Nadeem et al., 2018). Although technological advances in the field of molecular plant breeding offer potentially large savings in the time and effort required to improve "orphan crops," a greater emphasis on improvement by application of MAS is required. Implementing these approaches will depend on available resources, especially in less-developed countries, where there is significant and urgent need to develop crops for marginal lands. The lack of such resources, both economic and human capital, may delay application of newer techniques, such as MAS, to orphan crops and specifically legumes, although it is just these countries would benefit from such technology (Acquaah, 2012).

The prerequisite conditions for applying MAS are easily met for the major crops where select "elite" lines and inbreeding are available along with extensive genomic resources. However, MAS schemes have been less exploited for underutilized and "orphan" crops, particularly polyploids with complex breeding systems (see later for marama), where plants are taken from the wild with little knowledge of their characteristics. Despite recent advances in molecular plant breeding, identifying gene combinations responsible for significant crop improvement is a major challenge, even in the main crops. Moose and Mumm (2008) suggest that better integration of different research disciplines and activities is required, leading to improved knowledge of genome organization and function of genes, a solid foundation in analysis of large data sets together with statistics to estimate genetic effects, a strong background in plant biology, and experience with field-based breeding practices. Collaboration between groups and continuity in funding is required. Although at an early stage, these methods are being applied to neglected crops, as they are likely to contribute significantly to development of valuable new crop plants and are now being actively exploited to improve yield and resiliency of many underutilized crops, in particular "orphan legumes." An example of what might be achieved with underutilized legumes is chickpea, which is widely grown and has benefited from intensive scientific breeding (Millan et al., 2006). Chickpea breeding is focused on increasing yield by combining genes leading to improved resistance/tolerance to pests and diseases in elite germplasm, using marker-assisted selection (MAS) to target the desired genes, together with genetic mapping using highly polymorphic, codominant microsatellite-based markers (Sarmah, Acharjee, & Sharma, 2012).

The genomes of a subset of "orphan crops" have been sequenced and applied in molecular breeding programs to improve their yield and distribution (Varshney, Close, Singh, Hoisington, & Cook, 2009). The application of sophisticated crop improvement to these underutilized species depends, however, on the status of germplasm resources, current agronomic practices, and the ease of introgressing new characteristics into existing varieties. Many initiatives are underway to bolster the scientific breeding of these orphan species such as the sequencing and assembly of 101 orphan crop genomes allied with the training of plant breeders by the African Orphan Crops Consortium Initiative (AOCC, africanorphancrops.org) and the Alliance for the Green Revolution for Africa (AGRA) to accelerate improvement of neglected and unimproved species of importance for local communities in Africa (Sogbohossou et al., 2018).

2 | AIM OF REVIEW

The aim of this review is to examine the state of exploitation of "orphan legumes" by focusing on understanding the biology and ecology of marama bean which has the seed characteristics that have led to frequent suggestions that it should be domesticated and that it has the potential to be a new alternative crop for resource-poor farmers in southwest Africa. Many of the challenges that have been, or are currently faced with the other orphan crops are also present in the improvement of marama bean. Marama bean has not been domesticated but is extensively collected from the wild, raising the possibility of severe depletion of wild populations. However, marama has previously been grown experimentally in an agronomic setting (focusing only on seed yield in the short term), outside of Africa and the climate to which it is adapted, apparently with little success, and recently in the area where it is endemic (Chimwamurombe, 2016). Work already done on the plant's agronomy (Ramolemana, Machacha, Lebutswe, & Tsopitto, 2003; Ramolemana, Machacha, Lebutswe, Mosekiemang, & Tekane, 2007), physiology (Mitchell, Keys, Madgwick, Parry, & Lawlor, 2005), and molecular characteristics needs to be built on to progress domestication. Much has been written about marama's potential (Cullis, Chimwamurombe, Barker, Kunert, & Vorster, 2018), and it is now important to evaluate the current situation, from a wide perspective, to consider what the limitations to domestication are, to suggest specific targets for plant and crop improvement, and to identify what methods might best be employed to achieve the long-term aim of development of a viable crop for the target environment. Therefore, all the steps from identifying useful germplasm to selecting the first varieties of marama, while ensuring local acceptance of it as a new crop, will all be important in the effort to domesticate it. This will include surveying and evaluating the extant germplasm and developing an ongoing breeding program for this species. An aspect of developing a breeding program is the potential for teaching and training, and wider community development. This review also considers current scientific collaborations, particularly between the Namibia University of Science and Technology, Case Western Reserve University, and the University of Pretoria, aimed to ultimately provide improved yield (seed and tubers) for resource-poor farmers in southern Africa as a new food crop.

3 | WHY MARAMA BEAN?

Marama bean, which belongs to the subfamily of legumes, the Cercideae, a defining character of which is the inability to nodulate (LPWG, 2017), has long been recognized (National Research Council 1979, 2006) as a potential crop due its seed and tuber. The large seed (cotyledons and embryo) contains considerable protein (29%–34%) and oil (35%–43%), a combination which is unique: Soybean has *ca* 36% protein and 20% oil and peanut *ca* 25% and 44%, respectively. The seed has been consumed and used as a cosmetic for, probably, many millennia by people living on the western and southern fringes of the Kalahari where marama is endemic. In addition to marama bean's value as a protein source in subsistence diets, the proteins may have potential in modern pharmaceuticals. The oil has medicinal and cosmetic as well as nutrient potential, and might have industrial use. Young tubers were used by people of the region as a food and as a source of water, particularly in drought conditions. This storage organ is high in carbohydrates, including starches of interest to the food processing industry (Nepolo, Llyod, & Chimwamurombe, 2015). The dormant tuber is the means by which marama bean survives the winter, when the aboveground vegetative parts are dead. It also becomes dormant in periods of severe water shortage when the aboveground organs may die. As seeds and tubers have been used as a food, their adoption as a staple should not be problematical.

There has not been a concerted, sustained effort to select marama bean from wild populations with a view to develop it as a crop, nor to develop appropriate agronomic management, nor any sustained breeding effort to maximize the yield potential. Since there are two potential sources of yield, the seed and the tuber, these two characteristics will be considered largely separately. Although the interdependence of seed and tuber has not been examined (but clearly are intimately related), it makes sense to consider both for varietal development and improvement. The conservation status of marama is poorly assessed and the plant, because of its distribution in relatively small populations, might be subject to overexploitation. Evaluation of marama from an ecological and conservation perspective should also accompany attempts at exploitation (Lenné and Wood 2011).

4 | SELECTION AND BREEDING IN A DOMESTICATION PROGRAM

The approach taken to introduce marama, a perennial species, into agriculture can be modeled on earlier improvement programs in trees (Grattapaglia & Resende, 2011) and other perennials, such as blueberry (Blischak, Kubatko, & Wolfe, 2016), and ideally involves a series of interrelated procedures. There needs to be a selection of plants from diverse and identified field populations, with the potential to increase seed yield, per plant and per area of soil surface. These seeds need to be incorporated into field and breeding trials need to be established in target locations with suitable climate, to give the best, uniform, environmental conditions within each trial allowing the analysis of genotypic differences between plants. Analysis of the collected data on growth, development and yields of plants (with focus on seed and tuber yield), and molecular characteristics associated with these traits over time (throughout the trials) to provide the basis for selection of "elite lines" with identifiable phenotypic features suitable for a crop. These data will enable further marker-assisted selection of plants, which will be used in a crossing program followed by further cycles of evaluation. The aim of taking a wild-growing plant species into cultivation is generally based on the benefits of perceived end products—as in the case of marama. However, enthusiasm should be based on sound information about the species, from molecular to ecological aspects. Earlier attempts to domesticate marama were directed at exploitation and were not well founded on basic science. A more scientific approach is required. This review therefore outlines aspects of marama bean's biology. Ecology and physiology are emphasized in a review by Lawlor (2018) and molecular aspects are reviewed in Cullis et al. (2018).

5 | ENVIRONMENT, PHENOTYPE AND LIFECYCLE, MOLECULAR GENETICS

5.1 | Environment

Marama bean grows mainly on the western fringes of the Kalahari where annual rainfall (exclusively in summer) is 250–500 mm. Rainfall is often sporadic, with heavy storms, causing flooding, and extended dry periods. Years with almost no rain are relatively frequent. Marama bean grows, in summer, on very sandy soils with extremely little organic matter, or nitrogen and phosphorus. Maximum temperatures during daylight in the main period of growth are 32°C (range 28–37°C). Solar radiation during the 3 hr either side of midday is considerable, *ca* 2,000 μmol m^{-2} s^{-1}. Thus, the plant is adapted to an extreme environment to which current crops are not adapted, even when irrigated suggesting that marama would have a particular, even unique role, in the agroecology of the region.

5.2 | Phenotype and life cycle

Marama bean is a perennial, tuberous geophyte, with two potential units of economic yield, the seed and tuber (Jackson et al., 2010). The underground tuber, which when old (probably many decades) may be *ca* 250 kg, becomes dormant in winter when the vegetative, aboveground organs die. In spring, just before or at the start of the rainy season, several shoots emerge from the apex of the tuber, rapidly developing into vines (up to 6 m in length) which run along the soil surface, held up from it by short "pegs." Numerous bilobed leaves are produced alternately along the length of the vines. In the early morning, the leaf is completely open and fully exposed to the sun. At that time, conditions are most suitable for photosynthesis: high humidity and cool temperature and relatively low solar radiation. Later, when the air is drier and temperature and radiation flux greater, the upper surfaces of the leaf lobes fold together and the complete leaf assumes a vertical position and closely tracks the sun's position so that the leaf is minimally exposed to the sun's rays. This mechanism depends on the functions of pulvini and is crucial for survival of marama in the hot, dry conditions under a very large solar radiation flux, which cause photodamage to fully exposed leaves (see Lawlor, 2018; Travalos and Karamanos, 2008).

Plants in the second growth season may start to flower: Racemes of large, yellow, zygomorphic, heterostylous (the only report in the Fabaceae) papilionate flowers, with a sticky, nonpapilate stigma (first report in Caesalpinioideae: see Hartley, Tshamekeng, & Thomas, 2002), are produced on long peduncles from leaf axils. The flowers are insect-pollinated but details of the insect species responsible, their frequency, and success as pollinators, is very limited. It may be crucial to use of marama bean as a crop if pollinators are very specific and restricted to the areas where marama bean grows naturally. Marama is an outcrossing species: A diallelic self-incompatibility system (first report in Caesalpinioideae), with pollen tube inhibition in the style, is a major barrier to self-fertilization. These unique features suggest that marama bean has evolved a particular breeding system, perhaps related to the environmental conditions. Possibly, breeding systems play a large role in the evolution of plant adaptation to environment (Charlesworth, 2006), and marama bean should be considered in this context. The proportion of flowers fertilized and resulting in pods is very small and variable. Pods contain from 1 to 4 (exceptionally 6) seeds per pod, most frequently 2. Although the seed is large (*ca* 2 g with testa), the few seed per plant results in a very low seed yield per plant and per land area. In late summer, the pods mature and seed is shed. The leaves and vines die with the onset of winter. Seeds have a thick testa, which greatly slows water loss. Seeds may lie dormant for considerable periods until the onset of the rains stimulate germination. This is rapid, with formation of a radicle which penetrates into the soil, followed by leaves: Tuber formation follows very soon as a swelling of the radicle so that by the end of the first year a viable organ several centimeters long is produced. The tuber grows rapidly and may persist for many seasons: It is also capable of surviving, in a dormant state, prolonged drought periods. In terms of the biological survival of marama, there may be interactions between the availability of nutrients and water, size and storage in the tuber and the size of the vegetative growth above ground together with the amount and composition of the seed produced, which are traded off against longevity and dormancy of the tuber, and its ability to survive adverse conditions.

5.3 | Genotypic and environmental effects

There is considerable variation in the size and apparent vigor of plants in the field and also when cultivated, even when accounting for possible age differences. Variation is ascribed to genetic and environmental factors, but distinguishing between them is difficult. Plants grown at the University of Pretoria farm were of different size, with very different vegetative growth rates even though they were of the same age and were irrigated (Figure 1). Assessing the genetic basis for difference in size and vigor is an important aspect of selecting improved marama bean which can only be done if environmental effects are minimized. This would be a central part of a selection and breeding program for under- and undeveloped species and has been the focus of considerable work which is summarized by Acquaah (2012), explaining how domestication and a breeding program for a perennial, outbreeding species such as marama bean is structured and developed. Development of molecular markers is considered by Collard and Mackill (2008) and their use in genome mapping by Burghardt, Young, and Tiffin (2017). Association of different aspects of vegetative growth, based on careful phenotypic analysis, would greatly improve later selection of elite plants for use in further breeding programs. Since marama is an obligate outcrossing species, the development of improved lines for distribution could usefully follow the selection schemes used for forest trees (Funda & El-Kassaby, 2012), where one of the goals is to produce large quantities of seeds while maximizing genetic gain and minimizing economic costs. Additionally, the development of a "seed orchard," and the use of molecular markers to identify the parents of seeds produced, would then be fed into the design of field plantings. Markers could also indicate the distance of pollen travel (see breeding system of marama), an important attribute in designing field plots.

FIGURE 1 Two plants growing at the University of Pretoria farm under irrigation. Two plants, one in the foreground covering more than six times the area of the one in the background. The foliage represents a single season growth

5.4 | Genome and genetic diversity

Marama bean has a relatively small genome, currently determined at about one gigabase based on Feulgen staining (Takundwa, Chimwamurombe, & Cullis, 2012) and the coverage of the genome in next-generation sequencing (C. Cullis, unpublished data), which still requires confirmation by flow cytometry. Chromosome number was determined to be 42 (Takundwa, Chimwamurombe, & Cullis, 2012), suggesting that marama is a hexaploid (since the basal haploid chromosome number in legumes is 7). However, the timing of the genome duplications is unknown. Polyploidy is a very significant mechanism in the evolution of plant species and adaptation to environment (Spoelhof et al., 2017) and is likely to be of major importance in adaptation of marama to its environment. The immediate effects of polyploidy on phenotypes, compared to the diploid progenitors, include increased size of cells and organs, greater vigor and biomass and altered development (Blischak et al., 2016; Spoelhof et al., 2017). Many important crops are polyploids but despite the economic importance, the effects on quantitative phenotypic traits have been little studied. Studies on marama could therefore contribute considerably to such understanding.

Many of identified "orphan" crops, including marama, are now being sequenced through the African Orphan Crops Consortium (http://africanorphancrops.org). Marama bean, through activities including course based undergraduate research at Case Western Reserve University, has a preliminary genome assembly and whole-genome Illumina sequence data for 58 diverse individuals (Cullis et al., 2018). These data have been used to compile the chloroplast genome and identify diversity in this segment of the overall genome (Kim & Cullis, 2017). This genomic resource will be the basis of developing genetic maps and useful DNA markers for eventually applying MAS to marama. The genomic data have been already used to identify genes related to various important proteins, such as the cysteine proteases and their inhibitors (Cullis et al., 2018), as well as for identifying possible genes underlying self-incompatibility by comparative analysis with the incompatibility genes from other species. These candidate genes now need to be confirmed as active in the control of fertilization. Molecular markers for this characteristic would be important in developing the breeding program, for selection of plants for trials of the fertility of plants in the field. Early studies of diversity between and within geographically isolated populations of marama showed that there is diversity within populations which is as large as that between geographically isolated populations (see Hartley et al., 2002; Nepolo, Takundwa, Chimwamurombe, Cullis, & Kunert, 2009; Takundwa, Chimwamurombe, Kunert, & Cullis, 2012). This is expected of an outcrossing species which is a self-incompatible, and heterostylous. Selection of marama bean phenotypes required for cultivation is dependent on identifying sufficient diversity in the germplasm, which may be difficult given the heterogeneity in plants and environments from which the material may be obtained. Breeding is also likely to be difficult since pollinators and their range are still undefined.

Analysis of marama bean's reproductive biology will be of scientific value and is necessary if breeding and improvement of the germplasm is to progress. The first hurdle is that marama is self-incompatible, thereby eliminating that staple underpinning of most improvement programs, the inbred line. Self-incompatibility also affects the agronomic strategies required to get a mixed population of genotypes to ensure maximal seed set. Compounding this characteristic is that marama bean is a hexaploid. Therefore, implementing molecular marker strategies will be more complex, if only in the identification of useful allelic polymorphisms, rather than differences in duplicate loci. An initial screening of SSRs identified many polymorphisms within a limited germplasm and clear polymorphisms within an individual (Takundwa, Chimwamurombe, Kunert, & Cullis, 2010).

The molecular genetic diversity among some populations in Namibia has been determined both by SSR variation (Nepolo, Chimwamurombe, Cullis, & Kandawa-Schulz, 2010; Nepolo et al., 2009; Takundwa, Chimwamurombe, Kunert, et al., 2012) and chloroplast variation (Kim & Cullis, 2017) from the whole-genome sequence data from 58 different individuals across the Namibian germplasm (Cullis et al., 2018). These data and the SSR data confirm earlier studies (Lawlor, 2018) showing that there is a great deal of variability within a population. However, there may be specific attributes, such as seed number per pod that has a more restricted distribution. Therefore, as detailed later, there is a need to document the phenotypic as well as the molecular diversity in order to select the most appropriate germplasm to introduce to the initial breeding population.

5.5 | Desirable phenotypes and available diversity

The ultimate desirable characteristic for all crops is large yield and for perennial crops sustainability of yield over a long period. To evaluate the changes required to domesticate a plant species and increase the yield sustainably, it is essential to assess the potential limitations to yield production to provide focus to the research and development effort. The morphological components (visible phenotype) of a crop, such as a marama bean, determine seed yield. They may be summarized, over a growing season, as:

$$\begin{aligned}
&\text{Seed mass unit land area}^{-1}\\
&\quad = \text{number of plants unit land area}^{-1}\\
&\quad \times \text{number of shoots plant}^{-1} \times \text{number of flowers shoot}^{-1}\\
&\quad \times \text{number of pods flower}^{-1} \times \text{number seed pod}^{-1}\\
&\quad \times \text{mass seed}^{-1}
\end{aligned}$$

Marama also forms a single tuber, yield of which is given as:

$$\text{Tuber mass unit land area}^{-1} = \text{number of tubers unit land area}^{-1} \times \text{mass tuber}^{-1}$$

Each component of seed and tuber yield is genetically and environmentally determined and even in developed crops there is uncertainty about the relative contributions of each. Assessment of the factors determining yield increases in modern cereals suggests about half is genetic and half environmental: For marama at a very early stage of domestication, this partitioning may not apply and evaluation is desirable. High-quality, analytical, agronomic experiments are necessary, on which detailed crop-physiological analysis is carried out. The genetic factors underlying seed and tuber production operate at several levels of organization within the plant and are unlikely to be single factors operating alone. Establishing the relationships requires molecular genetic techniques applied to known phenology and processes which may play an important role in domestication. The analysis of genetic variation which can be separated from the environmental effects on plant morphology, size, etc., is required.

5.6 | Growing conditions for trials

Cultivation requires optimization, uniformity and stability (as far as these are possible) of the environment in relation to the different genetic components important in yield production. This is potentially difficult for marama bean seed production, as it is adapted to particular conditions, but is capable of growth and survival under a range of cultivated conditions (e.g., even under glass with supplementary light in the UK (Mitchell et al., 2005), as shown by several trials (Ramolemana, et al., 2003). Very sandy, and so freely draining, neutral to alkaline soils are most suitable. Summer rainfall in warm to hot regions, under strong radiation is essential. Excessive rainfall or irrigation is detrimental. Dry, cool winters are essential as tubers rot readily in moist soils. Marama bean appears to require limited nutrition (Ramolemana et al., 2003; Ramolemana et al., 2007; P. Chimwamurombe, field observations): Care with nutrition in field trials is important (see Lawlor, 2018 for details on cultivation). Uniform conditions in trials are essential if genetically determined differences in growth and yield are to be well founded.

5.7 | Plant material for cultivation

In the case of marama bean, plants must be established from seed, as vegetative propagation (e.g., rooting of vines) does not occur. Propagation from the meristem area of the tuber may be possible but has not been examined in detail. To establish a marama crop, where no “elite lines” exist, seed has to be obtained initially from wild heterozygous populations or from existing planted material, as at the Thusano Lefatsheng Trust, Mmankgodi, Botswana, field trials (see Lawlor, 2018). The provenance of the seed should be determined (both the wild population and ideally individual, marked plants). Samples from geographically separate populations would enable any differences between them to be determined, although phenotypic variation between geographically separate populations is regarded as small, in-depth studies have been few and limited in scope.

5.8 | Establishment of the trial

To disentangle the genetic from the environmental factors determining growth and yield, careful control of plant establishment is needed. If seed is sown directly, its viability is important otherwise the trial will be flawed. Pregermination and selection before planting is an alternative. The number of plants per unit land area in past field trials ((Ramolemana et al., 2003, 2007) has been based on knowledge of the spread of plants on the wild. Trials would be able to provide information on which to base this important parameter.

5.9 | Phenotyping and yield components

There has been relatively short term, limited work on this topic under field conditions (Ramolemana et al., 2003; P. Chimwamurombe, personal observation). Detailed phenotyping of many plants, as would be ideally done when evaluating trial material (Lawlor, 2018), is time-consuming and the application of nondestructive newer techniques for high-throughput phenotyping (Rasheed et al., 2017) considered. As yet, in no trials on marama has phenotype been adequately related to genotype. During the selection of advantageous genetic characteristics from wild-sourced plants, which is essential to develop marama as a crop, the different elements in the analysis—phenotyping and genotyping—must be extremely closely related.

5.10 | Tubers

Measuring tuber size (mass, dimensions) and growth (change in size over time) is clearly difficult, requiring destructive harvesting. Since tubers will be harvested after the season growth has been completed, the correlation between final tuber size and other field-related characteristics (e.g., leaf movements, water status, aboveground biomass) determined. Measurements made in this way can be related to (and calibrate) the phenotypic data records.

5.11 | Root system

It is expected that rapid formation of the very young seedling's root system is crucial for water and nutrient acquisition, allowing for maximum photosynthetic production and so for the growth of the tuber and shoot. A feed-forward effect on plant size and survival and reproductive capacity in the subsequent year(s) would be expected. There are indications of genetic variation in these early establishment traits—but knowledge of the fine root system is conspicuously absent. It is desirable to analyze this trait under best agronomic practice, perhaps combined with destructive measurements of tubers, and then related to genomic studies.

5.12 | Vines and leaves

As marama has very long, branched vines which radiate out from the top of the tuber (Figure 1), a single plant covers a large area of ground surface (up to *ca* 30–50 m^2 may not be unusual). However, there is considerable variation in the number, length, branching, and appearance of vines in the field, even of neighboring plants. Growth in length of vines is rapid under well-water conditions and decreases with water deficit, so it is a useful indicator of growth conditions, but little is known about variation in extension rate or final size. Variation in leaf number and size is apparent in plants from the same area, but as with other morphological features the cause—genetic or environmental—is not known and requires analysis. It is difficult to separate the effects of conditions, for example, during very early growth on later development, from true genetic differences.

Considering its growth habit, where marama depends on the position of vines just above the soil surface and the movement of leaflets and leaves to minimize the incidence of solar radiation, it is unlikely that marama could be grown similarly to cultivated vine crops such as grape or kiwi. Alternatively, a major aim could be the selection of a more erect (bushy) habit, thus allowing more plants per unit area, with potential increase in yield. Although this habit has not been observed in natural conditions and genetic factors controlling vine growth are unknown, improvement of bambara groundnut did involve the selection of bushy-type plants from wild populations (Ahmad et al., 2015). Variation in leaf number and size is apparent in plants from the same area, but as with other morphological features the cause—genetic or environmental—is not known and requires analysis.

5.13 | Inflorescence and flower formation

Inflorescences develop from leaf axils on mature parts of vines, and a plant may develop many, so the floral display is very obvious. Each inflorescence has several branches carrying many flowers. Clearly, the number of flowers is likely to be a major determinant of seed yield. Capacity to form many flowers, few of which are fertilized and form mature seed, is perhaps a general evolutionary adaptation to resource-limited environments (water, nutrients, pollinators, see Hartley et al., 2002). If this is the case, then overcoming an evolved trait such as low seed set, and the resultant small seed production, may prove a challenge. Some plants with many flowers (Figure 2) may have multiple pods indicating that it is the host plant that is controlling seed production rather than either the availability of pollen or pollinators. This will obviously be a fertile research area. Breeding by crossing will also be challenging as artificial pollen transfer between compatible plants or inflorescences or flowers will be required.

5.14 | Pods and seeds

Variation in number of pods per inflorescence, number of seeds per pod, and seed size has been observed. It is not known if the number of pods per inflorescence, or per vine or per plant or seed per pod is affected by the environment or strictly controlled genetically. Initial observations (C. Cullis, unpublished data) suggest that seed per pod is a genetically determined characteristic because pods on a plant tend to have the same number of seeds, for example, a plant will have one seed in all its pods or two seeds in all its pods. If this is confirmed, then a marker(s) associated with a larger number of seeds per pod can be identified and used to select breeding lines. This will be very advantageous and shorten the time to release of higher yielding germplasm. Detailed phenotyping will show if there is a possible constancy of seed per pod and if there is a relation to other yield components; for example, larger number of seed per pod

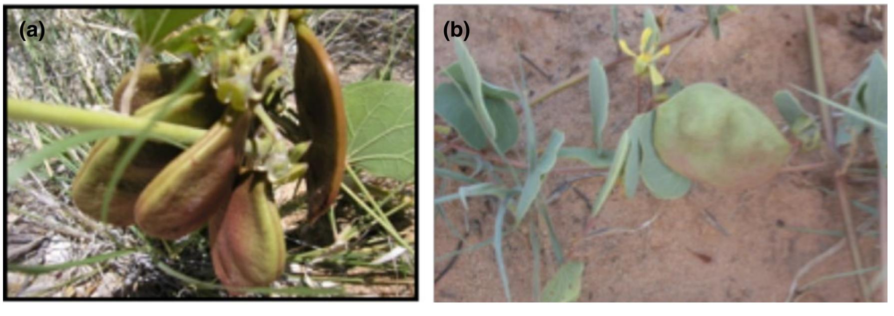

FIGURE 2 (a) Multiple pods on a single inflorescence. (b) Single 2-seeded pod

might be compensated by fewer pods per vine or vines with pods. Finally, marama pods can remain closed or be dehiscent. Evidence from other crops shows that selection, often over a very long period, fostered retention of the seed-bearing organ on the vegetative plant and was crucial for domestication (Dong & Wang, 2015), reducing losses before and during harvest. This feature depends on suppression of the formation of the abscission zone, for which the genes responsible are known (Dong & Yang, 2015), so molecular markers may be readily available to aid selection of this trait in marama.

5.15 | Tuber yield

This is likely to be a valuable component of economic yield. There is large variation in the growth rate and phenotype of the tuber produced within the progeny of a single maternal plant (C. Cullis, unpublished data; Figure 3). Differences in growth were also observed in the field in natural populations. Young tubers are edible and the starch from tubers may have important food applications. However, since marama is a perennial plant resprouting each year from the tuber to flower and produce seed, harvesting of the tuber would require an interruption of seed yield since replanting would not result in seed until the second year. A possible agronomic practice where tubers are harvested every year in between plants that remain for seed over decades might allow both components of economic yield to be collected, but the natural diversity in growth, tuber development, and appropriate time for harvest need to be determined. Since the tuber is not dependent on pollination, high-yielding lines could be produced without concern for ensuring a mixture of incompatibility groups in the field and result in a more stable yield. However, the relationship between tuber size or morphology and seed yield is unknown. Therefore, it is possible that selection for these two characteristics may need to be done separately for distinct lines, but here again molecular markers may be an essential underpinning to accelerate progress. A possible parallel would be the selection in *Linum* for oil or fiber flaxes, where varieties with a dual purpose of providing both seed and fiber have been difficult to produce (You et al., 2017).

5.16 | Pest and disease resistance

The self-incompatibility characteristic excludes the possibility of marama being grown as a homogeneous monoculture. However, there is still likely to be variability in resistance to pests and diseases. Surveying the natural populations for damage, especially by insects (lepidopteran larvae eat pods and seeds), will identify both the sources of damage and resistant phenotypes. Gene expression studies on developing pods, for example, of plants that have been damaged by pod/seed foragers will determine if the levels of, for instance cystatins, are protective (Cullis & Kunert, 2017; Cullis et al., 2018). The extreme damage caused by red spider mite (*Tetranychus urticae*) in covered cultivation is unlikely to be a problem in the field.

5.17 | Soil microbiome

Marama can grow in inhospitable regions (Figure 4). Although a legume, marama does not form nitrogen fixing nodules (Dakora, Lawlor, & Shibuga, 1999; LPWG 2017), but in spite of this, the plants can grow vigorously in extremely nutrient-poor soils and can form large tubers of more than 250 kg (Cullis et al., 2018). Possibly, the nitrogen supply involves the soil microbiome, but not through the traditional nodulation interaction for legumes, so identifying its contribution to marama's growth will be an important facet of any domestication process. The interaction of the plant genomics and the soil microbiome should inform any need to seed the “commercial” fields with a microbiome dressing to ensure maximal growth and, perhaps, reduce the prevalence of soil diseases. The sampling of the soil microbiome across the natural distribution of marama and any association with the growth rate of plants will be important in considering the need for seed dressing or other manipulations of the soil microbiome in proposed agronomic practices.

5.18 | Looking forward

Marama bean is a wild-growing perennial, adapted to grow in nutrient-poor soils under semidesert conditions of high temperature and solar radiation, with summer rain fall. Its seed has large contents of protein and oil, and tubers contain starches: Both may be eaten. For these reasons, marama has frequently identified as a plant which should be cultivated, particularly in drought-prone environments: The previous analysis supports this view. However, achieving this is not likely to be as easy as has often been assumed. The case for domesticating marama rather than using other more

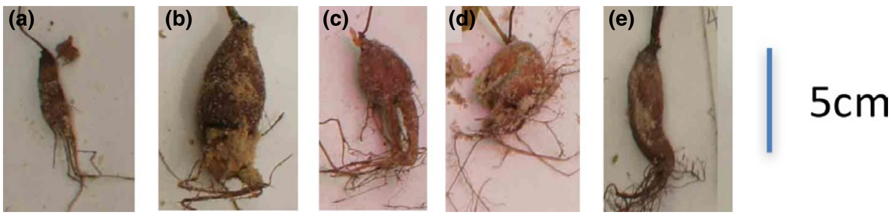

FIGURE 3 Tuber variation. These tubers were the result of a growth in sand with no added nutrients in 8 cm pots. All were grown from seeds from a single plant that shown in the foreground in Figure 1

FIGURE 4 Marama growing alongside a road in Namibia, GPS coordinates S23 42 57 E 19 13 6

advanced crops such as soybean or peanut is that the environmental niche occupied by marama is not suitable for either soybean or peanut—they will not grow in this environment, due to extreme aridity.

In this regard, the above analysis has identified a number of factors which may be challenging in any domestication process and need to be overcome if a viable marama bean crop is to be achieved. Factors include plant structure, which requires adaptation to agriculture, and poor yield per plant. Breeding of an outbreeding perennial, with little-understood floral biology, will further require adoption of established methods, including application of molecular techniques. A likely important aspect particularly of evaluating wild populations of marama is also assessing them, from different aspects, and fostering conservation which is important as the areas where it grows are subject to increased population growth and land and natural resource exploitation.

In general, crop domestication involves two major steps. The first step focuses on the development of an altered plant architecture and useful harvest traits, allowing the transformation of a wild ancestor into a "landrace" form compatible with farming. The second step is the development of specific cultivars of known genotype (Basu et al., 2007). For marama domestication, a first step might include the identification of, and selection for, a more bushy plant habit. This would permit growth as an erect plant on a much smaller area (Keegan & Van Staden, 1981) rather than as a creeper with scandent stems covering very large areas. The underutilized orphan legume bambara groundnut may be an excellent example for marama domestication (Bamshaiye, Adegbola & Bamishaiye, 2011; Doku, 1996). Bambara groundnut is, like marama, drought-tolerant and also grows well in poor soils, but like marama, greatly neglected within the research community. Bambara groundnut growth habit varies from bushy and standing erect to spreading and ground-hugging like marama. Domestication of bambara groundnut has led to the predominance of bushy and semibushy genotypes although the ancestor is an extreme spreading type, a loss of dormancy and for a number of other traits (Ahmad et al., 2015). Ground-hugging bambara groundnut is further grown by smallholders as a subsistence crop while the bushy type, which is planted in larger-scale farming, is easier to harvest and also tend to mature earlier (National Research Council, 2006).

As with other "orphan crops" a full breeding program for marama bean would require considerable investment of scientific resources, infrastructure and financial support. Such a breeding program should, in our opinion, progress in phases, each of which would have achievable, and verifiable aims. A cost–benefit analysis for marama should consider its value as a crop for environments to which it is adapted, and specifically the socioeconomic area of southwest Africa. Analysis should also include not just provision of a crop for "industrial" agriculture but for resource-poor farmers, who would be involved in field evaluations associated with breeding, for example, and would benefit from the financial and technological inputs. The adoption of advanced management methods is also an important aspect of developing such a program and would have spin-off effects in many aspects of the related science, technology, and management. Exploratory studies on, for example, examining marama in field trials, assessing the plant's morphology, physiology, and molecular features have been initiated. Because marama is a perennial plant, long-term trials are, however, required, which will necessitate a long-term commitment.

A limited breeding program based in a research and higher education institution may be coupled with teaching and community development, which are long-term development goals. A particular feature of such a program is the possibility of using it as a platform for collaborations, in many ways both intra- and interinstitution, and within a country and geographic region. Such a program considering research, development, and application at all levels, from plant biology, environment, to agronomy and marketing has been implemented in undergraduate and graduate education and research collaborations between Case Western Reserve University, The Namibia University of Science and Technology and the University of Pretoria. In this context, the current molecular studies, including the development of whole-genome sequences of many marama individuals have been the result of undergraduate and graduate course work at Case Western Reserve University. The steps required would benefit from increased resources, to allow the initiation of new areas of scientific analysis, such as floral biology, and the integration of different known facets of the plant's biology and environmental responses. However, the leverage of resources devoted to laboratory and field courses has, and can continue to, provide a basic funding source for progress in the domestication process.

In summary, taking marama bean into cultivation is not a trivial endeavor and certainly more demanding than earlier simplistic approaches aimed at quick exploitation assumed. However, by taking a stepwise approach with clear aims and utilizing existing technology, the probability of success is increased. The ancillary benefits are likely to be rapid and considerable. Turning wild-growing marama into a component of agriculture, particularly for southern Africa, is, in our opinion, a valid and valuable endeavor.

CONFLICTS OF INTEREST

The authors have no conflicts to declare.

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