

DOI: 10.1002/fes3.422

OPINION



Perspective on the present state and future usefulness of marama bean (*Tylosema esculentum*)

Christopher Cullis¹ | Percy Chimwamurombe² | Karl Kunert³ | Juan Vorster³

Correspondence

Christopher Cullis, Department of Biology, Case Western Reserve University, Cleveland, OH, USA. Email: cac5@case.edu

Funding information

Case Western Reserve University; Kirkhouse Trust; NRF South Africa

Abstract

Recent focus on indigenous knowledge of plant species that have long been consumed by mankind, but not having a prominent place in organized agriculture, has raised the profile of what steps are needed to bring such potentially useful plant species into formal agriculture practice. Many of these orphan plants have been cultivated, albeit usually without much improvement. Tylosema esculentum (marama bean) is unusual; in that, it has not been grown as a crop but only collected from wild stands. The nutritious seeds have been consumed, and other potentially useful products from the marama seeds and tubers have been identified. The marama bean grows under inhospitable environments, particularly hot arid conditions, and, if domesticated, could be grown in environments where there are no high-yielding alternatives. The perspective of this article is to view the current status of the marama bean domestication and the possible pathways to bring this plant into wider agricultural use. The scientific basis for an improved crop yield needs to be coupled with the identification of possible added value characteristics for growers. These include acceptability as a food, possible industrial use, the development and distribution of improved seed, and the recruitment of growers to plant marama as a crop. The authors' international collaboration has already made advances in some of these areas. We discuss the current ongoing developments and existing gaps in moving this plant into formal agriculture, along with a plan for the future developments necessary for marama to provide food security under climate change, particularly in Africa. Even as the process of domestication of marama bean is proceeding, its unusual and potentially useful characteristics will greatly benefit the improvement of other legume crops needed to provide adequate nutrition to about 35% of the world population living in semi-arid to arid regions of the developing world.

KEYWORDS

Climate-resilient crop, domestication, legume, marama

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. Food and Energy Security published by John Wiley & Sons Ltd.

¹Department of Biology, Case Western Reserve University, Cleveland, Ohio, USA

²Department of Natural and Applied Sciences, Namibia University of Science and Technology, Windhoek, Namibia

³Department of Plant and Soil Sciences, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Hillcrest, South Africa

1 | INTRODUCTION

The food supply is dominated by a small number of crops, with less than 20 plant species providing more than 95% of the world's calorie intake. Over recent decades, there has been a focus on the development of underutilized crops to supplement the food supply, in particular to improve food security in regions of insecurity (Fernie & Yan, 2019; Hunter et al., 2019; Maphosa & Jideani, 2017; National Research Council, 1979, 2006; Smýkal et al., 2018; Tadele & Assefa, 2012; Vidigal et al., 2019). Most of the species that are subject of improved attention and development, such as cowpea and bambara groundnut, are already used in formal agriculture, albeit usually under low input conditions with minimally improved germplasm.

In Southern Africa, longer periods of drought and changing rainfall patterns, with increasing temperatures are predicted (USAID, 2012). Global temperatures are expected to rise by 1.4°C to 3°C by 2050, which are a particular major threat to crop production in Southern Africa. Farmers will have, therefore, to deal with such changing climate to remain productive to avoid food shortages with fewer products produced locally. Specifically, Namibia, the driest country in sub-Saharan Africa (https://www.eld-initiative.org/en/where-we-work/africa/namibia/), will possibly have very low rainfall and will be very vulnerable to the impacts of future climate change. A country like Namibia has, therefore, to find methods to adapt to these changes.

Diversification of these neglected more droughttolerant under-utilized legume crops might offer alternatives for increased food and nutrition security among resource-poor farmers (Hunter et al., 2019). However, such under-utilized neglected crops have so far received limited research efforts and breeding activities directed towards their genetic improvement, compared to the major food crops such as wheat and rice. Unlocking the potentials of these under-utilized crops might be crucial towards the attainment of a sustainable environmental and nutritional food security. Most orphan legumes still fail as a food crop, in particular due to the severe drought conditions in many parts of Southern Africa. Legumes, such as common bean (Phaseolus vulgaris L.) and cowpea (Vigna unguiculata L.), have previously attracted some attention for growth in Southern Africa (Porch et al., 2013) with common bean, for example, an important legume crop worldwide. However, both abiotic and biotic stress limits bean yield to <600 kg ha⁻¹ in low-income countries and results in food insecurity (Porch et al., 2013). Additionally, beans, like common bean, are less suitable, in our view, for very dry countries, such as Namibia. They require 400-500 mm rainfall under rainfed conditions (Department of Agriculture, Forestry and Fisheries, South Africa, 2010).

However, farmers in Namibia also now have access to new genetically changed crop varieties of cowpea. They were created by seed irradiation to obtain more tolerance to drought and pests (Schlechter, 2019). These new cowpea varieties have higher yield produced in a short growing period, with flowering starting after 30 days, and plants being fully mature after 2.5 months. Cowpea cultivation also has the benefit that nitrogen is added to the soil by the plant, limiting the necessity of the application of industrial rather expensive nitrogen fertilizers. However, even these orphan food legumes still fail under severe drought conditions. The remaining alternatives are, therefore, orphan legumes that can still survive even where these other leguminous food crops generally fail. The climatic conditions, rainfall and temperature, where the marama samples were collected are shown in Figures 1 and 2.

In this perspective paper, we present our view that it will be specifically important to further invest into marama bean research to ultimately obtain improved relatively uniform high-yielding marama seeds. To support our view, we will, therefore, discuss our current research achievements and provide evidence why inclusion of marama into alternative cropping systems is a highly interesting research idea to address future food security particularly in Southern Africa. We will also discuss the steps necessary for farmers to cultivate marama, and finally, we will discuss how in any domestication process unusual and potentially useful characteristics of marama could also greatly benefit the improvement of other legumes.

2 | WHY SHOULD WE INVEST RESEARCH ACTIVITIES INTO MARAMA BEAN?

What advantages does marama bean have to justify a future research investment? Marama (Tylosema esculentum) is a perennial legume that has been identified as a possible additional food legume (Cullis et al., 2018, 2019; Cullis & Kunert, 2016; National Research Council, 1979, 2006). The bean interestingly provides a contrasting example to current legume crops in that it can produce some yield even in the driest years, but it is still exclusively wild-harvested. Marama has further a high nutritional value containing high levels of protein (Omotayo & Aremu, 2021). The fatty acids are unsaturated, and 87% are a combination of oleic acid, linoleic acid, and palmitic acid. The bean also contains significant amounts of vitamins (A, B3, B6, folic acid, B12, and E) and minerals (iodine, iron, and zinc) (Omotayo & Aremu, 2021). The nutritional composition of marama seeds vis-a-vis cowpea, soybean, and bambara groundnut has been detailed in Gulzar and Minnaar (2017). Marama has a protein content similar to soybean

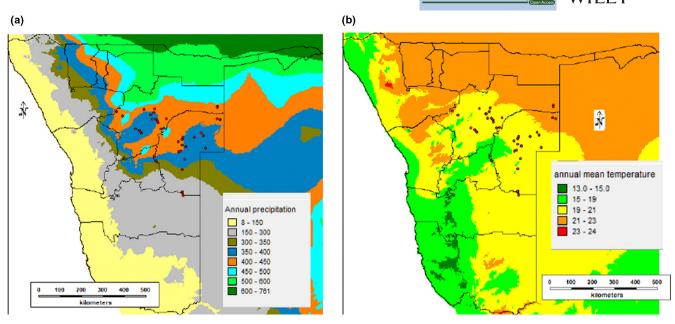


FIGURE 1 (a) Annual precipitation across Namibia. (b) Annual mean temperatures across Namibia. • - denotes collection sites

and greater than either cowpea or bambara. Marama protein differs from other legumes in that the 7S (vicilin) and 11S acidic subunits are absent (Amonsou et al., 2012). The marama proteins have a high tyrosine content with apparently fewer disulfide linkages.

Marama is further native to dry areas with little seasonal rainfall (Figures 1 and 2), and, being a legume, even if it does not nodulate, is particularly important in subsistence agriculture where farmers have little access to chemical fertilizers and water. The bean is a basal legume growing in an inhospitable climate. Perhaps the lack of nodulation is a response to the energetic stress imposed by supporting symbiotic nitrogen fixation in such an environment. The separation of marama from the nodulating legumes is also apparent when the nucleotide sequence of the leghemoglobin gene from marama is compared to that of other plants, where the version in marama is closer evolutionary to that found in non-legumes than to that from nodulating legumes. In addition, marama bean has, in our view, the following advantages:

- 1. The bean can act as an additional or alternative crop to cowpea and other legumes when the environmental conditions are not conducive. This is particularly important in light of the current and forecast severe drought conditions, for example in Namibia. The bean still produces a crop when everything else fails. The current yield for the bean in Namibia is 500 kg/hectare, compared with a range of Namibia cowpea yield of 600 kg-1200 kg/hectare (Horn & Shimelis, 2020).
- 2. The bean has low input and can be, therefore, grown without added fertilizers in poor soils.

- 3. No irrigation is required, as the bean uses stored water from the tuber which is also a starch reservoir.
- 4. The bean is easy to grow, not labor intensive with a continuous production of seeds throughout the growing period.
- 5. The bean can be harvested when convenient; it does not have a sensitive window when harvest must take place.
- 6. Growth habit of the bean is an advantage, in particular in hot areas.
- 7. The bean has a superior nutritional value.
- 8. Marama also contains a protease inhibitor (elastase inhibitor), which has a potential for use in pharmaceuticals (Elfant et al., 1985). In the related species *Bauhinia bauhinioides*, a potentially similar protease inhibitor has useful anti-thrombotic effects (Oliveira et al., 2021). This protease inhibitor is, however, the major antinutritional compound in marama seeds which has to be inactivated by boiling which might affect future consumer acceptance, and this trait has, therefore possibly, to be eliminated in the future. No toxic hemagglutinins have been so far identified (Bower et al., 1988).

The preceding list relates to marama as a potential stand-alone crop. This importance would be enhanced by development of a range of products from the bean that would provide an income for the growers if they had excess beans not required for their own consumption. The markets for such value-added products could include marama milk (with analogy to soy and almond milks), snack foods, and porridges (Faria et al., 2011). Also of interest is application in both the pharmaceutical

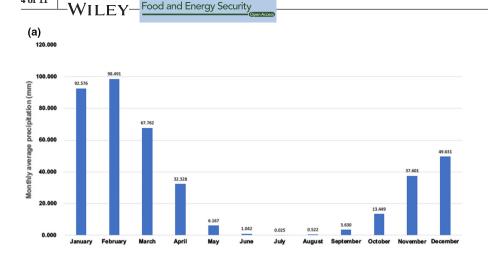
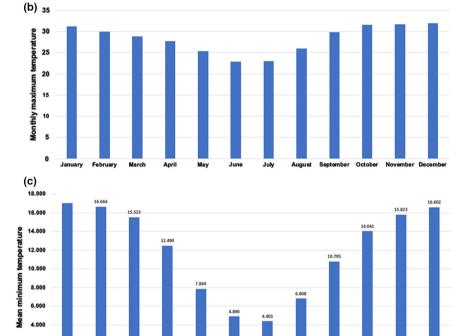


FIGURE 2 (a) Monthly average precipitation at marama bean collecting sites in Namibia. (b) Monthly average maximum temperature at marama bean collecting sites in Namibia. (c) Monthly average minimum temperature at marama bean collecting sites in Namibia



and cosmetics industries due to the proteinase inhibitor (elastase inhibitor), found in high concentrations in the seed (Elfant et al., 1985) and in few other plants (Oliveira et al., 2021). A wide range of potentially useful phytochemicals are also present in the bean seeds (Omotayo & Aremu, 2021).

3 | FUTURE MARAMA BEAN DEVELOPMENT

What is still missing and, therefore, will require, in our view, further research investment to develop marama bean into a new legume crop? Although marama bean has great potential as both a food security and cash crop,

it still does not have any initial selection for productivity under formal cultivation. The bean was first considered as a target for domestication more than 40 years ago (Keegan, 1981), but little has been advanced since then. However, the development of new methodologies for genomic sequencing, plant transformation, and specifically gene editing holds now the promise for reducing the time necessary for providing domesticated germplasm to farmers and to start growing marama as a formal new legume crop in organized agriculture, within the subsistence farming environment. These efforts are in line with previous suggestions that "de novo domestication" or "re-domestication" is a viable route to exploit the genetic diversity of wild species, shown, for example, in potato (Fernie & Yan, 2019; Zhang et al., 2021).

20483694, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/fes3.422 by South African Medical Research, Wiley Online Library on [04.04/2023]. See the Terms and Conditions (https://onlinelibrary.com/doi/10.1002/fes3.422 by South African Medical Research, Wiley Online Library on [04.04/2023]. See the Terms and Conditions (https://onlinelibrary.com/doi/10.1002/fes3.422 by South African Medical Research, Wiley Online Library on [04.04/2023]. See the Terms and Conditions (https://onlinelibrary.com/doi/10.1002/fes3.422 by South African Medical Research, Wiley Online Library on [04.04/2023]. See the Terms and Conditions (https://onlinelibrary.com/doi/10.1002/fes3.422 by South African Medical Research, Wiley Online Library on [04.04/2023]. See the Terms and Conditions (https://onlinelibrary.com/doi/10.1002/fes3.422 by South African Medical Research, Wiley Online Library on [04.04/2023]. See the Terms and Conditions (https://onlinelibrary.com/doi/10.1002/fes3.422 by South African Medical Research, Wiley Online Library on [04.04/2023]. See the Terms and Conditions (https://onlinelibrary.com/doi/10.1002/fes3.422 by South African Medical Research, Wiley Online Library on [04.04/2023]. See the Terms and Conditions (https://onlinelibrary.com/doi/10.1002/fes3.422 by South African Medical Research (https://onlinelibrary.com/doi/10.1002/fes3.422 by South (https://on and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons

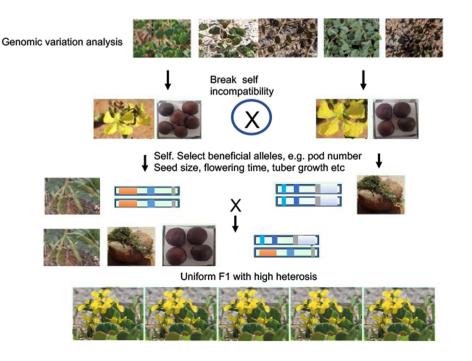
Desirable characteristics from the wild germplasm, available through Genebank accessions, have been introduced through crosses with cultivars followed by backcrossing and selection for the desirable trait, or alternatively, the desirable sequences have been introduced through gene editing. With marama bean, unfortunately no cultivars are available at the outset. As an outcrossing ancient hexaploid, the marama reproductive period requires at least 2 years per generation. A direct breeding scheme is further unlikely to provide improved potential cultivars within a reasonable timeframe.

An alternative route to domestication is provided in Figure 3. A wide range of plant phenotypic diversity for selectable characteristics is generally available in the "wild germplasm." For example, variation in plant size, which is related to vine growth rate and flower number, is available within the marama wild germplasm (Cullis et al., 2018, 2019; Takundwa, 2014). However, since marama is an obligate outcrosser, the genetic control of these characteristics is currently unavailable. Another important characteristic for yield is the number of seeds per plant. Our group has observed that individuals can have a large number of flowers. But seed setting is only in a minority of these flowers, although occasionally multiple flowers in a single inflorescence can set seeds. The reason for this low fertility is still unknown - the question still remains if it is due to a failure of pollination (e.g. a lack of pollinators), low abundance of compatible pollen, or an inability to support a large seed set. The last of these does not appear to be likely for marama as some individuals can have 6 or more pods set and develop from a single inflorescence (see Figure 3).

3.1 | Fertility determination

Two possible methods of answering the unresolved question of seed set would be, in our view, either by hand pollination, to determine if the success rate can be improved (it would be necessary to determine the compatibility of the pollen donor), or by mutating the self-incompatibility genes through gene editing and thereby permitting selfpollination (as suggested in Figure 3). For marama bean, an interesting research activity would be, therefore, the identification of the genes for self-incompatibility. According to previous studies, in vivo and in vitro diallel crossing experiments have already demonstrated that a diallelic heteromorphic self-incompatibility system exists in the bean. The major site of pollen tube inhibition in the intramorph crosses was thereby in the style (Hartley et al., 2002; Omotayo & Aremu, 2021). Such inhibition is a barrier in the development of varieties with large numbers of seeds for distribution from traditional breeding schemes. However, if this incompatibility can be overcome, and inbred lines can be developed, then production of more uniform marama hybrid seeds will be possible. The advantage of breaking the self-incompatibility bottleneck will also extend to the crop development with no issue of required appropriate pollinators if the crop is distributed to other arid regions of the globe. A set of genotypes to understand this characteristic has been already identified at two locations in Southern Africa, one is at the University of Pretoria Experimental Farm in South Africa, individual plants have been studying. Six individuals have been already growing on the farm for many years. All plants produce seeds, with no other marama plant individuals in

FIGURE 3 Scheme for developing improved cultivars for marama bean as a new crop. Genomic analysis undertaken to identify self-incompatibility genes can then be used to disable this pathway through gene editing by CRISPR. Selfed populations can then be grown to identify high-yielding individuals and the mode of inheritance of these characteristics determined. Subsequently, selected lines incorporating different desirable characteristics can then be crossed to give a uniform F1 with (hopefully) high heterosis



the locality. The other set of genotypes is in Namibia. An identified set of four individuals at this location that form a group that can cross-fertilize. We already have mainly unpublished Illumina whole genome sequence for each of the individuals from these locations, although the data for 5 individuals are available at https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA779273, PRJNA779273.

The recent development of new technologies using carbon dots, or carbon nanotubes (Demirer et al., 2020; Demirer, Zhang, Goh, et al., 2019; Demirer, Zhang, Matos, et al., 2019) for introducing foreign nucleic acids into plant cells has opened now also new possibilities for developing inbred lines in self-incompatible species. This can also be, in our view, an initial route to follow for marama. The marama incompatibility genes will be identified through a combination of bioinformatic interrogation of the current marama genome assembly and the large number of next-generation sequence data for multiple individuals, coupled with transcriptomic data from bean flowers, with the transcriptomic data yet to be collected. Following identification, interfering RNAs or gene editing constructs will need to be designed and delivered to the styles through this new carbon dot technology, followed by self-pollination. The performance of these selfed seeds will be finally evaluated, as would any possible hybrid vigor in crosses between the various selfed populations.

In parallel, the identification of the self-incompatibility controls will also permit the selection of compatible parents among the most vigorous native marama plants. The performance of these crosses will be compared with any artificial hybrids developed through biotechnology. It has, however, to be stressed that the selfing regime proposed above does not produce any bio-engineered (GMO) marama plants. The inhibition of the self-incompatibility mechanism is transient in the style and will, therefore, not be subject to any gene editing regulations. They will be equivalent to varieties developed using marker-assisted selection.

Finally, flowering time can, in our view, also be targeted. Natural marama populations have very different flowering periods. Marama individuals growing in close proximity have been observed to be at all stages of flowering, with some individuals having flowers just beginning to form, to bean plants that have completed the cycle and have mature seed pods present. Within one area, we further observed that groups of established marama plants had essentially completed flowering with many mature pods present, while adjacent plants, although growing vigorously, had not even started flowering. Identifying early flowering genotypes might reduce the time between generations if two flowering cycles per year could be managed.

3.2 | Plant architecture

Plant architecture is a key agronomical factor determining crop yield and is a major target for crop domestication. Should, therefore, the current architecture of marama bean be modified as part of a future research activity? The bean is a creeping vine in the wild with long stems which can grow to more than 5 meters in a single season. In addition, the flowers tend to be located towards the termini of the stems. This ground cover phenotype makes seed collection difficult. For other plants, an erect dwarf habit has been selected to improve the viability of the species as a crop. For example, the transition of plant architecture from the prostrate tiller of typical African wild rice (Oryza barthii) to the erect tiller of African cultivated rice (Oryza glaberrima) was a key step during domestication of African rice (Hu et al., 2018). Similarly, spontaneous mutations are the source of erect compact type plants with high number of branches arising from the base, commonly called as "bushy mutants" in chickpea (Sandhu et al., 2010). Two routes are currently available to discover whether this is a viable strategy for modifying the architecture of marama bean. The first step is a survey of the natural variation to determine if such a marama variant exists within the germplasm. The second route is to generate a mutant population to determine if there is such a viable mutant phenotype. An already observed variation in the internode length of marama bean appears to be genetically controlled (Takundwa, 2014). However, it is also necessary to increase the stem rigidity to have them remain semi-erect when associated with a greatly reduced elongation for a more productive marama phenotype. Irradiation has been already successful applied in generating mutants in marama bean (Takundwa et al., 2015), but the mutant screen was focused on early germination traits and, unfortunately, not final plant architecture. Since the initial parameters for inducing non-lethal mutations have already been determined (Takundwa et al., 2015), a research action into irradiating a larger population of seeds could be, therefore, highly informative. Seeds grown should be further evaluated to observe whether there are any growth habit variants arising. Application of the gene editing technology (Jang, 2019; Khlestkina & Shumny, 2016) will, in our view, also be an interesting approach to modify the marama bean equivalents of those important genes involved in changing plant architecture, as already identified in African rice (Hu et al., 2018) and cowpea (Sandhu et al., 2010). Application of these molecular tools will, in our opinion, rapidly identify possible routes to generating the appropriate architecture for improved sole cultivation.

3.3 Intercropping

An alternative interesting route for the development of wider production of the bean is also the application of marama bean as an intercrop, rather than a primary crop. This application will take advantage of the prostrate high vegetative marama growth already available and not require the modification of the growth habit. The current marama growth habit consists of a deep tap root. The lowest portion of the root produces side roots and also develops into the tuber (Figure 4a), although the tuber can develop closer to the surface (Figure 4b).

However, this deep rooting very likely does not interfere with the acquisition of resources (nutrients and water) for any shallow rooting crops, such as maize, with which it can be co-cultivated (Figure 5). The marama vines do not root but the groundcover is substantial providing shade to reduce the soil temperature as well as a barrier to soil moisture evaporation. Since the bean is a perennial once established, it would provide these advantages while still generating seeds in virtually every season, when the more developed crops fail due to lack of water. Finally, although marama is a legume, it does not nodulate. Therefore, the mechanism(s) that are employed to mobilize nutrients to support the rapid vegetative growth of the bean in poor soils may also improve the growth of the primary crop with which marama is co-cultivated as can also be seen in Figure 5.

3.4 **Tuberous root productivity**

At present, the tuber has received little research attention with respect to its possible contribution to food security or its growth characteristics. Previously, Cullis et al. (2018) suggested that the tuber might be a unit of productivity while the perennial marama bean plant matures to produce seeds. Seeds are not produced until the second year at the earliest. An attempt to cultivate the bean previously indicated that it requires up to 5 years before optimal yield is obtained (Powell, 1987). The root of marama plant grows to size as big as average potato tuber within a period of 6 months, which is after the first annual growth cycle. The majority of the fresh weight of marama tubers is water (>85%) while the remainder was mainly protein (2.4%), starch (5.6%), and ash (2.2%). In a comparison with potato and sweet potato, the quality of marama tubers was broadly comparable to sweet potato in most nutritional characteristics, and marama has a similar energy value and starch digestibility to potatoes (Adeboye & Emmambux, 2021). Given the data for first-year tubers, a possible interesting question is if the seeding rate in the field can be twice, or more, than that for the final seed production crop, with half the plants harvested for tubers each year (and perhaps even replaced for a second or third year of harvest)? A further interesting research question is whether there is any relationship between marama vegetative growth and tuber size? If such a relationship was discovered, then this could be a useful selectable parameter for identifying genotypes for the tuber-producing crop.

The development of the tuber also appears to be of two types, either a deep rooted one or one that is close to the surface (Figure 4). The profile of the development of the tuber needs to be determined since this would be a major factor determining the timing and/or the genetic control of tuber development, which would be most productive for tuber harvesting. In our view, a tuber that develops close to the surface would be preferable for ease of harvesting with the minimal disruption of surrounding plants.

Marama bean market 3.5

The aspect of any future marama bean commercialization can, however, not be ignored. Any improved variety of the bean that can be distributed to resource-poor farmers will not promote their adoption unless there are clearly recognized products and also a value chain by which these products can be marketed. Therefore, in order to turn the bean into a commercially viable agricultural crop, since this is currently not a significant crop within the food chain, it is crucial to identify and develop value-added products that can be sold. At present, there is, for example, not a marama seed market. Such market has, in our view, to be developed by possibly first providing seeds free to smallholder subsistence farmers to encourage uptake of this nascent crop. Our experience is that an increasing number of farmers in

FIGURE 4 Different tuber morphologies. (a) Approximately 70 kg tuber the upper surface of which was about 70 cm below ground level. (b) an approximately 200 kg tuber the upper surface of which was just below the ground level





FIGURE 5 Potential intercropping of marama and maize. This also demonstrates the relative vigor of marama and maize under water stress conditions although this was during a relatively wet year of 2020. The marama has already flowered, set seeds and has ripe pods present, one of which is arrowed

Namibia and also other developing countries with similar Namibian climatic conditions are indeed requesting seeds. Marama bean flour has, for example, the potential as a functional food ingredient (Maruatona et al., 2010), while the functional properties of raw and roasted bean flour have also been assessed (Jideani et al., 2009). Other potential products might include, in our view, the marama bean butter, oil, milk, biscuits, and possibly snack foods, in addition to the use of marama flour as a fortification ingredient in porridge as suggested elsewhere (Jackson, 2017). A range of other disciplines have, however, to be included in the successful development of these products and their introduction into the marketplace. The market conditions for bean products, focused on the economical, social, and cultural conditions in Southern Africa, have previously already been analyzed. Faria et al. (2011) concluded that "assuring the sustainability of local peoples' livelihood while creating a larger market is only achievable through community organizations supported by a broad marketing strategy and using cultivated marama beans."

4 | CURRENT RESOURCES

Numerous resources to support the de novo domestication effort have already been acquired. Large numbers of seeds from across the Namibian germplasm have been collected and the diversity across populations determined (Nepolo et al., 2009; Takundwa et al., 2010). Subsets of these seeds are available for mutagenesis studies to determine if these will identify the desired architecture mutants. In addition,

seeds have been provided to other countries in Africa as well as India for farmers to test their productivity and suitability as a cultivated crop. These seeds have been resourced from farmers who are already successfully cultivating marama bean for this initial distribution with the appropriate permissions from the Namibian authorities.

Microbiomes established in the soil near, or even inside, the roots play some important roles in plant growth (Lucaciu et al., 2019). Marama bean grows on poor soils and does not interact with Rhizobium to provide fixed nitrogen via nodules. The microbiome associated with the marama bean root system must, therefore, play a role in the mobilization of nutrients and provides, in our view, an opportunity to identify potential novel bio-fertilizer components. This would, however, require additional research investment. We have already collected soil samples adjacent to marama plants and the microbial (bacterial and fungal) populations characterized by phylogenetically informative ribosomal RNA sequences. Samples include representative collections from different regions of Namibia. Comparisons between the communities adjacent to and remote from bean plants are currently being investigated.

Across this collected germplasm, Illumina whole genome sequences for 78 individuals, with PacBio sequence data of one of these as well, are also already available. A genome assembly has been thereby developed for the individual which has 100X Illumina and low pass PacBio sequence. Due to the complexity of the genome, the size of the scaffolds for this assembly is low, with the majority of the longest scaffolds being from the chloroplast and mitochondrial genomes. These data have been already used to characterize the structure and diversity of the chloroplast genome (Kim & Cullis, 2017) and the marama mitochondrial genome (Li & Cullis, 2021). The assembly is sufficiently robust to identify genes of interest, using heterologous sequences, such as those that may underlie the self-incompatibility characteristic, the presence of genes involved in nodule formation, and the cysteine protease inhibitor. Within this set of genomic sequence data are representatives of progeny from 3 different parents so that as more polymorphisms are identified, the families can be useful in longer range assembly of the genomes. The candidate genes for self-incompatibility can be tested using the parental DNAs and progeny from two isolated populations (of 4 and 6 plants respectively) to determine if the inheritance of these regions is consistent with them determining (or being part of the mechanism for) self-incompatibility.

The genomic sequence data will provide many molecular markers, especially single nucleotide polymorphisms, for detailing the genetic variability of the current marama populations. It will also be useful in identifying the mobility between populations since many of the groups of plants are widely separated, so understanding the relationships within allocation might be important in understanding the specifics of the local environment supporting marama establishment and growth. The molecular markers can finally be used for genome-wide association studies to inform the selection of breeding lines to generate improved germplasm.

Finally, seeds of marama bean are distributed to smallholders in Namibia to initiate growing them in organized agriculture outside of the seed multiplication effort (Chimwamurombe & Khulbe, 2011). This distribution needs, however, to be expanded and associated with a value chain to provide validation data for an expansion of the cultivation effort to be successful.

LOOKING FORWARD 5

Marama bean has, in our view, already a well-documented potential as a possible future new food security crop that can be grown in regions where there are no other competing alternatives. However, this plant has potential through both the seed and tuber to become more than just a subsistence product available from wild populations. Currently, the bean is only collected from untended extant populations as a food (and primarily as a seed "crop"), particularly in times of food shortages. For enhanced production to be achieved, a coordinated effort with substantial financial investment is, however, needed to develop and expand the current pipeline for improved seed. In the event of such improved material becoming available, the infrastructure to provide income for growers (as an incentive to take up producing the crop) for any marama bean surplus to growers' food needs has to be developed. Based on the foregoing discussion, the short-term application as an intercrop will enable the supply chain to be developed while the possibility of producing an altered architecture to increase overall production is undertaken in the longer term.

The application of new molecular techniques for identifying desirable genotypes should also reduce the time for developing agronomically productive varieties as noted elsewhere (Fernie & Yan, 2019; Zhang et al., 2021). Ideally, a crop could be developed, in our view, from wild plants in a four-step process. Initially, the evaluation of novel plant architecture and the ease of manipulating the essential characteristics would be targeted (years 1-2). Then, the de novo domestication by combining (or modifying) the genes affecting the key traits would be accomplished (years 3-5). Since the bean does not flower in the first year after planting, simple introgression through crossing is likely to take longer than in an annual species. Then, achieving the stability of the ideal traits within breeding lines by modification of specific pathways (erect habit, overcoming selfincompatibility) would take a further 5 years (years 5–10). Finally, the combinations of all the designs into seeds and multiplication of these for distribution a further 5+years would be needed (years 10-15+). During the development of superior bean varieties, the recruitment of farmers willing to try growing the crop, the agronomic constraints on seed and/or tuber production, and the viability as an intercrop need to be addressed. Finally, the value chain for delivering the crop to market will, in our view, also have to be developed. This could probably be done by using both the wild collected material and the limited seeds from farmers, who are already formally cultivating the plant that will be processed to provide sample goods. All of these steps are possible, and as noted above, the initial work has laid the framework and basis for a successful de novo domestication.

Although the focus is currently on the arid regions of southwest Africa, a successful domestication of marama bean would, in our view, also be useful in other regions where it could become a viable food security crop in areas of low or unpredictable rainfall. With the current changes associated with climate change, the more resources available to ensure the food supply with adequate nutrition in areas of unstable food security the better.

FUNDING INFORMATION

Kirkhouse Trust to PC, Undergraduate teaching resources from Case Western Reserve University to CAC, NRF South Africa through incentive funding to rated researchers KK and JV.

ACKNOWLEDGEMENTS

Support was received from the Kirkhouse Trust, Case Western Reserve University and the NRF South Africa. Students in the course BIOL301 over a period of years supplied some of the supporting data.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Christopher Cullis https://orcid. org/0000-0002-9768-7763

Karl Kunert https://orcid.org/0000-0002-7740-3508 Juan Vorster https://orcid.org/0000-0003-3518-3508

REFERENCES

- Adeboye, A. S., & Emmambux, N. M. (2021). Composition, functional and nutritional quality of marama (*Tylosema esculentum*) storage root. *Journal of Food Science and Technology*, 58, 4391–4402. https://doi.org/10.1007/s13197-020-04924-2
- Amonsou, E. O., Taylor, J. R. N., Beukes, M., & Minnaar, A. (2012). Composition of marama bean protein. *Food Chemistry*, *130*, 638–643. https://doi.org/10.1016/j.foodchem.2011.07.097
- Bower, N., Hertel, K., Oh, J., & Storey, R. (1988). Nutritional evaluation of Marama bean (Tylosema esculentum, Fabaceae): Analysis of the seed. *Economic Botany*, *42*, 533–540. https://doi.org/10.1007/BF02862798
- Chimwamurombe, P. M., & Khulbe, R. K. (2011). Domestication. In A. Pratap, & J. Kumar (Eds.), *Biology and breeding of food legumes* (pp. 19–34). CABI.
- Cullis, C., Chimwamurombe, P., Barker, N., Kunert, K., & Vorster, J. (2018). Orphan legumes growing in dry environments: Marama bean as a case study. *Frontiers in Plant Science*, 9, 1199. https://doi.org/10.3389/fpls.2018.01199
- Cullis, C., & Kunert, K. J. (2016). Unlocking the potential of orphan legumes. *Journal of Experimental Botany*, 68(8), 1895–1903. https://doi.org/10.1093/jxb/erw437
- Cullis, C., Lawlor, D. W., Chimwamurombe, P., Bbebe, N., Kunert, K., & Vorster, J. (2019). Development of marama bean, an orphan legume, as a crop. *Food and Energy Security*, 8(3), e00164. https://doi.org/10.1002/fes3.164
- Demirer, G. S., Zhang, H., Goh, N. S., Gonzalez-Grandio, E., & Landry, M. P. (2019). Carbon nanotube-mediated DNA delivery without transgene integration in intact plants. *Nature Protocols*, *14*(10), 2954–2971. https://doi.org/10.1038/s41596-019-0208-9
- Demirer, G. S., Zhang, H., Goh, N. S., Pinals, R. L., Chang, R., & Landry, M. P. (2020). Carbon nanocarriers deliver siRNA to intact plant cells for efficient gene knockdown. *Science Advances*, 6(26), eaaz0495. https://doi.org/10.1126/sciadv.aaz0495
- Demirer, G. S., Zhang, H., Matos, J. L., Goh, N. S., Cunningham, F. J., Sung, Y., Chang, R., Aditham, A. J., Chio, L., Cho, M. J., Staskawicz, B., & Landry, M. P. (2019). High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plants. *Nature Nanotechnology*, 14(5), 456–464. https://doi.org/10.1038/s41565-019-0382-5
- Department of Agriculture, Forestry and Fisheries, South Africa (2010). Dry bean production guideline. Retrieved from https://www.nda.agric.za/docs/Brochures/prodGuideDryBeans.pdf
- Elfant, M., Bryant, L., & Starcher, B. (1985). Isolation and characterization of a proteinase inhibitor from Marama beans. *Proceedings of the Society for Experimental Biology and Medicine*, 180, 329–333. https://doi.org/10.3181/00379727-180-42184
- Faria, M., Mabaya, E., & Jordaan, D. (2011). Markets for marama beans in southern Africa: Linking sustainable products with sustainable livelihoods. *Development Southern Africa*, 28(4), 477–492. https://doi.org/10.1080/0376835X.2011.605566
- Fernie, A. R., & Yan, J. (2019). De novo domestication: An alternative route toward new crops for the future. *Molecular Plant*, *12*(5), 615–631. https://doi.org/10.1016/j.molp.2019.03.016
- Gulzar, M., & Minnaar, A. (2017). Underutilized protein resources from african legumes. In S. R. Nadathur, J. P. D. Wanasundara, & L. Scanlin (Eds.), Sustainable protein sources chapter 12 (pp. 197–208). Academic Press. https://doi.org/10.1016/B978-0-12-802778-3.00012-3

- Hartley, M. L., Tshamekeng, E., & Thomas, S. M. (2002). Functional heterostyly in Tylosema esculentum (Caesalpinioideae). *Annals of Botany*, *89*(1), 67–76. https://doi.org/10.1093/aob/mcf006
- Horn, L. N., & Shimelis, H. (2020). Production constraints and breeding approaches for cowpea improvement for drought prone agro-ecologies in sub-Saharan Africa. *Annals of Agricultural Sciences*, 65, 83–91. https://doi.org/10.1016/j.aoas.2020.03.002
- Hu, M., Lv, S., Wu, W., Fu, Y., Liu, F., Wang, B., Li, W., Gu, P., Cai, H., Sun, C., & Zhu, Z. (2018). The domestication of plant architecture in African rice. *The Plant Journal: For Cell and Molecular Biology*, *94*(4), 661–669. https://doi.org/10.1111/tpj.13887
- Hunter, D., Borelli, T., Beltrame, D. M. O., Oliveira, C. N. S.,
 Coradin, L., Wasike, V. W., Wasilwa, L., Mwai, J., Manjella,
 A., Samarasinghe, G. W. L., Madhujith, T., Nadeeshani, H. V.
 H., Tan, A., Ay, S. T., Güzelsoy, N., Lauridsen, N., Gee, E., &
 Tartanac, F. (2019). The potential of neglected and underutilized species for improving diets and nutrition. *Planta*, 250(3), 709–729. https://doi.org/10.1007/s00425-019-03169-4
- Jackson, J. C. (2017). Technology and nutrition opportunities for healthful foods from Morama beans, an emerging crop in Botswana. In G. V. Barbosa-Cánovas, G. María Pastore, K. Candoğan, I. G. Medina Meza, S. C. da Silva Lannes, K. Buckle, & R. Y. Yada (Eds.), Global food security and wellness, Chapter 7 (pp. 125–140). Springer Science + Business Media New York. https://doi.org/10.1007/978-1-4939-6496-3_7
- Jang, G. J. Y. H. (2019). CRISPR/Cas-mediated genome editing for crop improvement: Current applications and future prospects. *Plant Biotechnology Reports*, 13, 10.
- Jideani, V., Wyk, J., & Cruywagen, M. (2009). Physical properties of Tylosemia esculentum and the effect of roasting on the functional properties of its flour. *African Journal of Agricultural Research*, 4, 1208–1219.
- Keegan, A. B. V. J. (1981). Marama bean, Tylosema-Esculentum, a plant worthy of cultivation. South African Journal of Science, 77, 387.
- Khlestkina, E. K., & Shumny, V. K. (2016). Prospects for application of breakthrough technologies in breeding: The CRISPR/Cas9 system for plant genome editing. *Genetika*, 52(7), 774–787.
- Kim, Y., & Cullis, C. (2017). A novel inversion in the chloroplast genome of marama (Tylosema esculentum). *Journal* of *Experimental Botany*, 68(8), 2065–2072. https://doi. org/10.1093/jxb/erw500
- Li, J., & Cullis, C. (2021). The multipartite mitochondrial genome of Marama (Tylosema esculentum). *Frontiers in Plant Science*, 12(2864), 787443. https://doi.org/10.3389/fpls.2021.787443
- Lucaciu, R., Pelikan, C., Gerner, S. M., Zioutis, C., Kostlbacher, S., Marx, H., Herbold, C. W., Schmidt, H., & Rattei, T. (2019). A bioinformatics guide to plant microbiome analysis. *Frontiers in Plant Science*, 10, 1313. https://doi.org/10.3389/fpls.2019.01313
- Maphosa, Y. & Jideani, V. (2017). The role of legumes in human nutrition. Functional food improve health through adequate food, ed. Hueda M. IntechOpen. https://doi.org/10.5772/intechopen.69127
- Maruatona, G. N., Duodu, K. G., & Minnaar, A. (2010). Physicochemical, nutritional and functional properties of marama bean flour. *Food Chemistry*, 121(2), 400–405. https://doi.org/10.1016/j.foodchem.2009.12.054
- National Research Council. (1979). *Tropical legumes: Resources* for the Future. The National Academies Press. https://doi.org/10.17226/19836

- Nepolo, E., Takundwa, M., Chimwamurombe, P., Cullis, C., & Kunert, K. (2009). A review of geographical distribution of marama bean [Tylosema esculentum (Burchell) Schreiber] and genetic diversity in the Namibian germplasm. *African Journal of Biotechnology*, 8(10), 2088–2093.
- Oliveira, C., Valois, M. V., Ottaiano, T. F., Miranda, A., Hansen, D., Sampaio, M. U., Oliva, M. L. V., Abreu, M., & Humberto, F. (2021). The recombinant plant Bauhinia bauhinioides elastase inhibitor reduces rat thrombus without alterations in hemostatic paramers. *Scientific Reports*, 11, 13475. https://doi.org/10.1038/s41598-021-92745-4
- Omotayo, A. O., & Aremu, A. O. (2021). Marama bean [*Tylosema esculentum* (Burch.) a. Schreib.]: An indigenous plant with potential for food, nutrition, and economic sustainability. *Food & Function*, 12, 2389–2403.
- Porch, T. G., Beaver, J. S., Debouck, D. G., Jackson, S. A., Kelly, J. D., & Dempewolf, H. (2013). Use of wild relatives and closely related species to adapt common bean to climate change. *Agronomy*, *3*(2), 433–461.
- Powell, A. M. (1987). Marama bean (Tylosema esculentum, Fabaceae) seed crop in Texas. *Economic Botany*, 41(2), 216–220. https://doi.org/10.1007/BF02858969
- Sandhu, J. S., Gupta, S. K., Singh, I., Gill, B. S., & Bhardwaj, R. (2010). Genetics of bushy growth habit and its implications in chickpea improvement. *Indian Journal of Genetics and Plant Breeding*, 70. 3.
- Schlechter, D. (2019). Namibian farmers introduced to new cowpea and sorghum crop varieties New Era Live. Retrieved from https://www.smartfood.org/project/namibian-farmers-introduced-to-new-cowpea-and-sorghum-crop-varieties/
- Smýkal, P., Nelson, M. N., Berger, J. D., & Von Wettberg, E. J. B. (2018). The impact of genetic changes during crop domestication. *Agronomy*, 8(7), 119.
- Tadele, Z., & Assefa, K. (2012). Increasing food production in Africa by boosting the productivity of understudied crops. *Agronomy*, *2*, 240–283. https://doi.org/10.3390/agronomy2040240

- Takundwa, M. (2014). Domestication in Marama bean (Tylosema Esculentum): Agronomy, phenotypic and molecular characterization for its improvement.
- Takundwa, M., Chimwamurombe, P., Kunert, K., & Cullis, C. (2010). Isolation and characterization of microsatellite repeats in Marama bean (Tylosema esculentum). *African Journal of Agricultural Research*, 5(7), 561–566.
- Takundwa, M. M., Ruzvidzo, O., Nambahu, F., Kawadza, D. T., Chatukuta, P., & Chimwamurombe, P. M. (2015). Characterization of gamma-irradiated seeds of a wild Namibian marama bean (Tylosema esculentum) with microsatellite markers. *International Journal for Biotechnology and Molecular Biology Research*, 67(7), 10. https://doi.org/10.5897/IJBMBR2014.0207
- USAID 2012. Climate Change Adaptation in Southern Africa.

 Retrieved from https://www.climatelinks.org/sites/default/
 files/asset/document/Southern%20Africa%20Climate%20Inf
 o%20Fact%20Sheet_FINAL.pdf
- Vidigal, P. R., Romeiras, M. & Monteiro, F. (2019). Crops diversification and the role of orphan legumes to improve the sub-Saharan Africa farming systems. doi:https://doi.org/10.5772/intechopen.88076
- Zhang, C., Yang, Z., Tang, D., Zhu, Y., Wang, P., Li, D., Zhu, G., Xiong, X., Shang, Y., Li, C., & Huang, S. (2021). Genome design of hybrid potato. *Cell*, *184*(15), 3873–3883 e3812. https://doi.org/10.1016/j.cell.2021.06.006

How to cite this article: Cullis, C.,

Chimwamurombe, P., Kunert, K., & Vorster, J. (2023). Perspective on the present state and future usefulness of marama bean (*Tylosema esculentum*). *Food and Energy Security*, *12*, e422. https://doi.org/10.1002/fes3.422