

Review

Utilization of Plant Genetic Resources of Bambara Groundnut Conserved Ex Situ and Genetic Diversification of Its Primary Genepool for Semi-Arid Production

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Abstract: Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a nutritious and drought-tolerant crop that is native to Africa. Currently, it possesses a cosmopolitan distribution across the semi-arid agro-ecologies of Africa and its cultivation has extended to other regions, particularly in the drier parts of Asia and Indonesia. Due to historic neglect, research on the crop and policy prioritization in national breeding programs is lagging; hence, varietal development is in its infancy. Farmers rely on traditional landraces, which are characterized by low productivity and yield. Breeding for moisture stress adaptation and high yield is crucial to improve its productivity and adequate genetic diversity is desired to strengthen resiliency for climate adaptation and nutritional and food security. Nevertheless, thousands of plant genetic resources (PGRs) conserved in several genebanks are still not being fully utilized to reintroduce lost diversity into farmers' fields and pre-breeding activities. The exploitation of ex situ conserved PGRs has been minimal due to a lack of extensive genetic characterization. Thus, to harness utilization of the PGRs in genetic improvement programs, holistic strategies including the traditional genomic resources and participatory-led trait discoveries are needed to bridge the gap between conservation and utilization of ex situ germplasm. This review focuses on the PGRs of bambara, methods to widen genetic diversity, genomic resources, and high-throughput phenotyping to enhance the utilization of genetic resources in pre-breeding programs, and approaches to identify useful adaptive features for yield and drought.

Keywords: pre-breeding; genetic variation; plant introductions; bambara groundnut; plant genetic resources; genomics; core sets; focused subsets; drought adaptation



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1. Introduction

The global population is expected to reach 9.7 billion by 2050 <https://www.worldometers.info/world-population/> accessed on 27 December 2021; hence, there is a need to increase agriculture production for food security. Although more food will be required to be produced, competing interests, such as rapid urbanization, prompt the need for sustainable intensification of agriculture on land that is available [1]. To date, global food systems have managed to reduce hunger by increasing the crop yield of a few crops but have not adequately addressed malnutrition as evidenced by the rise of non-communicable diseases and all forms of malnutrition [2]. The situation has been exacerbated by the lack

of a diversified agricultural portfolio that can boost production systems and enable the diversification of crops [3]. In production systems, agriculture is dominated by relatively few major crops (maize, wheat, soybean, and rice), which contribute to unhealthy diets, threaten agrobiodiversity, offset the ecological balance, and predispose farming systems to climate shocks [4]. Nevertheless, several crops, mostly the wild relatives of cereals, roots, pulses, and fruits, despite their adaptive resiliency, nutritional qualities, and low input requirements, are underutilized and often receive less priority in research [5]. This translates to low dietary diversification, especially among the poor populations in the developing world, where diets are more energy-dense than nutrient-dense, with few fresh fruits, legumes, nuts, fats, and oil to constitute a balanced diet [6].

In addition, global climate dynamics have revealed that dryland areas are expanding rapidly in semi-arid regions and occupy more than 50% of the total area under dryland expansion [7]. Meanwhile, studies have indicated that in the lower altitudes, climate change has resulted in reduced yields of maize and wheat whilst in the higher altitudes, the yield has increased [8]. It then becomes imperative to consider what can be grown and where to ensure an adequate food supply and sustainable diets and at the same time build resiliency to climate change. Bambara groundnut is a warm-season annual diploid ($2n = 2x = 22$) orphan crop, which has the potential to contribute to climate and nutritional resiliency amid the ongoing climate change and unsustainable food consumption patterns [3].

Bambara groundnut is native to Africa, and is currently grown in most drier parts of sub-Saharan Africa (SSA) and other continents (Asia, Indonesia, South America), where it was recently introduced for climate change resiliency and dietary diversification [9,10]. The ability of the crop to grow in harsh conditions makes bambara an important legume crop in semi-arid Africa, where it ranks third after groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*). Notwithstanding, the extension of cultivation of bambara becomes important to elevate the status of bambara from an underutilized crop and consolidate its global importance.

Seeds are the economic part of importance for bambara and contain 49–63.5% carbohydrate, 15–25% protein, 4.5–7.4% fat, 5.2–6.4% fiber, 3.2–4.4% ash, and 2% mineral [11]. The seeds can be eaten as boiled immature pods or as dried grain that is normally consumed during the off-season whilst the nitrogen- and phosphorous-rich stalks acquired after harvesting can be fed to livestock [12]. This makes bambara a strategic crop for household food security among the rural community. In value addition, dried seeds are an important ingredient in the production of flour and plant-based milk [13]. To promote its incorporation into mainstream diets, many recipes have been developed through the Crops for the Future (<http://cropsforthefutureuk.org/> accessed on 7 January 2022), including biscotti, noodles, and various snacks [14]. Despite its potential, bambara is produced as a secondary crop grown under subsistence farming, mainly by women [15], and often with no inputs and as traditional landraces. Thus, coupled with efforts to boost the production of bambara, there is a need to strengthen genetic resources conservation and utilization for varietal development to support seed systems, value chain development, and strengthen agrobiodiversity.

Research on the genetic improvement of bambara groundnut is lagging and improved varieties are inexistent in the market, which places considerable limits on its productivity. It has been established that formal seed distribution systems, farmers' selections, germplasm conservation, and utilization are not well supported in many national programs [16]. The role of subsistence farmers as the major custodians of preserving its genetic diversity on their farms has been acknowledged. However, placing more dependency in the hands of farmers (without adequate financing, political will, and infrastructure) to safeguard against diversity loss of bambara groundnut predisposes the crop germplasm resources to genetic erosion through preferential cultivation of landraces in farming systems [17]. In this case, innovative approaches, such as community seed banks complemented by seed fairs, become imperative to preserve genetic diversity and enable seed exchange by the local farmers. In the same manner, international, regional, and national genebanks, as the

formally established sources of PGRs, play a crucial role in the collection, preservation, and distribution of genetic materials that are vital to the creation and expansion of variability in breeding programs [18]. This necessitates the need for stronger linkages between genebanks and breeding programs to bridge the gap between conservation and plant breeding.

Plant genetic resources (PGRs) are the basis of plant breeding and food, and to meet the current and future food demand, they should be properly conserved and utilized [19]. Thousands of crop accessions are conserved *ex situ*; however, for many crop species, including bambara, the utilization of the conserved materials for crop improvement purposes has been minimal due to a lack of robust characterization of the storage and the bulkiness of the samples [19,20]. This review outlines the plant genetic resources of bambara conserved in genebanks, genetic diversity and strategies to facilitate the use of the PGR in pre-breeding programs, methods to enrich genetic diversity in bambara pre-breeding, and breeding for drought adaptation.

2. Centers of Origin of Plant Genetic Resources of Bambara Groundnut

West Africa, between northern Nigeria and Cameroon, is indicated as the bambara groundnut center of origin as supported by the discovery of wild bambara varieties, which are confined to the area stretching from Nigeria to Sudan [16]. The cultivated forms are distributed throughout the tropics mainly in SSA, with Cameroon believed to be a center of diversity [21,22]. It is also believed that two separate domestication events occurred in western, and southern and eastern Africa, resulting in a major and minor center of diversity, respectively [23]. Inferences on the IITAs collection through passport data of its germplasm also reveal the existence of two subpopulations (Figure 1). Subpopulation I consists of West and Central African accessions, whilst subpopulation II consists of the Southern and Eastern African accessions, including Madagascar. These two major subgroups have also been revealed in population structure studies based on simple sequence repeat (SSR) and diversity array technology (DArT) markers [24]. Considering the prevailing climate change impacts, this genetic disconnection of the germplasm is advantageous given the contrasting (humid or dry) environments where bambara is grown, to identify germplasm resources for adaptation to prevailing and future climate conditions.

In bambara groundnut, 6145 PGRs (Table 1) are conserved at various international, regional, and national genebanks [9]. Whilst in farming systems, the bambara groundnut landraces cultivated are often a mixture of various genotypes, in genebanks, the conserved PGRs are seeds that were purified through a single plant approach from populations of landraces, wild relatives, and improved varieties that were collected from various centers of domestication farmers' planting fields. The PGRs act as a duplicate collection of PGRs stored in national genebanks to safeguard genetic resources from erosion and natural disasters, such as floods, drought, fire, etc., for immediate and future use [25]. The subsequent access, utilization, and exchange of conserved PGRs for commercial and non-commercial purposes are facilitated through the provisions of the International Plant Treaty and UN Convention on Biological Diversity (or Biodiversity Convention) and national regulations, which are regulated through the standard material transfer agreement (SMTA) [26].

The International Institute of Tropical Agriculture Genetic Resource Conservation (IITA-GRC) genebank in Nigeria's germplasm collection conserves the highest number (2186) of bambara accessions from 25 countries (<https://my.iita.org/accession2/collection.jsp?id=8> accessed on 27 December 2021). Due to the diverse geographical representation of the collection places of origin of the conserved PGRs, higher genetic diversity is, therefore, expected to be harbored in that collection than in national germplasm collections [27]. The ORSTOM in France is the holder of the second biggest collection of bambara groundnut, and the accessions were collected from Cameroon [28]. On the other hand, it is difficult to accurately ascertain the size of PGRs for bambara conserved in national genebanks due to the underrepresentation of accessions in the collections. About six national genebanks (Republic of South Africa, Botswana, Kenya, Ethiopia, South Sudan, and Côte d'Ivoire stated in Majola et al. [29]) report the number of accessions of less than ten accessions.

Further, across the major crops (including maize, wheat, common bean) that are conserved in international and regional genebanks, the collection of wild relatives is relatively low [26]. Similarly, this challenge has also been reported in ex situ collections of bambara, with wild relatives accounting for only 1% of the collection at IITA [9]. To reduce this collection gap, the IITA-GRC has embarked on exploration activities to collect additional diversity in Cameroon [30].

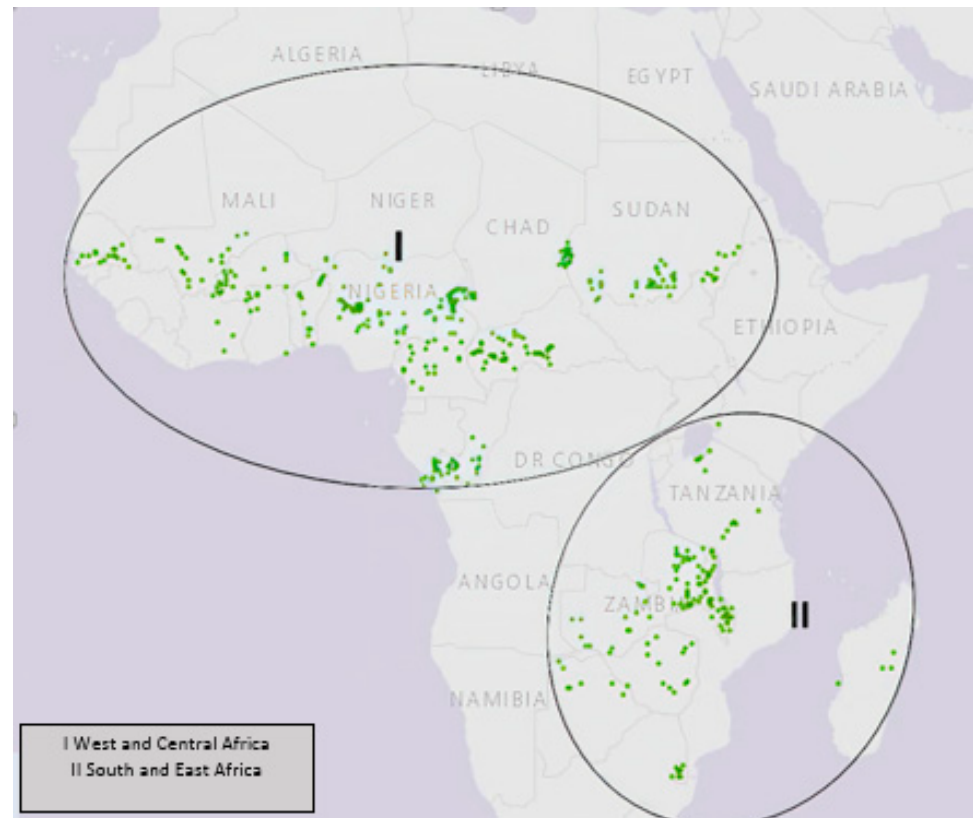


Figure 1. Centers of domestication of bambara groundnut accessions in IITA-GRC. Source: <https://www.genesys-pgr.org/> accessed on 3 January 2022.

Table 1. Plant genetic resources of bambara groundnut held in various genebanks.

	Institution Name	Accession No.	% of Total	Landrace	PGR Type % Wild Type	Unknown
1	IITA-GRC—Nigeria	2031	33	100	<1	*
2	ORSTOM—France	1416	23	100	*	*
3	Department of Agriculture	338	6	2	*	98
4	Plant Genetic Resources Research Institute—Ghana	296	5	*	*	100
5	National Plant Genetic Resource Centre—Tanzania	283	5	81	<1	18
6	National Plant Genetic Resource Centre—Zambia	232	4	100	*	*
7	Unspecified *	1549	25	59	1	40
	Total	6145	100			

* Denotes unspecified type of PGR and country. ORSTOM: Office de la Recherche Scientifique et Technique (now named the Institut de Recherche pour le Développement). Source [9].

3. Traits of Importance in Bambara Groundnut Improvement

Bambara groundnut is a leguminous crop, which belongs to the family Fabacea and genus *Vigna*. Two botanical varieties are recognized in bambara: *V. subterranea* var. *subterranea* (cultivated varieties) and *V. subterranea* var. *spontanea* (wild varieties) [21]. The

genetic diversity in bambara has been reviewed, and moderate to high variability levels based on agronomic and morphological descriptors, which allows selections for various traits, were reported [31]. A myriad of agronomic traits of importance in bambara has been identified, such as nutrition, yield potential, photoperiod, drought tolerance, and adaptation to marginalized soil conditions [32]. Therefore, it will be prudent to capitalize on the genetic variability present in the accessions, identify superior parents, mate them to widen genetic variability, and synchronize traits to constitute some elite cultivars.

Characteristically, legumes are self-pollinating crops that have a narrow genetic base [33]. Breeders' use of few donor lines in breeding programs can narrow a crop's genetic base, but in the case of bambara, the narrow genetic base can be exempted from these practices as currently, no improved varieties have been released for the market [34]. Since the genetic diversity at the genomic level of bambara is narrow [31], there is a need to widen the variation using conventional and biotechnological approaches.

The widely cultivated varieties of bambara groundnut have been identified through selective breeding for yield, adaptation to farmers' local growing conditions, and the preferences of the socio-linguistic groups among other traits. Studies of national plant genetic collections and genebank collections revealed considerable variability in seed yield, which can facilitate selections for further breeding activities [34,35]. The production of bambara in SSA takes place across diverse agro-climatic zones, including tropical rain forests and dry savanna [36]. However, various abiotic and biotic constraints limit its yield and productivity. Although the constraints have been well documented in several studies, the major challenges tend to emanate from limited progress on financing, technical, institutional, and policy reforms to enhance the production of bambara. As a result, the production and productivity of bambara have remained low over the years, and on average, yields of about 0.85 t/ha are obtained [16] from a meager planting area of about 370,953 ha, with total production quantities of 228,920 tons in 2019 (<https://www.fao.org/faostat/en/#data/QCL> accessed on 12 December 2021). Nevertheless, substantial differences between actual and potential yield ranging between 0.3 and 3.0 t/ha, respectively, have been recorded for different genotypes and in different environments [16]. This is partly due to its photoperiod sensitivity, which influences pod setting and often results in some best-performing high-yielding accessions from one locality to yield poorly in another [37]. Therefore, closing this yield gap offers prospects to increase production and contribute to greater utilization of genetic resources by farmers and scientists.

Nutritionally, bambara seeds are dense and contain high levels of carbohydrate and substantial levels of protein as mentioned earlier. The high level of essential amino acids (e.g., methionine, lysine, etc.) and richness in fiber, calcium, iron, and potassium make the bambara seed a "complete seed", which can complement the large cereal-based staples [12]. However, the presence of anti-nutritional factors, such as condensed tannins, phytic acid phosphate, polyphenol, and trypsin inhibitor, in bambara and other legumes has been noted [38]. Previous reports suggest that dark-colored seeds, especially the red-seeded type, contain higher iron and tannin levels than the light-colored or cream type [39]. Methods to reduce anti-nutritional factor levels, which include roasting, soaking, boiling, etc., exist [12] but are effective to a limited extent. In Africa, malnutrition is rampant, and micronutrient interventions are urgently required. Therefore, research aimed at leveraging the nutritional properties of bambara while improving food safety is required.

Bambara is mainly grown in poor soils with low fertility by resource-poor farmers who lack adequate inputs and irrigation. The low water footprint, ability to grow under marginalized soil conditions with no inputs, and nitrogen fixation ability has led to renewed interests by various stakeholders to promote bambara groundnut production [31]. Bambara is produced either in a pure stand or in inter-cropping systems, of which the latter allows subsistence farmers to maximize land use and improve soil fertility as a result of its symbiotic nitrogen fixation [40]. In as much as the crop is drought tolerant, the landraces have varied reactions to water stress [39]; hence, the stability of PGRs in different

environments is highly crucial and genotypes that produce high yields under water deficit conditions are ideal for ensuring that farmers' achieve better yields and food security.

Bambara is a crop of social, economic, and cultural importance in most parts of Africa, where it contributes to household food security, income generation, and strengthening of community values. To enhance its production and productivity in farming systems, it becomes crucial that farmers' indigenous knowledge of adaptive traits and preferred end-use traits are considered in crop improvement. To date, farmers' needs assessments have been widely conducted through participatory approaches to identify the production, variety preferences, and bottlenecks to bambara production [36]. The findings indicate the choice of an ideotype should consider a high yield (prolific, stable, big seed size) and seed color and the most preferred are cream types, drought tolerance, nutrition [13,16], and cooking time [10]. Therefore, when designing genetic improvement programs of such crops, a participatory plant breeding framework should ideally be integrated into the variety development strategy to enhance the chances that target end-users' preferences and breeders' strategic traits inclusively guide the selections.

4. Strategies for Utilization of Ex Situ Germplasm in Pre-Breeding Programs

Genebanks conserve thousands of accessions of crop species for various stakeholders, including breeders' utilization of the resources for the improvement of particular traits. The utilization of the PGRs depends on the identification of promising accessions based on the characterization and evaluation of quantitative and qualitative agronomic attributes, and molecular and biochemical profiles. However, due to the bulkiness of collections and the subsequent phenotyping time and cost implications, the direct utilization of PGRs by plant breeders in pre-breeding has been minimal [41]. The bulkiness of samples poses direct challenges to genebanks' efficiency in regeneration, characterization, and decision-making of what genetic material to provide to user requests. To promote the use of conserved PGRs, strategies are required to enhance the utilization of conserved PGRs.

4.1. A Core Collection or Core Set Approach

The concept of the core set was initially proposed by Frankel [42] and refers to a reduced subset (a proportion of about 10%), which possesses the maximum representativeness of the overall diversity of a germplasm collection of crop species. In germplasm management, the core collection presents a straightforward approach that involves applying sampling techniques when determining the diversity within species to reduce the entire collection to the desired subset of the germplasm entry number. Genetic diversity is a crucial pre-requirement underpinning conservation, protection, and utilization of biodiversity for crop improvement programs. Information derived from the descriptor sets for morphological and agronomic characters, biochemical and sequence data from molecular markers, and other enabling tools (passport data, agro-climatic data, and algorithms) [43,44] is crucial to the assembly of core sets and focused identification of germplasm strategy (FIGS) implementation. Software tools, e.g., PowerCore, Core Hunter 3, etc., can be utilized to sample diverse and representative subsets from large germplasm collections with minimal redundancy [45,46]. Consequently, the core collection method has been adopted in most major and minor crops to improve efficiency in the management and handling of germplasm resources. Core sets are useful in facilitating studies in systemic and evolutionary biology, identification of breeding populations, and germplasm for introduction into new environments. Figure 2 summarizes the plant genetic resources, proposed core collections and their variations, strategies for characterizing PGRs and enabling tools to develop core sets, and possible applications and benefits derived from core sets.

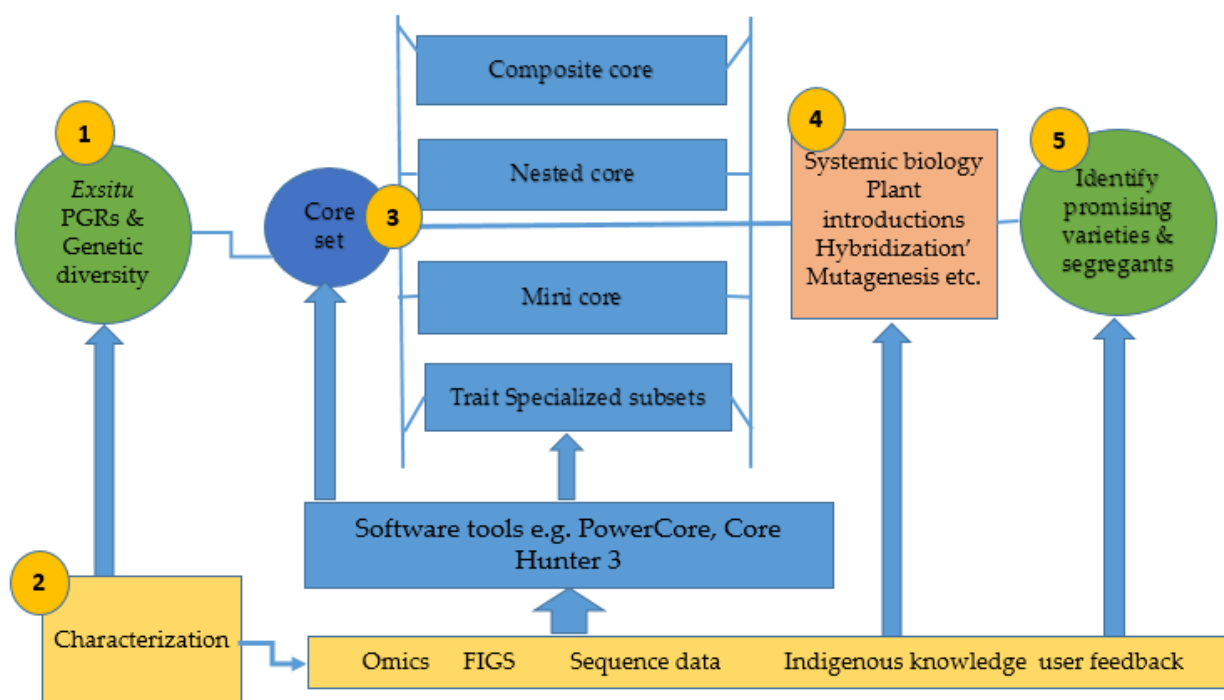


Figure 2. 1: Bambara plant genetic resources; 2: characterization approaches to establish genetic diversity and software tools to develop core sets; 3: strategies for mining PGRs; 4: deployment of core sets in various applications; and 5: target end products for the variety improvement process. Source: author.

Although core sets are reduced germplasm accessions, some crops contain relatively large numbers of conserved PGRs, leading to core collections that contain high numbers of accessions, which can be difficult to manage. In this regard, a mini core set, which represents about 1% of the core set size, can be developed as a subset of the core collection [47]. Due to its size, the mini collection can be assembled for trait-specific subsets, e.g., large seed size, disease resistance, or nutritional composition, which reduces the time required for screening in pre-breeding programs targeting a specific breeding objective. Further, mini collections allow multi-environment testing of genotypes. However, developing individual subsets for various traits can be expensive to maintain. Henceforth, the nesting of samples with different attributes in a single core set can be an ideal way to ensure maximum genetic representativeness of the original predefined core set panels [44]. Alternatively, the core and mini collections that were developed through stand-alone approaches using phenotypic traits, molecular markers, and eco-geographic data can be merged to constitute a composite core set. Combining the entries from individual core sets, which were identified from phenotypic or molecular diversity estimates, can enhance the chances of capturing maximum genetic diversity of a species in a composite core set as compared to when individual approaches are deployed [48].

In the legumes breeding programs of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), about 90 core sets were developed and several agronomic traits were identified [49]. In bambara, where trait improvement activities are in their infancy, trait-specific core sets have not been adequately developed and few reports are available (Table 2). With limited funding investments in orphan crops, ideally, efforts will be required to develop working collections for wider adaptation to ensure researchers can exchange and utilize the identified germplasm across various and/or similar agro-ecologies.

Table 2. Some of the developed core sets of bambara groundnut.

	Initial Accession No.	Core Subset Accession No.	Traits	Marker/Breeding System	References
1	223	24	Not indicated	AFLP	
2	123	24	Not indicated	SSR	[24]
3	Not indicated	12	Plant architecture, drought, photoperiod	SSD	[50]

AFLP: amplified fragment length polymorphism; SSR: simple sequence repeats; SSD: single plant descent.

4.2. Focused Identification of Germplasm Strategy

The focused identification of germplasm strategy (FIGS) is one of the recent strategies proposed to facilitate the use of conserved PGRs in pre-breeding programs, especially for adaptive features [43]. To facilitate its implementation, the strategy relies on passport data, which is usually captured during germplasm collection missions detailing the accession's collection sites. Nevertheless, the correct geolocation data should be complemented by phenotypic trait data to unveil the desired genetic materials, thereby indicating the importance of adequate characterization of PGRs.

The FIGS approach is built around the concept of linking trait data with climatic data, with an assumption that traits are associated with the prevailing agro-climatic variables in which they are found [20]. Therefore, it is suggested that by tracing the origins of the accessions and matching their adaptive features with the environmental conditions that shaped the trait, it would then be possible to unlock its genomic footprints using machine learning predictions [51]. To harness the use of PGRs in crop improvement and production, the FIGS approach can lead to introductions of germplasm resources in new environments and unlocking of adaptive traits for biotic and abiotic stresses, for instance, pest, disease, drought, and cold tolerance [51]. In lentils, this approach successfully led to the development of a subset of lentils for the anticipated "wetter" and "drier" regions of its production due to the influence of climate change [43]. In bambara, there are no reports available to date on the use of the FIGS to develop specific subsets.

4.3. Genomic-Enabled Conservation and Utilization of PGRs

Molecular markers are now routinely used in breeding programs of major and some minor crops for genomic analysis. These include PCR and array-based markers that enable detection of genetic diversity at either the genotype or gene level, which is not detected through traditional methods alone. The genomic information is important to establishing genetic diversity and can be used for predicting gene to phenotype associations and facilitating marker-assisted selection [52]. Marker-assisted selection is crucial when plant breeders intend to make an indirect selection of traits based on a marker that is tightly linked to the agronomic trait of interest [53]. Ideally, useful markers for marker-assisted breeding (MAB) should be polymorphic, reproducible, co-dominant, and cheap [54]. Bambara accessions have been genotyped using several markers, such as AFLP, restriction fragment length polymorphism (RFLP), SSR, inter sequence repeats (ISSRs), and random amplified polymorphic DNA (RAPD) [31]. However, the high cost and low reproducibility of the markers impact negatively on their integration in breeding programs. Nevertheless, over the last decades, advances in next-generation sequencing (NGS) platforms, such as genotyping by sequencing (GBS), have facilitated genome-wide single nucleotide polymorphism (SNP) markers' discovery in genomic-assisted breeding for crop improvement.

Genotyping by sequencing is an NGS technique used for SNP markers' discovery and screening in plant genomes, with and without an existing reference genome [53]. The DArTseq platform based on Illumina NGS generates two variants of markers, i.e., the dominant silicoDArT and co-dominant SNP markers [55]. Therefore, at present, in plant breeding programs, SNP markers are more preferable due to their high genomic abundance, amenability to high-throughput analysis with a relatively low genotyping error rate, and capability of simultaneously identifying SNPs and genotyping [56]. The GBS technique is

deemed a novel approach to germplasm characterization since it allows a large number of accessions to be genotyped in a short time at a relatively cheaper cost [57]. Further, SNP marker applications in germplasm conservation and utilization in pre-breeding programs are versatile and include genetic diversity and population structure analysis, genetic purity and identity analysis, linkage and quantitative trait loci mapping, allele mining, marker-assisted recurrent selection, and genomic selection [54]. The IITA-GRC, which houses the largest collection of bambara accessions, and Crops for the Future, UK adopted the GBS approach to fingerprint their collection and identify duplicates in the germplasm collections [16].

Besides marker deployment for genetic diversity analysis and fingerprinting, the use of markers in bambara have been extended to applications that include the construction of genetic maps (Table 3).

Table 3. Selected marker systems and applications in bambara groundnut.

Population	Marker	Application	References
Bi-parental (VSSP11 × Dip C)	67 AFLPs and 1 SSR marker	Construct first linkage map of 516 cM and 20 linkage groups	Basu et al. (2007)
Bi-parental population	marker bgPabg-596774	MAS for yield-related components	Ahmad et al. (2011)
-	SSR and DArT	Genetic diversity and linkage map construction.	Khan et al. (2016)
Bi-parental F5 (DipC × Tiga Nicuru)	165 gene expression markers	Map construction (920.3 cM) for domestication traits	Chai (2014)
F2 (IITA686 × Ankpa4)	SSR and DArT		Ho et al. (2017)
F3 (Tiga Necaru × DipC)	SSR and DArT		Khan et al. (2016)

To enable MAS, the construction of genetic maps with ample markers is crucial to identify the genetic regions or quantitative trait loci for important agronomic traits. Few maps exist in bambara; however, Ho et al. [58] proposed that in the absence of a complete bambara reference genome, the common bean, adzuki bean, or mung bean genomes can be temporarily adopted as “pseudo” maps for bambara. The linkage-based QTL mapping and association mapping approaches are the two methods commonly used to determine the genomic regions controlling the targeted traits. So far, QTL studies reported in bambara involve bi-parental populations as indicated in Table 3. However, challenges associated with quantitative trait loci markers from bi-parental populations often arise due to a lack of expression or instability in different cultivar backgrounds, which presents a limitation in the application of the markers in MAB [59]. As an alternative, and facilitated by high-throughput NGS, various populations besides bi-parental lines can be deployed to dissect the genetic mechanisms underlying complex governing biotic, abiotic, and quality traits. For instance, the genome-wide association study (GWAS) approach utilizes historical recombination events in naturally occurring germplasm panels to identify QTLs and validation candidate genes regulating the quantitative traits [60].

The draft reference genome of bambara groundnut is available in the National Center for Biotechnology Information (NCBI) database and in this assembly, 535.05 Mb was assembled into scaffolds and 31,707 protein-coding genes were predicted [61]. With developments in genomic resources and efforts in developing a complete reference genome and increased phenotyping, it can be anticipated that GWAS, genomic selection (GS), and identification of candidate genes can be implemented [14]. As a drawback, segregating populations of bambara are still few, which limits full exploitation of genomic-enabled applications, especially the validation of developed markers. Therefore, there is a need for several divergent populations for various traits and to facilitate variety development [16]. Nevertheless, genomic information remains relevant for other applications, which are less dependent on the phenotypic information and population structure of populations, for instance, environment association analysis (EAA). Unlike in GWAS, EAA, also termed genome environment association, requires georeferenced genetic materials to model associations between genetic

sequences and environmental attributes to perform marker–trait associations and identify candidate genes conferring local adaptation to stresses [62]. The EAA can help to identify functional polymorphisms and genetic variants associated with local adaptation to diverse climatic and edaphic factors [63]. Therefore, it offers an opportunity to mine, select, and utilize genetic resources adapted to abiotic and biotic stresses, especially to build resiliency to climate change.

4.4. Information Management and User Feedback

Documentation of PGR collections helps to ensure that vital information (e.g., passport data, stable indicators, and phenotypic data, etc.) is safeguarded against loss by human personnel [52]. This information is important as it provides a basis for decision-making on conservation and user requests for germplasm material. Therefore various information management systems, from sophisticated databases to simple MS excel and MS pro lists, are utilized for storing information and facilitating public access to accessions' information. Further, as part of the standard material transfer agreement (SMTA), requestors are urged to submit information from the characterization or evaluation of the accessions requested. This information is essential to strengthening knowledge of traits and performance in multi environments, and promoting collaborations amongst researchers. In turn, this strategy can lead to increased use of PGRs as information is made readily available. However, as observed, minimal feedback is often received from the requestors, making it difficult to determine the stability of accessions across environments [51].

Although several genebanks can handle accessions of the same crop, an additional challenge to accessing information in accessions has been directly attributed to institutions' standalone databases [51]. In the case of bambara, accession information can be publicly accessed through the GENESYS platform (<https://www.genesys-pgr.org/c/bambara> accessed on 3 January 2022), whilst several national genebanks also contain accessions, but some databases tend to be less coordinated. To avert this challenge, there are current propositions to link the databases of genebanks [25], for better information management and utilization conservation of PGRs.

5. Widening the Genetic Variability of the Bambara Groundnut Primary Gene Pool

The cultivated bambara groundnut (*Vigna subterranea*) is a progenitor of *Vigna subterranea* var. *spontanea*, its wild relative. Wild relatives are often more genetically diverse than the cultivated forms, and their use in breeding programs is crucial to broaden the genetic diversity of breeding populations. Some level of compatibility exists between the wild type and cultivated bambara as intraspecific hybridization between a wild type (VSP11) and a domesticated variety (DipC) was performed successfully and paved the way for genetic studies of the domestication traits in bambara [21,64]. Nevertheless, the use of wild relatives in breeding programs is minimal due to the challenges of incompatibility, the introduction of undesirable traits during recombination, and poor agronomic performance [65]. In this regard, pre-breeding efforts to identify traits or genes from unadapted donor parents and introgression into elite germplasm are crucial to improving the productivity of bambara and desirable traits. Pre-breeding activities require time and financial investments; nevertheless, long-term investments by national and private institutions are required for sustainable bambara genetic improvement. The Crops for the Future institute has been spearheading hybridization efforts in bambara and has successfully developed some of the first segregating populations of bambara [14]. In bambara genetic improvement, the segregants represent a positive milestone towards understanding trait inheritance mechanisms, varietal release, and marker validation studies.

Bambara is closely related to cowpea (*Vigna unguiculata*), and although attempts were made to perform an interspecific hybridization among the two legumes, the findings were rather inconclusive [66]. Unlike other legumes, such as soybean, common bean, chickpea, cowpea, peanut, etc., where improved varieties have been bred, the diversity in bambara landraces remains largely untapped as a source of alleles for introgression; hence,

the primary gene pool can harbor unexploited/abundant genes for crop improvement. Notwithstanding, there are no reports of the secondary and tertiary gene pools of bambara. Consequently, the variation and selection processes in the traditional breeding of bambara have been confined to represent its primary gene pool. Domestication of wild bambara (*V. subterranea* var *spontanea*) to the now cultivated forms (*Vigna subterranea* var *subterranea*) resulted in alteration of the plant architecture to a compact or bunch growth habit and reduced leaf sizes and shell thickness whilst selecting for increased stem numbers [21]. In both genomics and evolution, knowledge of domestication traits is important to understand genes that contribute to important agronomical plant-adaptive features and traits.

5.1. Hybridization

Bambara groundnut is a self-pollinating species that is propagated sexually through seeds. Different mechanisms of reproduction between the cultivated and wild varieties of bambara have been reported, of which the cultivated bambara is a highly self-pollinating crop with very low outcrossing rates, whilst the spreading types (wild) can be cross-pollinated by ants [67]. Given the highly cleistogamous nature of the crop, male sterility is important in bambara breeding. Attempts were made in the past to induce male sterility through the application of boric acid to permit pollination by nearby plants using ants but without success due to incorrect concentration rates, which caused premature dropping of the flowers [68].

Classic plant breeding for the development of varieties relies on Mendelian principles and hybridization techniques in the development of true seed [69]. Hybridization was performed to develop crosses by emasculation of the female flower and pollinating its stigma by rubbing pollen from the anther [40]. Unsuccessful crossing of bambara groundnut varieties is usually the result of small flower sizes and the position and fragility of the peduncles [67]. The success rate of crosses in bambara is very low, but further losses in seed numbers from the successive filial generations, e.g., F_1 , F_2 , might occur due to failure to set pods, poor germination, and the photoperiod sensitivity of the progenies [70].

Nevertheless, the development of recombinant inbred lines (bi-parental or magic populations) and segregating populations is crucial for new variety release and the implementation of genetic studies for QTL mapping and marker validation for MAS [71]. Knowledge of the mode of reproduction and inheritance mechanism is essential to direct breeding programs. Preliminary genetic studies on the internode length, growth habit, stem per plant, and seed color in segregating populations suggested the involvement of dominance and codominance inheritance with few genes [21], implying a simple backcrossing strategy can be used for trait introgression. However, the most economically important traits are quantitatively inherited; hence, genotype \times environment (G \times E) reduces the effectiveness of early selection in trait improvement. Therefore, different populations might be required, thereby warranting the need for continuous development of segregating and recombinant populations.

5.2. Mutation Breeding

A mutation is a sudden heritable change in an organism that can be natural, although the nature of mutation is often spontaneous or represents a rare event. In crop improvement programs, mutation breeding is a technique deployed to create genetic variation through gamma radiation, X-ray, ultraviolet rays, and chemical mutagens, etc. targeted on seeds, plant organs, whole plantlets, and pollen to alter traits in an otherwise elite or adapted variety [72]. Mutation breeding through gamma radiation and chemicals has successfully led to the development of 72 groundnut cultivars in highly cleistogamous species, such as groundnut (*Arachis hypogea*) [73]. In bambara, gamma radiation mutagenesis contributed to a broadened variability of yield components, such as plant height, branches per plant, pod yield, and seed weight [74]. In another report, by exploiting ethyl methane sulphonate (EMS) to induce mutations, mutant lines with improved yielding abilities under moisture stress were identified [75]. However, no varieties were released from these populations.

The limitations associated with the trait improvement technique emanate from the need for several cycles of evaluations and observations to detect the mutants, which can prolong the time to variety release.

Besides variety development, there is renewed interest in classical mutagenesis to complement NGS techniques (Targeting-Induced Local Lesions in Genomes experiments (TILLING and EcoTILLING) for reverse genetic screens [76]. TILLING and EcoTILLING are reverse strategies that involve traditional mutagenesis for induced mutant and natural polymorphism discovery [77]. The reverse genetic screening strategy is the opposite of the widely deployed forward genetics approach and employs a candidate gene approach to investigate the change in the structure of the gene and the resultant phenotypic change after mutation induction [78]. EcoTilling is considered to be an efficient and rapid technique for identifying DNA polymorphisms. For instance, in rice, it enabled characterization of the SNPs of a drought candidate gene, of which 31 SNPs were identified to facilitate a genome-wide SNP haplotype, and from an initial 900 genotypes, a subset of 20 drought-tolerant genotypes was assembled [79].

5.3. Plant Introductions as a Breeding Strategy for Variety Development

Plant PGR introductions serve as important genetic resources for enhancing the local genetic variability. The challenge of narrow genetic diversity in grain legumes has been widely established. Consequently, improved varieties of bambara groundnut with optimum and desired agronomic (better pod filling, high yield, photoperiod neutral) traits are unavailable for farmers to propagate [31]. For genetic improvement, PGR introductions are a useful source of novel genes that can be tapped into for varietal development through hybridization and selections [80]. In turn, these introductions, in the form of wild relatives and domesticated forms, can enhance gene flow and contribute to the diversification of the primary gene pool. Therefore, *ex situ* PGRs provide an opportunity to widen the breeding bases of public and private breeding programs. If introductions are directly released as varieties, the selections for promising varieties can be made over few generations, which can offer an economical and quick way to achieve crop improvement. Molosiwa [24] developed a subset of 35 diverse bambara accessions from the IITA-GRC collection for evaluation and identification of potential entries for release in Botswana. Recent reports indicate that outside of Africa, specifically in Indonesia and Asia, introductions of bambara are being evaluated in multi environments, thereby opening prospects for the identification of areas as future centers of its cultivation [9,37]. However, with introductions, there is a need to ensure phytosanitary measures to curb biosecurity threats due to the carryover of pest and disease strains that can be introduced into new areas. Outside of the breeding programs, plant introductions play an important role in biosystematics and understanding the centers of origin and evolution of plant species.

6. Breeding for Drought Adaptation

Bambara is produced under marginalized agro-ecological conditions, where prevailing conditions are drier and rainfall distribution is characterized by short and erratic rain, with both intermittent and terminal drought. The Sahel region is one of Africa's semi-arid environments where bambara groundnut is produced [30]. Similarly to the south of Africa, bambara is produced in the marginalized semi-arid areas of Namibia and Botswana's dry and hot environments. Based on a climatic suitability simulation study by Azam-Ali [81], bambara showed prospects for cultivation in some areas of each continent, implying that its production can have a global footprint of importance amid the limitations placed by climate change on crop productivity. Bambara is deemed to be a drought-tolerant crop as it can still produce some yield under low moisture compared to other major crops and legumes [82]. Nonetheless, like any other crop, the crop suffers yield losses due to prolonged absence of moisture. High sensitivity to moisture stress tends to be greater during the reproductive stages (pod filling and flowering) than vegetative stages [83]. Studies have established that an average of approximately 49% yield losses can occur due to drought conditions [16].

Mabhaudhi and Modi [39] established that the sensitivity of different genotypes to water stress varies. Therefore, there is a need to consider drought tolerance among traits of importance to improve the yielding abilities of the crop and to identify candidates that possess high levels of tolerance for possible use as parents in breeding programs.

6.1. Selection for Drought Tolerance

Breeding for drought tolerance can be achieved through empirical (selecting directly for yield) or physiological breeding (selection for secondary traits). Yield is a primary trait used for selecting for drought tolerance, thus the empirical breeding approach emphasizes breeding for drought tolerance by directly aiming for yield and yield components [73]. It is perceived that this approach likely confers a two-fold advantage, whereby the cultivars that produce higher yields under optimal conditions will produce higher yields during seasons of above-normal rainfall, whilst during periods of low moisture, they are likely to produce fairly higher yields [84]. Findings on the common bean (*Phaseolus vulgaris*), a leguminous crop, revealed that despite yield penalties under terminal drought, cultivars that produced higher yields under optimal conditions produced average yields under drought stress-imposed conditions [85]. Although grain yield can be used to identify drought-tolerant individuals, consideration should also be given to morpho-physiological traits (physiological breeding) that confer adaptation to a particular environment.

In bambara, the studies of Karikari and Tabona [86] identified canopy spread, root: shoot ratio, 100 seed weight, and seeds per pod as informative traits that can be used for indirect selection of drought-tolerant bambara groundnut lines in Botswana, a semi-arid environment. Seed color is an indicator of drought tolerance in bambara, with reports suggesting that the brown seed coat likely possesses higher drought tolerance than the cream- or lighter-colored seed coats [39], a factor that was linked to the presence of a high tannins content in the darker seed coat colors. Multiple traits are associated with grain yield; however, to be used as a selection criterion, the trait should be highly heritable, genetically correlated with grain yield under drought conditions, and relatively easy to measure to facilitate indirect selections by breeders [71,85].

6.2. Drought Response Mechanisms

Knowledge of the drought response mechanisms conditioning resistance on a set of bambara groundnut genotypes is important for strategizing the breeding approach [36]. Bambara groundnut exhibits the escape, tolerance, and avoidance mechanism [39]. The timing of drought occurrence varies and can be intermittent or terminal, with different severities and durations. Drought escape is considered important for agro-regions where the rainfall period is short, typical of arid and semi-arid production areas, i.e., terminal drought, as it allows the crop to complete its life cycle before acute soil and water deficits develop [86]. However, when yield is the breeding objective, earliness often results in yield penalties due to photosynthetic adjustments [16].

Drought tolerance is facilitated by reduced tissue water through an increase in cell elasticity, a decrease in water deficit with a low tissue water potential through osmotic adjustment, and an increase in cell elasticity and decrease in cell sizes [36]; hence, it is deemed effective for curbing the effects of intermittent drought spells. Avoidance ensures that the crop can maintain a relatively high tissue water potential and root architecture, and has been associated with legume crops' ability to withstand moisture stress [36]. Beebe et al. [85] established that a deeper rooting depth and root hydraulic conductivity provide drought tolerance in legumes. In bambara, variation in the root architecture exists between genotypes from drier areas and wetter areas, whereby the former possesses a deep tap root and the latter a shallow lateral taproot [36,86–88]. This variation is essential for physiological breeding. Noteworthy, classical breeding through hybridizations and selections under optimal and drought-stressed conditions have, to some extent, contributed to the development and identification of tolerant varieties in some major crops. So far, some notable studies conducted that have targeted drought traits in bambara have focused on

(i) understanding drought responses [39] (ii) transcriptomic approaches to understanding response mechanisms [89], (iii) evaluating drought tolerance indices [90], and (iv) constitutive and selective indices for drought escape [86].

Breeding for drought tolerance is complicated as drought can be confounded with other environmental (heat) variables and is under the influence of multiple genes, which tend to be quantitatively inherited, making selections difficult. Therefore, when investigating drought traits, omics technologies need to be integrated with the conventional approach to increase the accuracy of selections. Through transcriptome approaches, genes conferring upward and downward drought tolerance in bambara have been investigated, and several ABA signaling genes have been reported [89]. In addition, for QTL mapping, a candidate marker bgPabg-596774 was identified for the selections of the number of pods, number of nodes, pod weight, number of seed, seed weight, and biomass dry weight traits and can be used in MAS under water stress [91]. As a limitation, challenges imposed by the instability of QTLs require validation of the marker prior to MAS in different recombinant cultivar backgrounds, which are not readily available in most breeding programs.

In drought phenotyping, the presence of homogenous testing conditions, non-destructive techniques, and precise phenotyping are crucial for making accurate selections. Therefore, for researchers to ensure homogeneity of the testing conditions, drought traits need to be studied under rainout shelters and in columns to enhance the reliability of the results. Recent advances in phenotyping allow high-throughput and non-destructive phenotyping, for instance, in root imagery techniques [92]. Alternatively, indirect estimates of the root architecture can be derived from canopy temperature depression, which represents the amount of water extracted from the soil and that which is lost as evapotranspiration, and thus can serve as an indirect predictor of the root size and depth [93].

7. Future Work and Conclusions

Next-generation sequencing, phenotyping, and bioinformatics facilitate large-scale screening of accessions, which can pave the way for modern breeding approaches to accelerate varietal development in bambara groundnut. Additional segregating populations must be developed. An assembly of a complete reference genome for bambara is required to facilitate other genomic data applications. The collection of bambara PGRs in various genebanks needs to be consolidated since the collections in each genebank are small and fragmented, as each genebank hosts approximately >50% of the total accessions reported. There are chances that the same accessions might be held in several genebanks, which causes redundancy. The diversity present in some national collections is unknown and underrepresented due to few accessions. Therefore, efforts are needed to encourage continued deposits of national PGR collections into the IITA-GRC collection. Subsequently, there is a need for robust characterization of PGRs through traditional and high-throughput phenotyping to support the utilization of plant genetic resources in pre-breeding programs. Simultaneously, it is also essential to assemble core collections and trait-specific subsets to expedite genetic improvement programs for bambara. Strategically, the utilization of various approaches including EAA and FIGS in developing composite and nested core sets for the mining of PGRs is important.

Bambara is widely grown for its nutritional properties, low water footprint, ability to grow in marginalized environments, soil replenishment of N, and social-cultural significance. Genetic diversity assessment at local production levels provides insight into the level of variability present within national genebank collections and farmers' cultivated material. Genetic enrichment using hybridization and mutagens is important to develop cultivars of bambara. In the absence of adequate genetic variability, the narrow genetic diversity in the locally cultivated germplasm necessitates the introduction of accessions from some agro-ecologies to widen the genetic base of base populations. Participatory approaches should be a basis for informing traits of importance, in addition to the strategic traits for various stakeholders. Genomic-assisted breeding should complement conventional breeding strategies to facilitate genomic analysis in bambara. The effectiveness of strategies

in mining PGRs for pre-breeding activities relies on the availability of well-characterized collections, with geographical indexing, genomic data, and feedback. Core collections and trait-specific subsets are crucial for the effective utilization of PGRs. Climate change is anticipated to worsen drought, heat, flood, and the emergence of diseases and pests, thus plant introductions can contribute to additional genetic variation that is useful for the development of varieties that are tolerant of unknown future biotic and abiotic stresses. The centers of cultivation of bambara have extended beyond Sub-Saharan Africa, thus adaptive features, particularly for drought and yield, are important to promote wider adoption of the crop to new agro-ecologies.

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