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Current Status and Future Needs of the US National Plant Germplasm System *Vigna* Germplasm Collection

1. Introduction

The genus *Vigna* contains several species that are important in world agriculture. They are warm season legumes and are grown in tropical, sub-tropical and temperate climates. Globally, they are grown on more than 10 million hectares annually and provide a significant portion of the dietary protein in many developing countries. They are mainly cultivated as a vegetable crop or legume/pulse crop for human consumption. Among them, cowpea (*V. unguiculata*), mung bean (*V. radiata*) and urd bean (*V. mungo*) are the most widely cultivated crops. Additionally, adzuki bean (*V. angularis*), bambara groundnut (*V. subterranea*), moth bean (*V. aconitifolia*) and rice bean (*V. umbellata*) are also important in daily diets, especially in Africa. Additionally, *Vigna* species are cultivated for livestock forage and as cover crops to suppress weeds and soil erosion, or as green manure to enrich soil fertility (Parida et al., 1990; Fery, 2002; Martins et al., 2003; Rodrigues et al., 2013). *Vigna* is a leguminous genus whereby they naturally enrich soil through symbiotic biological nitrogen fixation.

On a dry weight basis cowpea contains approximately 22 to 30% crude protein in grain and foliage (Bressani, 1985; Nielsen et al., 1997), whereas its haulms contain 13 to 17% of crude protein with a high digestibility and low fiber content (Tarawali et al., 1997), making it a valuable source of both grain and fodder (Fatokun et al., 1992; Samireddypalle et al., 2017). Further, *Vigna* species provide dietary fiber and protein in several edible forms, such as tender green shoots and leaves, immature/green pods and seeds, and dry seeds. Their high protein content helps complement carbohydrate-rich diets comprised mainly of roots, tubers, or cereals of many in Africa. *Vigna* species are successful in harsh environments such as daytime high temperatures in the range of 35-40ºC, sparse rainfall and marginal soils (Fery, 2002). Because of these desirable features, *Vigna* crops historically and currently have been chosen by many resource-limited farmers in developing countries, as well as in Western and Asian cultures from ancient times and in the New World since the Columbian Era.

1.1. Biology, origin, and taxonomy of genus *Vigna*:

The genus *Vigna* belongs to family Fabaceae and is native to the tropical and sub-tropical regions of both old and new worlds (Maxted et al., 2004; Tomooka et al., 2002 and 2009). The species are predominantly self-pollinating, although cross-pollination by bumblebees and other insects occurs up to 10% (Vaz et al., 1998). The plant growth habit ranges from erect/semi-erect bush to prostrate/trailing with profuse vegetative growth. The *Vigna* species used for commercial production are annuals. However, many perennial species exist in the genus. The species display a large variation in stem pigmentation, flower color, size and color of pods and seeds, all of which are used as descriptors to gather useful morphological data.

The genus consists of more than 100 species including some that were formerly *Phaseolu*s. The species have predominantly originated in Africa and the Indian subcontinent. Nine domesticated species including *V. aconitifolia* (moth bean), *V. angularis* (adzuki bean), *V. mungo* (urd bean or black gram), *V. radiata* (mungbean), *V. reflexo-pilosa* (creole bean), *V. subterranea* (bambara groundnut), *V. umbellata* (rice bean), *V. unguiculata* (cowpea) and *V. vexillata* (tuber cowpea) have been documented. Further, the genus contains both cultivated and wild species. About 80% of the species are diploid ($2n = 2x = 22$), a few are aneuploids ($2n = 2x = 18$, 20 or 24) and *V. reflexo-pilosa* is the only known amphidiploid ($2n = 4x = 44$) (Parida et al., 1990; Yang et al., 2014).

Genus *Vigna* is divided into five subgenera (Verdcourt, 1970; Marechal et al., 1978) and further into sections, species and subspecies primarily based on morphological traits. The five subgenera are *Ceratotropsis, Haydonia, Lasiospron, Plectrotropis* and *Vigna*. Cultivated *Vigna* species occur in *Ceratotropsis, Plectrotropis* and *Vigna*. Subgenus *Ceratotropsis* forms the Asian *Vigna* group and contains mung and urd beans along with other Asiatic *Vigna* species. The tuber cowpea, *Vigna vexillata*, belongs to subgenus *Plectrotropis* whereas, cowpea and bambara groundnut belong to subgenus *Vigna*, with cowpea in section *Catjang* (DC) Verde. and bambara groundnut in section *Vigna* (Tomooka et al., 2009). The remaining subgenera contain many of the indigenous African *Vigna* species. The taxonomy of genus *Vigna* is complex and is yet to be resolved completely. Recent advances in *Vigna* genomics should help clarify the taxonomic structure of the genus. Further, several recent studies have identified domestication related QTLs suggesting that important domestication traits are controlled by a small number of major QTLs with a few minor QTLs (Chaitieng et al., 2006; Isemura et al., 2007, 2010, 2012; Kaga et al., 2008; Kongjaimun et al., 2012a & b; Lo et al., 2018 and 2019).

1.2 Plant breeding and its products

Cowpea, mung bean and urd bean breeding and evaluation research programs in the US are being conducted by several land grant universities. A search of CRIS Reports in September 2020 provided a snapshot of current projects involving *Vigna* species, though just a few are on breeding. This included recipient Universities in Alabama, Arkansas, California, Colorado, Connecticut, Delaware, Florida, Georgia, Guam, Hawaii, Indiana, Kansas, Kentucky, Louisiana, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Montana, Nebraska, New Mexico, North Carolina, Oklahoma, Puerto Rico, Rhode Island, South Dakota, Tennessee, Texas, Utah, Vermont, Virginia and Wisconsin. It also included several USDA-ARS projects and one at the Micronesia State Agricultural Experiment Station. Historically, the University of Arkansas, University of California Davis and Riverside campuses, and Texas A&M University have been actively involved in cowpea improvement research for several decades. Currently, the University of Arkansas cowpea research program has been active in cultivar development, germplasm evaluation for drought and application of genomic technologies for breeding improved cultivars. Similarly, the University of California-Riverside cowpea research program has been developing improved varieties for California dry grain, fresh pod and cover crop purposes. Forage legume programs at the University of Florida and Texas A&M University have been evaluating cowpeas for fodder potential. Cowpea, mung bean and urd bean evaluations and breeding activities are also being conducted by other universities as summarized in Table 1.

The University of California-Riverside (UCR) has a long history with breeding improved dry-grain blackeye cowpea cultivars and complementary management systems that increase profitability through increased yield, grain quality, and decreased production costs (Ehlers et al., 2002). More recently, the UCR research group has been involved in developing modern genomic resources, including a high-density genotyping array (Muñoz-Amatriaín et al., 2017) and the first cowpea genome assembly with annotations of genes, repeats and genetic variants (Lonardi et al., 2019).

Most of the currently grown cowpea cultivars were developed by the University of Arkansas, University of California-Davis, University of California-Riverside and the USDA. The USDA has released 13 cowpea cultivars and 10 germplasm/breeding lines in the past 30 years. The green cotyledon genetic locus in cowpea was discovered by the USDA researchers and was the basis of the first commercially successful cowpea cultivars with a "persistent green" seed phenotype (Fery 1999; Fery et al., 1999; Ehlers et al., 2002). Additionally, Harrison et al., (2014) selected three cowpea lines from landraces: US-1136 (PI 664531), US-1137 (PI 664532) and US-1138 (PI 664533) that are known to have high biomass yield, high nitrogen fixation rate in the soil (220 kg ha^{-1}) and root knot nematode (RKN) resistance.

Globally, cowpea cultivars released from IITA are being grown in about 68 countries. They are high yielding with early or medium maturity combined with consumer-preferred traits such as large seeds, seed coat texture and color. Many of the cultivars have high levels of resistances to common diseases, insect pests, nematodes, and parasitic weeds. In 2019, the Nigerian Biosafety Management Agency (NBMA) approved the commercial release of genetically modified cowpea, pod borer-resistant cowpea (PBR cowpea) event AAT709A, to farmers in Nigeria (ISAAA, 2019). Additionally, IITA trains farmers through its Farmer Field School (FFS) projects in improved pest management practices of cowpea. The IITA and other African germplasm is used extensively in the University of California-Riverside cowpea research program for cultivar improvement as well as for genomic research.

1.3 International and domestic crop production:

Globally, the most widely grown *Vigna* crops include cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*), urd bean (*Vigna mungo*), adzuki bean (*Vigna angularis*) and moth bean (*Vigna aconitifolia*) with production distributed mainly in Asia and Africa. Some other *Vigna* species such as *Vigna subterranea (*bambara groundnut*)* and *V. umbellata* (rice bean) are grown locally in several African countries and in India for domestic consumption. Among the *Vigna* crops, only cowpea and mung bean are of economic importance in the United States and are produced regionally either as food, forage, or ground cover.

1.3.1. International production:

Annual worldwide production of the various *Vigna* species is about 32 million metric ton (Mt), and virtually all this production is in Asia and Africa (Rawal and Navarro, 2019). Brazil is also a major producer of cowpeas with an estimated annual production of 1 million metric tonnes, but they do not disaggregate cowpeas from other grain legumes.

Cowpea ranks as the most cultivated *Vigna* crop with an annual dry seed production of 7.2 Mt and an average yield of 578 kg/ha from a total harvested area of 12.5 million hectares in 2018 (FAOSTAT, 2018). Nigeria, Niger, Burkina Faso, Tanzania and Cameroon are the top producers (excluding Brazil) with Nigeria alone contributing about 54% to global production. Besides the sub-Saharan countries, Myanmar has emerged as a new producer of cowpea in East Asia (Rawal and Navarro, 2019).

Mung bean and urd bean are mainly grown in Asia, especially in India, Myanmar, Bangladesh, China, Pakistan, Thailand and Sri Lanka. India is the largest producer as well as consumer of mung bean and urd bean. Myanmar and India are also the world's leading producers of urd bean. Myanmar is an important exporter of mung bean and urd bean to other south east Asian countries including China, Vietnam and Singapore. China produces mung bean mainly for domestic consumption as sprouts and noodles. Recently, Australia has been producing mung bean for export markets, especially to India, Phillipines, the UK and the USA. The World Vegetable Center, formerly known as the Asian Vegetable Research and Development Center in Taiwan, coordinates mung bean improvement research among the Asian countries through the International Mungbean Improvement Network.

Adzuki bean is currently cultivated in China, Japan, South Korea and Australia. China is the largest producer as well as consumer of adzuki bean with some exports to South Korea and Japan (Rawal and Navarro, 2019). Additionally, Canada and Australia have started producing adzuki bean primarily for export markets in Asia. Bambara groundnut is a popular local crop in arid regions of Africa with significant production in Mali, Burkina Faso, Cameroon, Niger and the Democratic Republic of Congo (Rawal and Navarro, 2019). Lastly, moth bean and rice bean are mostly grown in the tropical areas of the Indian subcontinent mainly for human consumption as well as animal fodder (Rawal and Navarro, 2019).

1.3.2. Domestic production:

Prior to the second world war, in the USA cowpea was mainly grown as a forage crop for cattle. Hence, the name cowpea, which is attributed to Thomas Jefferson in a communication to John Taylor of Caroline in 1797 "*I have also received all the good kinds of field pea from England, but I count a great deal more on our southern cowpea*". Of late, cowpea is now grown on a relatively large scale for different edible forms such as a processing vegetable crop (southernpea), as a dry bean (blackeye pea, blackeye bean, field pea) and as a market garden crop (field pea, crowder pea, purple hull pea). Additionally, various types of cowpeas are popular home garden items in the southern states from Texas through the Carolinas and Virginia. While Georgia, Arkansas and Tennessee are leading producers of processing peas, Texas and California are the leading producers of dry beans. In 2019, cowpea was grown on about 13,700 acres (5,544 hectares) in the USA with an annual dry seed production of about 26 million lb (11.8 million kg) with an average yield of about 1900 lb/ac (2100 kg/ha) (USDA-NASS, 2020). Manufacturers usually have a contract with growers for different forms, or growers may sell them in local farmers markets as fresh vegetable.

In addition to cowpea, mung bean is also grown in the US, mainly for consumption as bean sprouts. Asian restaurants and commercial processors use about 9 million kilos of mung bean seed annually for sprouts. Much of this is imported with a small domestic production in Oklahoma and Missouri. About 15-20 million pounds of mung bean are consumed annually and nearly 75% of this is imported (Food Source Information, CO).

2. Extent of crop vulnerability and threats to food security:

2.1 Genetic uniformity and varietal life span:

Pod/seed yield, seed size and quality are the primary traits of choice for cowpea, mung bean and urd bean cultivar development. In turn, such focus on a small set of traits risk narrowing of genetic diversity, making these crops genetically more vulnerable to unforeseen pests and pathogens. The varietal life span of cowpea and mung bean cultivars is extremely long in the US. Some popular cultivars developed in the 1990s are still being cultivated. For example, in California the predominant cowpea variety under production still is CB46, which was released in 1991 (Helms et al., 1991), though newer varieties, CB27 with increased heat tolerance (Ehlers et al., 2000) and CB50 with a whiter seed coat, larger seeds and improved resistance to *Fusarium* and root knot nematodes (Ehlers et al., 2009) have been released. Overall, there does not seem to be an imminent threat to the continued cultivation of existing cultivars. However, it would be rational to develop new cultivars with genetically diverse sources available in the USDA *Vigna* collection to buffer against unforeseen biotic or abiotic stresses.

2.2 Current biotic and abiotic threats to cowpea, mung bean and urd bean production:

Several insect pests, nematodes, fungal pathogens, bacteria and viruses infect the crops resulting in significant yield losses. Globally, diseases and insects are the main constraints to cowpea, mung bean and urd bean production. Substantial crop losses are also caused by abiotic stresses such as unreliable rainfall, drought and soil salinity (Zonneveld et al.,, 2020; Singh et al.,, 1999). Additionally, parasitic weeds such as *Striga* and *Alectra* cause significant yield losses in cowpea in Africa. Chemical control, where possible, is expensive, adding to the production costs, thereby reducing the grower's profits even more. Also, chemical control measures lead to environmental pollution. The development of disease, insect pest and drought resistant, short-duration cultivars is critical for sustainable production.

In the US production regions, the common insect pests include aphids, pod borers, bruchids, root knot nematodes and the circulio beetle. A list of the economically important diseases and insect pests is in Table 2.

2.3. Accessibility of plant genetic resources and limitations:

The productivity and profitability of *Vigna* crops can be increased by exploiting the vast amount of genetic diversity available in the germplasm collections. Genetic characterization of the germplasm resources for economically important biotic and abiotic stresses is necessary to identify sources than can be incorporated into cultivar development programs (Table 3). High throughput phenotyping tools (UAVs/drones) and new genomic technologies can greatly enhance the ability of researchers to identify desirable germplasm sources to use in breeding programs. A significant limitation for *Vigna* crop improvement research in the US has been the lack of funding and infrastructure investments. Currently, in the US, most research programs are actively conducting only cowpea breeding and genomics research and practically none for the other *Vigna* crops.

There is an urgent need to bring awareness among leaders of land-grant universities, other public and private agencies and industry partners, of the many desirable traits of the *Vigna* crops to promote research and infrastructure funding support for domestic purposes. Similar efforts are needed to promote awareness of *Vigna* crops among growers for cultivation in a diversified and sustainable production systems. The *Vigna* germplasm collection held in the USDA genebank continues to be the most important resource for breeders and other researchers to help develop improved cultivars for US growers. The germplasm is provided free of charge world-wide for research and educational uses.

3. Status of NPGS collection to reduce genetic vulnerability:

3.1 Germplasm collections and *in situ* reserves:

3.1.1 Holdings:

The US *Vigna* genetic resources are maintained at the Plant Genetic Resources Conservation Unit (PGRCU) genebank managed by the US Department of Agriculture - Agricultural Research Service (USDA-ARS) in Griffin, GA. The germplasm collection includes cowpea, mung bean, urd bean, bambara groundnut, and several related species (Tables 4 and 5). Much of this collection is maintained also under long-term seed storage conditions at the National Laboratory for Genetic Resources Preservation (formerly the National Seed Storage Laboratory) in Ft. Collins, CO as a backup. The working collection consists of 13,065 accessions (Table 4), of which 10,991 (84%) are available for research and educational uses. A total of 12,871 accessions (91%) are backed up at Ft. Collins, with an additional 278 accessions backed up at the Svalbard Global Seed Vault. Distribution inventories are maintained at 4ºC at 25% humidity, and an additional inventory intended for long term preservation is maintained at -18ºC. Viability testing has been performed on 11,540 accessions (88%). Viability tests are conducted on all newly regenerated samples to determine a baseline for seed viability before they are put in the -18ºC (Table 4).

Cowpea and mung bean are the most significant *Vigna* collections in the USDA gene bank. The cowpea collection includes 8,242, accessions of which 6,862 are currently available for distribution. Similarly, the mung bean collection includes 4,232 accessions of which 3,797 are available for distribution (Table 4). Passport information along with associated characterization/evaluation data of the *Vigna* germplasm is available from the Germplasm Resources Information Network-Global (GRIN-Global) website (http://www.ars-grin.gov). Germplasm can be ordered online and a small sample (25 seeds) is distributed free of charge for both public and private research and educational institutions. Data or other related information from the use of the germplasm are solicited and uploaded onto the GRIN-Global database. Collaborative characterizations led to the identification of unique traits in cowpea, such as heat and chilling tolerance (Ehlers et al., 2002; Hall 1992; Ismail et al., 1997 &1999), and resistance to pests such as root-knot nematodes, cowpea curculio beetle, and *Fusarium* wilt (Ehlers and Hall 1997 ; Hall et al., 1997). Sandhu and Singh (2020) evaluated a large collection of USDA mungbean PIs and observed a magnitude of useful traits for cultivar improvement.

Additionally, University of California-Riverside (UCR) holds about 6000 cowpea accessions with substantial overlap with the USDA collection. A large portion of the accessions in the USDA cowpea germplasm collection were obtained via UCR/USDA Specific Cooperative Agreements which greatly enhanced the adequacy of the USDA collection to meet U.S. cowpea germplasm needs. The world's largest and most diverse collection of cowpeas is maintained at the International Institute of Tropical Agriculture (IITA) genebank in Nigeria. The germplasm collection (http://my.iita.org/accession2/collection.jspx?id=1) includes approximately 16,000 accessions from about 90 countries. The collection represents about 70% of African germplasm and nearly half of the global diversity. Although the IITA collection overlaps with the USDA collection, many IITA accessions still are lacking in the USDA collection. Globally the IITA collection is a very important resource, and a major effort needs to be made to store duplicate samples in the USA, preferably at the National Laboratory for Genetic Resources Preservation (NLGRP) in Fort Collins, Colorado.

Currently, a large collection of mung bean germplasm is stored at the World Vegetable Center in Taiwan. Consideration should also be given to storing duplicates of this collection at the NLGRP.

The National Bureau of Plant Genetic Resources (NBPGR), India, maintains large collections of mung beans and urd beans. The urd bean hybridizes quite easily with the mung bean, and it should be considered part of the mung bean gene pool. Again, it would be a long-term benefit to storing duplicates of both collections in the USA.

3.1.2 Genetic coverage and gaps:

The *Vigna* germplasm collection in the Griffin gene bank provides a reasonable coverage of most of known genetic diversity of genus *Vigna*, with a few gaps, mostly with some of the native species in Africa and Asia. There are opportunities to work with the USDA Plant Exchange Office to collect native *Vigna* species. The various *Vigna* collections described in the previous section represent a broad sampling of much of the world's gene pools. However, there may still be centers of diversity that need sampling, particularly in southeast Asia and south-central and west Africa. It is well-known that duplications among the global collections exist. For example, it is believed that about a third of the USDA and UCR cowpea germplasm overlaps with that of IITA. Hence, it would be useful to consolidate the passport, other descriptor data to compile unique accession lists. Genotyping technologies would help greatly to distinguish duplicates from unique accessions.

3.1.3 Acquisitions:

No efforts to acquire new germplasm from foreign countries have been carried out in recent years. However, as mentioned earlier, there are several wild and native *Vigna* species in Africa and Asia which may be of interest to acquire and introduce into the NPGS *Vigna* collection. The USDA Plant Exchange Office solicits proposals for germplasm collections and helps coordinate with local researchers for collection of native germplasm. Thus, there is a possibility for acquiring new *Vigna* germplasm from foreign countries in the future. Also, efforts must be made to compare NPGS cowpea *germplasm* list with that of IITA, to fill gaps with reciprocal exchange of materials. Other cowpea germplasm collections exist in Brazil, Botswana, Burkina Faso, China, Germany, Greece, Italy, India, Mexico, Senegal, Sudan, Tanzania, Uganda, Zambia, Zimbabwe and perhaps other locations. It is worthwhile to collaborate with these genebanks for reciprocal exchange of materials to fill gaps in the US cowpea collection.

3.1.4. Maintenance:

The USDA-ARS managed PGRCU in Griffin maintains the US *Vigna* collection. The seed storage unit is well equipped with dedicated staff to care for the maintenance and distribution of germplasm. Maintenance of accessions is generally straightforward. Most of the collection has significant quantities of seed in cold storage. All available germplasm is stored at -18ºC for long-term storage as well as a sample stored at 4ºC and 25% humidity as the working collection for routine and regular distributions. The seed storage unit is connected to an emergency alert system in case of an interruption of electrical power and to back-up generators. The facility remains locked and is only accessed by the unit staff.

3.1.5 Regeneration:

All regenerations are conducted in field plots (1x20 ft. row) at the Westbrook Farm, UGA-Griffin campus. Occasionally, a few of the collaborators help in regenerations of some materials. For regular annual regenerations, the curator uses information on the quantity of seed available, germination percentage and length of interval between regenerations as the main criteria for replenishing fresh seed into the collection so viable collections are maintained. Most *Vigna* species produce large quantities of seed. Pods are harvested as they mature (turn brown/black) at several times during the season and seeds are bulked at the end of the season. The seeds are dried to about 8-10% moisture before they are transferred to the cold storage facilities for long-term preservation. All pertinent regeneration information is available on GRIN Global.

3.1.6 Distributions and outreach:

The default distribution seed amount is 25 seeds for all *Vigna* species. Table 4 shows the total number of domestic and international distributions during 2019. When required, all international distributions were examined and issued a phytosanitary certificate by APHIS inspectors before they were shipped. Throughout the year, many students and teachers from nearby schools, colleges, and universities, researchers from US and foreign countries and stakeholder groups visit the germplasm bank to learn about the *Vigna* collection. Additionally, the curator coordinates with the *Vigna* CGC members with germplasm needs and characterizations.

3.2 Associated information:

3.2.1 Genebank and/or crop-specific web site(s):

For the US *Vigna* collection, the GRIN-Global database system (https://www.ars-grin.gov/npgs/index.html) is the only comprehensive source of information. Several US land-grant universities maintain cowpea germplasm collections, evaluation data and related genomic information. For example, the University of Arkansas conducts evaluation of cowpea germplasm for cultivar improvement and released several cultivars. Similarly, the University of California-Riverside and University of California-Davis campuses not only maintain germplasm but also released cultivars and developed advanced genomic technologies for cowpea improvement. University of Florida is also actively conducting genomic and breeding research for developing cowpea as a profitable forage/cover crop. Additionally, several international institutes and other agencies maintain large repositories of *Vigna* germplasm. Among them, the International Institute of Tropical Agriculture (IITA), Nigeria (http://www.iita.org) and the World Vegetable Center (formerly AVRDC), Taiwan (http://www.avrdc.org) maintain several *Vigna* species collections for public research and educational uses. A list of these agencies is provided in Table 6. Similarly, https://legumeinfo.org/ has genomic information of some important *Vigna* species for interested researchers. Lastly, the web link, https://www.ars.usda.gov/southeast-area/griffin-ga/pgrcu/ provides information about the *Vigna* curator and contact details for assistance with the US collection.

3.2.2 Passport information:

The GRIN-Global database used by the National Plant Germplasm System (https://www.ars-grin.gov/npgs/index.html) provides passport information of all *Vigna* germplasm in the US collection for public access. The passport data consists of general description of collection site information including its GPS coordinates, donor name, accession/PI, collector numbers and other identifiers, backup status, other narrative descriptions of plant, pod and seed traits and related digital images, where available. Also, it has a link to the Plant Introduction (PI) books detailing when the materials were introduced into the US collection and other pertinent information. Based on the passport data, Gillaspie et al., (1996) developed a US working core collection of 720 accessions for cowpea. The US mung bean core collection contains 410 accessions (Table 4).

3.2.3 Genotypic characterization data:

The International *Vigna* Genome Project was initiated to sequence the genomes of 15 wild *Vigna* species (Sakai et al., 2013; Sakai et al., 2016) to provide an International Foundation to develop genomic technologies for *Vigna* improvement.

The University of California-Riverside group developed a mid-density SNP genotyping assay, published a new genetic linkage map (Muchero et al., 2009) and began to apply SNP genotyping for assessment of seed purity, validation of crosses, and to compile marker-trait associations for agronomic and consumer-related traits. More recently, the genotyping platform was upgraded to a 50,000 SNP assay (Muñoz-Amatriaín et al., 2017), which was utilized in Feed the Future Innovation Labs to assist with genetic materials relevant to California and African breeding operations. This included the entire IITA cowpea core collection of 2050 accessions. With the recent development of a high quality, annotated cowpea genome sequence (Lonardi et al., 2019) and pan-genome (CowpeaPan; https://phytozome-ext.jgi.doe.gov/cowpeapan/), marker sets for traits that have been genetically mapped provide positions within which candidate genes exist. The evolving body of information is being disseminated through public portals including the Department of Energy Joint Genome Institute's Phytozome (phytozome.jgi.doe.gov), the Legume Information System (legumeinfo.org), the Pulse Crop Database (pulsedb.org) and the National Center for Biotechnology. This information has been used for the development of a ~2700-SNP genotyping assay for the Excellence in Breeding Platform (cgiar.org/research/program-platform/excellence-in-breeding-platform/) to ensure that a cost-efficient and uniform set of cowpea SNPs will be provided on a mid-density platform. It will be advantageous to germplasm users for this type of information to be available for accessions in the National Plant Germplasm System.

At Iowa State University, using genome-wide association studies (GWAS), Sandhu and Singh (2020) evaluated a subset of 482 accessions of mungbean and developed genetic markers for several quantitative traits related to yield enhancement. Further, they reported FERONIA, a known flowering pathway gene as the candidate gene for QTL with largest effect on days to flowering. Their evaluations of accessions included both phenotypic and genotypic characterizations and identified several useful traits and marker association for future cultivar improvement.

3.2.4 Phenotypic evaluation data:

Without adequate characterization data, breeders would not know which accessions to choose as desirable parents for cultivar development.

Standards for phenotypic characterization of *Vigna* accessions have been published by IBPGR (1983 and 1985). They include a range of attributes called descriptors. Currently, descriptor data is accumulated from annual regenerations for plant, pod and seed traits in addition to capturing digital images of pods and seeds. Where available, additional information such as resistance to diseases/pests and other quality parameters are included, in the database. Germplasm characterization data are also solicited from researchers receiving materials from the USDA. The Germplasm Resource Information Network-Global (GRIN Global) "http://www.ars-grin.gov", a database of descriptor information for each plant introduction in the USDA system, has made it much more efficient to access information regarding the collection. This information can be easily accessed, and plant introductions containing desired characteristics can be ordered for use in research or cultivar development. Further, efforts should be made to establish synchrony/uniformity in trait ontologies across the repositories. IITA has a set of trait ontologies for cowpea, and LIS is using something similar that is developing in parallel. The lack of uniformity hampers effective sharing of information. Sandhu and Singh (2020) evaluated about 3000 mungbean accessions from the USDA collection and compiled a subset of 482 accessions for replicated field evaluations. They reported positive correlations among several phenotypic triats such a days to flowering, plant height, Fusarium wilt resistance, 100-seed weight and several other qualitative traits. Their observations would help in choosing of appropriate parental germplasm for mungbean cultivar development.

3.3 Plant genetic resource research associated with the NPGS:

3.3.1 Goals and emphases:

PGRCU actively supports research collaborations with public and private entities by freely providing necessary germplasm to understand and develop knowledge of the collection for effective and efficient use by breeders and other researchers. One of the current cooperative research projects involves the evaluation of a select set of cowpea accessions for drought/salt tolerance. This project is conducted by Dr. Ainong Shi, Cowpea breeder at the University of Arkansas in Fayetteville. Further, it is anticipated that the recent efforts to rejuvenate the *Vigna* CGC will generate additional collaborations for future germplasm evaluations and use in research programs.

3.3.2 Significant accomplishments:

The NPGS *Vigna* collection is one of the most comprehensive and genetically diverse collections in the world. Several studies also highlighted the genetic diversity, patterns and distribution of genetic variation to provide knowledge about the genetic structure of the genus and species compatibilities (Fang et al., 2006; Xu et al., 2010; Boukar et al., 2018; Iseki et al., 2018; Zonneveld et al., 2020). Further, these studies led to the identification of desirable accessions for cultivar improvement. The distribution and use of germplasm from the US national collection resulted in many improved cultivars for the growers and the germplasm collection is truly a national treasure.

3.4 Curatorial, managerial and research capacities and tools:

3.4.1 Staffing:

There is one full-time technician supported with S-009 funds to assist the curator with germplasm curation activities. However, the technician is also responsible to help the curator with managing the US national peanut collection. The curator and the technician work together to maintain plants in the field with regular operations such as irrigation, weeding and chemical sprays. They also coordinate field planting, harvesting and post-harvest activities involving cleaning seeds, recording descriptor data, and submitting the seeds to the seed storage unit.

3.4.2 Facilities and equipment:

Adequate field and greenhouse space are available for annual regenerations. Adequate work area is also available for postharvest cleaning of seeds and bagging. A full-fledged analytical laboratory is available for measuring protein content and other nutritional components. The laboratory is also equipped to conduct PCR, if needed. See Table 7 for details of the analytical laboratory equipment available in the PGRCU. Adequate manual support is available in the unit for planting, managing the crop, and harvesting operations.

3.5 Fiscal and operational resources:

Reasonable and acceptable levels of fiscal and operational resources via project fund allocation are available for curation activities. However, additional technician to help with management of *Vigna* collection would be desired.

4. Other genetic resource capacities:

Vigna germplasm evaluation and enhancement efforts in the US is conducted exclusively by horticultural plant breeders at land-grant universities (Table 1). The research projects varied from forage cropping systems to cover crops to evaluation of germplasm and breeding to develop cultivars. Virtually all research projects are with cowpea except two with mung and urd beans. Germplasm evaluation and breeding research is being conducted at all these universities (Table 1). Much of the cowpea research is focused on development of improved technologies and methods to enhance forage nutritional quality and development of precision genomic technologies to produce superior cultivars.

5. Prospects and future developments:

Genetic vulnerability is not seen as a critical problem for the *Vigna* crops grown in the US at this time. The economic *Vigna* species (*V. unguiculata* (cowpea) and *V. radiata* (mung bean)) grown in the U.S. are not considered to be particularly vulnerable to any type of fungal or bacterial pathogens, nematodes, insect pests, other than *Fusarium* and aphids in California and circulio beetle and nematodes in the South. However, some viruses can be extremely devastating to *Vigna*, and many are seed borne or can be easily spread by insect vectors or infected plant debris. Overall, biotic and abiotic stresses are of priority for evaluation of *Vigna* germplasm. Along with this, to extend cowpea production for grain, cover, or forage into cooler climates within the U. S., an emphasis on enhanced chilling tolerance from seedling emergence onward would be beneficial.

In comparison with many of the agronomic crops, cowpea and mung bean are relatively minor crops and neither is a staple food in the U. S. Although cowpea is grown mainly for dry seeds for human consumption, there has been growing interest for cowpea as a forage crop in the USA, due to its nutritious biomass, and as a cover crop for weed control and soil enrichment. US cowpea breeders face many challenges in developing new cultivars suitable for modern, highly specialized production and marketing systems. Sources of new traits continue to be identified in cowpea germplasm, and the traits defined at high genetic resolution with the new genotyping resources available for identifying marker- trait associations. However, reliable phenotyping data is essential for identifying marker-trait associations. US cowpea breeding programs can capitalize on the broad genetic diversity that exists within cowpea germplasm and recently developed genotyping tools develop superior cultivars for grain, forage, and cover crop purposes.

In summary, below is a list of recommendations by the *Vigna* CGC for sustainable and profitable production of *Vigna* crops in the USA.

1) Increase awareness of *Vigna* crops among farmers, especially cowpea and mung bean, as protein rich, drought tolerant, low input profitable crops.
2) Identification of additional germplasm accessions for resistances to common insect pests, pathogens, and drought for use in cultivar enhancement programs.
3) High throughput phenotyping to complement new genomic tools to aid marker-assisted breeding.
4) Development of genome editing technologies for trait specific cultivar development.
5) Development of sustainable agronomic production practices, especially for management of weeds.

6) Characterization of core sets for nutritional components and other consumer preferred traits.

7) Establishment of local or regional supply chain systems from harvesting to post harvest storage and market accessibility.

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7. Appendices:

Table 1: US institutions conducting research with *Vigna* crops

No.	Institution	Research Area	Researcher(s)
1	University of Arkansas	Cowpea breeding	Ainong Shi
2	University of California	Cowpea genomics/breeding	Tim Close, Phil Roberts and Bao Lam Huynh (Riverside)
		Cowpea germplasm/breeding/pod shattering	Paul Gepts and Sassoum Lo (Davis)
		Cowpea insect response	Eric Schmelz (La Jolla)
		Bambara groundnut and soil microbiome	Ann Hirsch (Los Angeles)
3	Colorado State University	Cowpea breeding and genomics	Maria Munoz-Amatriain
4	Cornell University	Cowpea stress traits	Magdalena Julkowska
5	University of Florida	Cowpea forage/cover crop	Esteban Rios
6	University of Georgia	Apomixis in cowpea	Peggy Ozias-Akins
7	Iowa State University	Mung bean and urd bean germplasm evaluation/breeding	Arti Singh
8	Mississippi State University	Physiological evaluation of cowpeas	Casey Barickman
9	Noble Research Institute	Cowpea forage/cover crop	Yun Kang
10	South Dakota State University	Forage cropping systems with cowpea	Pete Sexton
11	Middle Tennessee State University	Organic forage cropping system with cowpea	S. Cui
12	Tennessee State University	Cowpea and mung bean evaluation/breeding	Matthew Blair
13	Texas A&M University	Forage legume research with cowpea	Gerald Smith and Monte Rouquette
14	USDA-ARS, Fargo, ND	Short season cowpea breeding	Mike Grusak
15	Utah State University	Cowpea evaluation for beetle resistance	Frank Messina
16	Virginia State Univiversity	Mung bean evalautions	Harbans Bharadwaj
17	University of Washington	Cowpea insect response	Adam Stenbrenner

Table 2. Economically important disease and insect pests of *Vigna* crops in the US

Disease/insect pest	**Crops affected**	**Vector/causal agent**
Mung bean yellow mosaic virus (MYMV)	Mung bean Urd bean Moth bean	white fly (*Bemisia tabaci)*
Cowpea mosaic virus	Cowpea	Aphid (*Aphis craccivora*)
Powdery mildew	Mung bean Urd bean	*Erysiphe polyponi DC*
Ceracospora leaf spot	Cowpea	*Cercospora canescens*
Choanephora pod rot	Cowpea	*Choanephora cucurbitarum*
Southern blight	Cowpea	S*clerotium rolfsii*
Rhizoctonia stem canker or damping-off	Cowpea	*Rhizoctonia solani*
Bacterial blight and canker	Cowpea	*Xanthomonas axonopodis pv. vignicola*
Root-knot nematodes	Cowpea Mung Bean	*Meloidogyne* spp.
Pod borer	Cowpea	*Helicoverpa* *Maruca vitrata*

Beet Armyworms	Cowpea	*Spodoptera exigua*
Cowpea bruchid/Cowpea weevil	Cowpea	Callosobruchus *maculatus*
Cowpea curculio	Cowpea	*Chalcodermus aeneus*
Southern green stink bug	Cowpea	*Nezara viridula*
Brown stink bug	Cowpea	*Euschistus servus*

Table 3: Cowpea germplasm sources with desirable traits
(Modified from Singh, B. B. 2016 with USDA PI #s)

Diseases and parasitic weeds	Resistant lines	USDA PI #
Anthracnose & Bacterial pustule	TVu-201	PI 579010
	TVu-408	PI 579107
	TVu-697	PI 598339
Brown blotch	TVu-201	PI 579010
	TVu-1977	PI 225922
Scab	TVu-1977	PI 225922
Bacterial blight	TVu-1190	PI 579394
	TVu-1977	PI 225922
Septoria	TVu-456	PI 579128
	TVu-483	PI 579148
	TVu-1977	PI 225922
Web blight	TVu-317	PI 406286
	TVu-2483	PI 579938
	TVu-4539	PI 580015
Phakospora rust	TVu-612	PI 583521
	TVu-4540	PI 406290
Root knot nematode	TVu-264	PI 293579
	TVu-401	PI 579101
	TVu-1560	PI 579607
Major viruses	TVu-201	PI 579010
	TVu-1190	PI 579394
Striga and Alectra	TVu-12415	PI 581440
	IT90K-76	PI 632850
	IT90K-82-2	PI 632853
	IT90K-277-2	PI 632848
	IT90K-372-1-2	PI 632851
	IT97K-499-35	PI 642160
	IT97K-819-118	PI 642161
Insect-Pests		
Aphid	TVu-57	PI 406284
	TVu-3000	PI 353159

Leafhopper	TVu-1190 (moderate resistance)	PI 579394
Flower thrips	TVu-2870 (moderate resistance)	PI 353024
Maruca pod borer	TVu-946 (low resistance)	PI 598327
Pod sucking bugs	TVu-1977 (moderate resistance)	PI 225922
	TVu-7274 (moderate resistance)	PI 580384
Bruchid weevil	TVu-11952	PI 581030
	TVu-11953	PI 581031
Drought tolerance	TVu-11979	PI 581055
	TVu-11986	PI 581062
	IT89KD-374-57	PI 639260
	IT89KD-288	PI 598343
Heat tolerance	IT93K-452-1	PI 642157
	IT97K-499-35	PI 642169
	IT97K-499-35	PI 642169
Multiple disease resistance	IT82E-16 (red)	PI 582537
	IT82E-18 (tan)	PI 582393
	IT82E-32 (red)	PI 582394
	IT82D-752 (tan)	PI 582446
	IT82D-789 (light brown)	PI 582451
	IT83S-818 (white blackeye)	PI 582492
	IT85F-867-5	PI 582962
	IT86D-1010 (white blackeye)	PI 532807
	IT89KD-288	PI 598343
Grain and fodder yield	IT97K-499-35 (high)	PI 642160
	IT86D-719 (high)	PI 598346

Table 4: Status of the US *Vigna* Collection (June 2020)

Crop	Genus/Species	Total accessions	Available	Unavailable	Backed up	In -18 C	Germ Tested	Distributed items, 2019	Domestic distributions 2019	Foreign distributions 2019	Svalbard backup
Bambara Groundnut	1 / 1	98	35	63	95	53	65	74	19	55	0
Cowpea	1 / 7	8242	6862	1380	8200	888	7226	1545	1441	104	250
Mung bean	1 / 5	4232	3797	435	4232	4074	3931	143	114	29	13
Other *Vigna* species	6 / 23	493	297	196	354	493	318	104	47	57	25

Table 5: *Vigna* Collection of other species in the US national Collection (June 2020)

Species*	**Total PIs**	**Number Available**	**Backed Up**
Ancistrotropis peduncularis	1	1	1
Cochliasanthus caracalla	6	6	6
Helicotropis stenoloba	3	2	3
Leptospron adenanthum	8	6	8
Sigmoidotropis speciosa	2	0	1
Vigna aconitifolia	56	51	56
Vigna angularis	295	129	157
Vigna angularis var. nipponensis	2	1	2
Vigna lasiocarpa	1	1	1
Vigna longifolia	1	1	1
Vigna luteola	14	12	14
Vigna marina (Burm.) Merr.	1	0	1
Vigna membranacea	2	1	2
Vigna minima	1	1	1
Vigna mungo	1	1	1
Vigna mungo var. mungo	303	289	303
Vigna oblongifolia	5	5	5
Vigna oblongifolia var. oblongifolia	8	7	8
Vigna oblongifolia var. parviflora	3	3	3
Vigna reflexopilosa subsp. glabra	1	1	1
Vigna schimperi	1	1	1
Vigna spp.	24	17	24
Vigna trilobata	2	2	2
Vigna umbellata	41	36	41
Vigna vexillata	15	9	15
Totals	797	583	658

*Synonymous with *Vigna*

Table 6: Important *Vigna* genetic resources repositories and information networks

- US National Plant Germplasm System
  - (https://www.ars-grin.gov/npgs/index.html)
- International Institute of Tropical Agriculture (IITA), Nigeria
  - (http://www.iita.org)
- World Vegetable Center (AVRDC), Taiwan
  - (http://www.avrdc.org)
- Global online platform for plant genetic resources (GENESYS)
  - (https://www.genesys-pgr.org/)
- National Bureau of Plant Genetic Resources, India
  - (http://www.nbpgr.ernet.in)
- The European Vigna Database
  - (https://www.genebank.at/en/ecpgr-vigna.html)
- International Mungbean Improvement Network
  - (https://avrdc.org/intl-mungbean-network)
- US Vigna: A cowpea working group
  - (https://www.usvigna.org/)
- Legume Information System (LIS)
  - (http://legumeinfo.org)
- The Legume Federation
  - (https://www.legumefederation.org/en/)

Table 7. Analytical Laboratory Equipment available in the PGRCU, Griffin

Equipment	Model #	Manufacturer	Use
Protein analyzer	Rapid N Exceed	Elementar	Protein content
TD-NMR	mq-one	Bruker Optics	Seed oil content
GC-Mass Spec	7890A-5977	Agilent Technologies	Fatty acid composition, flavor analysis
HPLC	1100 w/ RID, DAD, FLD	Agilent Technologies	Sugar content, amino acids, secondary compounds
Real-time PCR	QuantStudio 3	Thermofisher	Gene expression, SNP genotyping