# Chapter 7 Cowpea

Ousmane Boukar, Christian A. Fatokun, Philip A. Roberts, Michael Abberton, Bao Lam Huynh, Timothy J. Close, Stephen Kyei-Boahen, Thomas J.V. Higgins and Jeffrey D. Ehlers

### 1 Introduction

Cowpea (*Vigna unguiculata* (L.) Walpers) is a commonly grown and consumed grain legume in sub-Saharan Africa (SSA). It is particularly well adapted to the dry savanna region of SSA where many other crops could fail or perform very poorly due to water stress caused by irregular and short duration rainfall as well as poor soil fertility. The grains, which are the main product of the crop, contain between 22 and 30% protein thus making it a good source of quality food especially among the rural dwellers and urban poor. Cowpea grains are consumed in different forms. They are eaten boiled, fried (as akara), or steamed (as moi moi). In addition to the high protein content, cowpea grains are high in complex carbohydrates. Cowpea haulms (dried leaves, stems, and pod walls) are also a good source of quality fodder for livestock especially ruminants. In some parts of East Africa, notably Kenya and Tanzania, young succulent leaves of cowpea, also characterized by high protein and mineral nutrient contents, are picked and eaten as pot herbs.

O. Boukar (🖂)

C. A. Fatokun International Institute ot Tropical Agriculture (IITA), PMB 5320, Ibadan, Oyo State, Nigeria e-mail: c.fatokun@cgiar.org

P. A. Roberts Department of Nematology, University of California—Riverside, 900 University Avenue, Riverside, CA 92521, USA e-mail: philip.roberts@ucr.edu

M. Abberton International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria e-mail: mabberton@cgiar.org

International Institute of Tropical Agriculture, Cowpea Breeding Unit, Sabo Bakin Zuwo Road, Kano, Kano 3112, Nigeria e-mail: o.boukar@cgiar.org

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Cowpea is grown mainly as an intercrop along with sorghum and millet in the dry savannas but is also intercropped with maize in the moist savannas. Only very few farmers in SSA grow cowpea as a sole crop. It is grown in wide spacing when intercropped such that plant population density is usually low, perhaps around 1000 plants/ha or even less. However, when grown sole the population density is much higher and this is reflected in higher grain yield. Cowpea, like many other legumes, is able to contribute to the sustainability of the soil in SSA farmers' fields. Being a legume, cowpea is capable of fixing atmospheric nitrogen in its root nodules hence it has little or no need for nitrogen fertilizer application. It can fix up to 240 kg/ha and leave between 60 and 70 kg/ha in the soil after harvest (Rachie 1985). The following crop can therefore benefit from this left over nitrogen.

Cowpea is grown in no less than 45 countries across the globe on about  $14.5 \times 10^6$  ha. A total of 6.2 million metric tons (MMT) of grains are produced annually implying an average yield of 454 kg/ha. Nigeria and Republic of Niger produce about 45 and 15% of total world cowpea followed by Burkina Faso with about 6%. The bulk of cowpea production as well as consumption are in West Africa. Another major producing country is Brazil, but the quantity they produce is not correctly reported in the Food and Agriculture Organization (FAO) statistics. The projected annual production rate of growth for cowpea in SSA is expected to be 3%, which means  $8 \times 10^6$  t by 2020 (Abate et al. 2012). Demand, however, will increase at the rate of 5% per year in West Africa and this has implications for the people in West Africa especially Nigerians. Demand for cowpea grain is expected to decline in Kenya and South Africa during this same period (Abate et al. 2012). Cowpea does not feature in international trade but trade between neighboring countries such as Niger

B. L. Huynh

T. J. Close

S. Kyei-Boahen International Institute of Tropical Agriculture (IITA), Av. Eduardo Mondlane 326, 2nd Floor Room 210, Nampula 709, Mozambique e-mail: s.boahen@cgiar.org

T. J.V. Higgins CSIRO Agriculture Flagship, Clunies Ross Street, Black Mountain, Canberra, ACT 2601, Australia e-mail: tj.higgins@csiro.au

J. D. Ehlers Department of Agricultural Development, Bill and Melinda Gates Foundation, 500 N. 5th Ave N, Seattle, WA 98102, USA e-mail: jeff.ehlers@gatesfoundation.org

Department of Nematology, University of California—Riverside, 3401 Watkins Drive, Boyce Hall 1463, Riverside, CA 92521, USA e-mail: baolam.huynh@ucr.edu

Department of Botany and Plant Sciences, University of California, 900 University Avenue, Riverside, CA 92521-0124, USA e-mail: timothy.close@ucr.edu

and Nigeria takes place. There is an annual deficit of over  $0.5 \times 10^6$  t in Nigeria and supplies from Niger and Cameroon have made up for this shortfall. There is a need for expansion in the production of cowpea if the projected deficit is to be adequately forestalled. The bulk of the growth in cowpea recorded over years is attributable to increase in land area cultivated to the crop. Technologies that will lead to increased productivity per unit area of land now need to be developed and promoted if food security is to be ensured.

There is a need for the application of agrochemicals especially insecticides to the cowpea crop. Farmers who grow cowpea in intercrop usually do not give any protection to the crop against insect pests and apply no fertilizer. However, the few farmers who grow cowpea as a sole crop try to apply insecticides to provide protection against insect pests that otherwise cause significant grain yield losses. In many instances, the insecticides applied may not be effective against all of the insects that limit the crop's productivity. Different insects attack cowpea plants at various stages of the crop's life cycle. Aphids (Aphis craccivora) attack cowpea and cause the most damage when the plants are in the seedling stage while flower bud thrips (Megalurothrips sjostedti) cause flower buds to abort prematurely thereby preventing them from reaching anthesis. The legume pod borer (Maruca vitrata), the most cosmopolitan of cowpea insect pests, damages flowers and developing pods and seeds. A complex of pod-sucking bugs (e.g., Clavigralla tomentosicollis, Anoplocnemis curvipes, and Riptortus dentipes) feeds on both mature and immature pods and seeds leading to shrinking, deformity, and nonviability of the seeds. Such deformed seeds are not fit for consumption and therefore not marketable. Cowpea weevil (Callosobruchus maculatus) feeds on stored seeds, which is why most farmers sell off the seeds shortly after harvest at fairly low and noncompetitive prices to avoid storage losses caused by the weevil. From the foregoing, it is obvious that insects are capable of wreaking immense damage to productivity of cowpea if not adequately controlled. For now, the application of insecticides seems to be the only method for control of some of the cowpea pests.

Generally, the traditional farmers' cowpea varieties are late maturing (>90 days to flowering) and characterized by spreading growth habit. On the other hand, most of the improved varieties are erect to semierect in growth habit and could be early (60–65 days) or medium maturing (75–80 days). The early maturing erect cowpea lines are well suited to sole cropping and could be planted at high population density, while the spreading type seems to be more adapted to intercropping systems. Studies have shown that some spreading-type cowpea lines such as 'Dan Ila' are able to withstand shading better than non-spreading types (Terao et al. 1997). Farmers in the dry savanna areas still grow their traditional varieties because even when insects have caused grain yield losses these varieties still are able to get fodder which they harvest and sell for income or use as quality feed for their livestock.

The response of cowpea plants to photoperiod has been described as being typical of quantitatively short-day implying that photoperiod beyond a critical value can only delay but not prevent flowering (Njoku 1958; Lush et al. 1980). While most of the farmers' traditional varieties belong to this category, that is, day-length sensitive, there are some lines which are day neutral (i.e., length of days does not influ-

ence time to flower). Most of the improved cowpea varieties being grown presently are day neutral in addition to being erect or semierect in growth habit.

#### 2 Origin and Systematics

Cowpea is an indigenous crop in SSA. It has been reported that the immediate progenitors of cultivated cowpea such as V. unguiculata ssp. dekindtiana/spontanea are widely distributed across Africa including Madagascar (Ng and Singh 1997). Ng and Maréchal (1985) suggested that cultivated cowpea moved from West to East Africa from where it was taken to Europe. It was recognized by the Romans as far back as 2300 before present (BP). It probably moved from Europe to India in 2200 BP and to the Americas by Spanish and Portuguese traders in the seventeenth century. The greatest amount of genetic diversity in cultivated cowpea has been found to exist in West Africa especially the dry savanna regions of Cameroon, Niger, Nigeria, Burkina Faso, Benin, and Togo. However, the origin of wild cowpea has been traced to southern Africa particularly the area covering from Namibia, Transvaal to Swaziland. It is in this subregion of Africa that the highest amount of genetic diversity for wild V. unguiculata and V. rhomboidea has been detected (Ng and Singh 1997). The wild cowpea lines found in southern Africa usually have small seeds when compared with those found in West Africa such as subspecies dekindtiana/spontanea, which have slightly larger seeds. This probably confirms the claim that V. unguiculata ssp. dekindtiana/spontanea is an immediate progenitor of cultivated cowpea that is characterized by a large-seed size. The different wild cowpea relatives were previously regarded as independent species but Maréchal et al. (1978) merged all of them into a single species (V. unguiculata). Further, taxonomic efforts have subdivided these into various subspecies and varieties. Examples are V. unguiculata ssp. unguiculata var. spontanea, ssp. dekindtiana var. dekindtiana, ssp. pubescens, and ssp. protracta var. protracta among others. The taxonomy of the genus Vigna and particularly cowpea and its relatives was recently reviewed by Pasquet and Padulosi (2012). There is yet to be a well-defined and universally agreed classification of the wild cowpea relatives. So far, there are no distinct classifications into primary, secondary, and tertiary gene pools for cowpea. Varying levels of difficulties are encountered when crossing some of the wild V. unguiculata subspecies with cowpea and even among themselves. For example, embryo rescue was necessary for a successful cross between a cowpea line and a line of V. unguiculata ssp. pubescens (Fatokun and Singh 1987). That the wild cowpea relatives have hardly been used in the genetic improvement of the crop may have contributed to the low interest in defining the crop's gene pools. Desirable genes conferring resistance to many insect pests that cause damage to cowpea yield are present in some wild Vigna species such as V. vexillata, which have resistance to aphids, Maruca pod borer, and some others. However, strong incompatibility barriers prevent successful crossing of vexillata and cowpea thus making it impossible to transfer such useful genes to cowpea through conventional breeding methods.

#### 3 Varietal Groups

Cultivated cowpea and its cross-compatible wild relatives belong to the section *Catiang* of the genus *Vigna*. All of the cultivated cowpea lines are classified as V. unguiculata subspecies unguiculata. Cultivated cowpea is subdivided into four cultivar groups (cv.-gr.), namely, Biflora, Textilis, Sesquipedalis (vard-long- bean), and Unguiculata/Melanophthalmus (Westphal 1974; Marechal et al. 1978). Each of these cultivar groups is distinct from the others. For example, Textilis is characterized by long peduncles, which are a good source of fiber used in the textile industry, while Sesquipedalis, the vard-long bean, has long, fleshy, and pendulous pods. The yard-long bean whose pods can be as long as 90 cm or more is consumed as a vegetable especially in Asia. Yard-long bean with long pods may have evolved from regular cowpea due to selection pressure exerted in Asia, where its consumption as a vegetable is very popular. Despite the length of the pods, the number of seeds per pod is usually not more than is found in cowpea which belongs to cultivar group cv.-gr. Melanophthalmus. The cv.-gr. Unguiculata/Melanophthalmus comprises the cultivated cowpea with most number of accessions. The protein-rich grains are the most economically important part of the crop hence seeds are large and crowded in the pods. This probably explains why cowpea is also referred to as crowder bean in some communities. Dual purpose varieties are noted for their grain and fodder yield and should be attractive to people in East Africa who consume cowpea leaves as vegetables as well as livestock farmers in the dry savanna regions of SSA. Many cowpea farmers in the dry savanna areas of SSA get almost the same amount of income from sales of fodder as from grains.

## 4 Genetic Resources: Conservation and Utilization

The collection, conservation, characterization, documentation, and distribution of genetic resources (germplasm) are important, and the diversity of germplasm gathered in ex situ collections, or gene banks, is a key underpinning of current and future breeding programs.

The most extensive collection of cowpea germplasm (15,371 accessions) is held by the Genetic Resources Center (GRC) of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The IITA collection contains germplasm from 90 countries but around half of the collection (7912 accessions) is derived from West Africa, the center of diversity for the crop. This collection, along with others in the Consultative Group for International Agricultural Research (CGIAR) gene banks, was designated by FAO as held "in trust" for the international community, a status reinforced under Article 15 of the International Treaty on Plant Genetic Resources for Food and Agriculture, which entered into force in 2004. Germplasm from this collection is distributed to all those requesting it for research and breeding for food, feed, and agriculture, free of charge under the standard material transfer agreement. Cowpea seed is conserved under medium-term storage at 5°C for the active collection, from which seeds will be distributed, and in long-term storage at -20 °C. Other significant collections are held in the USA with the US Department of Agriculture (USDA), Griffin and with the University of California Riverside (UCR), (around 10,000 and 6000 accessions, respectively). It should be noted that IITA and these two USA collections have a combination of duplicate and unique cowpea accessions.

Collection of genetic resources is accompanied by "passport data" giving details of collecting site and other information recorded by collectors as well as accession identifiers. Germplasm is characterized primarily by a number of morphological "descriptors" of plant features which are relatively constant under different environments. A descriptor list developed by the International Board for Plant Genetic Resources (IBPGR 1983) and modified by IITA and Bioversity International (Dumet et al. 2010) is used for the characterization of cowpea germplasm; this includes vegetative and floral parts and seed. Progress in the development of improved cowpea varieties that would be well adapted to different agroecologies in the tropics depend largely on the genetic resources being conserved. Besides adaptation to different agroecologies, many new varieties with specialty traits and consumer preferences may also be developed using these resources (Table 7.1).

The core collection of a germplasm set is often developed to capture a high proportion of the diversity in a number of accessions that can be more manageably phenotyped, typically 5–10% of the total collection. In many cases, both agromorphological characters and molecular markers are used to develop this. IITA has developed a "core-collection" of 2062 accessions of cultivated cowpea using geographical, agronomic, and botanical descriptors (Mahalakshmi et al. 2007). In a further refinement of this core collection concept, under the auspices of the Generation Challenge Program (GCP) of the CGIAR (http://www.generationcp.org/research/ research-projects), a "mini-core" collection of cowpea was developed with 374 accessions. The continuing development and application of genomic tools, including a draft cowpea genome sequence, will underpin new approaches to the characterization of cowpea genetic resources and enhance their utility for breeders.

Hearne et al. (2012) studied a subset of the core collection comprising 86 accessions plus 10 gene bank accessions, representing 84 countries of origin. Fourteen SSR markers were used in the study, based on high polymorphism rates for two alleles per marker and good technical resolution, to assess the levels of inbreeding and heterogeneity within this group. The study revealed that inbreeding was not as complete as previously assumed and that up to five plants per accession would provide more accurate measures of diversity (Hearne et al. 2012).

A recent study of cowpea diversity analyzed the genetic relatedness of 433 cowpea landraces collected from 56 countries and 46 accessions of wild cowpeas using a set of >1200 genome-wide single-nucleotide polymorphism (SNP) markers (Huynh et al. 2013a). Among the landraces, 323 were from North, West, Central, East, southeastern, and southern Africa, and 99 were representative of the rest of the world. The wild cowpea accessions represented three countries in West Africa and five countries in eastern and southern Africa. The genotyping was conducted using the 1536-SNP cowpea Illumina GoldenGate assay (Muchero et al. 2009a).

Resistant/tolerant	Germplasm accessions	References
Diseases	F	
Fusarium wilt	TVu 109-2, TVu 347, TVu 984, TVu 1000	Singh et al. (1983)
Scab	TVu 853, TVu 1404, TVu 1433.	Singh et al. (1983)
Septoria	TVu 456, TVu 483-2, TVu 486, TVu 1433, TVu 11761, TVu 12349	Singh et al. (2002)
Bacterial blight	TVu 347, TVu 410, TVu 483-2, Danilla (Nigerian landrace)	Singh et al. (1983)
BICMV	TVu 2480; TVu 2657, TVu 3433	Taiwo et al. (1982); Bashir (1992)
CABMV	TVu 401, TVu 1582	Bashir (1992)
CPMV	TVu 227, TVu 345, TVu 612, TVu 2331	Patel (1982)
CPMoV	TVu 3901	Allen et al. (1982)
Striga and Alectra	B301; TVu 14676	Lane et al. (1997); Ouedraogo et al. (2012)
Insect pests		
Aphid	TVu 36, TVu 62, TVu 408, TVu 410, TVu 801, TVu 2896, TVu 3000	Singh et al. (1983)
Flower bud thrips	TVu 1509, Sanzi (Ghanaian land race)	
Leafhoppers	TVu 59, TVu 123, TVu 662,	Singh et al. (1983)
Bruchid	TVu 2027, TVu 11952, TVu 11953	Singh et al. (1983)
Drought	TVu 11979, TVu 14914, Danilla	Watanabe et al. (1997), Agbi- codo (2009)

Table 7.1 Cowpea germplasm accessions with desirable traits

*TVu* tropical *Vigna unguiculata* are germplasm lines available at the genetic resources center of IITA

*BICMV* Blackeye cowpea mosaic virus, *CABMV* Cowpea aphid-borne mosaic virus, *CPMV* Cowpea mosaic virus, *CPMoV* Cowpea mottle virus

The diversity analysis using Bayesian inference identified two distinct cowpea gene pools in Africa, one centered in western Africa and a second gene pool centered in eastern Africa. Each gene pool was most closely related to the wild cowpeas collected from the same geographic region. These results indicate that a process of divergent domestication has occurred leading to the formation of the two gene pools. Genetic variation was found to be slightly higher among the group of accessions from non-African countries than among the African accessions, and accessions from Asia and Europe were more related to those from western Africa while accessions from the Americas were closer to the eastern Africa gene pool (Huynh et al. 2013a). The overlap in distribution and the chronological sequence of domestication events is difficult to interpret precisely due to the lack of historical records of human involvement in domestication and geographical movement of early cowpea forms. However, these diversity studies are valuable in guiding introgression decisions in breeding programs and for enhancing utilization of cowpea germplasm collections.

Availability of seed for distribution depends on number of seeds, their quality, and health status. At IITA, the viability of seed under storage is maintained at 85% or above and regeneration of accessions is carried out when viability falls below this level to ensure availability of sufficient seed of good quality for distribution.

An important aspect of the conservation and distribution of cowpea seed is health testing, particularly virus indexing. Guidelines for the regeneration of cowpea were developed by Dumet et al. (2008).

The cowpea breeding program at IITA makes extensive use of the cowpea core collection from its GRC. In the last few years, accessions from the core collection have been evaluated for a wide range of traits by the Cowpea Breeding Unit. These include resistance to biotic stresses: Striga, aphids, bacterial blight, fusarium, smut, brown blotch, and several viruses. Accessions were also evaluated for grain protein and mineral content (Boukar et al. 2011) and drought tolerance (Fatokun et al. 2012a). For a number of these traits, important sources of genes were identified, and following crossing, lines were advanced for international trials in many countries of SSA where lines with superior performance have been identified and released.

Cowpea has a number of related subspecies and species, which may be valuable sources of important agronomic traits. The IITA's GRC maintains about 2000 accessions of cowpea wild relatives. The genetic resources of these wild relatives are not well represented in ex situ collections. The cultivated cowpea and some of its wild relatives belong to the section *Catiang* of the genus *Vigna*. Previous activities aimed at introgressing desirable genes through hybridization from some wild Vigna species have shown that crosses are possible only among members of this section. However, varying levels of compatibility have been observed when crosses were made between cultivated cowpea and some of these wild relatives. Some wild Vigna species such as *V. vexillata* have been found to show high levels of resistance to the major insect pests (cowpea aphid, flower bud thrips, legume pod borer, pod-sucking bugs, and cowpea bruchid) that cause immense grain-vield reduction in cultivated cowpea (Singh et al. 1992). The efforts devoted to making interspecific crosses between cowpea and vexillata did not yield any hybrid (Fatokun 2002). In addition, experience has shown that seeds of wild cowpea relatives are very small, with hard testa and unattractive color and texture. Breeders have tended to shy away from utilizing the crop's wild relatives. However, recent developments in the new genomic tools for cowpea may change this attitude.

To avoid risk of loss of valuable germplasm it is good practice to "safety duplicate" in another gene bank preferably in another country. The "safety backup" for many important collections is the Svalbard global seed vault in Norway. The current status of this vault was reviewed by Westengen et al. (2013) and 14,099 of the IITA cowpea collection are currently held at Svalbard with the great majority of these also safety duplicated in another gene bank in addition to that of IITA.

In 2008, the Global Crop Diversity Trust commissioned IITA to lead a survey and expert consultation on the development of a strategy for cowpea conservation, the results of which are summarized in Dumet et al. (2012). This highlighted the need for capacity development of national systems, particularly in SSA.

The line B301 was collected from Botswana, and it has been a major source of genes for resistance to Striga and Alectra. It has therefore been used extensively as a donor for resistance to these two parasitic flowering plants. An improved cowpea breeding line TVx 3236 with tolerance to flower bud thrips was selected from a segregating population that resulted from a cross involving TVu1509 (Singh et al. 1992).

#### 5 Major Breeding Achievements

The productivity of cowpea in SSA farmers' fields is very low with mean grain yield of less than 400 kg/ha, whereas in the USA yield is >5000 kg/ha. Several factors, notably an array of insect pests, diseases, and drought, militate against high grain yield in SSA. That cowpea is grown in SSA by peasant farmers who are resource poor and unable to procure the necessary pesticides to protect their crops in the field and grain in storage, contributes to the low productivity of the crop. Most of the breeding activities therefore focus on how to increase productivity by developing improved varieties with high yield potential and resistance to the various abiotic and biotic stresses. IITA has an active cowpea breeding program and very strong collaboration with cowpea breeders in the various National Agricultural Research Systems (NARS) of SSA. Besides, a number of advanced research institutions and universities collaborate with IITA scientists in all aspects of cowpea research. The cowpea germplasm lines available in the genetic resources center of IITA have continued to be the major source of genetic diversity upon which breeders depend for a continuous generation of improved breeding lines.

Thus far, only cultivated cowpea germplasm lines have been exploited in the development of improved varieties. Several improved cowpea breeding lines have been developed, many of which have been evaluated across various agroecologies in different countries and those with good performance and that are attractive to farmers and consumers have been released as varieties in various countries (Table 7.2). Through genetic improvement, the majority of cowpeas now adopted in SSA farmers' fields are varieties that are erect in growth habit and are day neutral. The grain yield of traditional cowpea varieties is inherently low. In addition, because they spread, farmers plant them at wide spacing thereby resulting in fewer plants per hectare as compared to the erect or semierect improved varieties. Table 7.2 lists cowpea varieties from IITA breeding nurseries that were released globally. Many of the varieties combine resistance to diseases, Striga, Alectra, and flower bud thrips.

Generally, the key achievements in breeding have focused on introgression of traits dealing with biotic and abiotic stresses to cowpea yield, combined with improved agronomic qualities of enhanced grain size, grain quality, including seed-coat color and texture, plant architecture, and time to maturity. Examples of genetic improvements in West African country breeding programs are provided by new cowpea variety releases in Burkina Faso and Senegal, with support from the US Agency for International Development (USAID) Bean/Cowpea and Dry Grain Pulses Collaborative Research Support Program (CRSP) programs. Institut Senegaliais Recherches Agricoles (ISRA) of Senegal has released a series of varieties over the past 20 years, which combines targeted biotic stress resistances with enhanced yield and grain qualities preferred by consumers and shortened maturity times to hedge against drought years. These include Melakh, Mouride, Yacine, Pakau, and in 2013 three new lines with large white grain types. These varieties have increased yields over the national average by up to about 20% and typically combine resistance or tolerance to one or more cowpea insect pests such as flower thrips or

Table 7.2 List of cowpea varieties rele	Table 7.2 List of cowpea varieties released in different countries. (Adapted and updated from Singh et al. 2002)	nd updated from Singh et al. 2002)	
Country	Variety released	Country	Variety released
Angola	TVx 3236	Argentina	IT82D-716
Australia	IT82E-18 (as Big Buff)	Belize	VITA-3, IT82D-889, IT82E-18
Benin Republic	IT81D-1137	Bolivia	IT82D-889, IT83D-442
	IT84S-2246-4,IT95K-193-12		
Botswana	ER-7, TVx 3236	Brazil	VITA-3, VITA-7, TVx 1836-013J
Burkina Faso	TVx 3236, KN-1		
	IT99K-573-2-1, IT98K-205-8	Myanmar	VITA-4 (Yezin-1)
Cameroon	IT81D-985 (BR1)	Central African Republic	VITA-1, VITA-4, VITA-7, VITA-5
	IT81D-994 (BR2)		TVx 1948-01F, IT81D-1137, IT83S-818
	TVx 3236		IT82E-18, IT81D-994
	IT88D-363 (GLM-92)		
	IT90K-277-2 (GLM-93)	Colombia	IT83S-841
		Cote d'Ivoire	IT88D-361, IT88D-363
Cuba	IT84D-449 (Titan)	Democratic Republic of Congo.	IT89KD-349, IT89KD-389
	IT84D-666(Cubinata-666)		IT89KD-355
	IT86D-314 (Mulatina-314)		
	IT86D-368 (IITA-Precoz)		
	IT86D-782 (Tropico-782)		
	IT86D-792 (Yarey-792)		
	IT88S-574-3(OR 574-3)		
Equatorial Guinea	IT87D-885	Ethiopia	TVx 1977-01D, IT82E-16, IT82E-32
Egypt	Tvu 21, IT82D-716	Fiji	VITA-1, VITA-3
	IT82D-709, IT82D-812		
	IT82E-16	Gambia	IT84S-2049 (Sosokoyo), IT83S-728-13

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Table 7.2 (continued)			
Country	Variety released	Country	Variety released
Ghana	IT82E-16 (Asontem)		
	IT83S-728-13 (Ayiyi)	Guinea Conakry	IT81D-879, IT83D-340-5
	IT83S-818 (Bengpla)		IT82E-16, IT85F-867-5 (Poku Togboi),
	TVx 1843-1C (Boafa)		IT85F-2805, IT83S-990,
	TVx 2724-01F (Soronko)		IT87S-1463, IT84S-2246-4
	IT87D-611-3 (Nhyira), IT87D-2075 (Tona)		
Guinea Bissau	IT82E-889, IT82D-889	Haiti	VITA-4, IT87D-885
India	VITA-4, TVx 1502	Jamaica	VITA-3, ER-7, IT84S-2246-4
Lesotho	IT82E-889, IT87D-885		IT84E-124
	IT82E-16, IT82E-32		
		Liberia	IT82D-889, TVx 3236, VITA-5
Malawi	IT82D-889, IT82E-16		VITA-4, VITA-7
	IT82E-25, IT99K-494-6		
		Mali	TVx 3236, IT89KD-374 (Korobalen)
Mauritius	TVx 3236		IT89KD-245 (Sangaraka), IT97K-499-
			35 (Jiguiya), IT93K-876-30, IT82D- 812, IT83S-818, IT85F-2020
		Mozambique	IT82E-16
			IT00K-1263, IT97K-1069-6
Nepal	IT82D-752 (Aakash)		
	IT82-889 (Prakash)		
		Nigeria	TVx 3236, IT81D-994, IT86D-719

Table 7.2 (continued)			
Country	Variety released	Country	Variety released
Niger	IT89KD-374, IT90K-372-1-2		IT84S-2246-4, IT90K-76
	IT97K-499-35, IT97K-499-38		IT86D-721, IT88D-867-11
	IT98K-205-8, IT99K-573-1-1, IT96D-610		IT82E-60, IT89KD-374, IT90K-277-2
			1T90K-82-2, IT89KD-
			288,119/K-499-53, 1189KD-391, IT99K-573-1-1
			IT99K-573-2-1
		Paraguay	IT86D-1010, IT87D-378-4
			IT87D-697-2, IT87D-2075
		Philippines	IT82D-889
Sierra Leone	TVx 1990-01E, IT86D-721	Senegal	TVx 3236
	IT86D-719, IT86D-1010		
	IT82E-32, TVx 3236, Tvu 1190	Somalia	TVx 1502, IT82D-889
	VITA-3		IT82E-32
		Sudan	IT84S-2163
South Africa	IT90K-59		(Daha ElGoz=Gold from Sand)
	IT82E-16 (Pannar 311)		
		Swaziland	IT82D-889 (Umtilane), IT82E-18
Sri Lanka	IT82D-789 (Wijaya)		IT82E-27, IT82E-71
	IT82D-889 (Waruni)		
	TVx 309-01EG, VITA-4	Thailand	VITA-3, IT82D-889
	TVx 930-01B (Lita)	Uganda	TVx 3236, IT82E-60
	IT86D-1010		
Suriname	IT82D-889, IT82D-789	USA	IT84S-2246-4, IT84S-2049 (for nema-
			Muc Icaiatailuc)

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Country	Variety released	Country	Variety released
Tanzania	TKx 9-11D (Tumaini)		
	TVx 1948-01F (Fahari)		
	IT82D-889 (Vuli-1), IT85F-2020	Yemen	TVx 3236, IT82D-789, VITA-5
	IT99K-7-21-2-2-1, IT99K-573-1-1		
	(Vuli AR1), (Vuli AR2)	Venezuela	VITA-3, IT81D-795
			IT82D-504-4, TVx 1850-01E
Togo	VITA-5, TVx 3236		1
	IT81D-985 (VITOCO)	Zambia	TVx 456-01F, TVx 309-01G
			IT82E-16 (Bubebe)
		Zimbabwe	IT82D-889

pathogens such as virus or bacterial blight with higher innate yield potential. In Burkina Faso, the Institut de l'Environnement et de Recherches Agricoles (INERA) has released a series of cowpea varieties with larger grain size and resistance to the parasitic weed Striga plus resistance to aphids and viruses. Interestingly, the variety Melakh, which was bred and is now widely grown in Senegal, was found to be an excellent variety in areas of Burkina Faso with similar agroecologies, and this variety has also been released by INERA in Burkina Faso. This example of a line developed in Senegal being evaluated and released in Burkina Faso demonstrates the advantages of collaborative breeding programs and material exchange between scientists from different countries of the subregion.

In the USA, cowpeas are bred to meet markets for use as both a vegetable and as a dry bean. In California, the breeding program has focused on developing improved blackeye dry grain cowpea types. The focus for cowpea improvement at UCR has been to introgress resistance to Fusarium wilt and root-knot nematodes into highyielding backgrounds with improved grain quality. A recent example is California Blackeye No. 50 (CB50) released in 2009, which has improved grain size and quality (brighter white seed-coat color) combined with resistance to *Fusarium* wilt races 3 and 4 plus strong resistance to the root-knot nematodes Meloidogyne incognita and *M. javanica* (Ehlers et al. 2009). An earlier variety of blackeye cowpea, California Blackeye No. 27 (CB27), which was released in 1999, was bred to combine *Fusarium* wilt and root-knot nematode resistance with heat tolerance (Ehlers et al. 2000). In the southern USA, a successful breeding focus has been to incorporate the persistent green seed-coat trait into high-yielding cowpea varieties for canning or as fresh-shelled peas for freezing. This breeding focus was described in Ehlers et al. (2002) and is based on the green cotyledon and green testa traits which result in a persistent green seed color. The green cotyledon trait is conditioned by a single recessive gene, with the symbol gc (Fery and Dukes 1994), and several successful varieties have been released including Bettergreen, Charleston Greenpack, Petite-n-Green, and Green Dixie (Ehlers et al. 2002).

#### 6 Specific Goals in Current Breeding

The goals of cowpea genetic improvement change with time and usually depend on agreed priorities set by stakeholders. The stakeholders include farmers, extension agents, NGOs and donor representatives, seed companies, consumers, and researchers. Current cowpea breeding goals also vary with the target production areas but are based on enhancing yield and grain quality, largely through introgression of biotic and abiotic stress tolerance and resistance. The low productivity of cowpea in the subregion is of major concern and efforts are directed primarily at addressing the factors that appear responsible. In recent times, nutrition and health conscious individuals and organizations seek cowpea varieties with higher protein content than the levels in many of the available varieties which is around 25%. Breeders

aim to develop varieties that overcome some of the identified production constraints as follows.

#### 6.1 Resistance to Insect Pests

From the seedling stage to time of harvest and even seed storage there are major insect pests that damage cowpea. Aphids *(Aphis craccivora)* attack cowpea plants in the field and if not controlled they can kill the plants especially the seedlings. They are particularly troublesome when there is a short spell of drought after seedling emergence. Earlier, a single dominant gene conferred resistance to aphids but those varieties have now succumbed to the insect. New races of the insect have evolved, so new sources of genes for resistance are being sought among cultivated and wild cross-compatible cowpea relatives. A few lines among wild cowpea have been found to be aphid-resistant. Molecular markers will be deployed to better understand the resistance and to facilitate marker-assisted selection (MAS).

In California, emphasis is also on cowpea aphid resistance and tolerance to Lygus bug using resistance and tolerance traits identified in African cowpea germplasm lines from IITA, including IT97K-556-6 for aphid resistance and IT93K-2046-1. The aphid resistance in IT97K-556-6 has been shown by quantitative trait loci (QTL) mapping to be inherited by one minor and one major QTL on cowpea linkage groups 1 and 7, respectively (Huynh et al. 2015). In California, Lygus bugs cause two types of yield loss in cowpea: First, feeding on young floral buds causes these buds to drop which drastically reduces pod set and grain yield, and second, feeding by Lygus bugs during pod and grain development leads to pitted and discolored grains. Conventional breeding of Lygus-tolerant blackeye pea is underway by pedigree selection from crosses between the African donor line and California Blackeye varieties, and field phenotyping for tolerance in insecticide protected and unprotected plot designs to assess grain yield and quality under natural Lygus bug infestation. The Lygus tolerance determinants have yet to be mapped and SNP-tagged within the cowpea genome, currently precluding MAS approaches for breeding.

A significant challenge for breeders is to better define traits for flower thrips resistance and resistance to pod-sucking bugs. Phenotypic screening efforts are underway in West African cowpea programs to provide genetic mapping data for QTL discovery for these traits. The critical yield losses caused by these insect groups make them a priority focus in cowpea breeding. In the case of flower thrips, Omo-Ikerodah et al. (2008) identified DNA markers associated with QTLs that have effects on resistance to flower bud thrips in a biparental mapping population derived from a cross that had Sanzi, the land race from Ghana, as one of the parents. Many other improved breeding lines with resistance to diseases, drought, Striga, and pests were derived from crosses that involved above listed (Table 7.1) and other germplasm lines.

### 6.2 Tolerance to Drought and Low Soil P

Cowpea is grown mainly in drought-prone areas of SSA. Compared to many other crops, cowpea is regarded as relatively drought tolerant. This notwithstanding, depending upon severity, drought can still cause yield reduction in cowpea and the variation observed among germplasm lines indicates the present level of drought tolerance in the crop can be enhanced (Fatokun et al. 2012a). Molecular markers have been identified that are associated with OTLs for drought tolerance (Agbicodo 2009). A series of QTLs have been identified following the analysis of different recombinant inbred lines (RIL) segregating for drought tolerance. Seedling-stage drought induced delayed senescence traits were identified in cowpea genotype IT93K-503-1 and others in both greenhouse and field phenotyping experiments, and reproducible OTLs for this trait were mapped in the cowpea genome (Muchero et al. 2008, 2009b). More recently, the staygreen phenomenon, a trait which enhances delayed senescence, biomass, and grain vield under drought stress, was characterized in cowpea through genetic mapping using SNP genotyping, field and greenhouse phenotyping, and linkage disequilibrium association mapping in conjunction with biparental OTL mapping (Muchero et al. 2013). Seven loci were identified; out of which five exhibited pleiotropy for delayed senescence, biomass, and grain vield. In particular, three of these putative staygreen OTLs were resolved at 3.2 cM or lower map distances and provide important targets for introgression through marker-assisted selection (MAS). In addition, co-location of these QTLs with those governing early vegetative delayed senescence provides a rapid screening approach by phenotyping plants at the seedling stage for drought response (Muchero et al. 2013). These markers will be useful in marker-assisted recurrent selection (MARS) for drought tolerance in cowpea.

Like most other legumes cowpea has a need for phosphorus to be able to form nodules adequately and fix nitrogen. The soils in SSA are generally low in phosphorus and farmers who grow cowpea usually do not apply fertilizer to their crops. Improved breeding and germplasm lines have been evaluated for tolerance to low soil P and differences were detected among them which are indications that lines with a need for low levels of soil P can be developed.

#### 6.3 Heat

Heat stress in cowpea disrupts flowering and pod set. It is an abiotic stress for which genes for tolerance are available as targets for molecular breeding approaches. A set of five heat tolerance QTLs were identified through QTL mapping in a biparental RIL population developed from the heat-tolerant variety CB27 as one of the parents (Lucas et al. 2013a). These QTLs provide resources for incorporating heat tolerance into other elite heat-sensitive cowpea varieties using MAS.

## 6.4 Dual Purpose

Although grain is the most economically important product of cowpea, in some parts of SSA such as Kenya, Tanzania, and Mozambique, young green and succulent leaves are relished as pot herbs while the haulms are a source of quality fodder for livestock in the dry savannas of West Africa. The young leaves are known to contain high levels of protein while many farmers derive income from selling dried cowpea fodder. The development of dual purpose varieties for leafy vegetables as well as for grain could meet the needs of many more people across the African region. Some attention is being devoted to selecting lines with this dual attribute that will serve as a source of grains and leaves for human food and animal feed. Improved dual purpose breeding lines have been identified in the IITA cowpea breeding program and shared with collaborators for evaluation in their countries for acceptability to farmers and consumers.

### 6.5 High Protein Content in Grains

The protein content of the grain is a major reason why cowpea is popularly consumed at home in several SSA communities. It is also why cowpea is commonly referred to as poor man's meat. In SSA, the cost of meat is prohibitive and not affordable in the quantity that is needed for a balanced diet. Depending on the variety, cowpea grains contain between 18 and 29% protein with a potential for 35% (Duke 1981). Among 79 cowpea varieties studied by Evans and Boulter (1974), protein content ranged from 21 to 34%. In another study involving 100 lines, Nielsen et al. (1993) found that protein content ranged from 22.9 to 32.9% with a mean of 28.6%. Boukar et al. (2011) identified the following germplasm lines as having high protein content-TVu 408, TVu 526, TVu 1820, TVu 2356, TVu 2508, TVu 2723, TVu 2880, TVu 3638, TVu 8810-1—which could be used in the development of improved breeding lines. The cowpea protein consists of 90% salt-soluble globulins and 10% water-soluble albumins (Duke 1981). The anti-nutritional factors found in cowpea grains such as hemagglutinins and trypsin inhibitors are heat labile and can be inactivated easily by heating, thus making cowpea protein readily digested and absorbed. This makes cowpea protein suitable for infants and formulations of baby foods containing cowpea should be encouraged and commercialized in SSA.

### 6.6 Resistance to Diseases

Many diseases afflict cowpea plants in the field. There are fungal, bacterial, and viral diseases that attack the plants. Since farmers do not apply chemicals to protect their cowpea crops the diseases are best controlled by planting varieties that are resistant. Among the most devastating of fungal diseases is ascochyta blight caused

by *Ascochyta phaseolorum* Sacc., which is seed-borne (Emechebe and Shoyinka 1985). The disease causes severe defoliation and lesions on stem and pods and can lead to the death of susceptible plants. Line TVu 11761 was identified as a potential source of resistance to this disease (Singh et al. 2002). Brown blotch is another major fungal disease of cowpea in SSA. The causal organism is *Colletotrichum capsici* (Emechebe and Florini 1997). All plant parts above soil level show symptoms of the disease in susceptible lines. Such symptoms include failure of seeds to germinate, damping off of seedlings, girdling of stem and branches, and flower abortion, among others. Treating seeds with fungicides, such as benomyl or carbendazim, before sowing helps reduce incidence of the disease. However, the development of resistant varieties appears the most attractive option for most SSA farmers. Scab, smut, and Septoria caused by *Elsino phaseoli, Protomycopsis phaseoli,* and *Septoria vignicola*, respectively, are also important fungal diseases of cowpea and cause yield reductions.

The most important bacterial disease of cowpea is bacterial blight caused by *Xanthomonas campestris* pv. *vignicola*. It is a disease that has been reported on cowpea in different parts of the world. Disease symptoms include large irregular foliar lesions with yellow margins, stem cankers, and preemergence and postemergence seedling mortality (Emechebe and Florini 1997). Some germplasm lines have been found that are resistant to the disease and the genes conferring resistance have been transferred to several improved varieties. However, in view of the high rate of mutation in the bacterium, it is necessary to continue identifying additional sources of resistance.

Many viruses attack cowpeas and these can only be controlled by sowing resistant varieties. The cowpea aphid-borne mosaic virus (CABMV) and bean common mosaic virus (BCMV-BIC), both of which are seed-borne and occur worldwide, are two economically important cowpea pathogens (Huguenot et al. 1997). In addition to these two viruses, Hampton et al. (1997) reported that cucumber mosaic cucumovirus (CMV), cowpea mosaic (CPMV) and cowpea severe mosaic (CPSMV) comoviruses make up the most devastating viruses of cowpea. They are also seedborne. Worrisome in cowpea production is the occurrence of mixed infections of these viruses. Mixed infections cause drastic disease symptoms and even death of plants in the field. Since there are no chemicals to control these pathogens the development of resistant varieties is the only option for their control. Sources of genes for resistance to several of the viruses have been identified and many have been incorporated in released varieties.

A recent review of the important biotic stress resistance traits with molecular marker-based associations is provided in Huynh et al. (2013b). Genetic map positions in the cowpea genome and linked, flanking SNP markers have been identified for resistance to root-knot nematodes, *Fusarium* wilt, *Macrophomina phaseolina* (ashy stem blight or charcoal rot), bacterial blight, several cowpea viruses (cowpea mosaic virus, cowpea severe mosaic virus, blackeye cowpea mosaic potyvirus), foliar thrips, cowpea aphid, and parasitic weed *Striga gesnerioides*. These resources have helped to better define the breeding targets in several US, African, and Asian cowpea-breeding programs. In California, the focus remains on *Fusarium* wilt and root-knot nematode resistance.

# 6.7 Large Seed Size

In recent times, consumers have shown a preference for cowpeas with large grain size. Breeding activities have been initiated towards developing varieties that meet this preference. Experience has, however, shown that small seed size in cowpea is dominant to large seed size hence there is need to embark on backcrossing to transfer the genes for large seed size to preferred varieties.

# 6.8 Adaptation to Intercropping

Most of the improved cowpea varieties that have been released have erect or semierect growth habit. They are, therefore, well adapted to sole cropping. Many farmers in SSA still prefer to intercrop cowpea with sorghum, millets, and other cereals. This habit is difficult to change because each farmer has access to a small land area where he plants all the crops that provide the family with food and some income. When intercropped, cowpea plants are readily shaded by the taller cereals. The traditional varieties which farmers grow under this cropping system spread on the ground and remain there until the cereals are harvested when they now receive more sunlight, flower, and set pods. New varieties can be developed that can adapt to intercrop conditions such that their flowering and podding are not adversely affected by shading.

### 6.9 Striga Resistance

In Africa, most programs are targeting Striga resistance, in combination with drought-tolerance traits as well as virus and insect resistance. For example, in Burkina Faso, any new variety released must contain Striga resistance (Drabo personal communication 2014).

# 7 Breeding Methods and Specific Techniques

Cowpea breeding methods are similar to those of other self-pollinated crops such as peanut, soybean, wheat, and barley. Cowpea breeders depend upon the germplasm available in the different collections described earlier. The IITA GRC is a source of genes of interest in both domesticated and wild cross-compatible cowpea relatives. Several decades of breeding effort by different institutions also provide the chance to build on the numerous improved breeding lines. The IITA cowpea breeding nursery distributes many breeding lines annually for testing, adoption as released varieties or as parents to be used in the importing countries' breeding programs. Most

cowpea breeding activities are focused on development of improved varieties with farmers' and consumers' preferred traits. Breeding programs include the development of populations segregating for desirable traits and from which selections are made for both simple and complex characteristics (e.g., disease resistance, drought tolerance, grain yield). Additional activities include determination of inheritance, creation, and evaluation of new genetic variability and production of specific genotypes.

Cowpea variety development programs follow the conventional breeding steps for self-pollinated crops (pure-line breeding, pedigree breeding, single-seed descent, etc.). Parents characterized by important traits of interest are chosen and used to generate genetically variable populations through artificial hybridization followed by selection. Appropriate selection pressure that favors identification of desired traits such as imposition of disease pathogens, insect pests, drought, heat, low phosphorus, etc. is exerted on the segregating populations. In addition, the lines to be selected are assessed for agronomic and quality characteristics. As segregating populations advance, homozygosity also increases. The lines selected at this stage on the basis of good performance are homozygous and ready for replicated performance trials across multiple locations and cropping seasons. Seeds of the best one to five percent of lines with superior agronomic performance or quality characteristics are multiplied under controlled conditions for variety release and to maintain their genetic purity.

As in all breeding programs, the exact techniques used in cowpea cultivar development vary widely. In the case of simply inherited traits such as disease resistance, the backcross method is used to introgress the associated gene(s) into existing cultivars that are lacking the trait. When several traits are being moved from two or more parents, hybrids from single, double, three-way, or other complex crosses are advanced through any of several methods that support the acceleration of homozygosity. Commonly used procedures include bulk population, the pedigree method, single-seed descent and modifications of these methods as necessary. The ultimate product of all the methods is a group of homozygous lines, which only vary in the time frames during which selection pressures are applied. Cowpea breeders have relied primarily on pedigree breeding to combine favorable traits from two parents and by recurrent backcrossing to introgress a major trait from a donor line into an elite recurrent parent which is usually a preferred current variety. Considerable success has been achieved by both approaches to improve cowpea grain quality and grain and biomass yield by the combining of traits determining grain size, texture and color, drought and heat tolerance, and resistance to a range of pests and diseases.

Cowpea being primarily self-pollinated, hybridization between parents usually involves emasculation (removal of anthers) from flowers of one parent (female or seed parent) and artificial transfer of pollen from the alternate (male) parent. Cowpeas are easier to cross than many other grain legumes due to the large size of the flowers and to the fact that the keel is straight, beaked, and not twisted. Cowpea flowers have few floral nodes per raceme and tend to have a lower rate of abortion than many other species. Rapid and effective methods of hand emasculating and crossing cowpeas were described by Myers (1996).

Although emasculation and pollination can be carried out all day, hybridization of cowpea is less effective when the temperature is high. Night temperatures greater than 20 °C reduce microsporogenesis leading to formation of indehiscent anthers and pollen with low viability (Warrag and Hall 1983; Ahmed et al. 1992). Thus, moderate temperature and increased humidity appear to increase the percentage of pod set following hand-emasculated crosses. In general, the rate of such pod set-ting varies enormously with environmental conditions, genotype, and manipulative techniques.

In IITA, crossing activities are conducted in mesh houses or greenhouses to allow: (1) good control of insect pollen vectors, major pests, and diseases; (2) good plant development (water, fertilizer, etc.); and (3) easy manipulation of plants during crosses. When planted in the screenhouse, most cowpea lines tend to climb and stakes are, therefore, needed which also help position the flowers at a height that is comfortable for the person making the crosses. In the planning of crossing activities, photosensitivity and number of days to flowering of the parental genotypes are always considered to avoid asynchronous flowering. Use of different planting dates, removal of developing flowers and fruits, using black polythene to cover plants from afternoon to next morning for a number of days (to reduce length of days) and the use of cuttings are some of the techniques to ensure synchronous flowering by the parental lines.

A hybrid plant reproduces to form a segregating population (segregation and recombination of genes). Development of a new variety usually involves inbreeding of a segregating cowpea population for three to seven generations, during which selection is applied and individuals in the population become increasingly homozygous (true breeding). Narrow crosses between closely related parents normally require fewer generations of inbreeding than wide crosses (very different parents) to become true breeding.

In IITA, screenhouses are used for rapid advancement of cowpea breeding populations with the possibility of 3-4 generations per year. When photoperiod-sensitive parents are involved in the crosses, breeding programs can only have 2-3 generations per year. Generally, a high level of homozygosity is attained from  $F_5$  to  $F_7$  since variation within rows derived from single selected plants will be relatively small. Bulked  $F_c/F_\tau$  populations are grouped according to maturity, plant type, seed quality, and resistance to major pests and diseases. Farmers' involvement through participatory variety selection is encouraged. Homozygous materials are evaluated in initial evaluation trials (IET) at 2-3 locations without replication. Selected lines from IET are tested in preliminary variety trials and advanced variety trials (AVT) in 3 replications across 4 locations representing different agroecological zones: (a) Ibadan (7° 25' N, 3° 37' E) derived savanna with bimodal rainfall (1500 mm); (b) Samaru (11° 10' N, 7° 38' E) in the northern Guinea savanna (1000 mm rainfall); (c) Minjibir (12°08' N, 8°40' E) in Sudan savanna with about 700 mm rainfall; and (d) Toumnia (13° 58' N, 9° 01' E) or Malamadori representing the Sahel with about 350 mm rainfall. High-performing lines from AVT are compiled into cowpea international trials which are sent to collaborators for testing in their local environments. The best lines are released as varieties or used in their breeding programs for further improvement.

In some cases, this traditional approach to cowpea breeding which is based on phenotypic selection of progenies carrying the desired traits is still continuing in most programs. This is particularly the case where molecular markers for genotyping are unavailable, or where the marker-trait locus associations have not been identified. The advent of new marker technologies for genotyping, explained in detail in the next few paragraphs, is expected to facilitate a more efficient breeding approach than the previous reliance on phenotypic selection which is timeconsuming. Thus, marker-assisted pedigree breeding (MAPB) and marker-assisted backcrossing (MABC) have been incorporated into several cowpea breeding programs by taking advantage of the new marker-driven selection tools. For MAPB, the design of an "ideotype" as the breeding goal is a useful first step in targeting the genotype of the combined set of favorable alleles to be donated by the two parents. Ehlers et al. (2012) provided a detailed example of this approach based on experience with two African cowpea populations derived from "elite" × "elite" crosses produced by ISRA, Senegal (Mouride×IT84S-2246-4) and INERA, Burkina Faso (IT93K-503-1  $\times$  IT84S-2246-4). The ideotype design is the full combination of the target OTLs all in the homozygous condition for the favorable alleles. Molecular breeding software programs (see next section) can then be used to analyze the genotypic data to identify and rank families or individual plants in progenies with the appropriate molecular scores (ideotype = maximum score or 100%).

In MABC, the marker genotyping application has two components, namely "foreground" selection for the presence of the target trait QTL using linked flanking markers at the trait QTL, and "background" selection using genome-wide markers to select for individual plants with the highest recurrent parent genotype profile. In the California Blackeye cowpea-breeding program, transferring aphid, nematode, and *Fusarium* wilt resistances into improved versions of the current varieties CB27, CB46, and CB50 is being achieved with a MABC approach. For example, the QTLs identified by Pottorff et al. (2012, 2014) for resistance to *Fusarium* wilt races 3 and 4 are being transferred through MABC into new lines. CB46 is race 3 resistant but lacks race 4 and aphid resistances, so markers for the resistance loci can be used to select the donated favorable alleles for resistances to aphid and *Fusarium* race 4 and also confirm the presence in the background of the favorable haplotypes for *Fusarium* race 3 and root-knot nematode resistances. In Africa, several breeders have started using MABC to add Striga and aphid resistances into elite local varieties through collaborations with advanced research institutions.

A third breeding approach is MARS, which is being tested in four African cowpea breeding programs at INERA, Eduardo Mondlane University, IITA, and ISRA in partnership with UCR. MARS has been used with success in some cereal breeding programs (Charmet et al. 2001). The goal of MARS is to combine multiple favorable traits from two parents in a complementary manner, in which early generation progenies with partial combinations of the full set of traits are intercrossed for as many as three cycles of recombination to maximize the pyramiding of QTLs for multiple traits into advanced breeding lines (Charmet et al. 2001). This approach overcomes the limitations which occur in pedigree breeding with manageable progeny sizes, especially when the favorable alleles at several QTLs need to be combined. The geometric increase of a target locus becoming homozygous for the unfavorable allele with each generation, renders individual homozygous positives for all target QTLs rare or absent (Ehlers et al. 2012).

A series of MARS populations were developed from crossing elite parents with complementary trait sets (Suvita 2×IT97K-499-35, IT84S-2246-4×IT98K-1111-1, CB27×IT97K-499-35, IT93K-503-1×Mouride) relevant to each target environment in the four SSA cowpea breeding programs, with a focus on drought tolerance, seed size, and color, yield gain and biotic stress resistance (Striga and rootknot nematode). About 300 F2 seeds were derived from each biparental cross followed by selfing to produce about 300  $F_{2:3}$  or  $F_{2:4}$  families. Each of the  $F_{2:3}$  or  $F_{2:4}$  populations was phenotyped in different field sites for yield and other agronomic traits. Leaf samples of  $F_2$  individuals or  $F_{2\cdot3}/F_{2\cdot4}$  bulks from the field were genotyped by KBioscience (www.lgcgenomics.com) using a customized list of polymorphic SNPs generated by SNP Selector (www.breedit.org) based on distance in cM between genome-wide markers, and between markers at known trait positions. QTLs were discovered at F2:3 or F2:4 generations. For recombination cycles, QTL indices were computed by OptiMAS (http://moulon.inra.fr/optimas/), and members of highest QTL-index families were then genotyped, selected, and intercrossed to recombine favorable alleles. The outcome of this MARS breeding plan is that advanced lines have been produced that are homozygous for the favorable alleles of the target QTLs for yield, seed size, heat tolerance, staygreen, and resistance to root-knot nematodes and Striga. Currently the 20-30 advanced lines with the OTLs confirmed by SNP-genotyping are in a 2-year performance testing phase in production field trials, from which new variety releases are expected, and which will provide elite lines for use as parents in further cowpea improvement.

#### 8 Integration of New Biotechnologies in Breeding Programs

High levels of resistance to several insects and diseases exist in wild *Vigna* species, but cross incompatibility with cultivated lines is the biggest bottleneck limiting their exploitation for cowpea improvement through conventional breeding. Biotechnological approaches were suggested as ways to overcome these limitations. If useful genes can be isolated from wild *Vigna* species, a genetic transformation system is a prerequisite for their deployment in cultivated cowpea. Initial genetic transformation efforts using *Agrobacterium tumefaciens* as the gene vector were conducted by Garcia et al. (1986, 1987). This was followed by embryo imbibition with or without subsequent electroporation (Akella and Lurquin 1993; Penza et al. 1992).

In all these cases, the development of transgenic cowpea calli or chimeric plantlets from leaf discs, axilliary buds, or embryos were obtained but no mature transgenic plants could be generated. Microprojectile bombardment (biolistics) was also used by several researchers to achieve the introduction of foreign DNA into cowpea leaf tissues and embryos and to obtain high levels of transient expression of the ß-glucuronidase transgene, but regeneration of plantlets from the transformed cells was not possible (Kononowicz et al. 1997). The development of transformation systems using either microprojectile bombardment or *Agrobacterium* cocultivation gave some promising results with the coculturing of de-embryonated cotyledons with *A. tumefaciens* resulting in selection of four plants on hygromycin (Kononow-icz et al. 1997). This last approach helped in the development of a system that was the first to be reproducible and that obeys Mendelian rules of inheritance (Popelka et al. 2006). Critical features of this system include suitable explants from cotyle-donary nodes or embryonic axes and a tissue-culture regime without auxins, but which includes a cytokinin at low levels during shoot initiation. There are now several reports showing experimental evidence for reproducible gene transfer to cowpea including genes for resistance to pod borer (Higgins et al. 2012) and cowpea weevil (Solleti et al. 2008) as well as for weed control (Citadin et al. 2013) and a range of model genes to evaluate the technology (Citadin et al. 2011).

The development of cowpea with a *Bt* gene was carried out successfully in the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia. Field testing of these lines has been carried out in Nigeria, Burkina Faso and Ghana in a Pod Borer-Resistant (PBR) Cowpea Project led by the African Agricultural Technology Foundation (AATF) and supported by USAID. A selected *Bt* cowpea line with near complete resistance to Maruca pod borer is being used to introgress the *Bt* gene into farmer preferred varieties. Selection using molecular markers will expedite the rapid development of cowpea varieties with resistance to Maruca and incorporating other traits preferred by farmers. Encouraging results have been obtained by the relevant breeding programs in SSA, and the AATF is working towards commercializing and making the PBR cowpea available to farmers in SSA.

In addition to genetic transformation, molecular breeding for cowpea is also well advanced but requires a genotyping capability that is cost effective and efficient so that genotyping results can be generated and interpreted quickly enough to make breeding selection decisions for crossing or targeted phenotyping. The genomics revolution has had important positive impacts on modern cowpea breeding. SNP genotyping platforms were developed based on genic SNPs developed from expressed sequence tags. They have high rates of polymorphism among the primary cowpea germplasm sources in the target breeding programs. Muchero et al. (2009a) developed a 1536-SNP Illumina GoldenGate assay which included about 1100 genetically mapped SNPs. This platform was used for extensive QTL discovery and to develop a consensus genetic map for cowpea constructed from six RIL populations (Muchero et al. 2009a).

The cowpea consensus map has been improved several times (Diop et al. 2012; Lucas et al. 2011) and the current version constructed from 11 RIL populations and two breeding populations is available online via HarvEST:Cowpea (Close and Wanamaker 2001). The 1536-SNP platform and the genetic maps were used to identify the genomic positions of the many QTLs for important cowpea traits described earlier. The QTL discovery has been based on extensive phenotyping for agronomic as well as abiotic and biotic stress resistance traits in the genotyped biparental RIL populations and cowpea diversity panels (Huynh et al. 2013b; Muchero et al. 2013; Lucas et al. 2013b; Pottorff et al. 2014). The SNP calls associated with the favorable alleles at each QTL provide the marker haplotypes needed for positive trait selection for use in foreground selection. The genome-wide markers across the 680 cM genetic map, spaced on average, every 0.6 cM, provided the resource for background selection across the genome in the MABC breeding efforts.

Application of the SNP marker resource for cowpea breeders was further advanced by converting the mapped SNP markers to a flexible genotyping platform, using the Kompetitive Allele Specific PCR (KASP) technology of LGC Genomics (formerly KBiosciences). This platform enables choice in which and how many SNP markers the breeder would like to use in a given breeding project and flexibility in the number of DNA samples per genotyping run. This translates into a more costefficient genotyping capability than the fixed GoldenGate platform. A new 60,000 SNP genotyping platform has been developed using the Illumina Infinium iSelect technology (Close et al. 2015). The cowpea breeding programs with a strong focus on molecular breeding have taken advantage of outsourcing the genotyping work. In the programs at UCR, INERA, SARI, Eduardo Mondlane University, IITA, and ISRA, the molecular breeding is underpinned by the outsourcing operation (Ehlers et al. 2012). Leaf samples (leaf punches placed in 96-well plates and dried with silica gel for preservation) are collected in the greenhouse or field, then expressshipped to the genotyping facility (LGC Genomics in the UK or the USA for the KASP platform), where DNA is extracted and genotyped with a preselected subset of informative SNP markers. The data are made available usually within 4 weeks, which can then be interpreted to make breeding selection decisions for crossing or progeny selections.

Improvements in the workflow for molecular breeding have included development of some in-house software programs for SNP selection and data analysis. They are used in conjunction with the CGIAR IBP (Integrated Breeding Platform) Breeding Management System software programs for analyzing the genotype and phenotype data for QTL tracking and in the MARS, MAPB, and MABC breeding schemes.

#### 9 Seed Production

Improved seeds constitute one of the most important farm inputs needed for increasing agricultural production. High quality seeds of improved varieties should, therefore, be available to farmers to ensure sustainable crop production. However, it has been observed that there is not much enthusiasm on the part of large seed companies to engage in grain legume seed enterprises because of low margin of profit, as farmers could recycle their own saved seed for up to 5 years (Abate et al. 2012). These authors reported that more than 70% of farmers use their own saved seed across the thirteen countries where the Tropical Legumes II (TL II) project is being implemented. Kenya is the only exception where saved seed supplied just over 34% of farmers' needs. In Mozambique, only 12% of adopters of improved cowpea varieties bought improved seed from agro-dealers, with the rest using their own recycled seed (Fatokun et al. 2012b).

Strategies to ensure some level of production of good seeds include strengthening community-based and farmer-level seed production systems. Generally, the National Agricultural Research Institutes are responsible for the production of breeder and foundation seeds. Individual farmers and farmers' groups, agricultural universities, and small private seed companies also produce foundation seeds. The private sector and farmers' groups are generally responsible for certified seed production. In some countries, small-scale farmers and the public sector with the use of contract farmers also produce certified seeds. Other quality seeds are also produced by farmers' associations generally supported by NGOs. In Nigeria for example, breeder and foundation seeds of cowpea are produced mainly by research institutes such as IITA and the Institute for Agricultural Research while certified seeds are produced by seed companies (e.g., Maina Seeds, Alheri Seeds, Jikur Seed), Agricultural Development Projects, some NGOs, out-growers, and National Program for Food Security community seed growers.

Access to quality seed is a crucial factor in the adoption of improved technologies by farmers. Use of improved, modern varieties was generally low across some SSA countries following baseline studies conducted at the beginning of phase I of the TL II project (Abate et al. 2012). It was also reported by these authors that unavailability of improved seed and, in some cases, lack of access to credit were major bottlenecks for improved variety adoption. Fatokun et al. (2012b) noted that in Mozambique over 70% of non-adopters of improved cowpea indicated lack of access to improved seeds as the major constraint. In Nigeria, 71% of male-headed households complained about lack of cash availability to purchase seeds and other inputs. In their investigations of the cowpea seed subsector in Nigeria, they found that access to quality, and affordability, of improved seeds was of concern. Most cowpea producers (60% in Kano and 86% in Borno States of Nigeria) get information on availability of seeds of improved varieties through Ministries of Agriculture extension agents. Few producers get information from seed companies, research institutes' staff, fellow farmers, and NGOs in Kano State. These observations have implications for the adoption of new varieties.

The cowpea seed system receives very little attention from the formal seed industry consisting of public sector research institutions, seed companies, and organizations (the National Agricultural Seed Council) in almost all the countries of SSA. A larger proportion of the smallholder farmers' seed needs are therefore met by the informal sector. With the low level, or even absence, of the involvement of large-scale seed companies, it is important to strengthen the informal sector and use it as a means of providing resource-poor farmers with quality seeds of improved varieties of crops at affordable prices. Concerted efforts are being made to promote the dissemination of seeds of improved cowpea varieties in many SSA countries. Farmer-to-farmer seed diffusion was jointly promoted by IITA, IAR and Kano State Agricultural and Rural Development Authority to disseminate new cowpea varieties (IT90K-277-2 and IT93K-452-1) in the late 1990s. About 8 kg of cowpea seeds were given to each primary farmer selected to establish a 0.4 ha of seed farm. The 300 kg of seeds produced by each of the primary farmers (foundation seeds step 1 = FS 1) were distributed/sold to 12 secondary farmers. Each secondary farmer in turn established a 0.4 ha seed farm (FS 2) and the 300 kg produced by each farmer gave a total of 3600 kg which was enough to plant  $1444 \times 0.4$  ha of commercial crop (Utoh and Ajeigbe 2009).

This strategy was found to be faster and cheaper for seed dissemination than previously used methods. In Nigeria, community seed production was promoted by the National Program for Food Security. Farmers were trained in seed production strategies and linked to seed companies and research institutions for renewal of seed stocks. The role of extension agents is very important in seed production and adoption of new improved varieties. In southern Borno State, the Promoting Sustainable Agriculture in Borno State program implemented by IITA and national partners selected and trained seed producers and assisted them with establishing community-based seed multiplication schemes in 30 communities that covered three agroecological zones (Fatokun et al. 2012b). The TL II project helped to establish an awareness creation system for improved varieties through field days, demonstrations, seed fairs, agricultural shows, dealing with farmers' research groups/ farmer field schools, and distribution of small packs of seed samples. The small seed pack strategy, developed in partnership with the private sector, was helpful in getting seeds of improved varieties to many more farmers. Marketing seed in small quantities of 1- or 2-kg packs that are within the reach of smallholder farmers was found to be both profitable to a small private seed company and attractive to farmers (Fatokun et al. 2012b). Over 12,000 farmers were reached with this method over a 3-year period (2010-2012) and this further popularized some improved cowpea varieties.

Improved market linkages have encouraged seed producers to increase seed production to supply a growing market. Market development for cowpea seed resulted in increased production and sales of cowpea, making significant contributions to improving livelihood and poverty reduction. Over 188 MT of seed was sold by seed producers in Nigeria, 31.5 MT in Mali, and 93.7 MT sold in Niger, within the first phase of TL II project (Fatokun et al. 2012b). This market is now established and paying good prices for seeds, a situation likely to be sustained.

Based on our experience with the TL II project, strengthening of communitybased organizations, in particular the farmers' groups and associations, through training and support for quality certified and foundation seed production, reinforced with postharvest processing, storage, distribution, and marketing will ensure that quality the seed of newly developed and released varieties will be available to a majority of farmers.

Another major constraint to seed trade in cowpea is the susceptibility of the seeds to the bruchid weevil (*Callosobruchus maculatus*). This insect is capable of destroying all seed stored by farmers within 6 months. No cowpea variety has resistance to this insect pest. In order to protect the seeds from damage by the insect, farmers use a number of methods which are mostly not effective. In recent times, researchers at Purdue University, USA have come up with a simple and cheap

chemical-free method for storing cowpea seeds. The technology is commonly referred to as Purdue Improved Cowpea Storage (PICS). With this method farmers and seed retailers are able to store cowpea seeds longer than hitherto and this should encourage them to produce and keep seeds for planting in the following cropping season.

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