Chapter 10
Cowpea, a Multifunctional Legume

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Abstract Cowpea [*Vigna unguiculata* (L.) Walp.] is an important warm-season legume grown primarily in the semi-arid tropics. The majority of cowpea is grown by subsistence farmers in west and central sub-Saharan Africa, where its grain and stover are highly valued for food and forage. Despite its economic and social importance in developing parts of the world, cowpea has received relatively little attention from a research standpoint. To a large extent it is an underexploited crop where relatively large genetic gains can likely be made with only modest investments in both applied plant breeding and molecular genetics. A major goal of many cowpea breeding and improvement programs is combining resistance to numerous pests and diseases and other desirable traits, such as those governing maturity, photoperiod sensitivity, plant type, and seed quality. New opportunities for improving cowpea exist by leveraging the emerging genomic tools and knowledge gained through research on other major legume crops and model species. The use of marker-assisted selection and other molecular breeding systems for tracking single gene traits and quantitatively inherited characteristics will likely increase the overall efficiency and effectiveness of cowpea improvement programs in the foreseeable future and provide new opportunities for development of cowpea as a food staple and economic resource.

10.1 Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the most important food and forage legumes in the semi-arid tropics that includes parts of Asia, Africa, Southern Europe, Southern United States, and Central and South America (Singh 2005; Timko et al. 2007a). It is truly a multifunctional crop, providing food for man and livestock and serving as a valuable and dependable revenue-generating commodity for farmers and grain traders (Singh 2002; Langyintuo et al. 2003). The cowpea plant is a herbaceous, warm-season annual requiring temperatures of at least 18 °C throughout all stages of its development and having an optimal growing

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temperature of about 28 °C (Craufurd et al. 1997). Seeds of cultivated cowpea types weigh between 80 mg and 320 mg and range in shape from round to kidney-shaped. The seed pods contain between eight and 18 seeds per pod and are cylindrical and curved or straight. The seed coat varies in texture (e.g., smooth, rough, or wrinkled), color (e.g., white, cream, green, buff, red, brown, black), and uniformity (e.g., solid, speckled, or patterned). Seeds of the most well-known cowpea types, such as "blackeye pea" and "pinkeye," are white with a round irregularly shaped black or red pigmented area encircling the hilum that gives the seed the appearance of an eye.

Following germination, emergence of the cowpea seedling from the soil is considered epigeal. This type of emergence makes the seedling more susceptible to injury since the plant cannot regenerate buds below the cotyledonary node. The first two true leaves are opposite, sessile, and entire, whereas the remaining leaves are alternate, petiolate, and trifoliate. Structure of the mature plant varies depending on genotype, growth temperature, and the photoperiod in which the plant grows. The major plant growth habits are erect, semi-erect, prostrate (trailing), or climbing. Most cowpea plants are indeterminate in growth habit. However, some of the newly developed early maturing varieties have a determinate growth phenotype. Early flowering cowpea genotypes can produce a crop of dry grain in 60 days, while longer season genotypes may require more than 150 days to mature, depending on photoperiod.

According to Fery (1985), the inflorescence is axillary and formed of a peduncle 10 to 30 cm long, at the end of which there is a rachis with each node bearing a pair of flowers and a cushion of extrafloral nectaries that contribute to the attraction of insects. Cowpea primarily is self-pollinating. In cultivated forms, the flowers open at the end of the night and close in late morning, with the dehiscence of the anthers taking place several hours before the flower opens. Although considered autogamous, outcrossing rates as high as 5% have been recorded, and therefore some care needs to be taken to avoid outcrossing during the production of breeder and foundation seeds. Two or three pods per peduncle are common, and often four or more pods are carried on a single peduncle if growing conditions are very favorable. The presence of these long peduncles is a distinguishing feature of cowpea, and this characteristic also facilitates hand harvesting.

Cowpea is primarily a short day plant or, in some instances, day-neutral (Ehlers and Hall 1996; Craufurd et al. 1997). Floral bud initiation and development is sensitive to photoperiod in many cowpea accessions, and in some genotypes the degree of photoperiod sensitivity (i.e., the extent of delay in flowering) is influenced by temperature (Wein and Summerfield 1980; Ehlers and Hall 1996). In West Africa, selection for differing degrees of photosensitivity or differences in extent of juvenile growth has occurred in different climatic zones resulting in genotypes where pod ripening occurs at the end of the rainy season in a given locale, regardless of planting date that often varies due to the variable onset of wet seasons (Steele and Mehra 1980). This attribute allows pods to escape damage from excessive moisture and pathogens.

10.2 Economic, Agronomic, and Social Importance

V. unguiculata is known by a variety of names world-wide, with cowpea being among the most prevalent in the literature. In the English speaking parts of Africa it is known as cowpea whereas in the Francophone regions of Africa, the name "niébé" is most often used. Local names for cowpea also include "seub" and "niao" in Senegal, "wake" in Nigeria, and "luba hilu" in the Sudan. In the United States, it is typically referred to as blackeye beans, blackeye peas, and southern peas. On the Indian subcontinent it is called "lobia" and in Brazil it is "caupi."

The seed, or grain as it is sometimes referred to, is the most important part of the cowpea plant for human consumption. The seeds are most often harvested and dried for storage and consumption at a later time, either after cooking whole or after being milled like a flour product and used in various recipes (Nielsen et al. 1997; Ahenkora et al. 1998). As such, cowpea plays a critical role in the lives of millions of people in the developing world, providing them a major source of dietary protein that nutritionally complements low-protein cereal and tuber crop staples. The nutritional profile of cowpea grain is similar to that of other pulses with a relatively low fat content and a total protein content that is two- to fourfold higher than cereal and tuber crops. Similar to other pulses, the storage proteins in cowpea seeds are rich in the amino acids lysine and tryptophan when compared to cereal grains, but low in methionine and cystiene when compared to animal proteins. Total seed protein content ranges from 23% to 32% of seed weight (Nielson et al. 1993; Hall et al. 2003). Cowpea seeds are also a rich source of minerals and vitamins (Hall et al. 2003) and among plants have one of the highest contents of folic acid, a B vitamin necessary during pregnancy to prevent birth defects in the brain and spine (http://www.cdc.gov/ncbddd/folicacid/).

In the southeastern parts of the United States, portions of West Africa, Asia, and in the Caribbean, consuming fresh seeds and green pods is preferred to the cooked dry seeds (Nielsen et al. 1997; Ahenkora et al. 1998). In many parts of Africa and Asia, in addition to the seeds, the fresh or dried leaves are also consumed as a side dish or as part of a stew and provide significant nutritional value. In addition to human consumption, cowpea leaves and stems (stover) are also an important source of high-quality hay for livestock feed (Tarawali et al. 1997, 2002). Cowpea fodder plays a particularly critical role in feeding animals during the dry season in many parts of West Africa (Singh and Tarawali 1997; Tarawali et al. 1997, 2002). Although protease inhibitors have been found in the seed, the use of cowpea grain does not apparently present any serious nutritional problems in animal nutrition and has been used an alternative to other more costly grain protein sources of animal feed (Singh et al. 2006).

Dry grain production is the only commodity of cowpea for which production estimates are generated on a worldwide basis. According to the United Nations Food and Agricultural Organization (FAO), approximately 4 million metric tons (mmt) of dry cowpea grain are produced annually on about 10 million ha worldwide (www.faostat.fao.org/faostat). Worldwide cowpea grain production has gone

from an annual average of about 1.2 mmt in the 1970s to approximately 3.6 mmt per annum (during the five-year period spanning 1998 to 2003). This increase in production is partly tied to long-term drought in the Sahelian zone of West Africa that has resulted in many farmers in this part of Africa shifting their production to cowpea because of its drought tolerance (Duivenbooden et al. 2002). Singh et al. (2002) suggest that cowpea production and acreage are actually higher than FAO estimates, with worldwide production of 4.5 mmt on 12 to 14 million ha, because the FAO estimates do not include the acreage and production figures in Brazil, India, and some other countries.

About 70% of cowpea production occurs in the drier Savanna and Sahelian zones of West and Central Africa, where the crop is usually grown as an intercrop with pearl millet (*Pennisetum glaucum*) or sorghum (*Sorghum bicolor*). In these regions, cowpea is less frequently planted in monoculture or intercropped with maize (*Zea mays*), cassava (*Manihot esculenta*), or cotton (*Gossypium* sp.) (Langyintuo et al. 2003). Other important cowpea production areas include the lower elevation areas of eastern and southern Africa, low elevation areas in South America (particularly in Peru and northeastern Brazil), parts of India, and the southeastern and southwestern regions of North America.

Nigeria is the largest producer and consumer of cowpea grain with approximately 5 million ha under cultivation with an annual yield estimate at 2.0 mmt (Singh et al. 2002). After Nigeria, Niger and Brazil are the next largest producers with annual yields estimated at 650,000 mt and 490,000 mt, respectively (Singh et al. 2002). Cowpea grain production in Central America and in east and southern Africa are likely underestimated since these regions also produce significant quantities of common beans (*Phaseolus vulgaris*) and the two are often not distinguished during collection of production statistics. Commercial trading of dry cowpea grain and hay are particularly important to the local and regional economies of West Africa (Singh 2002, 2005; Langyintuo et al. 2003). Most of the cowpea grain sold at large commercial markets in large urban centers of coastal West Africa is produced further inland where climates are drier and favorable to production of high-quality grain. Cowpea production in the United States is estimated at 80,000 mt, with the majority of the production in Texas, California and the southern states of Alabama, Arkansas, Georgia, Louisiana, Missouri, and Tennessee (Fery 2002; Timko et al. 2007a).

Compared to other legumes, cowpea is known to have good adaptation to high temperatures and resistance to drought stress (Hall et al. 2002; Hall 2004). For example, Hall and Patel (1985) reported cowpea grain yields of as much as 1000 kg ha^{-1} of dry grain in a Sahelian environment with low humidity and only 181 mm of rainfall. At present, few other legume crop species are capable of producing significant quantities of grain under these conditions. Cowpea is also a valuable component of farming systems in areas where soil fertility is limiting. This is because cowpea has a high rate of nitrogen fixation (Elawad and Hall 1987), forms effective symbiosis with mycorrhizae (Kwapata and Hall 1985), and has the ability to better tolerate a wide range of soil pH when compared to other grain legumes (Fery 1990). Cowpea is also well recognized as a key component in crop rotation schemes because of its ability to help restore soil fertility for succeeding cereal

crops (Carsky et al. 2002; Tarawali et al. 2002; Sanginga et al. 2003). In addition, well-adapted, early maturing cowpea varieties capable of producing seed in as few as 55 days after planting often provide farmers with the first source of food from the current harvest sooner than any other crop (Hall et al. 2003).

In the developing world where soil infertility is high, rainfall is limiting, and most of the cowpea is grown without the use of fertilizers and plant protection measures (i.e., pesticides or herbicides), a wide variety of biotic and abiotic constraints also limit growth and severely limit yield (Singh 2005; Timko et al. 2007a).

While cowpea is inherently more drought-tolerant than other crops, water availability is still among the most significant abiotic constraints to growth and yield. Erratic rainfall at the beginning and towards the end of the rainy season adversely affects plant growth and flowering resulting in a substantial reduction in grain yield and total biomass production. The use of early maturing cultivars helps farmers escape the effects of a late season drought, but plants exposed to intermittent moisture stress during the vegetative or reproductive stages will perform very poorly.

Cowpea is susceptible to a wide range of bacterial, fungal, and viral diseases and a large variety of insect pests (Singh 2005; Timko et al. 2007a). The major insect pests of cowpea are aphids (*Aphis craccivora*), thrips (*Megalurothrips sjostedti*), Maruca pod borer (*Maruca vitrata*), a complex of pod sucking bugs (*Clavigralla* spp., *Acanthomia* spp., *Riptortus* spp.), and the storage weevil *Callosobruchus maculatus*. Of these, thrips and *Maruc*a cause major damage in sub-Saharan Africa. There are some location-specific insect pests such as *Lygus* in the Americas, bean fly in Asia and East Africa, and ootheca beetles in wetter regions of the tropics.

Nematodes are important constraints in some areas (Roberts et al. 1996, 1997) and parasitic weeds such as *Striga gesnerioides* and *Alectra vogelii* are a major limitation to cowpea production in Africa (Timko et al. 2007b). *Striga* causes severe damage to cowpeas in the Sudan savanna and Sahel of West Africa, whereas *Alectra* is more prevalent in the Guinea and Sudan savannas of West and Central Africa and in portions of eastern and southern Africa. *Striga* infection in cowpea is more devastating in areas with sandy soils, low fertility, and low rainfall. Both parasites are difficult to control because they produce a large number of seeds and up to 75% of the crop damage is done before they emerge from the ground.

Major opportunities exist for breeders to develop cowpea cultivars with tolerance to a wide range of abiotic factors (e.g., drought, low soil fertility, high salinity), resistance to a variety of diseases, pests, and parasites, and agronomic characteristics (e.g., plant growth habits, flowering times, maturity dates) specifically adapted to agroecological production zones and crop product utilizations (i.e., dual-purpose grain and hay production).

10.3 Taxonomic Relationships

Cowpea [*Vigna unguiculata* (L) Walp.] is a dicotyledonous crop in the order Fabaceae, subfamily Faboideae (Syn. Papillionoideae), tribe Phaseoleae, subtribe Phaseolinae, genus Vigna, and section Catiang (Verdcourt 1970; Maréchal et al.

1978). It contains 22 chromosomes (2n = 2x = 22). The genus *Vigna* is pantropical and highly variable. In addition to cowpea, other members include mungbean (*V. radiata*), adzuki bean *(V. angularis*), blackgram (*V. mugo*), and the bambara groundnut (*V. subterranea*). The genus was initially divided into several subgenera based upon morphological characteristics, extent of genetic hybridization/reproductive isolation, and geographic distribution of species (Maréchal et al. 1978). The major groupings consist of the African subgenera *Vigna* and *Haydonia*, the Asian subgenus *Ceratotropis*, and the American subgenera *Sigmoidotropis* and *Lasiopron*. Under the scheme proposed by Maréchal et al. (1978) cultivated cowpea was placed in the subgenus *Vigna*, whereas mungbean and blackgram were placed in the Asian subgenera.

V. unguiculata subspecies *unguiculata* includes four cultigroups: *unguiculata*, *biflora* (or *cylindrica*), *sesquipedalis*, and *textilis* (Ng and Maréchal 1985). *V. unguiculata* subspecies *dekindiana, stenophylla,* and *tenuis* are the immediate wild progenitors of cultivated cowpea and form the major portion of the primary gene pool of cowpea. Members of subspecies *dekindiana, stenophylla,* and *tenuis* are also considered part of this gene pool. A secondary gene pool is constituted by other wild subspecies like *pubescence* that do not readily hybridize and show some degree of pollen sterility and require embryo rescue (Fatokun and Singh 1987). Observations from recent attempts to cross *V. vexillata* and *V. radiata* with *V. unguiculata* (Barone et al. 1992; Gomathinayagam et al. 1998) indicate that these may constitute a tertiary gene pool for cowpea.

10.3.1 Origin and Diversity of Cultivated Forms

The precise origin of cultivated cowpea has been a matter of speculation and discussion for many years. Early observations showed that the cowpeas present in Asia are very diverse and morphologically different from those growing in Africa, suggesting that both Asia and Africa could be independent centers of origins for the crop. However, the absence of wild cowpeas in Asia as possible progenitors has led some to question whether the Asian center of origin is valid. All of the current evidence suggests that cowpea originated in southern Africa, although, it should be noted that it is difficult to ascertain where on the continent the crop was first domesticated. Based on the distribution of diverse wild cowpeas along the entire length of eastern Africa, from Ethiopia to Southern Africa, Baudoin and Maréchal (1985) proposed east and southern Africa to be the primary region of diversity, and west and central Africa to be the secondary center of diversity. These researchers also proposed Asia as a third center of diversity. More recent studies strongly indicate that the highest genetic diversity of primitive wild forms of cowpea can be found in the region of the African continent currently encompassed by Namibia, Botswana, Zambia, Zimbabwe, Mozambique, Swaziland, and South Africa, with among the most primitive species observed in the Transvaal, Cape Town, and Swaziland (Padulosi 1987, 1993; Padulosi et al. 1990, 1991). Based on this latter observation, Padulosi and Ng (1997)

suggested that southern Africa may be site of origin of cowpea with subsequent radiations of the primitive forms to other parts of southern and eastern Africa, and subsequently to West Africa and Asia. The small seed size of wild cowpeas likely facilitated their dispersal by birds throughout East and West Africa contributing to the diversity and development of secondary wild forms. Human selection for larger seeds and better growth habits from natural variants in wild cowpeas likely led to diverse cultigroups and their domestication in Asia and in Africa (Steele 1976; Ng and Padulosi 1988; Ba et al. 2004; Ng 1995).

Based on analysis of chloroplast DNA polymorphisms, Vaillancourt and Weeden (1992) discovered that a loss of a *Bam*HI restriction site in chloroplast DNA (haplotype 0) characterized all domesticated accessions and a few wild (*Vigna unguiculata* ssp. *unguiculata* var. *spontanea*) accessions. Based on these data, they suggested that Nigeria was the center of domestication in West Africa. In contrast, studies based on analysis of amplified fragment length polymorphism (AFLP) profiles led Coulibaly et al. (2002) to propose domestication in northeastern Africa. Currently, the wild cowpea, *Vigna unguiculata* ssp. *unguiculata* var. *spontanea,* is thought to be the likely progenitor of cultivated cowpea (Pasquet 1999; Pasquet and Baudoin 2001). Using a new set of chloroplast DNA primers, Feleke et al. (2006) evaluated 54 domesticated cowpea accessions and 130 accessions from the wild progenitor. They confirmed the earlier observation of Vaillancourt and Weeden (1992) that domesticated accessions, including primitive landraces from cultivar groups biflora and textilis, are missing the *Bam*HI restriction site in chloroplast DNA, suggesting that this mutation occurred prior to domestication. However, 40 var. *spontanea* accessions distributed from Senegal to Tanzania and South Africa showed the alternative haplotype 1. Whereas this marker could not be used to identify a precise center of origin, its very high frequency in West Africa was interpreted as a result of either genetic swamping of the wild/weedy gene pool by the domesticated cowpea gene pool or as the result of domestication by ethnic groups focusing primarily on cowpea as fodder.

It is likely that the cowpea was first introduced to India during the Neolithic period (Pant et al. 1982) and was certainly there before the Christian era, since it has a Sanskrit name in writings dated to 150 BC (Steel and Mehra 1980; Ng and Maréchal 1985). It is at that point that human selection led to it being modified to a form different from that present in Africa. Cowpea probably moved to West Asia and parts of Europe between 800 and 300 BC (Ng and Maréchal 1985; Tosti and Negri 2002). Cowpea is well adapted to parts of southern Europe, including Italy, Spain, Portugal, and Turkey but less adapted to the western parts of Asia and continental Europe (Tosti and Negri 2002). Little variability and selection has taken place relative to South Asia and South East Asia, where small seeded and vegetable cowpeas were developed. Asia is often considered a secondary domestication site for the crop. "Yardlong beans," a unique cultivar group (Sesquipedialis) of cowpea that produces very long pods widely consumed in Asia as a fresh green or "snap" bean, apparently evolved in Asia and is rare in African landrace germplasm.

Spanish explorers are likely responsible for introducing cowpea into the New World, bringing seed to the West Indies in the 16th century (Purseglove 1968). The

plant presumably was introduced into Central and South America at about the same time and made its way to the continental United States by 1700 (Purseglove 1968).

10.3.2 Molecular Phylogeny and Genome Organization

The development and use of biochemical-based analytical techniques and molecular-marker technologies, such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs) , amplified fragment length polymorphisms (AFLPs), and microsatellites or simple sequence repeats (SSRs), have greatly facilitated the analysis of the structure of plant genomes and their evolution, including relationships among the Legumioseae (Choi et al. 2004; Yan et al. 2004; Gepts et al. 2005) This in turn has contributed significantly to our current understanding of the cowpea genome organization and evolution.

Using RFLP analysis, Fatokun et al. (1993a) analyzed 18 *Vigna* species including five of the subgenus *Ceratotropis* to determine the taxonomic relationship between the subgenus *Ceratotropis* and other subgenera. These investigators showed that a high level of genetic variation exists within the genus, with a remarkably higher amount of variation associated with *Vigna* species from Africa relative to those from Asia. Their data supported the taxonomic separation of the Asian and Africa genera as proposed by Maréchal et al. (1978) and underscored the previously held viewpoint that Africa is the likely center of diversity for *Vigna*. In general, the placement of species and subspecies based upon molecular taxonomic procedures by Fatokun et al. (1993a) substantiated prior classifications based on classical taxonomic criteria, such as morphological and reproductive traits.

Genetic variation in 23 accessions of five species within the subgenus *Ceratotropis* was subsequently reinvestigated by using RAPD analysis by Kaga et al. (1996a). Based on the degree of polymorphism at 404 informative loci, these investigators were able to separate the accessions into two main groups differing by approximately 70% at the molecular level. Within each of the main groups, the accessions could be further divided into five subgroups whose composition were in complete agreement with their taxonomic species classifications.

Sonnante et al. (1996) examined isozyme variation between *V. unguiculata* and other species in the subgenus *Vigna* and showed that *V. unguiculata* was more closely related to *V. vexillata*, a member of the subgenus *Plectotropis*, than to any other species belonging to section *Vigna.* This is not surprising since *V. vexillata* is thought to be the intermediate species between African and Asian *Vigna* species. Vaillancourt and Weeden (1996) reached a similar conclusion about the relatedness of these species. Based on an analysis of variation in chloroplast DNA structure (Vaillancourt and Weeden 1992) and isozyme polymorphisms (Vaillancourt et al. 1993), it was suggested that *V. vexillata* and *V. reticulata* were the closest relatives of *V. unguiculata*. While the close relationship between *V. unguiculata* and *V. vexillata* proposed by Vaillancourt and Weeden (1996) is consistent with previous observations (Maréchal et al. 1978), *V. reticulata* was placed in a different cluster based upon RFLP analysis (Fatokun et al. 1993a).

Polymorphisms in 21 different enzyme systems were used by Pasquet (1999) to evaluate the relationship between 199 accessions of wild and cultivated cowpea differing in breeding system and growth characteristic (i.e., annual vs. perennial growth habit). Based on these allozyme data, perennial subspecies of cowpea (spp. *unguiculata* var. *unguiculata*) were shown to form a coherent group closely related to annual forms (ssp. *unguiculata* var. *spontanea*). Among the 10 subspecies studied, *V. unguiculata* var. *spontanea* and ssp. *pubescens* were the closest taxa to cultivated cowpea. Most recently, Ajibade et al. (2000) used inter simple sequence repeat (ISSR) DNA polymorphism analysis to study the genetic relationships among 18 *Vigna* species. They showed that closely related species within each subgenera clustered together [e.g., *V. umbellata* and *V. angularis* (subgenus *Ceratotropis*), *V. adenantha* and *V. caracalla* (subgenus *Sigmoidotropis*), and *V. luteola* and *V. ambacensis* (subgenus *Vigna*)]. Cultivated cowpea grouped closely with the wild subspecies of *V. unguiculata*, and the entire species was separated from its most closely allied species *V. triphylla* and *V. reticulata*. ISSR polymorphism analysis split *Vigna* into groupings that differed in their composition from previous classifications. For example, the subgenus *Vigna* was split into three lineages, with *V. unguiculata/reticulata/friesorum* forming one group, *V. luteola/ambacensis* forming a second, and *V. subterranea* being far from the other two. *Ceratotropis* split into two sections, with three species (*V. radiata*, *V. mungo*, and *V. acontifolia*) in one section and two species (*V. angularis* and *V. umbellata*) in a second section. While such groupings had been suggested previously (Maréchal et al. 1978; Fatokun et al. 1993a; Vaillancourt and Weeden 1996), it should be noted that ISSR analysis was not as effective at resolving genetic distance relationships at the subgeneric level as it was at resolving relationships at the species level and below. Therefore, the authors note that their conclusions regarding subgeneric classifications should be taken with some caution. There is still considerable need to develop appropriate strategies and molecular techniques to resolve exact taxonomic relationships among members of this important genus.

Repetitive DNA sequences have been shown to represent a substantial fraction of the nuclear genome of all higher plant species and to account for much of the variation in genomic DNA content observed among species (Flavell et al. 1994). Many of the repeat sequences found in plant genomes appear to have originated through the activity of transposable elements (transposons) that move either by first forming an RNA intermediate (i.e., retrotransposons [Boeke et al. 1985]) or by direct DNA transposition intermediates (i.e., transposons [Federoff 1989]). To gain insight into the genomic organization and evolution of species within *Vigna*, Galasso et al. (1997) examined the genomic organization and distribution of Ty1-*copia* type retrotransposons in seven different species and subspecies of *Vigna* and several related leguminous plants. Gel blot analysis of genomic DNA from *V. unguiculata*, *V. luteola*, *V. oblongifolia*, *V. ambacensis*, and *V. vexillata* probed with radioactively labeled probes to the reverse transcriptase gene amplified from *V. unguiculata* ssp. *unguiculata*, *V. unguiculata* ssp. *dekindtania, V. luteola*, and *V. vexillata* showed variable hybridization patterns and intensities generally correlating with their previously defined taxonomic position. Fluorescence in situ hybridization analysis of the

distribution of the Ty1-*copia* type sequences showed that these elements represented a major fraction of the cowpea genome and were dispersed relatively uniformly over all of the chromosomes. Little or no hybridization was found associated with centromeric, subtelomeric, and nucleolar organizing regions of the chromosomes, indicating that these portions of the genome may not be suitable sites for transposition. Comparisons of retrotransposon structural similarity between *Vigna* and other genera of legumes generally supported the subdivision of the tribes Phaseoleae and Vicieae, with greater homology being seen between members of the Cicereae and Phaseoleae than *Cicer* species and those from the Vicieae (Galasso et al. 1997).

Ba et al. (2004) used RAPD analysis to characterize genetic variation in domesticated cowpea and its wild progenitor, and their relationships. Twenty-six domesticated accessions representing the five cultivar groups and 30 wild/weedy accessions, including accessions from West, East, and southern Africa, were evaluated. Twenty-eight primers generated 202 RAPD bands. One hundred and eight bands were polymorphic among the domesticated compared to 181 among wild/weedy cowpea accessions. Wild accessions were more diverse in East Africa, which is the likely area of origin of *V. unguiculata* var. *spontanea*. *V. unguiculata* var. *spontanea* is thought to have spread westward and southward, with a loss of variability that is counterbalanced in southern Africa by introgressions with local perennial subspecies. Although the variability of domesticated cowpea was the highest ever recorded, cultivar groups were poorly resolved, and several results obtained with isozyme data were not confirmed here. However, primitive cultivars were more diverse than evolved cultivars, suggesting two consecutive bottlenecks within domesticated cowpea evolution. These data support the single domestication hypothesis and further underscore the gap between wild and domesticated cowpea and the widespread introgression phenomena between wild and domesticated cowpea. Furthermore, the findings demonstrated that there is a widely distributed cowpea crop-weed complex all over Africa consistent with previous studies using other molecular marker tools (Pasquet 1999; Coulibaly et al. 2002). Taking into account that there appears to have been a single domestication event, the genetic similarity of some of these wild accessions to the domesticated group would be the result of post-domestication gene flow between wild and domesticated forms due to their sympatric distribution.

10.4 Classical Genetics and Breeding

Significant long-term genetic improvement efforts of cowpea have taken place within national laboratories and universities in several West African countries, India, Brazil, and the USA. Within the Consultative Group on International Agricultural Research (CGIAR), the International Institute of Tropical Agriculture (IITA) based in Ibadan, Nigeria, has the global mandate for improving cowpea cultivars. IITA develops and distributes a range of improved cowpea breeding lines to

65 countries. The accomplishments of some of these programs have been described recently (Ehlers et al. 2002a; Singh et al. 2002; Hall et al. 2003; Singh 2005; Timko et al. 2007a).

10.4.1 Germplasm Collections

Cowpea germplasm is maintained in collections around the world with varying levels of accessibility and documentation. The largest collections are held by the IITA with more than 14,000 accessions. The collection can be accessed via an electronic database maintained through the CGIAR-SINGER system (http://singer.cgiar.org). The United States Department of Agriculture (USDA) maintains a collection with ca. 8,000 accessions. Access to this collection is through the USDA Germplasm Resources Information Network or GRIN system (www.ars-grin.gov). The University of California-Riverside has a collection with ca. 5,000 accessions accessible on a Microsoft Access database. There is also a large collection of Mediterranean and African landraces (ca. 600 accessions) held at the Istituto di Genetica Vegetale at Bari, Italy (www.ba.cnr.it). Other centers maintaining seeds of wild and cultivated cowpeas include the following: Agricultural University-Wageningen (Wageningen, The Netherlands), Botanical Research Institute (Pretoria, South Africa), Le Jardin Botanique National de Belgique (Meise, Belgium), International Plant Genetic Resources Institute (IPGRI) in Harare (Zimbabwe), Institut Français de la Recherché Scientifique pour le Développement en Coopération (ORSTOM; now IRD) in Montpellier (France), Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) in Goiana (Brazil), Zentralinstitut fur Genetik und Kultupflanzenforschung (GAT) in Gatersleben (Germany), and the National Bureau of Plant Genetic Resources in New Delhi (India).

In addition to the centers and facilities mentioned above, many national cowpea breeding programs in Africa (including programs in Botswana, Burkina Faso, Ghana, Kenya, Nigeria, and Senegal) also have substantial germplasm collections. The condition of some of these collections, which are important reserves of local diversity, could be improved with funding for germplasm maintenance and facility repair.

10.4.2 General Breeding Strategies

Most cowpea breeders employ backcross, pedigree, or bulk breeding methods to handle segregating populations because cowpea is a self-pollinating species and varieties are pure lines. Higher grain yields and improved grain quality are the primary breeding objectives of nearly all programs. In addition, most breeders seek to incorporate a wide range of abiotic and biotic stress resistance/tolerance characters. The constraints that direct individual breeding programs at the local and national program levels depend on the major diseases and pests encountered in their target environments. Several comprehensive reviews of cowpea breeding have been

published, among which the recent efforts by Hall et al. (1997), Singh (2005), and Timko et al. (2007a) are recommended.

The general strategy of most breeding programs is to develop a range of high yielding cowpea varieties adapted to different agroecological zones that possess regionally preferred traits for plant type, growth habit, days to maturity, and seed type. Some of the major breeding objectives for cowpea are summarized in Table 10.1. In general, the focus is on the development of extra-early maturing (60–70 days) and medium maturing (75–90 days), non-photosensitive lines with good grain quality and potential for dual-purpose use (i.e., food and fodder), either for use as a sole crop and as an intercrop in multiple cropping systems. Other traits targeted include resistance to major diseases, insect pests, and parasitic plants (*S. gesnerioides* and *A. vogelii*), tolerance to drought, heat, acidity and low fertility, and seed types with high protein content and low cooking time. For example, new extra-early cowpea varieties have been developed that have erect plant type, early maturity and resistance to major pests, and are capable of yields up to 2.5 tons ha^{-1} within 60 days compared to less than 1 ton/ha of the local varieties, which mature in 100 to 140 days. Similarly,

Table 10.1 The Major Breeding Objectives for Cowpea[1]

Breeding Objective	Selection/Improvement Criteria
High seed yield	Without inputs under intercropping conditions from 100 to 400 kg ha^{-1}
	With inputs under sole cropping conditions from 900 to 3000 kg ha^{-1}
Diverse types	Extra-early maturing (60–70 days) photo-insensitive grain type, for use as sole crop in multiple cropping systems and short rainy seasons
	Medium-maturing (75–90 days) photo-insensitive grain type, for use as a sole crop and intercrop
	Late-maturing (85–120 days) photo-insensitive dual-purpose (grain + leaf) types, for use as a sole crop and intercrop
	Photosensitive early-maturing (70–80 days) grain types, for intercropping
	Photosensitive and photo-insensitive medium-maturing (75–90 days) dual purpose (grain + fodder) types, for intercropping
	Photosensitive late-maturing (85–120 days) fodder type, for intercropping
	High-yielding, bush-type vegetable varieties
Resistance to biotic stresses	Insects: Aphid (*Aphis cracivorra*), Thrips ( ), leaf hoppers (*Empoasca* sp.), podborer (Maruca vitrata), *Clavigralla* spp., *Anoploenemis* spp., *Riptortus* sp., *Nezara viridula*
	Parasitic plants: *Striga gesnerioides* and *Alectra vogelii*
	Diseases: *Colletotrichum* sp., *Xanthomonas* sp., viral mosaics and mottling
Tolerance to abiotic stresses	Drought, high temperatures, low phosphorus, high BNF, and soil acidity; root architecture
Quality and acceptability of the seed	Size, color and texture of seed coat
	Protein content
	Mineral levels (Fe, Zn, Ca, K)
	Low cooking time

[1] Partly adapted and modified from Pasquet and Baudoin (2001)

a number of medium-maturing, dual-purpose cowpea varieties have been developed which yield over 2.5 tons ha^{-1} grain and over 3.0 tons ha^{-1} fodder in 75–80 days. In recent years, over 40 improved cowpea varieties have been released in 60 countries covering Africa, Asia, and Central and South America. Table 10.2 lists a few of the notable improved varieties released in different agroecological regions.

10.4.3 Breeding for Resistance to Biotic Stresses

For many bacterial, fungal and viral diseases, effective screening techniques have been developed that allow researchers to identify cultivars with potential sources of resistance (Ehlers and Hall 1997). In general, good progress has been made using conventional breeding techniques to move resistance to various bacterial, fungal, and viral diseases, parasitic weeds (*S. gesnerioides* and *A. vogelii*), and root-knot nematodes into farmer-acceptable germplasm. Resistance to these pathogens and parasites is usually governed by single genes that are often effective only in a restricted region due to pathogen/parasite variability and may be overcome in a relatively short period of time. Marker-assisted selection can be helpful in assembling more durable resistance by incorporating an array of resistance genes from other regions as discussed below.

Insect pests are a major problem in cowpea in cultivation (Singh and van Emden 1979; Daoust et al. 1985). Therefore, developing cultivars with sustainable resistance to insects is a key objective of many breeding programs worldwide. While in the developed world the problem of insect infestation and damage is easily controlled by treatment with insecticides, in many parts of the developing world access

Table 10.2 Improved Cowpea Varieties Released for Use in Africa, Asia and the Americas

Region	Variety/Breeding Line/Cultivar
Asia and Oceania	IT81D-897, IT82D-752, IT82D-789, IT82D-889, IT82E-18, IT93K-452-1, IT97K-1042-3, IT98K-1111-1, VITA-4, Victory, Breeze, Light,, Sky, Big Buff
East and Southern Africa	IT82E-16, IT82E-18, IT82D-889, IT85F-2020, IT86D-1010, IT87D-611-3, IT89KD-245, IT90K-59, IT90K-76, IT93K-2046-2, IT97K-568-18, IT97K-499, Hope, Pride, Gold from the Sand
West and Central Africa	TVx 3236, IT81D-985, IT81D-994, IT83S-818, IT83S-728-13, IT84S-2246-4, IT86D-719, IT86D-721, IT87D-453-2, IT89KD-245-1, IT89KD-288, IT88D-867-11, IT89KD-374-57, IT90K-76, IT90K-82-2, IT90K-277-2, IT90K-372-1-2, IT93K-452-1, IT97K-499-35, Melakh, Ein El Gazal, Mouride, Son of IITA, Korobalen, Ayiyti, Asontem, Bengpla, CRSP Niebe, Lori Niebe
North, Central, and South America	VITA-1, VITA-3, VITA-6, VITA-7, IT82E-18, IT82D-716, IT82D-789, IT82D-889, IT83D-442, IT83S-841, IT84D-449, IT84D-666, IT84S-2246-4, IT86D-314, IT86D-368, IT86D-782, IT86D-792, IT86D-1010, IT87D-697-2, IT87D-885, IT88S-574-3, TVx1836-01J, IT87D-1627, IT89KD-288, IT90K-284-2, IT91K-118-2, Titan, Cubinata, California Blackeye No.27, Bettergreen, Charleston Greenpack

to the insecticides themselves or the financial resources required to purchase the insecticides and the equipment required for proper application are not available. In addition, the use of insecticides is an environmental and human safety concern. The imposition of new and significantly more stringent restrictions on the use of some popular insecticides is likely forthcoming and therefore alternative approaches to insect control are needed, especially for cowpea, where the number of registered products for use is low.

The development of insect-resistant cowpea cultivars would have a significant impact on yield and food availability and nutritional status in many regions. Achieving this goal will not be easy since cowpea is attacked by a large number and diversity of insect pests throughout its life-cycle and attack by any one of the major pests can be devastating. Therefore, resistance to multiple pests would have to be developed to positively influence seed production/ yield without the use of insecticides. For example, if cultivars were developed with a high level of resistance to flower thrips, capable of protecting their floral buds from damage, any resulting flowers and pods on these plants would likely be destroyed by pod bugs and pod borers. However, resistance to individual pests can reduce the number of sprays needed to obtain optimal yields and would generally increase yields without insect protection in regions where pest pressure is moderate, as in the case of the Sahel.

Screening methods have been developed for several major insect pests of cowpea (Ehlers and Hall 1997). However, despite the evaluation of hundreds to thousands of cowpea accessions, plants with high levels of resistance to most of the most significant pests have not been identified. Among the pest for which good sources of resistance have been identified are the cowpea aphid (*Aphis cracivorra*) and leaf hoppers (*Empoasca* sp.). Low to moderate levels of resistance have been identified in several genotypes for flower thrips, pod bugs, and Maruca pod borer (Singh et al. 2002; Singh 2005). Recurrent selection is being used to combine these resistances, but progress in this area is hampered by the low heritability of the traits based on the field screening methods currently available. The identification of molecular markers for insect resistance would greatly facilitate the transfer and pyramiding of the resistance genes in preferred backgrounds.

Using a combination of field and laboratory screening, a number of cowpea breeding lines have been developed with combined resistance to cowpea yellow mosaic, blackeye cowpea mosaic and many strains of cowpea aphid borne mosaic, *Cercospora*, smut, rust, *Septoria*, scab, *Ascochyta* blight, bacterial blight, anthracnose, nematodes, *Striga, Alectra*, ahpid, thrips and bruchid. Among these, IT82D-889, IT83S-818, IT86D-880, IT86D-1010, IT84S-2246-4, IT89KD-889, IT90K-59, IT90K-76, IT90K-277-2, IT90K-284-2, IT97K-207-15, IT97K-499-35, and IT98K-205-8 are very promising (Van Boxtel et al. 2000; Singh et al. 2002; Lale and Kolo 2007).

10.4.4 Breeding for Tolerance to Abiotic Stresses

Using simple screening methods for heat and drought tolerance and root architecture, major varietal differences for all three traits have been identified and

incorporated into improved lines (Matsui and Singh 2003). The best drought-tolerant varieties are IT89KD-374-57, IT88DM-867-11, IT98D-1399, IT98K-131-1, IT97K-568-19, IT98K-452-1, and IT98K-241-2, and the best heat-tolerant lines are IT93K-452-1, IT98K-1111-1, IT93K-693-2, IT97K-472-12, IT97K-472-25, IT97K-819-43 and IT97K-499-38. Significant progress has also been made in developing cowpea breeding lines with enhanced nitrogen fixation and tolerance to low phosphorus. Some of the more promising lines are IT89KD-374-57, IT90K-372-1-2, IT98D-1399, IT99K-1060, IT97K-568-19, IT97K-568-11, IT00K-1148, IT97K-1069-6, IT03K-314-1 and IT03K-351-2.

10.4.5 Breeding for Improved Nutritional Quality

Under the Harvest Plus initiative funded by the Bill & Melinda Gates Foundation (http://www.gatesfoundation.org/default.htm) and others, a systematic breeding program to develop improved cowpea varieties with enhanced levels of protein and micronutrient contents was initiated in 2003. Since its inception, considerable progress has been made and approximately 2,000 genotypes (cultivars and breeding lines) have been evaluated revealing significant genetic variability in protein and micronutrient contents. Typical values are as follows: protein 21% – 30.7%; calcium 545 ppm – 1,300 ppm; iron 48 ppm – 79 ppm; zinc 23 ppm – 48 ppm; and potassium 12,750 ppm – 16,150 ppm. Among the genotypes tested, IT97K-1042-3, IT99K-216-48-1, and IT97K-556-4 appeared to have good levels of all attributes, whereas IT 97K-131-2 and IT86D-724 had the lowest concentration of most of the attributes. These data suggested that cowpea already has fairly high levels of these micronutrients compared to other crops, and there is also a good opportunity to further improve the nutritional attributes of new cowpea varieties.

In developed countries, cowpea is also being considered as a healthy alternative to soyabean as consumers look to more traditional food sources that are low in fat and high in fiber and that have other health benefits. Fat contents of cowpea seeds range from 1.4 to 2.7% (Nielson et al. 1993), while fiber content is about 6% (Bressani 1985). Protein isolates from cowpea grains have good functional properties, including solubility and emulsifying and foaming activities (Rangel et al. 2004), and could be a substitute for soy protein isolates for persons (especially infants) with soy protein allergies. Processed-food products using dry cowpea grain, such as cowpea-fortified baked goods, extruded snack foods, and weaning foods, have been developed (Phillips et al. 2003).

10.4.6 Breeding for Regional Preference in Seed Type

Diverse regional preferences make the breeding objectives very challenging. For example, only white- and brown-seeded varieties with rough seed coats are preferred in West and Central Africa because of the ease of removing the seed coats for local

food preparations. On the other hand, red or brown seeded varieties with smooth seed coats are preferred in East and Southern Africa and parts of Central and South America where cowpea is used as boiled beans for which removal of seed coat is not desirable. In Cuba and some of the other countries in Central America, black-seeded cowpea varieties are used as a substitute of black beans for local delicacies. The relative density of cowpea seeds ranges from 1.01 to 1.09, while hardness (crushing weight) ranges from 3.96 kg to 8.4 kg for IT89KD-288 and Aloka local, respectively. The seed coat content ranged from 5.7 % to 13.8 % in IT95K-207-15 and TVu 12349, respectively, and cooking time ranged from 27.5 minutes for IT90K-277-2 to 57.5 minutes for Aloka local. The seed hardness was positively correlated with cooking time (Singh 2005).

Varieties of cowpea with a "persistent-green" grain have been developed by breeding programs in the USA that are a versatile product for frozen vegetable applications (Ehlers et al. 2002a). Persistent-green cowpea grains are green colored when dry but when soaked in water for several hours closely resemble fresh-shelled cowpea that can be used in frozen vegetable products to add color and variety. Because persistent-green cowpea grain can be harvested and stored dry until rehydration and freezing, it is a quite convenient and economical frozen vegetable compared to other frozen vegetable crops that require highly coordinated harvesting and processing operations and expensive long-term frozen storage.

There is a need for late maturing dual purpose cowpea varieties in East and Southern Africa where cowpea leaves are an important vegetable and in West Africa where cowpea stovers are important fodder for the livestock, but most countries would like to have early and medium maturing varieties because cowpea is grown in low rainfall areas. Most of the Asian countries grow cowpea for green pods for vegetables and some grow exclusively for fodder.

10.5 Genetic Maps

Numerous attempts have been made to develop a comprehensive genetic map of cowpea (Fatokun et al. 1992; Fatokun et al. 1993b; Menancio-Hautea et al. 1993b; Menéndez et al. 1997; Li et al. 1999; Ubi et al. 2000). The most complete genetic map currently available was developed by Ouédraogo et al. (2002a) using a recombinant inbred population derived from a cross between IT84S-2049 and 524B (see Menéndez et al. 1997). IT84S-2049 is an advanced breeding line that was developed at IITA in Nigeria for multiple disease and pest resistance and has resistance to several races of Blackeye cowpea mosaic virus (B1CMV) and to virulent root-knot nematodes in California. Line 524B is a blackeye cowpea that shows resistance to *Fusarium* wilt and was derived from a cross between cultivars CB5 and CB3, which encompasses the genetic variability that was available in cowpea cultivars in California.

The map contains a total of 441 markers of which 432 were assigned to one of 11 linkage groups (LGs) spanning a total of 2,670 cM, with an average distance of

ca. 6 cM between markers. The markers comprise 242 AFLPs and 18 disease- or pest-resistance-related markers developed by Ouédraogo et al. (2002a) integrated with 133 RAPD, 39 RFLP, and 25 AFLP markers from the map of Menéndez et al. (1997). Among these marker loci, genes for a number of biochemical and phenotypic traits have been located on this map (see Table 10.3). Candidate resistance genes (termed resistance gene analogs or RGAs) were also placed by RFLP analysis in various locations on the integrated cowpea map, including LG2, LG3, LG5, and LG9. However, none of the RGA loci have yet to be associated with specific disease or pest resistance trait underscoring the need for additional disease and pest resistance phenotyping and mapping in cowpea.

V. vexillata (L.) A. Rich is a perennial wild relative of cowpea and has significant potential as a repository of genes for resistance to pests and diseases to which cowpea plants succumb. In fact, many *V. vexillata* lines have been identified as having high levels of resistance to several cowpea insect pests including the pod-sucking bug *Clavigralla tomentosicollis*, the bruchid *Callosobruchus maculatus*, and the pod borer *Maruca vitrata*, and it possesses high resistance to cowpea mottle carmovirus (CPMoV) (Thottappilly et al. 1994, Ogundiwin et al. 2002). However, the usefulness of this species in traditional breeding approaches for cowpea improvement is limited because there is strong cross incompatibility between these two species. It might be possible using molecular cloning approaches to identify and transfer these desirable genes. To facilitate accessibility of desirable genes in *V. vexillata* for cowpea improvement, maps of the wild cowpea *V. vexillata* have also been generated (Ogundiwin et al. 2000; Ogundiwin et al. 2005). The most recent version comprises 120 markers, including 70 RAPDs, 47 AFLPs, one SSR, and two morphological traits, namely, the CPMoV resistance locus and leaf shape (La), utilizing an F2 generation of the intra-specific cross Tvnu 1443 x Tvnu 73 (Ogundiwin et al. 2005). The map has 14 linkage groups, with 11 of the LGs containing at least three markers, ranging in size between 15.0 and 454.9 cM while the remaining three contained two markers each. The map covered 1,564.1 cM of the *V. vexillata* genome. The average distance between markers was 14.75 cM, ranging from 1.0 to 49.0 cM. Of 106 intervals between loci, 38 were below 10 cM. Thirty-nine quantitative trait loci (QTL) associated with nine morphological and agronomic traits (leaf length, leaf width, petiole length, peduncle length, pod length, internode length, number of seed per pod, 100 seed weight, seed/pod ratio) distinguishing both parents were resolved by composite interval mapping (CIM). The QTL detected on the linkage map accounted for between 15.62 and 66.58% of their respective phenotypic variation. Seven chromosomal intervals contained QTL with effects on multiple traits. Further efforts must be made to generate additional markers, thus leading to the development of a linkage map of *V. vexillata* that would assist breeders to improve cowpea to reach its full potential.

Several early studies involving comparative mapping in legumes showed high levels of conservation between the genomes of cowpea and mungbean (*V. radiata*) and mungbean and common bean (*Phaseolus vulgaris*) (Menancio-Hautea et al. 1993a; 1993b; Boutin et al. 1995). The genetic map of mungbean constructed by Menancio-Hautea et al. (1993a) consisted of 172 markers placed into

Table 10.3 Agronomic, growth habit, and disease and pest resistance trait loci currently placed on the cowpea genetic map of Ouédraogo et al. (2002a) and other traits mapped to probable non-analogous linkage groups[1]

Trait	Locus designation	Linkage group/reference map
Pod pigmentation	P	LG1; (LG1-Menéndez et al. 1997)
Resistance to *Striga gesnerioides*-Race 1	*Rsg2-1*	LG1
Resistance to *Striga gesnerioides*-Race 3	*Rsg4-3, Rsg1-1*	LG1
Root-knot nematode (*Meloidogyne incognita*) resistance	Rk	LG1
Nodes to 1st Flower (D1301a)	NTF	LG2; (LG2-Menéndez et al. 1997)
Dehydrin protein	Dhy	LG2; (LG7-Menéndez et al. 1997)
Resistance to cowpea mosaic virus	CPMV	LG2
Resistance gene analog (pathogen unknown)	RGA-438	LG2
Resistance gene analog (pathogen unknown)	RGA-468	LG2
Resistance gene analog (pathogen unknown)	RGA-490	LG2
Resistance to *Fusarium oxysporum*	*Fus*R	LG3
Cowpea severe mosaic virus resistance	CPSMV (*ims*)	LG3
Cowpea mosaic virus resistance	CPMV	LG3
Resistance gene analog (pathogen unknown)	RLRR3-4B	LG3
General flower color factor	C	LG4; (LG1-Menéndez et al. 1997)
Seed weight (OB6a)	SW	LG5; (LG5-Menéndez et al. 1997)
Resistance gene analogs (pathogen unknown)	RGA-434	LG5
Resistance to southern bean mosaic virus	SBMV(*sbc-1,2)*	LG6
Resistance to *Striga gesnerioides*-Race 1	*Rsg3-1, Rsg-994*	LG6
Resistance to blackeye cowpea mosaic virus	BlCMV	LG8
Resistance gene analogs (pathogen unknown)	RLRR3-4T	LG9
Traits mapped in other populations (likely nonanalogous linkage groups to map of Ouédraogo et al. 2002a)		
Resistance to cowpea aphid (*Aphid craccivora*)	*Rac1*	(LG1-Myers et al. 1996)
50% Flowering	50%FL	(LG7-Fatokun et al. 1993)
Seed weight	SW	(LG7-Fatokun et al. 1993)
Plant height	HT	(LG8-Fatokun et al. 1993)
Pod number per plant	PodN	(LG9-Fatokun et al. 1993)

[1] Adapted from genetic maps and data of Ouédraogo et al. (2002a) and Menéndez et al. (1997) that used the same genetic population. There is insufficient marker data to integrate LGs of the maps of Fatokun et al. (1993) and data from Myers et al. (1996) with the map of Ouédraogo et al. (2002a)

11 linkage groups and provided 1,570 cM coverage with an average distance of 9 cM between loci. Significant colinearity was recognized to exist between the cowpea and mungbean genomes (Menancio-Hautea et al. 1993b). Similarily, Kaga et al. (1996b) reported significant blocks of synteny when comparing the linkage map of azuki bean with those of mungbean and cowpea. Choi et al. (2004) combined genetic, phylogenetic, and DNA sequence comparison to examine the degree of conservation of genome microstructure between model legumes such as *M. truncatula* and *L. japonicus* and crop legumes including *G. max* (soybean), *P. sativum* (pea), *V. radiata* (mungbean), and *P. vulgaris* (common bean). These studies revealed extensive conservation of gene order and orthology between the crop and model legumes and also identified features of structural divergence between these genomes.

10.6 Molecular Markers and Marker-Assisted Selection in Cowpea Breeding

There is a clear need for leveraging modern biotechnological tools to complement conventional breeding in cowpea. Such efforts should focus on the development of molecular markers and protocols for use in marker-assisted selection (MAS) and marker-assisted breeding. Support for such endeavors should come from a cooperation of both public sources and private foundations and must integrate national and regional breeding programs (Timko et al. 2007b).

MAS relies on the identification of DNA sequences within or near genes controlling traits of interest that can then be used to track those genes in breeding populations where the phenotypes are difficult or time-consuming to observe. In practice, MAS allows a more efficient means of assembling alleles of interest in an improved cultivar, thereby increasing the overall efficiency and effectiveness of crop improvement programs (Moreau et al. 2000; Charcosset and Moreau 2004). The application of MAS can be relatively straightforward for genes conditioning large and easily scored phenotypic effects. Most important traits are governed by multiple genes, each having relatively small effects. These "quantitative traits" have been difficult to understand and to manipulate in conventional crop breeding programs. The term QTL, quantitative trait loci, refers to the chromosomal regions of genes that control quantitative traits.

Prior to applying MAS, a realistic assessment of the cost-benefit ratio in comparison with phenotypic assays performed in the field, greenhouse, or laboratory needs to be conducted (Dekkers and Hospital 2002; Dreher et al. 2003). In general, traits that are difficult or expensive to measure using phenotypic assays are good candidates for MAS. In some cases, MAS can allow smaller populations to be used, reduce the number of generations needed to reach a goal, or increase the accuracy of evaluations (Sharma et al. 2002). MAS offers the only practical method to combine multiple resistance genes into one cultivar when the genes mask the expression of one another, yet when together provide more durable resistance

(Kelly et al. 2003). Other advantages of MAS are that a single technology can handle selection of diverse types of traits (e.g., pest resistance and grain quality parameters) and that cultivars developed through the use of MAS are not subjected to negative stereotyping as transgenic cultivars (Dubcovsky 2004). Also, selection of traits conferring resistance to quarantined pests can be conducted using MAS, eliminating the need for transfer of quarantined pests and assessment of resistance in expensive quarantine facilities.

The use of MAS has yet to be implemented in cowpea, but some of the groundwork for its application is in place (Kelly et al. 2003). As noted above, a genetic map has been constructed (Ouédraogo et al. 2002a) and loci controlling important pest and disease resistance genes and agronomic traits have been placed on the map. In addition, markers closely linked to some resistance factors whose function has yet to be fully defined have been identified (Gowda et al. 2002; Timko et al. 2007b). Many of these traits are controlled by single genes and therefore are potentially good candidates for MAS. Currently, no QTL with linked markers have been identified for use in selecting for more complex traits such as grain yield.

Based on host differential response of various cowpea genotypes (cultivars and breeding lines) and genetic diversity analysis, at least seven distinct races of *S. gesnerioides* have been identified within the cowpea-growing regions of West Africa (Lane et al. 1996; 1997; Botanga and Timko 2006). Similarly, "resistance-breaking" strains of the root-knot nematode *Meloidogyne incognita*, cowpea aphid (*Aphis craccivora*), cowpea weevil (*Callosobruchis maculatus*), and Fusarium wilt (*Fusarium oxysporum* f. sp. *tracheiphilum*) have been recognized in specific cowpea production areas. Markers for genes conferring resistance to the various strains of these pests would allow efficient development of varieties with resistance that is more broadly effective using MAS.

Ouédraogo et al. (2001) found three AFLP markers linked to *Rsg2-1*, a gene that confers resistance to *Striga* Race 1 (SG1) present in Burkina Faso, and six AFLP markers linked to gene *Rsg4-3*, a gene that provides resistance to *Striga* Race 3 (SG3) from Nigeria (Fig. 10.1). Two of the AFLPs were associated with both *Rsg2-1* and *Rsg4-3*. Boukar et al. (2004) also reported two AFLP markers that are closely linked to *Rsg1-1*, a gene that also confers resistance to SG3 in Nigeria. Five markers were subsequently found linked to the *994-Rsg* gene on LG6 that also confers resistance to SG1 (Ouédraogo et al. 2002b).

Currently, two sequence confirmed amplified region (SCAR) markers suitable for use in MAS for *Striga* resistance have been developed (Timko et al. 2007b). One SCAR marker, designated 61R(E-ACT/M-CAA), was generated from an AFLP marker associated with resistance to SG1 on LG1 (Ouédraogo et al. 2002a). The second SCAR marker, designated as SEACTMCAC83/85, is linked to SG3 on LG1 (Boukar et al. 2004). Analysis has shown that both 61R and a modified version of it termed MahSE2 (Ouédraogo J, Ouédraogo M, and Timko MP, unpublished data) are effective in identifying resistance to *Striga* races SG1 and SG3, but are less well linked to race SG5. At present, these two markers are available for germplasm evaluation and efficacy testing on populations in the field. Work is also currently underway aimed at identifying markers lined to SG2 and SG4z.

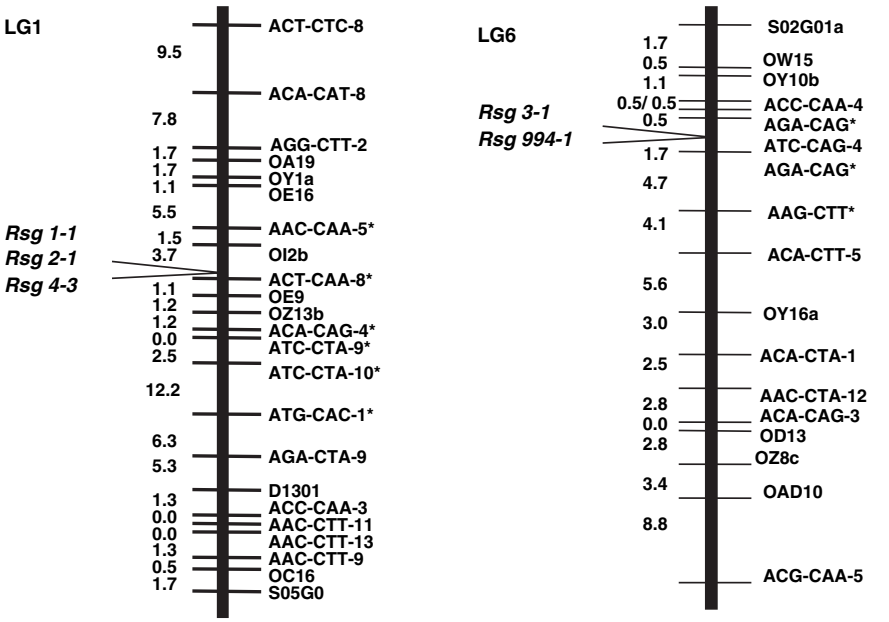

Fig. 10.1 Linkage of molecular markers to S. gesnerioides race-specific resistance genes in cowpea. Shown are the partial linkage maps of linkage groups LG1 (left) and LG6 (right) indicating the position of various AFLP, RAPD, and other molecular markers linked to S. gesnerioides race 1 and race 3 resistance genes. S. gesnerioides race 1 on LG1: Rsg2-1 and Rsg1-1; S. gesnerioides race 3 on LG1: Rsg4-3; S. gesnerioides race 1 on LG6: Rsg3-1 and Rsg994-1. Map distances are shown in cM. Adapted from Ouédraogo et al. (2001)

Breeding lines resistant to root-knot nematodes (*Meloidogyne* spp.) are well characterized in U.S. germplasm, and laboratory and field bioassays to assess resistance to root-knot nematodes in cowpea are effective and reasonably cost effective (Roberts et al. 1997; Ehlers et al. 2002b). Work underway to develop polymerase chain reaction (PCR)-based markers tightly linked to the *Rk* locus (that has multiple resistance specificities to *Meloidogyne* populations) should lead to more effective breeding for nematode resistance in cowpea (Roberts et al. 1996, 1997; Ehlers et al. 2002b).

Chida et al. (2000) obtained three RAPD markers flanking a gene conferring resistance to cucumber mosaic cucumovirus (*Cry* gene) that could be useful in MAS. Linkage analyses of these molecular markers showed that genetic distances of the markers CRGA5, D13/E14-350, WA3-850, and OPE3-500 to the *Cry* locus were 0.7, 5.2, 11.5, and 24.5 cM, respectively.

Insect resistance is a good candidate for MAS in cowpea because assessments of host plant resistance to insects are often difficult to conduct in the field or greenhouse. Most insect resistance factors in cowpea do not provide immunity to the pest and often have low heritability under field conditions. Field screenings that rely on natural insect infestations are subject to natural fluctuations in pest pressure.

When such variability is combined with incomplete resistance, field screens can lead to misclassification and selection of lines lacking the strongest resistance. For example, this has been the case with screening cowpea breeding lines and accessions for resistance to aphids, Lygus bug (*Lygus hesperus*), and pod-sucking bugs (such as *Nezara viridula, Clavigralla tomentosicollis, Riptortus dentipes*). In addition, colonies of insects may be difficult to rear without specialized facilities and trained entomologists to monitor the growth and uses. Such resources may not be available to cowpea breeding programs.

Resistance to the pod bug *Clavigralla tomentosicollis* has been identified in the wild cowpea (ssp. *dekindtiana*) germplasm line TVNu 151 (Koona et al. 2002). The development of effective markers for this trait would allow breeders to use MAS to introgress resistance into cultivated forms using a rapid backcrossing approach, based on the simultaneous selection for the resistance genes (markers) and against markers associated with unwanted wild germplasm characteristics such as small seed size and seed shattering. Clearly, such an approach would require a substantial increase in the number of markers available in cowpea and the development of high-throughput markers such as SSR and Single Nucleotide Polymorphism (SNP) markers.

The application of MAS for improvement of agronomic traits controlled by QTL is much more difficult. Expression of many quantitative traits (such as yield) reflects the influence of many (often interacting) developmental processes over a substantial period of time such as a full growing season. As noted earlier there has been little progress toward the development of markers linked to QTL useful in the selection of agronomic characteristics in cowpea. Progress has been faster in other related legumes (such as *Phaseolus*), and it is possible that some of this information may be leveraged since there is a significant degree of synteny between the bean and cowpea genomes (Kelly et al. 2003).

10.7 Current Genomic Resources

The development of tools for genomics-based research has proceeded rapidly in some legumes, whereas in others the development of such resources has lagged. Among those at the forefront are two species considered to be model legumes, Medicago (*M. truncatula*) and soybean (*G. max*), the latter of major economic importance in the United States. Other species such as alfalfa (*Medicago sativa*), common bean (*P. vulgaris*), pea (*P. sativum*), lentil (*Lens culinaris* Med.), chickpea (*Cicer arietinum*), and peanut (*Arachis hypogaea*), have also received considerable attention (Van den Bosch and Stacey 2003). Until recently, few genomic resources were available for researcher working with cowpea. However, this is likely to change as improvements in technology and reduced costs allow a broader examination of the plant kingdom.

One of the difficulties in developing genomic resources for some plant species stems from the fact that the genomes of many higher plants are relatively large and contain significant amounts of repetitive DNA surrounding the low-copy number

expressed regions of the genome (Rabinowicz et al. 1999). A number of experimental approaches have been developed that focus on targeted sequencing of gene-rich regions as an alternative to whole-genome sequencing (Palmer et al. 2003; Whitelaw et al. 2003; Bedell et al. 2005; Rabinowicz et al. 2005). Because of its relatively small genome size (estimated at 620 Mbp), cowpea is a good candidate for reduced representation cloning. To test this, a pilot study was carried out to determine whether methylation filtering technology (http://www.oriongenomics.com/) could be positively applied to analyzing the genespace of cowpea. The results of this pilot study showed that methylation filtering produced a 4.1-fold enrichment of gene-rich clones from cowpea genomic DNA libraries and estimated the size of the hypomethylated, gene-rich space of cowpea to be approximately 151 Mb (Chen et al. 2007). Based on these findings, a large scale analysis of the genespace was undertaken in which the nucleotide sequences of approximately 145,000 clones were determined from the forward and reverse directions, yielding a total of 268, 950 successful gene-space sequence reads (GSRs) with an average read length of 610 bp and an estimated raw coverage of approximately 160 Mb (Chen et al. 2007; Timko, MP unpublished results). A homology-based approach was applied for annotations of the GSRs, mainly using BLASTX against four public FASTA formatted protein databases (NCBI GenBank Proteins, UniProtKB-Swiss-Prot, UniprotKB-PIR [Protein Information Resource], and UniProtKB-TrEMBL). Comparative genome analysis was done by BLASTX searches of the cowpea GSRs against four plant proteomes from *Arabidopsis thaliana, Oryza sativa, Medicago truncatula,* and *Populus trichocarpa.* The results of the analysis, and information on the annotation of individual sequences, can be viewed at (http://cowpeagenomics.med.virginia.edu/). The data provide an excellent starting point for both marker development and comparative genomics.

In addition to the GSRs, other genomics tools are now becoming available. As part of a Generation Challenge Program grant, expressed sequence tags (ESTs) from drought-stressed and non-stressed drought-sensitive and tolerant cowpea lines are being generated (http://www.generationcp.org). A deep-coverage (14X) bacterial artificial chromosome (BAC) library and combinatorial pools of BACs are available from various cowpea cultivars and whole BAC and BAC end sequencing is underway (http://www.medicago.org/genome/BACregistry.php). A 6X BAC library has also been constructed from IT97K-499-35, the advanced line used for the genespace sequencing (M.P. Timko, unpublished). These new initiatives will certainly help in the further development of resources for both marker development and gene expression analysis. Given the rapidity at which sequence data, gene expression information, and other resources are being generated, it is clear that cowpea genomics is poised to begin making significant contributions to crop improvement.

10.8 Transformation Systems for Generating Transgenic Cowpea

Over the last two decades, a substantial number of research laboratories have worked diligently on the development of a reliable genetic transformation and in vitro plant regeneration system for cowpea (Anand et al. 2001; Van Le et al. 2002;

Machuka et al. 2002; Ikea et al. 2003; Avenido et al. 2004). Garcia and his colleagues (Garcia et al., 1986; 1987) were among the first to demonstrate successful transformation of cowpea, obtaining kanamycin-resistant callus, but were unable to achieve plant regeneration. Penza et al. (1991) attempted *Agrobacterium* co-cultivation using longitudinal sections derived from mature embryo slices but could not show evidence of stable integration of either selectable marker or reporter genes. Muthukumar et al. (1995) obtained four cowpea plants after co-cultivation of mature de-embryonated cotyledons and selection on hygromycin-containing media. However, DNA gel blot analysis could demonstrate integration of the *hpt marker* gene in only one of the presumptive transgenic plants, and transference of the marker could not be shown in subsequent generation. Ikea et al. (2003) also observed transformation in cowpea, but the transgenes were transmitted to only a small proportion of the progeny and there was no evidence for stable integration.

The results of the studies described above were at best inconclusive and, unfortunately, cowpea remained one of the last major grain legume species for which an efficient genetic transformation and regeneration system had yet to be developed. This changed in 2006 with the announcement by T.J. Higgins and his colleagues at the CSIRO in Australia that by adapting features of legume transformation systems, they have developed a protocol for *Agrobacterium*-mediated genetic transformation of cowpea that was reliable and modestly efficient in its recovery of transgenic cowpea plants (Popelka et al. 2006). More importantly, these researchers demonstrate for the first time stable transmission and expression of two co-integrated genes in the progeny of transgenic plants. Among the critical parameters in this transformation system are the choice of cotyledonary nodes from developing or mature seeds as explants and a tissue culture medium devoid of auxins in the early stages, but including the cytokinin BAP at low levels during shoot initiation and elongation. Addition of thiol-compounds during infection and co-culture with *Agrobacterium* and the choice of the bar gene for selection with phosphinothricin were also important. Transgenic cowpeas that transmit the transgenes to their progeny can be recovered at a rate of one fertile plant per thousand explants.

These results pave the way for the introduction of new traits into cowpea. Which traits will be selected for initial genetic manipulation will require some critical analysis and should be done in a manner complementary to existing breeding programs. Among the leading candidates are genes conferring strong resistance to insect pests which are a major constraint to productivity and affect post-harvest seed security. These include the use of *Bacillus thurengensis* (Bt) toxin (e.g., Cry1Ab, Cry1C, and CryIIA proteins) against the *Maruca* pod borer (*Maruca vitrata*), the alpha-amylase inhibitor gene from common bean for control of cowpea weevil (*Callosobruchus maculatus),* the soybean cysteine protease inhibitor soyacystatin N (scN) and alpha-amylase inhibitor (alphaAI) from wheat with synergistic effects against the cowpea weevil (Amirhusin et al. 2004), and genes for various plant lectins and plant proteinaceous inhibitors (PIs) of insect proteinases (serine, cysteine, aspartic, and metalloproteinases) (Machuka et al. 2002; Machuka et al. 2002). The development and successful deployment of transgenic cultivars with genes conferring resistance to insects will be a major achievement.

10.9 Conclusions and Perspective

One of the major goals of cowpea programs is to combine resistances to numerous pests and diseases and other desirable traits such as those governing maturity, photoperiod sensitivity, plant type, and seed quality. Parental lines with many desirable traits, such as resistance to cowpea weevil, cowpea aphid, and the parasitic weeds *A. vogelii* and *S. gesnerioides*, along with resistances to bacterial blight, CABMV, and other pathogens, exist in different advanced breeding lines developed by cowpea breeding programs around the world. The release of new improved cowpea varieties in over 60 countries has led to a quiet revolution in cowpea cultivation throughout the tropics. From about 6.3 million ha and 1.1 mmt production in 1974, the global area and production under cowpea in 2004 were about 14.5 million ha and 4.5 mmt, respectively. The new cowpea varieties developed have been given special names like 'Victory' and 'Breeze' in Sri Lanka, 'Light' and 'Sky' in Nepal, 'Big Buff' in Australia, 'Hope' and 'Pride' in Tanzania, 'Gold from the Sand' in Sudan, 'Son of IITA' in Nigeria, 'Korobalen' in Mali, 'Ayiyti', 'Asontem' and 'Bengpla' in Ghana, and 'Titan' and 'Cubinata' in Cuba, etc. Millions of small holder farmers in the tropics are benefiting from the new improved cowpea varieties. The major impact has been in Nigeria where cowpea production has increased from 580,000 mt in 1981 to over 2.3 mmt in 2004 (Singh 2005).

Cowpea remains to a large extent an underexploited crop where relatively large genetic gains can be made with only modest investments in both applied plant breeding and molecular genetics. Because it is grown mostly by poor farmers in developing countries it has received relatively little attention from a research standpoint. Indeed, cowpea has been identified as an "orphan crop" that is recommended for increased public/donor support for biotechnology research (Naylor et al. 2004). The development of new genomics-based resources for cowpea will certainly assist in the future expansion of both marker-assisted selection and marker assisted-breeding. It will also contribute to the development of transgenic plants that can be used in the developing world in a safe, rational, and controlled manner. Future development of cowpea will also benefit from the application of knowledge being gained from basic genomics research on other legume crops and "model species".

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