Chapter 7
Advances in Cowpea Improvement and Genomics

B.B. Singh, Michael P. Timko, and Francisco J.L. Aragao

Abstract Cowpea is a diploid (2n = 2× = 22) self pollinating legume species with a genome size of 613 Mbp. Since the available genomic resources are not adequate, the use of genomics tool in cowpea breeding programme has been very limited. However, a modest beginning in developing genomic resources has led the basic foundation of use of genomic resources in cowpea improvement. In order to perform genetic and genomic analysis various markers like RFLP, RAPD, AFLP, SSR and SNP have been employed in several studies. QTLs for striga and aphid resistance have been identified and validated. However, QTLs for other agronomic traits and important diseases and pests are still to be explored. Eight linkage maps including one consensus map published so far describes a good progress in development of linkage maps. Further efforts are required to construct high-density genetic map for analyzing inheritance of target gene and localization of specific genomic regions for map based cloning. Efforts are also on sequencing of genome of this important crop. With identification of micro RNAs, ESTs, BACs and transcriptomic data sets, the cowpea genomics is gaining momentum. The need of integration of all these efforts will promote the cowpea improvement. This paper presents an overview on advances made in development of genomic resources, gene expression and regulation, marker assisted breeding and progress towards sequencing cowpea genome.

Keywords Vigna unguiculata • Molecular mapping • Marker assisted selection • Sequencing • QTLs • Genomic resources • ESTs • SNPs • Crop improvement

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Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is a widely cultivated diploid legume species with 2n = 22 chromosomes. It is cultivated in over 65 countries covering Asia and Oceania, the Middle East, Southern Europe, Africa, southern USA and Central and South America (Singh 2005). With about 25–30 % protein in the grains and 15–18 % protein in its haulms, cowpea is a major source of dietary protein and minerals to humans as well as livestock. The average yield of cowpea is less than 500 kg/ha due to several production constraints including spreading growth habit and late maturity of traditional varieties, numerous diseases, parasitic weeds, insects and low soil fertility as well as shading due to intercropping with cereals like maize, sorghum and millet (Singh et al. 2003a, b).

Efforts have been made in the establishment of genomic resources and their application for cowpea genetic improvement. The advances began with the development and use of molecular marker technologies for diversity analysis of germplasm and in molecular breeding activities, and now include genomic scale sequence characterization, bacterial artificial chromosome (BAC) and other megabase fragment-based genomic libraries, and protocols and platforms for genome-wide gene expression profiling. This chapter briefly describes the progress made in cowpea breeding and genomics.

Genetic Resources

The world largest collection of cowpea is maintained at IITA having over 15,700 accessions of cultivated ones drawn from over 100 countries including 560 accessions of wild relatives. These have been characterized and evaluated for desirable traits and being conserved and used in the breeding program at IITA and freely made available to national breeding programs. Systematic screening of the germplasm lines has revealed extreme variation in respect of many traits such as plant pigmentation, plant type, plant height, leaf type, growth habit, photosensitivity and maturity, nitrogen fixation, fodder quality, heat and drought tolerances, root architecture, resistance to major bacterial, fungal and viral diseases, resistance to root-knot nematodes, resistance to resistance to insect pests like cowpea aphid (*Aphis craccivora*), leaf hoppers (*Empoasca signata* and *E. dolichi*), legume bud thrips (*Megalurothrips sjostedti*), pod borers (*Maruca vitrata*), pod-sucking bugs (*Clavigralla* sp.), and bruchid (*Callosobruchus maculatus*), as well as resistance to parasitic weeds (i.e., *S. gesnerioides* and *A. vogelii*), pod traits, seed traits and grain quality (Ng and Singh 1997). Based on their multiple resistances, the germplasm lines TVu 201, TVu 408, TVu 410, TVu 1190, TVu 1977 and TVu 4577 have been extensively used in the breeding program. These were also distributed to international collaborators for broad based testing. The results from these trials indicated four lines, TVu-201, TVu-1190, TVu-1977 and TVu-4577 to be resistant to many

diseases and had very high yield potential. These were described as VITA-1, VITA-3, VITA-4 and VITA-5 (Vigna IITA-1, 3, 4, and 5) respectively and subsequently released in many countries. These VITA lines were also extensively used as parents for the initial crossing programs and development of segregating populations. The focus was primarily to develop multiple disease resistant breeding lines with high yield potential. Based on the good performance across many countries, five new lines were described as VITA numbers and released in many countries. These were TVx 1193-7D as VITA-6, TVx 289-4G as VITA-7, TVx 66-2H as VITA-8, TVx 1948-01F as VITA-9, and TVx 1836-013J as VITA-10. The breeding objectives were broadened from 1980 onwards to develop a diverse set of improved cowpea varieties differing in plant type, growth habit, maturity and seed type to suit the regional preferences and cropping systems.

Breeding Progress

The global mandate for cowpea breeding has been a challenging task to the scientists at IITA because the biotic and abiotic constraints and variety requirements for cowpea differ from region to region in respect of the seed color preference, use patterns, maturity and growth habit. Thus, no single cowpea variety could be suitable for all countries and regions. Therefore, efforts have been made to develop varieties which can suit to specific circumstances.

Development of '60-Day' Erect Type Cowpea Varieties

The traditional cowpea varieties as well as the improved varieties until 1980 were medium to late maturing and semi-spreading type. Later on a need was felt for developing extra-early erect plant type cowpea varieties to be grown in areas with short rainy seasons and as a niche crop in multiple cropping systems to expand the cowpea cultivation in non-traditional areas with an yield potential between 1.5 and 2.5 t/ha within 60–65 days maturity (Singh and Sharma 1996). These were collectively called "60-day" cowpea varieties. Beginning from 1982, a large number of "60-day" cowpea varieties have been developed. Some of the prominent varieties of this group that have been released and become popular in many countries. These are IT82E-9 (black), IT 82E-60 (white blackeye), IT82E-16 (red), IT82E-18 (tan), IT82E-32 (red), IT82D-752 (tan), IT82D-789 (light brown), IT82D-889 (red), IT83S-818 (white blackeye), IT85F-867-5, IT86D-1010 (white blackeye), IT93K-452-1(white blackeye), IT97K-1042-3 (red), IT98K-1111-1(white blackeye) and IT98K-205-8 (white small eye). Of these, IT82D-889, IT83S-818, IT85F-867-5 and IT86D-1010 are resistant to over eight major viruses and IT 98K-205-8 is resistant to major viruses as well as resistant to aphid, thrips, bruchid, *Striga* and *Alectra*.

Development of Medium and Late Dual Purpose Varieties

In addition to the extra-early varieties, a number of medium maturing varieties (75–80 days) with semi-erect plant type combining multiple pest resistance and diverse seed types were also developed and distributed to national collaborators. Some of the prominent varieties of this group that have been widely tested, released and become popular in many countries are VITA-1, VITA-3, VITA-4, VITA-5, IT84S-2163, IT84S-2246-4, IT84D-449, IT84D-666, IT85F-2020, IT86D-368, IT86D-719, IT87D-697, IT87D-1627, IT88S-574-3, IT89KD-374, IT90K-277-2, IT90K-372-1-2, IT97K-368-18 and IT98K-506-1. The photosensitive late maturing cowpea varieties are commonly grown and fit well as a relay intercrop in 'millet-sorghum-cowpea', systems in many countries in West Africa and serve as dual purpose varieties providing grain as well as fodder. However, these varieties are too late and often suffer serious yield loss due to terminal drought. Therefore, selected photosensitive varieties were used as parents and a new set of improved medium-late photosensitive as well as photo-insensitive varieties which mature between 90 and 110 days were developed. Some of these combine resistance to major diseases, aphid, bruchid as well as *Striga* and *Alectra*. The promising varieties in this group that have been released many countries are IT81D-985, IT81D-994, IT89KD-245, IT89KD-288, IT89KD-391 and IT99K-216-38-1.

Vegetable Types with Bushy Growth Habit

Several countries grow the yard long cowpea varieties as a vegetable crop but these cultivars need staking to keep pods from touching the ground and rotting which involves extra cost and thus restricts the area under cultivation. Therefore, by crossing the yard long varieties with early erect types, bush-type vegetable cultivars with 30-cm long succulent pods were developed which yield up to 18 t/ha green pods with 4–6 pickings starting at 45 days after planting. Some of the promising ones are IT81D-1225-10, IT81D-1228-14, IT81D-1225-15, and IT86D-880. These cultivars have semi-erect growth habit with extra-long peduncles (40–50 cm long), protruding well over the canopy and holding the pods above the ground. Picking green pods periodically reduces the weight on peduncles and they remain upright all the time. Frequent picking also stimulates further flowering and podding on the same peduncles, which ensures a continuous supply of green pods for a 6–7 week period after the start of picking, provided soil moisture is not limiting.

Breeding for Disease Resistance

Using the resistant germplasm lines as parents in the breeding program and a combination of field and laboratory screening methods, most of the improved cowpea

varieties have been developed for combined resistance to major diseases like Cercospora, smut, rust, Septoria, scab, Ascochyta blight and bacterial blight, Macrophomina, anthracnose. Breeding for resistance to all the diseases has been easy because of simple inheritance in all the cases (Abadassi et al. 1987). Some of the best breeding lines with multiple resistances to major fungal and bacterial diseases are TVx 3236, IT81D-1228-14, IT82D-716, IT90K-277-2, IT97K-556-4, IT98K-476-8, IT97K-499-39, IT97K-1042-3, IT97K-1069-5 and IT98K-205-8. Of these TVx 3236 is a major source of resistance to scab, IT81D-1228-14 is for resistance to bacterial blight and IT97K-556-4 is for resistance to powdery mildew. Several cowpea breeding lines have also been identified with combined resistance to several major virus diseases including cowpea yellow mosaic, blackeye cowpea mosaic, southern bean mosaic, severe mosaic and many strains of cowpea aphid borne mosaic. Among these, IT82D-889, IT83S-818, IT86D-880, IT86D-1010, T90K-277-2, and IT98K-205-8 are most promising and found to be virus resistant in many countries (Van Boxtel et al. 2000) Good progress has also been made in breeding for combined resistance to several nematodes. Some of the improved breeding lines with nematode resistance are IT84S-2049, IT84S-2246-4, IT89KD-288 and IT97K-556-4 (Singh et al. 2002). Among these, IT89KD-288 is a high yielding photosensitive variety with high level of resistance to nematodes in Nigeria as well as resistant to four strains of *Meloidogyne incognita* in USA (Ehlers et al. 2000).

Breeding for Resistance to Striga *and* Alectra *Resistance*

Parasitic weeds cause considerable yield reduction in cowpea in Africa. Of these, *Striga gesnerioides* is primarily prevalent in West Africa but *Alectra vogelii* is widely distributed throughout the east, east and southern parts of Africa. Complicating the identification of *Striga*-resistant germplasm is the variable nature of the parasite with at least seven distinct races (pathotypes) of *S. gesnerioides* having now been identified. These are designated SG1 (Burkina Faso), SG2 (Mali), SG3 (Nigeria and Niger), SG4 (Benin), SG4z (Zakpota region of Benin), SG5 (Cameroon), and SG6 (Senegal) (Botanga and Timko 2006).

A local landrace, B 301 from Botswana, was found to be completely resistant to *Striga* and *Alectra* in Burkina Faso, Mali, Cameroon, Niger and Nigeria but only moderately resistant to SG4z from the Zakpota region of Benin Republic. A few other lines such as IT81D-994, IT89KD-288, 58-57 and Gorom local were found to confer complete resistance to races SG1 and SG4z from Burkina Faso and Zakpota, Benin Republic and but highly susceptible to race SG3 from Nigeria and Niger. Race-specific resistance to both parasitic weeds is inherited monogenically (Singh and Emechebe 1990; Atokple et al. 1993, 1995) and by using the complementary resistant parents in crosses, a number of new varieties have been developed with combined resistance to *Alectra* as well as all of the known races of *Striga* (Singh 2005). The most promising new cowpea varieties are IT90K-59, IT90K-76, IT90K-82-2, IT93K-693-2, T97K-499-35, and IT97K-819-118, IT98K-205-8.

Some of these lines are also resistant to bacterial blight, aphid, bruchid, thrips, and viruses with much higher yield potential than the local varieties (Singh 2005; Carsky et al. 2003). These lines also serve as a false host for *S. hermonthica* reducing its seed bank in the soil when grown as intercrop or in rotation with cereals.

Breeding for Insect Resistance

Using the available sources of resistance from germplasm lines at IITA, several improved cowpea varieties have been developed with combined resistance to aphid, thrips and bruchid (Adjadi et al. 1985; Bata et al. 1987; Singh et al. 2002). Aphid resistance is controlled by a single dominant gene which confers very high level of resistance causing death and highly reduced fecundity of aphids. Bruchid resistance is controlled by two recessive genes characterized by slow and reduced emergence of bruchids from infested seeds (Adjadi et al. 1985). This greatly minimizes seed damage due to bruchids during storage. Resistance to thrips is moderate and controlled by two recessive genes. Among several resistant breeding lines developed, IT90K-76, IT90K-59, IT 89KD-288, IT90K 277-2 and IT98K-205-8 are already popular varieties in several countries. The resistance to aphid and thrips is due to specific antibiosis and the resistance to bruchid is considered to be due to a 7s-storage protein, "vicillin" in the resistant cowpea seeds (Yunes et al. 1998). These factors are highly specific to insects only and therefore, no harmful effect to humans.

Only low level of resistance has been bred for Maruca pod borer and pod bugs. This is because none of the cultivated cowpea germplasm lines and cross-compatible wild cowpeas are resistant to Maruca pod borer. A distant wild relative of cowpea *Vigna vexillata* has shown high level of resistance to Maruca pod borer and bruchid but all the efforts made at IITA to transfer Maruca resistance genes from *Vigna vexillata* to cowpea has not been successful (Fatokun 1997). Developed through conventional breeding approaches, the new field resistant lines require only 1 or 2 sprays of insecticide for normal yield of 1.5–2.5 t compared to 5–6 sprays needed for the susceptible varieties.

Breeding for Tolerance to Drought, Heat and Cold

Since cowpea is grown in varied environments it encounters stresses such as drought, heat and cold temperatures. Also, cowpea suffers due to high temperatures in the Sahelian region. Using simple screening methods for heat and drought tolerance and root architecture, major varietal differences for all the three traits have been identified and incorporated into improved lines (Singh and Matsui 2002). Good progress has also been made at University of California, Riverside on water use efficiency, heat tolerance and chilling tolerance (Hall et al. 1997; Ismail and Hall 1998). The best drought tolerant varieties are IT89KD-374-57, IT88DM-867-11, IT98D-1399, IT98K-131-1, IT97K-568-19, IT98K-452-1, 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, IT and IT97K-499-38.

Breeding for Enhanced N-Fixation and Efficient Acquisition of Phosphorus

Most of the cowpeas in West Africa are grown in sandy soils which have low organic matter and low-phosphorus. Therefore, efforts are being made to screen and identify cowpea lines with enhanced nodulation and nitrogen fixation as well as efficient acquisition and utilization of phosphorus from low-P soils and rock phosphates (Sanginga et al. 2000). Recent work at IITA have shown major varietal differences in cowpea for growth, nodulation and performance under low phosphorus. Some of the promising lines under low-P condition were IT90K-372-1-2, TN5-78, IT98D-1399, TN27-80, IT99K-1060, IT89KD-374-57, TN 256-80, IT97K-1069-6 and IT98K-476-8. Screening cowpea varieties for tolerance to aluminum has also indicated major varietal differences and cowpea varieties IT91K-93-10, IT93K-2046-1 and IT90K-277-2 appear to be tolerant to aluminum and they gave higher response to phosphorus fertilization when grown in soils with aluminum toxicity problems (Kolawole et al. 2002). It is expected that the ongoing research may lead to the development of new cowpea varieties which would perform well in marginal lands where soil fertility is low.

Breeding for Improved Nutritional Traits

Following the development of a diverse set of improved cowpea varieties with high yield potential and multiple pests resistance, a systematic improvement program for nutritional and health traits was initiated in 2003. To begin with all the existing high yielding varieties and advanced breeding lines were analyzed for physical properties and protein, minerals, antioxidants and cooking properties and a great deal of variability was observed (Nielsen et al. 1993; Singh 2001). The mean values ranged from 21 to 31 % for protein, 46–79 ppm for iron, 545–1,330 ppm for calcium, 23–48 ppm for zinc, and 12,750–16,250 ppm for potassium. The best varieties in respect of high protein and high iron, zinc, calcium and potassium were IT97K-1042-3 and IT98K-205-8. The IT97K-1042-3 was also best for antioxidant activity.

Genome and Genome Size

The size of the cowpea genome was initially estimated at 613 Mbp (Arumuganathan and Earle 1991) and more recently at 620 Mb (Varshney et al. 2009) making it one of the smallest among the legumes and at the lower end of plant genomes in general.

Initially, efforts aimed at developing genomic resources were hampered owing greatly to the fact that cowpea was an orphan crop with little socioeconomic importance in the developed world. Gradually this has changed with the recognition of the broader importance of the crop. Among the first attempts at characterizing the gene content and complexity of the cowpea genome was the work of Timko et al. (2008) who applied a reduced representational approach known as methylation filtration (MF) to overcome the presence of ubiquitous repetitive DNA and capture only the hypomethylated, gene-rich coding sequences in the genome of the African cowpea cultivar IT97K-499-35. Using MF these investigators were able to achieve a 4.1-fold enrichment for the gene-rich space of cowpea and generated 263,425 gene-space sequence reads (GSRs) that could be assembled into 41,260 unigenes representing 19,786 unique GenBank accession numbers (Chen et al. 2007). Additional information on the cowpea genespace can be found on the Cowpea Genomics Knowledge Base (CGKB) website (http://cowpeagenomics.med.virginia.edu/CGKB/index.pl). The CGKB website provides an annotated, well-organized, and rigorously analyzed dataset of sequences as a resource for cowpea researchers and pan-legume crop specialists including a list of over 1,000 predicted and confirmed simple sequence repeat (SSR) primer combinations that can and in some cases have already been used for diversity analysis and molecular mapping. Additional SSR primer combinations based on expressed sequence tags (EST) (Gupta and Gopalakrishna 2010) and GSR sequences (Xu et al. 2010) can also be found in the literature.

Genomic Resources

Molecular Markers

The recent marker repertoire has enhanced our understanding of cowpea's genome structure and organization. Several markers like RAPD, SSR, AFLP and ISSR have been used to reveal the genetic diversity in cowpea. RAPD technology was proved to be a useful tool in the characterization of the genetic diversity among cowpea cultivars by Zannou et al. (2008) Malviya et al. 2012; Nkongolo 2003 and Chen et al. 2008. SSR is the most frequently used marker in the genetic diversity analysis of cowpea.

The earliest cowpea SSR research was conducted by Li et al. (2001) by developing 27 SSR primers. After that, SSR research on cowpea for assessing genetic diversity from different areas, mainly Africa and Asia, has been carried out. Africa is the diversity center of wild cowpea, which was proved by Ogunkanmi et al. (2008) with SSR analysis. Asare et al. (2010) utilized SSR molecular marker to evaluate genetic diversity and phylogenetic relationships among 141 cowpea accessions collected throughout the nine geographical regions of Ghana. Badiane et al. (2012) assessed the genetic diversity and phylogenetic relationships among 22 local cowpea varieties and inbred lines collected throughout Senegal by SSR markers and developed a set

of 44 polymorphic primer combinations from cowpea genomic or expressed sequence tags. Sawadogo et al. (2010) evaluated the genetic diversity and phylogenetic relationships among cowpea genotypes used in breeding for resistance to *Striga gesnerioides* in Burkina Faso using simple SSR molecular markers. Very few primer combinations showed polymorphic bands capable of discriminating *Striga*-resistant from susceptible cultivars, which revealed a high efficiency of SSR markers. Lee et al. (2009) estimated the genetic diversity of 492 Korean cowpea landrace accessions using six SSR markers. Xu et al. (2010) assessed the genetic diversity of asparagus bean cultivars from different geographical origins in China by EST-derived and GSS-derived SSR markers.

AFLP is recognized as one of the most efficient molecular markers. Coulibaly et al. (2002) employed AFLP to evaluate genetic relationships within a total of 117 cowpea accessions to assess the organization of their genetic diversity. Fang et al. (2007) examined genetic relationships among 60 advanced breeding lines from six breeding programs in West Africa and USA and 27 landrace accessions from Africa, Asia and South America. AFLP markers with six near infrared fluorescence labeled *Eco*RI + 3/1bases/*MseI* + 3/1bases primers sets were used in the study. Tantasawat et al. (2010) estimated genetic diversity and relatedness of 23 yardlong bean (*Vigna unguiculata* spp. *sesquipedalis*) accessions and seven accessions of a hybrid between cowpea (*V. unguiculata* spp. *unguiculata*) and dwarf yardlong bean in Thailand by morphological characters, SSR and ISSR markers

Genetic Maps

The first attempts at linkage mapping used a variety of tools aimed at detecting molecular polymorphisms such as restriction fragment length polymorphism (RFLP) analysis, randomly amplified polymorphic DNA (RAPD) detection, etc., and were successful in providing a baseline for more detailed genomic analyses. Since the first cowpea genetic mapping attempts (Fatokun et al. 1993; Menéndez et al. 1997), there has been a progression of increasingly informative maps with a greater number of traits analyzed and greater depth of marker coverage (Table 7.1).

Ouédraogo et al. (2002) offered the most comprehensive coverage, integrating amplified fragment length polymorphism (AFLP), RFLP, and RAPDs markers with numerous phenotypic characteristics and biochemical traits, into 11 linkage groups (LGs) spanning a total of 2,670 cM, with an average distance of 6.43 cM between markers. The use of this genetic map and its derivatives allowed the development of effective molecular markers for use in marker assisted breeding and selection strategies aimed at incorporating resistance to various biotic constraints into local germplasm (Timko et al. 2007). A genetic linkage map based on segregation of simple sequence repeat (SSR) markers has recently been developed using a recombinant inbred (RI) population of 159 individuals derived from a cross between the breeding line 524B, a California Blackeye type, and 219-01, a perennial wild cowpea from Kenya (Andargie et al. 2011). This genetic map contains approximately 202

Table 7.1 Molecular maps developed in cowpea

Mapping population	Parents	Markers	Number of markers	Average distance (cM)	Genetic length (cM)	Linkage groups	Reference
F2	IT 84S-2246-4×NI 963	RFLP		7.70	680	11	Young (1999)
RILs	IT84S-2049×524B	AFLP, RAPD, RFLP	242	6.43	2,670	11	Ouédraogo et al. (2002)
RILs	Six pairs of parents	SNP	928	0.73	680	11	Muchero et al. (2009a)
RILs	524B×219-01	SSR	639	3.00	677	11	Andargie et al. (2011)
RILs	JP81610×TVnu457	SSR		3.96	852.4	11	Kongjaimum et al. (2012)
RIL	Zhijiang282×ZN016	SNP, SSR		1.98	745	11	Xu et al. (2011)
RIL	IT84S-2049×524B	RAPD, RFLP, AFLP		6.40	972	12	Menéndez et al. (1997)
RIL	13 pairs of crosses	SNP	1,107	Not given	680	11	Lucas et al. (2011)

markers placed in 11 LGs spanning 677 cM, with an average distance between markers of 3 cM. Since the cross involved both a domesticated and wild forms of cowpea, the investigators were able to map agronomic traits related to domestication such as seed weight and pod shattering, as well as floral characteristics.

The advent of new and improved technologies brought rapid and significant refinements in the cowpea genetic map. Among these technologies was the development of platforms for high throughput DNA and cDNA sequencing and single nucleotide polymorphism (SNP) detection. Using SNP assays, Muchero et al. (2009a) were able to map 928 expressed sequence tag (EST)-derived SNPs using an Illumina 1536 GoldenGate platform. This map represented a substantial improvement over previously available genetic maps (Menéndez et al. 1997; Ouédraogo et al. 2002) because it was not population specific and surveyed polymorphism at 1,536 identical loci in six recombinant inbred line (RIL) populations. Building upon this work, a new consensus map containing 1,107 EST-derived SNP markers (856 bins) has been recently reported by Lucas et al. (2011). This new map was developed by integrating 13 population-specific maps and contains 1,107 markers. It is noteworthy that not only these investigators were able to add 179 new markers, an almost 20 % increase in marker density compared to the earlier consensus map created by Muchero et al. (2009a), but the number of informative positions increased on an average by 19 bins per linkage group and the average distance between informative positions was reduced from 1.05 to 0.79 cM. The consensus genetic linkage map for cowpea is given in Fig. 7.1. The SNP-based maps have an additional value as the polymorphic loci mapped are associated with expressed genes (see Lucas et al. 2011). As a consequence of being associated with a known coding region rather that a random or repetitive sequence, it is possible to examine synteny of these gene positions among closely and more distally related legume species. The use of SNPs in syntenic comparisons both with closely related species and subspecies (such as *V. unguiculata* subsp. *sesquipedalis*) and more distally related genera such as *Glycine*, *Medicago*, and *Phaseolus* has been described by Varshney et al. (2009), Lucas et al. (2011).

QTLs

Mapping more QTLs of quantitative trait by analyzing the linkage between molecular marker and those traits are significant. Kongjaimum et al. (2012) identified one major and six minor QTLs for pod length. Andargie et al. (2011) identified the QTLs for agronomic traits related to domestication (seed weight, pod shattering) by SSR markers. Six QTL for seed size were revealed with the phenotypic variation ranging from 8.9 to 19.1 g/100 seeds. Four QTLs for pod shattering were identified with the phenotypic variation ranging from 6.4 to 17.2 %. The QTL for seed size and pod shattering mainly clustered in two areas of LGs 1 and 10. Fatokun et al. (1992) identified major QTLs for seed weight. Muchero et al. (2009b)

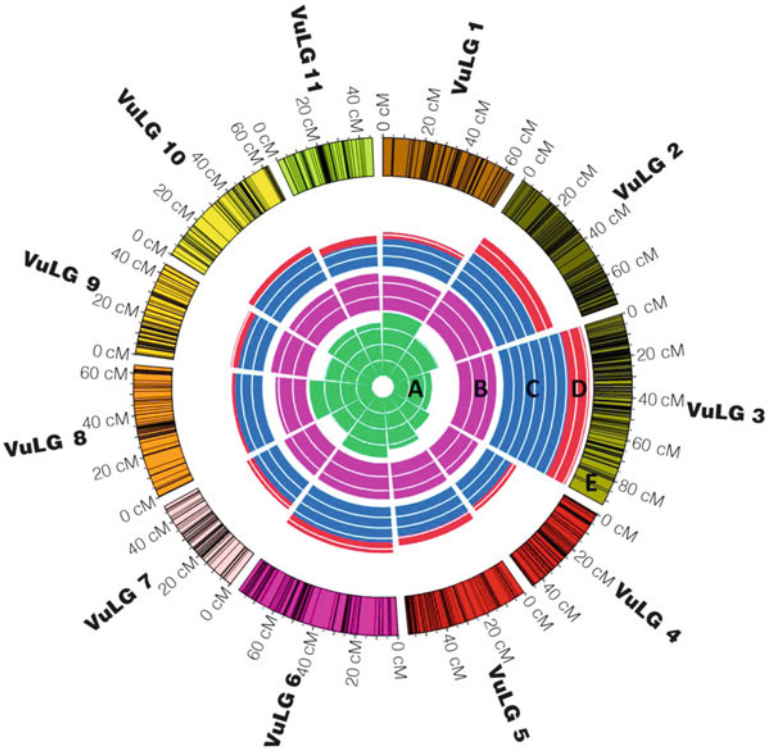

Fig. 7.1 Consensus genetic map of cowpea and parameters depicting map characteristics. (**a**) Average distance between bins (0.25 cM). (**b**) Average number of markers per bin (0.5 units). (**c**) Number of bins (25 units). (**d**) Number of markers (25 units). (**e**) Bin locations. (**c**) and (**d**) begin at the same radial position [Reprinted from Lucas MR, Diop NN, Wanamaker S, Ehlers JD, Roberts PA and Close TJ (2011) Cowpea–soybean synteny clarified through an improved genetic map. Plant Genome 4: 218–225. With permission from ACSESS-Alliance of Crop, Soil, and Environmental Science Societies. Copyright 2011 © Crop Science Society of America]

reported the mapping of 12 QTL associated with seedling drought tolerance and maturity. Regions harboring drought-related QTL were observed on linkage groups 1, 2, 3, 4, 6, 7, 9 and 10 accounting for between 4.7 and 24.2 % of the phenotypic variance. Further, two QTLs for maturity were mapped on linkage groups 7 and 8 separately from drought-related QTL.

A few QTLs of resistance to disease and insects have also been identified. For cowpea bacterial blight, Agbicodo et al. (2010) identified three QTLs, CoBB-1, CoBB-2 and CoBB-3 on linkage group LG3, LG5 and LG9, respectively. Besides, Muchero et al. (2011) identified the QTL for *Macrophomina phaseolina* resistance and maturity. Muchero et al. (2010) also identified three QTL for resistance to *Thrips tabaci* and *Frankliniella schultzei* based on an AFLP genetic linkage map.

ESTs

Among the areas where remarkable progress has been made in recent years is the significant expansion in the number a genomic and transcriptomic sequences (i.e., cDNA, expressed sequence tags, etc.) of cowpea origin available in public databases. Multiple cDNA libraries and approximately 190,000 cDNA sequences and 189,779 ESTs are publicly available from GenBank at NCBI. These represent various cowpea genotypes with the greatest proportion coming from sequence projects carried out by researchers at the University of California, and Department of Energy Joint Genome Institute, USA. For researchers, the cowpea EST assemblies are available through the HarvEST: Cowpea website (http://harvest.ucr.edu, HarvEST: Cowpea 1.27)

BAC Libraries

At least three different bacterial artificial chromosome (BAC) libraries have been produced for cowpea in recent years. The first was created from the IITA advanced breeding line IT97K-499-35 at the University of Virginia (now available through Amplicon Express, http://ampliconexpress.com/aexPremadeLib.php) and representing approximately 6× coverage of the cowpea genome. A 10× library has been constructed by George Bruening and Doug Cook (University of California, Davis; Varshney et al. 2009) from cowpea cultivar Blackeye 5 and used to generate approximately 36.7 Mbp of BAC end sequence (BES). Lastly, a second library from IT97K-499-35, consisting of approximately 60,000 BAC clones (yielding 17× genome coverage) was produced by Tim Close, Jeff Ehlers and Phil Roberts (University of California, Riverside). This library was subjected to automated, high-throughput, high-information-content fingerprinting (Luo et al. 2003) allowing Mingcheng Luo (University of California, Davis) and his colleagues to assemble a physical map of the cowpea genome. The current physical map is an assembly of 43,717 BACs with a depth of 11× genome coverage.

Small RNAs

Among the more recent developments impacting our understanding of the factors that control gene expression was the discovery of small non-coding RNAs in plants (sRNAs). There are two main types of sRNAs based on their biogenesis: microRNAs (miRNAs) and small interfering RNAs (siRNAs). miRNAs are 20–24 nucleotides long and generated by one of the Dicer-like (DCL) proteins from RNA precursors that fold into stem-loop structures MiRNAs regulate gene expression by directing mRNA cleavage or translational repression and have now been shown to be involved

in a variety of developmental processes and responses to various abiotic and biotic stresses (Jones-Rhoades et al. 2006; Sunkar et al. 2007; Brodersen et al. 2008). Several reports have appeared in the literature that examine miRNAs in cowpea. Lu and Yang (2010) used an *in silico* approach to identify 47 potential miRNAs in cowpea belonging to 13 miRNA families previously identified in other plant species. Among these, there were about 30 miRNAs predicted to target genes encoding transcription factors or enzymes participating in the regulation of development, growth, metabolism, and other physiological processes. In another study, 18 conserved miRNAs belonging to 16 families were identified. Paul et al. (2011) similarly used a comparative genomic approach and were able to identify 18 conserved *V. unguiculata* miRNAs belonging to 16 distinct miRNA families. Fifteen of the potential miRNAs were predicted to target transcription factors, and the investigators were able to experimentally validate seven of them as being present and up-regulated in roots during salt stress. In a related study, Barrera-Figueroa et al. (2011) used a combination of NextGen sequencing of sRNA and comparative bioinformatics to identify miRNAs in cowpea specifically associated with drought tolerance. These investigators were able to identify 157 miRNA genes that belong to 89 families including 44 which they were able to predict as drought-associated miRNAs. In addition, about 30 were up-regulated in drought condition and 14 were down-regulated. Many of the targets identified for these miRNAs were transcription factors associated with drought and other stress responses in cowpea.

Transcriptomic Data-Sets

A few transcriptomic datasets have been developed in cowpea indicating that many genes are expressed during drought, extreme temperature, nitrogen deficient conditions as well as during symbiosis and iron accumulation. Several transcripts known as CPRD (cowpea clones responsive to dehydration), CPRD 8, CPRD 14, CPRD 22 and VuNCED1 encode a 9-cisepoxycaratenoid dioxygenase responsible for abscisic acid (ABA) biosynthesis during drought, high salinity and heat stresses that are highly expressed (Iuchi et al. 1996, 2000). Recently, uncharacterized genes which are down-regulated in drought conditions were reported by Coetzer et al. (2010) by using suppression subtractive hybridization. Membrane stability and membrane lipids play greater role in tolerance against drought. Cystatin and aspartic protease are two important proteins related to membrane stability. The transcripts coding these proteins VuC1 and VuAP1 were isolated in drought tolerant cowpea cultivars subjected to water deficit and their expression localized in different organs (de Carvalho et al. 2001; Diop et al. 2004). The investigators reported that, the expression of the gene encoding phospholipase D1 (*VuPLD1*) was moderately increased in the drought tolerant cowpea cultivars (Maarouf et al. 1999), in that phospholipase D is a major lipid degrading enzyme in plants sensitive to drought. In heat stress conditions, analysis of transcripts expression showed 600 bands, among which 55 and 9 were up-regulated and repressed, respectively (Simoes-Araujo et al. 2002).

In other conditions such as in nitrogen deficiency, a decrease of *pur*5 transcript level which codes aminoimidazole ribonucleotide synthetase involved in purine synthesis. In symbiotic association with *Rhizobium*, the gene encoding for leghaemoglobin (*lbll*), a gene similar to the soybean leghaemoglobin *lbll* was found abundantly expressed in cowpea. These transcripts are useful resources for cowpea improvements.

Gene Expression Patterns and Their Regulation

To date only limited information is available on global transcription changes in cowpea plants during developmental and under normal physiological and aphysiological conditions such as biotic and abiotic stress conditions. Using the ~43,253 annotated unigenes obtained from sequencing of the MF gene space from cowpea a 385,000 feature long oligonucleotide-microarray (Roche–NimbleGen) was designed that represents each predicted gene coding sequence with 3–6 long oligos (60-mers) (Huang K, Mellor KE, and Timko, MP, unpublished data). This microarray was then used to examine global changes in gene expression in the roots of the cowpea cultivar B301 during compatible (susceptible) and incompatible (resistant) interactions with *S. gesnerioides* races SG4z and SG3 at 6 days and 13 days post-inoculation (dpi), early and late stages of the resistance response, respectively (Huang K, Mellor KE, and Timko, MP, unpublished data). A total of 111 genes were differentially expressed in B301 roots at 6 dpi, with this number increasing to 2,102 genes at 13 dpi. At 13 dpi during compatible (susceptible) interactions of B301 with SG4z a total of 1,944 genes were differentially expressed. Genes and pathways involved in signal transduction, programmed cell death and apoptosis, and defense response to biotic and abiotic stress were differentially expressed in the early resistance response, whereas at the latter time point enrichment was primarily for defense related gene expression, and genes encoding components of lignifications and secondary wall formation. In compatible interactions (B301–SG4z), multiple defense pathways were repressed including those involved in lignin biosynthesis, secondary cell wall modifications, while cellular transport process for nitrogen and sulfur were increased. These studies show that distinct changes in global gene expression profiles occur in host roots following successful and unsuccessful parasitism attempted by *Striga*. Induction of specific defense related genes and pathways define components of a unique resistance mechanism. Some genes and pathways up-regulated in the host resistance response to SG3 are repressed in the susceptible interactions suggesting that the parasite is targeting specific components of host defense.

Prior to the availability of a cowpea microarray platform, Das et al. (2008) were able to demonstrate that the Affymetrix soybean genome array is a satisfactory system for identification of single feature polymorphisms (SFPs) useful in the development of molecular markers for genetic mapping. Subsequently, the use of this heterologous platform was also shown to be useful in global gene expression

analysis. In order to elucidate cowpea response to root-knot nematodes, Das et al. (2010) examined the transcriptional changes in roots of resistant genotype CB46 and a susceptible near-isogenic lines (null-*Rk*) following infection with *Meloidogyne incognita* using a soybean Affymetrix GeneChip expression array. These investigators found that at 3 days post-inoculation (dpi) 746 genes were differentially expressed in incompatible interactions (infected resistant tissue compared with non-infected resistant tissue) and 623 genes were differentially expressed in compatible interactions (infected susceptible tissue compared with non-infected susceptible tissue). At later stages of nematode infection (i.e., 9 dpi) 552 genes were differentially expressed in incompatible interactions and 1,060 genes were differentially expressed in compatible interactions.

Using a different approach for monitoring global changes in gene expression, Coetzer et al. (2010) recently examined differential gene expression in drought stressed and unstressed cowpea plants by comparing the effects of water deprivation on drought tolerant (IT96D-602) and drought susceptible (Tvu7778) breeding lines developed at the International Institute of Tropical Agriculture (IITA). These investigators used suppression subtractive hybridization (SSH) to create forward and reverse cDNA libraries enriched for cowpea drought response genes. They then selected clones for sequence characterization and quantitative reverse transcription PCR based on the calculation of enrichment ratios using a statistical software pipeline they developed for the analysis (SSH screen 2.0.1; available from http://microarray.up.ac.za/SSHscreen). From the analysis they were able to identify a set of clones representing drought-induced cowpea genes as well as a group of genes significantly down-regulated by the drought stress genes. Among up-regulated category, genes were encoding a late embryogenesis abundant Lea5 protein, a glutathione S-transferase, a thaumatin, a universal stress protein, and a wound induced protein. Among the down-regulated category a lipid transfer protein and several components of photosynthesis were identified.

Marker Assisted Breeding

Marker assisted breeding was successfully employed in developing cowpea cultivars resistant to a parasitic weed, Striga gesnerioides. The SCAR and other PCR amplifiable markers were found capable of tracking most of the major race specific resistant genes to *S. gesnerioides* in West Africa (Boukar et al. 2004; Timko et al. 2007; Li et al. 2009) and the subsequent exploitation of one of these marker SSR 1 facilitated the positional cloning and characterization of the nuclear genes conferring resistance to the noxious pest (Li and Timko 2009). Besides *Striga gesnerioides*, markers were also found to be associated with the rust caused by *Uromyces vignae* An AFLP marker (E-AAG/M-CTG) was converted to a SCAR marker, named ABRSAAG/CTG 98, and the genetic distance between the marker and Rr1 gene was estimated to be 5.4 cM (Li et al. 2007). Myers et al. (1996) found one RFLP marker, bg4D9b, to be tightly linked to the aphid resistance gene (Rac 1).

The close association of rac1 and RFLP bg4D9b presented real potential for cloning this insect resistant gene. Inspite of such progress made, more concerted efforts are required to accelerate in marker assisted breeding to develop high yielding and disease resistant cultivars in cowpea.

Sequencing of Cowpea Genome

The first attempted full genome sequence was recently reported by Close et al. (2011). In this study genomic DNA from IT97K-499-35 (an improved breeding line combining genes for resistance to many diseases, insects and Striga) was shotgun sequenced using an Illumina GAII sequencer with TrueSeq chemistry in a paired-end format. The Illumina sequences (296,868 contigs with total length of ~186 MB, available at http://www.harvest-blast.org) were then assembled using SOAP denovo together with a combination of 260,642 cowpea gene-space random shotgun sequences (Timko et al. 2008) and 30,527 BAC end sequences (obtained from M.-C. Luo, UC Davis, http://phymap.ucdavis.edu:8080/cowpea), 54,123 cowpea Genome Survey Sequences (GSS) from dbGSS of GenBank http://and cowpea EST assembly to yield a draft cowpea genome assembly.

Genetic Transformations

The transformation systems developed have been used to introduce genes related to important agronomic traits into cowpea. The first report on the regeneration and stable transformation of cowpea expressing a gene of agronomic importance appeared in 2008 when a transgenic line that expressed some degree of insect resistance was generated (Solleti et al. 2008a, b). The establishment of an *Agrobacterium tumefaciens*-mediated transformation protocol using geneticin and supplementation of post-selection media with BA (Solleti et al. 2008b). The strategy was based on the use of the gene for alpha-amylase inhibiting protein (*aAI-1*) from common bean (*Phaseolus vulgaris*) as a means of conferring resistance against different insects. The efficiency of transformation in this case was enhanced by using multiple copies of the gene *vir*, co-culture of explants in the presence of thiol compounds and by sequential selection using geneticin (Solleti et al. 2008a). The work reported up to 82.3 % decrease in insect susceptibility in transgenic plants when exposed to pulse beetle (*Callosobruchus chinensis*) (Solleti et al. 2008a). This successful demonstration of cowpea resistance using *aAI-1* gene was followed by the report of another considerable resistance against *Maruca vitrata* by T_3 progenies after transformation of nodal cuttings with a plasmid harboring *Cry1Ab*, the now popular gene for protein toxin from *Bacillus thurigiensis*, using *nptII* as a selectable marker under the control of 35S of CaMV (Adesoye et al. 2008, 2010). These transgenic lines were generated by the T. J. Higgins's group at CSIRO (Australia).

A number of field trials have been going on in the last couple of years. Cowpea plants with high degree of resistance against *Maruca vitrata* and *Callosobruchus maculatus* have been subjected to field trials to test agronomic performance and insect resistance in Puerto Rico and Nigeria with promising results (T. J. Higgins, CSIRO personal communication/http://www.csiro.au/people/TJ.Higgins.html).

We have explored the interfering RNA (RNAi) mechanism to generate transgenic lines that are simultaneously resistant to the *Cowpea severe mosaic virus* (CPSMV) and *Cowpea aphid-borne mosaic virus* (CABMV) (data not published). In addition, plants are also extremely tolerant (more than three times the recommended commercial dose) to herbicides from imidazoline class. Another important candidate gene of great potential in improving cowpea is cystatin, a cysteine proteinase inhibitor with potential as a pest resistance conferring agent. We are currently trying to develop transgenic cowpea expressing chicken cystatin with a view to expressing insecticidal activity against bruchids.

Although cowpea is an important source of nutrients, including several amino acids, it is deficient in sulfur-containing amino acids, a trait common in most legumes. Several strategies have been devised to address this using transgenic technology in a number of legumes. Our group is using a transgenic approach to introduce methionine-rich protein in cowpea using the gene for δ-zein from maize.

In the last few years, significant progress has been made to establish different protocols and their application in the development of transgenic cowpea. There have been important findings that started with obtaining transgenic callus; from that came transgenic plants that exhibited mendelian segregation, culminating in recent findings that have led to the production of transgenic cowpea with agronomic traits. Currently, various research groups in countries including Australia, Brazil, India and Nigeria possess transformation systems that can be used to obtain useful genetically modified lines. Nevertheless, in our experience, only one out of 20 independent transgenic lines obtained has the potential to be introduced into a breeding program to generate a commercial variety. Consequently, despite of having suitable cowpea transformation systems, these technologies should be improved to accelerate the development of cowpea varieties with improved agricultural characteristics.

Conclusion and Perspectives

With the modest beginning, cowpea genomics is now progressing at a rapid pace. Molecular markers are essential resources for accelerating the breeding efforts for cowpea improvement. However, studies of molecular markers on cowpea are meager in comparison to other legumes like soybean and common bean. Therefore, it is necessary to make more serious research efforts in identification of molecular markers for cowpea breeding. Further a very few molecular markers have been found which were linked to resistance gene. There are a lot of diseases like rust, powdery mildew, fusarium wilt, and insect pests like bean weevil and pod borer for which there is need to identify more molecular markers linked to these disease and insect

resistance genes. QTL studies of quantitative traits in the crop are few, which need to be accelerated further for many important agronomic and economic characters such as yield, protein content, and maturity. The QTLs so identified would be useful for research on marker assisted breeding, mechanism of heterosis, genetic diversity, isolation and cloning of gene (s) associated with quantitative trait. The progress towards cowpea genome sequencing (Timko et al. 2008) in combination with the availability of genomic resources from other model legumes would help identify candidate genes that govern the agronomically important traits. After finalization of sequencing and the annotation of genome more efforts need to be done to understand the interactions between the small non coding RNA (small interfering RNA, micro RNA, trans-acting RNA, etc.) (Borsani et al. 2005). There is need for more studies to be done on cowpea proteome, metabolome, lipidome and ionome analyses. All these efforts are needed to complement to improve cowpea for higher production, resistance against key pests and diseases and quality.

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