Chapter 10
Genomics-Assisted Breeding for Drought Tolerance in Cowpea

Siva K. Chamarthi, Nouhoun Belko, Abou Togola, Christian A. Fatokun and Ousmane Boukar

Abstract The importance of cowpea, *Vigna unguiculata*, in human and animal nutrition and sustainability of soil fertility are recognized globally especially in sub-Saharan Africa (SSA) where the crop is mainly produced in the Savanna and the Sahelian agro ecologies. However, cowpea productivity is adversely affected by both biotic (insect pests, diseases, parasitic weeds, nematodes) and abiotic (drought, heat, low soil fertility) constraints. Appreciable progress has been made in the improvement of cowpea for resistance to some biotic stresses particularly diseases such as bacterial blight, ashy stem blight, marcophomina, parasitic weeds like Striga and Alectra and some insects like aphid, leaf and flower thrips among others. There is need for intensifying research activities with focus on improving cowpea resistance to abiotic stresses. As a crop grown commonly in arid regions, cowpea is subjected to seedling stage, midseason and terminal droughts. In the recent past, the amount of rainfall, during the cropping season in the dry savannah regions of SSA, is getting less. Consequently the cropping season is getting shorter occasioned by late commencement or early cessation of the rain. Farmers in the cowpea producing areas of SSA generally have no access to irrigation hence their crops are grown under rain-fed conditions. With the impending higher frequency of drought in the dry savannah region due to climate change, efforts should be made in developing climate resilient cowpea varieties that farmers will grow. Efforts have been made in enhancing tolerance to drought in some improved cowpea varieties using conventional breeding but progress has been slow in this regard. Drought tolerance is a complex trait and many genes are involved in its inheritance. Pyramiding of these genes in improved varieties would therefore, be desirable. Such varieties with pyramided genes are likely to be stable in performance over the years and across several locations in the savannahs. Recent developments in molecular biology could play significant role in the development of such resilient varieties. In a number of crops, molecular markers associated with resistance loci have been identified and are being used in marker assisted breed-

---

S. K. Chamarthi · N. Belko · A. Togola · O. Boukar (✉)
International Institute of Tropical Agriculture (IITA), Kano, Nigeria
e-mail: o.boukar@cgiar.org

C. A. Fatokun
International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria

V. R. Rajpal et al. (eds.), *Genomics Assisted Breeding of Crops for Abiotic Stress Tolerance, Vol. II*, Sustainable Development and Biodiversity 21,
https://doi.org/10.1007/978-3-319-99573-1_10

ing. Marker assisted backcrossing (MABC) is the choice when single traits that are simply inherited are to be moved to varieties with superior performance but lacking in the trait being transferred. Also, marker assisted recurrent selection (MARS) has shown promise in accumulating multiple genes in improved varieties of some crops. Some work has been initiated in cowpea on the use of MARS to pyramid resistance to Striga, yield and drought. Results obtained so far show the potential of this method in pyramiding desirable genes in cowpea. As more resources get committed to cowpea research a solid foundation would be established for the generation of molecular tools that should facilitate their routine application to the improvement of the crop.

Keywords Cowpea · Drought tolerance · MAGIC populations · MARS · Striga

10.1 Introduction

Cowpea (*Vigna unguiculata* L. walp.) belongs to the genus *Vigna* and family *Fabaceae*. It is one of the four cultivated species of the genus with the remaining three being *V. cylindrica, V. sesquipedalis* and *V. textilis*. It is a highly self-pollinating crop, diploid with $2n = 22$ and has an estimated genome size of 620 Mb (Chen et al. 2007). Its genome is similar to that of some other warm season legumes, particularly the common bean (*Phaseolus vulgaris* L.) (Vasconcelos et al. 2015). Although, cowpea is one of the most important crops in sub-Saharan Africa where it is considered drought-tolerant as compared to other legumes and cereals cultivated in the semi-arid regions, but it still encounters significant damage and yield losses due to severe and frequent droughts. Its grains and pods play an important role in human nutrition, while biomass provides good nutritious fodder to livestock (Ehlers and Hall 1997; Singh et al. 2003; Boukar et al. 2016). Its grains are rich in carbohydrates, protein and folic acid, and contain respectable amounts of some minerals (Boukar et al. 2011, 2016; Carvalho et al. 2017). The pods are also rich in protein, chlorophyll, carotenoids, phenolics and have high antioxidant activity, low concentrations of nitrates and raffinose family oligosaccharides (Karapanos et al. 2017). It is a great source of income for small holder famers and food vendors. Based on evaluation of 1541 germplasm lines, cowpea grains were estimated to contain on average approximately 25% protein, 53.2 mg/kg iron, while zinc, calcium, magnesium, potassium, and phosphorus content were reported to be 38.1, 826, 1915, 14,890, and 5055 mg/kg, respectively (Boukar et al. 2011). As a leguminous species, cowpea has the ability to fix nitrogen from the atmosphere, some of which is left in the soil for succeeding crops (Sanginga et al. 2000). As a relatively drought tolerant crop, cowpea is excellent for studying the genetic basis of drought tolerance.

Globally, cowpea production is projected at around 6.5 million metric tonnes annually on about 14.5 million hectares. Approximately 83% of the worldwide cowpea production is obtained in Africa, out of which, 80% production is in West-Africa. The world's major producer and consumer of cowpea is Nigeria (45% production), followed by Niger (15%), Brazil (12%), and Burkina Faso (5%). Fatokun et al. (2012a) reported that during 1980–2010, there was an increase of an average rate of 5%, with 3.5% annually in area and 1.5% in yield, implying that 70% of the total growth in cowpea production during this period is accounted for area expansion. Worldwide, the proportion of cowpea in total cultivated area under pulses increased from 10% in 1990 to nearly 20% in 2007 (Boukar et al. 2016). According to Fatokun et al. (2012a) and Boukar et al. (2016), demand for cowpea in West Africa is expected to grow at a faster rate of 2.68% per year than supply (2.55%) over the period 2007–2030. Average cowpea yield in farmer's field is very low due to several biotic and abiotic stresses. Among abiotic stresses, drought is the major constraint in cowpea production in the West African Sahel and dry Savannahs.

Climate change may lead to a higher frequency and severity of drought events as already being experienced in the dry savannah regions of sub-Saharan Africa (SSA). Drought is a major constraint to crop production in SSA where irrigation facilities are grossly inadequate. It has been forecasted that by 2050, water shortages will affect 67% of the world's population (Ceccarelli et al. 2004). Terminal drought is one of the most common environmental stresses that continues to be a challenge to sub-Saharan African farmers and plant breeders. Drought tolerance is a complex trait controlled by polygenes whose expressions are influenced by environmental factors. Therefore, unraveling its genetic basis is crucial for both breeding and basic research. Breeding for drought tolerance with conventional methods could be difficult and time consuming. This calls for concerted efforts by various players such as geneticists, breeders, molecular biologists, physiologists and agronomists among others. Due to rapid population growth particularly in the developing countries where climate change is likely to have greater devastating impact, conventional breeding procedures may not be adequate in developing improved varieties that can ameliorate the challenges. New tools involving molecular breeding have the potential to contribute positively to needed progress in developing appropriate technologies to combat effects of climate change. With the recent progress in genomics, it should be feasible to breed robustly drought tolerant varieties within shorter period. In this chapter, we describe recent developments in genetic and genomic resources, and molecular breeding in cowpea with emphasis on drought tolerance.

10.2 Drought Tolerance Phenotyping and Mechanisms in Cowpea

Breeding for drought tolerance and high yield under drought has not been as successful as for simply inherited traits in cowpea. This is mainly due to the complexity of abiotic factors and plant mechanisms involved in drought tolerance and the lack of simple, accessible and reliable trait-based phenotyping techniques to select drought-tolerant progenies from segregating populations (Singh and Matsui 2002). Drought tolerance in plants is a complex trait since various environmental parameters (air temperature and humidity, soil texture, moisture and fertility) and plant features and strategies (in shoots and roots) operate jointly to enable crop plants cope with drought stress. Therefore, it is important to discriminate and investigate these factors and mechanisms individually, study and understand their interactions and contributions to plant drought tolerance in a given environment or drought scenario, so they will be easy to manipulate by breeders and use in crop improvement (Vadez et al. 2013; Sinclair et al. 2015). Physiological phenotyping for drought tolerance is quite expensive, time consuming, and difficult to use for screening crop germplasm with large number of accessions. However, significant efforts and achievements were made in developing high throughput plant physiology screening methods for improving drought tolerance in cowpea over the past decades.

Singh et al. (1999a) developed a simple “wooden box screening technique” which eliminates the influences of the root system and allows nondestructive identification of plant shoot dehydration tolerance at seedling stage in cowpea. Two types of drought tolerance mechanisms at vegetative stage were identified and described by Mai-Kodomi et al. (1999a). Upon exposure to progressive soil water deficit stress, the Type 1 drought-tolerant lines (TVu-11986 and TVu-11979) stopped growth and conserved water in all the plant tissues, stayed alive for over two weeks without irrigation, and gradually the entire plant parts dried as the drought stress became intense and drastic. The type 2 drought-tolerant lines (Dan’Ila and Kanannado) continued slow growth of the trifoliates but with increased soil moisture deficit stress, their unifoliates senesced early and dropped off with their growing tips remaining turgid and alive relatively longer. Mai-Kodomi et al. (1999b) studied the inheritance of drought tolerance at seedling stage of cowpea. Three cowpea genotypes: TVu-11986 with Type 1 drought tolerance, Dan’Ila with Type 2 drought tolerance, and TVu-7778 as drought sensitive were crossed in multiple combinations, and the segregation pattern revealed that vegetative stage drought tolerance is a dominant trait and both Type 1 and Type 2 reactions are controlled by a single dominant gene but the genes are independent in the two types. The box screening method showed good correlation with drought tolerance at vegetative and reproductive stages, and was also efficient in evaluating and selecting drought-tolerant plants in different crop species (Singh et al. 1999b; Tomar and Kumar 2004; Slabbert et al. 2004; Ewansiha and Singh 2006).

Pot experiments in the screen-house and growth chamber showed that reduced plant leaf area, restricted canopy water loss, transpiration efficiency, delayed leaf senescence, prolonged and sustained stem greenness are important traits for enhancing cowpea growth and grain yield in drought-prone environments (Muchero et al. 2008; Belko et al. 2012, 2013). However, selection based on phenotype has been relatively slow and difficult mainly due to the unpredictable timing, intensity and occurrence of drought and considerable genotype-by-environment interactions and effects on phenotypic expression in the field. Cowpea has a noteworthy capability to survive drought by limiting its water loss or enhancing soil water uptake and use through various anatomical, morphological, biochemical and physiological strategies. Plants “escape” drought by changing phenological development and the period of a particular growth phase (Agbicodo et al. 2009). Some cowpea varieties evade terminal drought by early flowering (around 12 days), whereas other varieties respond by staying green for weeks and flower later when favorable conditions are re-established (Fatokun et al. 2012b).

Drought "avoidance" strategies are predominantly physiological and morphological alterations to withstand drought stress while maintaining relatively high tissue moisture. A few of the intrinsic and stress-induced mechanisms documented in cowpea include, but not limited to, higher root density or depth (Sicher et al. 2012), decreased leaf area, enlarged leaf waxiness and thickness (Singh and Raja Reddy 2011), reduced stomatal and lenticular conductance and leaf rolling (Fatokun et al. 2012b; Hall 2012). The crop shows very little variations in leaf water content under extreme drought; an isohydric phenomenon, associated with three drought evading mechanisms i.e. stomata closure, paraheliotropism, and high root hydraulic conductivity (Agbicodo et al. 2009). Drought "tolerance" traits are mainly associated with osmotic adjustments, which result from the synthesis and accumulation of compatible solutes in the cytoplasm as well as movement of solutes into the vacuoles (Warren et al. 2011; Khan et al. 2015; Blum 2017). These hydrophilic solutes, by replacing water molecules on membrane and protein surfaces, raise the cellular osmotic pressure and concomitantly the water potential gradient between soil and roots, which allows continued water influx via osmosis. Modifications and/or stabilization of cell walls and membranes also confer tolerance to drought (Lugan et al. 2010; Jin et al. 2016). To lessen the deleterious consequences of water shortage, plants use these mechanisms either independently or jointly.

Belko et al. (2014) evaluated the impacts of reproductive stage drought stress on the growth, development and yields of a diverse set of thirty early and thirty medium maturity cowpea cultivars under post-flowering water stressed (WS) and well-watered (WW) conditions in the field using stress tolerance selection indices e.g. stress tolerance index (STI) and geometric mean productivity (GMP). Overall, lines IT85F3139, IT93K-693-2, IT97K-499-39, IT93K-503-1, IT96D-610, IT97K-207-z15, KVx-61-1, KVx-403, KVx-421-25, and Mouride exhibited the highest grain yield in both WS and WW environments and were therefore identified as the most drought-tolerant lines based on their outstanding STI and GMP values. Vadez et al. (2012a) argued that despite the complexity of the plant's response to drought, simple hypotheses based on soil water availability and plant water-use pattern (water supply vs. demand) can be developed to guide selection of critical plant traits that are capital for adaptation to drought-prone regions. Hence, Belko et al. (2012) tested the hypothesis that water saving shoot traits is important for end-of-cycle drought tolerance and thereby discriminates drought tolerant and susceptible lines. Thus they phenotyped a wide range of cowpea genotypes for their variation in vegetative growth attributes and water-use patterns under different water regimes (WW and WS) and atmospheric vapor pressure deficit (VPD) outdoors, in glasshouse and growth chamber. However, gravimetric measurement of whole plant leaf canopy transpiration rate (TR) involves weighing pots and can be laborious and time consuming. Therefore, Belko et al. (2013) set the conditions (plant age, time of the day) and tested and validated a method in which plant transpiration rate (TR) can be indirectly assessed via plant canopy temperature (CT) in a high throughput mode using an infrared imaging system. Under WS conditions, canopy transpiration dropped at a lower fraction of transpirable soil water in drought tolerant than in susceptible lines. Tolerant lines also maintained higher transpiration efficiency (TE) and TR, and lower CT under severe water stress (Belko et al. 2012). Under WW conditions, cowpea plants grew and developed larger biomass under low VPD than under high VPD, with a consistent trend of lower leaf area and biomass in drought tolerant lines. Substantial differences existed among cowpea lines in their TR response to natural variation of VPD, with drought tolerant lines having significantly lower TR than sensitive ones, especially at times of highest VPD. Cowpea genotypes also varied in their TR response to progressively increasing VPD, with some tolerant lines displaying a clear VPD breakpoint at about 2.25 kPa, above which there was very little increase in TR whereas sensitive genotypes showed a linear increase in TR as VPD increased. Canopy temperature, estimated with thermal imagery, was highly correlated with TR and could therefore be used as proxy for canopy transpiration (Belko et al. 2013). Plant traits that control canopy water loss when soil water is available at vegetative stage such as low leaf area, low TR by stomata control, and reduced TR in response to high VPD discriminated between drought tolerant and sensitive cowpea lines and, therefore, are reliable indicators of terminal drought stress tolerance. A lower TR could limit plant growth and water use at vegetative stage, and allow drought tolerant genotypes to behave like unstressed plants late in the season when the soil water is progressively depleted. The water saving shoot characteristics of some cowpea genotypes are hypothesized to conserve more water in the soil profile, which is crucial for pod and grain filling and subsequently terminal drought adaptation.

Root-related traits i.e. deep, profuse, dense and extensive root systems are thought to be key in conferring drought tolerance to cultivated crop plants (Lynch 2007; Singh et al. 2010; Gowda et al. 2011). Although variation in root growth and morphology can increase the amount of water uptake under drought and root traits are used as surrogates for soil water extraction (Vadez et al. 2008, 2012; Gowda et al. 2012), the relationships between root traits and water acquisition and their contribution to yield formation under drought remain unclear across crop species (Vadez et al. 2007). Few investigations have been carried out on roots in cowpea and most have focused on the analysis of root growth and morphological differences using limited number of test lines (Matsui and Singh 2003; De Ronde and Spreeth 2007; Onuh and Donald 2009). Moreover, these studies did not address whether root attributes relate to soil water accessibility and use under drought, especially during critical pod and grain filling stages. A “root-box pin-board’ technique was developed to study the two-dimensional root system of large number of cowpea plants and progenies, and permitted characterization of major variations for root system architecture (deep and profuse vs. shallow and dense systems) in cowpea (Singh and Matsui 2002). More recently, Burridge et al. (2016) developed an integrated low-cost and high throughput visual, manual (shovelomics) and image-based (DIRT: digital imaging of root traits, an automated image analysis software) phenotyping technique for in situ field and laboratory evaluation of root phenes in cowpea. The method was used for quantitative evaluation of root architectural traits, and identification and selection of useful root phenes among 189 lines of cowpea diversity panel. Like in other major crops of economic importance (maize, common bean, soybean), several root phenotypes i.e. adventitious and basal root numbers and growth angle, tap root diameter at different soil layers, secondary or lateral root numbers and branching density, root nodules and diseases scores, significantly varied among tested cowpea genotypes. Genetic analysis was performed to evaluate the relationships between cowpea root traits and agronomic performance and tolerance to parasitic weeds in the field. It was found that plants with steep and profuse root system were better adapted to drought conditions while those with shallow and dense root system were tolerant to low phosphorus and Striga infestation (Burridge et al. 2016). The results therefore suggested the adoption of this integrated root phenotyping platform in the breeding program to improve cowpea adaptation to multiple constraints e.g. vegetative and reproductive stage droughts and Striga infestation.

10.3 Genetic Resources in Cowpea

Genes responsible for resistance/tolerance to several abiotic and biotic stresses have been identified through the germplasm screening, available in different countries. The International Institute of Tropical Agriculture (IITA) is maintaining more than 15,000 accessions of cultivated cowpea and over 2000 wild relatives, in its genetic resources center. Mining these resources has identified several potential donors, which can impart resistance to biotic and abiotic stresses. Several cowpea lines resistant/tolerant to abiotic and biotic stresses have been reported (Ferry and Singh 1997; Singh 2002; Boukar et al. 2013, 2015, 2016). Breeders will continue to rely on these genetic resources as sources of genes for desirable traits in cowpea improvement. They have the potential to provide genes for developing new varieties that will help in combating emerging problems that would arise due to climate change as well as human food and animal feed requirements. It is also worth noting that wild cowpea relatives have hardly been utilized in the development of new improved varieties. A lack of interest in the use of cowpea wild relatives can be attributed to the possibility of linkage drag that may occur from their use as parents. For example, wild cowpea relatives have very small seed size with smooth and unattractive seed coat colors. Several backcrosses to the recurrent parents would be needed in order to recover the cowpea seed size desired by consumers. However, with recent developments in genomics which can facilitate progress in breeding new varieties through marker-assisted selection, more interests may shift in favor of the available crop wild relatives as sources of new genes.

10.4 Genomic Resources in Cowpea

The development of genomic resources for cowpea has lagged behind compared with many other crops. However, because of the advantages associated with the new marker technology, concerted efforts are now being devoted to the development of genomic resources in cowpea. An appreciable amount of progress has already been made from these efforts (Muchero et al. 2009a, 2010, 2013; Amatriain et al. 2017). The molecular markers based genetic linkage maps for cowpea have been published, although not yet aligned with physical maps (Amatriain et al. 2017). These linkage maps have been utilized to identify quantitative trait loci (QTL) associated with morphological as well as stress related traits (Table 10.1) (Boukar et al. 2016). Major recent developments in cowpea genomics include sequence assemblies from 65× coverage whole-genome shotgun (WGS) short reads, a bacterial artificial chromosome (BAC) physical map, minimal tiling path (MTP) BACs, and assembled sequences from 4355 BACs using an improved variety (IT97 K-499-35) which has been released to farmers in several African countries due to its superior yield performance and resistance to *Striga gesnerioides*. Additionally, more than one million SNPs have been discovered from sequences of 36 diverse cowpea accessions supported by the development of a genotyping assay (Illumina Cowpea iSelect Consortium Array) for 51,128 SNPs. Five bi-parental RIL populations (Tvu-14676 × IT84S-2246-4, Sanzi × Vita7, ZN016 × Zhijiang282, CB46 × IT93 K-503-1, and CB27 × IT82E-18) were genotyped with this genotyping platform to produce a consensus genetic map containing 37,372 SNP markers. This genetic map has enabled the anchoring of 100 Mb of WGS and 420 Mb of BAC sequences, an exploration of genetic diversity along each linkage group, and synteny between cowpea and common bean (Amatriain et al. 2017). Updated versions of the cowpea consensus map are accessible via HarvEST:Cowpea (http://harvest.ucr.edu/). With the above listed genomic resources in cowpea, opportunities now abound for the fine mapping of QTLs, map-based cloning, assessment of genetic diversity, association mapping and marker-assisted breeding.

The first DNA marker based genetic linkage map for cowpea was published by Fatokun et al. (1993) followed by Menendez et al. (1997), Ubi et al. (2000) and Ouedraogo et al. (2002a) using RFLP, RAPD, AFLP, cDNA and morphological markers. However, cowpea genome resolution was poor based on these published maps. First attempt to improve these maps was carried out by Muchero et al. (2009a, b), using an Illumina GoldenGate assay having 1,536 EST-derived SNP markers. The authors genotyped a total of 13 recombinant inbred line (RIL) populations, which not only improved the map resolution but also made orthologous gene identification easier by increased synteny with soybean genome (Lucas et al. 2011). The most recent consensus genetic map described above (Amatriain et al. 2017) has a 4-fold increase in marker density and a four-fold increase in resolution (number of bins) over the consensus map of Lucas et al. (2011). This map has dense coverage of all eleven cowpea linkage groups, with 1.85 cM on LG1 being the largest gap.

Table 10.1 List of QTLs for drought and other related traits

Trait	Cross	Population type	Marker system	LG	No. markers/QTLs	PV%	References
Drought traits							
Root architecture traits	Genome wide association study	Diversity panel entries	SNP	All LGs	11 and 21	–	Burridge et al. (2017)
Drought-induced senescence and maturity	CB46 × IT93K503–1	RIL	AFLP	LG1, LG2, LG3, LG5, LG6, LG7, LG8, LG9, LG10	12	5–24; 25–29	Muchero et al. (2009b)
Delayed senescence, biomass, grain yield	Germplasms and IT93 K-503-1 × CB46.	Germplasms and RIL	SNP	–	3	–	Muchero et al. (2013)
Heat tolerance	CB27 × IT82E-18	RIL	SNP	LG2, LG3, LG6, LG7, LG10	5	12–18	Lucas et al. (2013)
Other related traits							
Aphid resistance	CB27 × IT97 K-556-6	RIL	SNP	LG1, LG7	2	4.8–65.7	Huynh et al. (2015)
Macrophomina phaseolina and maturity	IT93 K-503-1 × CB46	RIL	SNP/AFLP	LG2, LG3, LG5, LG6, LG11	9	6.1–40	Muchero et al. (2011)
Fusarium wilt resistance (Fot race 3 & 4)	CB27 × 24-125B-1, IT93 K-503-1 × CB46, CB27 × 24-125B-1, CB27 × IT82E-18.	RIL	SNP	LG1; LG8, LG9, LG3	1; 1	28; 18–47	Pottorff et al. (2012b, P2014)
Bacterial blight resistance	C-152 × V-16; DanIla × TVu7778.	F2:3/RIL	SSR/CISP/SNP	LG3, LG5, LG9; LG8, LG11	3; 3	10.63–30.58; 10–22	Dinesh et al. (2016), Agbicodo et al. (2010)
Golden mosaic virus	IT97 K-499-35 × Canapu T16.	F2	AFLP	–	3	–	Rodrigues et al. (2012)
Striga resistance	IT84S-2246 × Tvu14676; TVx 3236 × IT82D-849; IT93 K-693-2 × IAR1696.	F2	AFLP/SCAR	LG1	2, 4		Ouedraogo et al. (2001), (2002a, b), (2012), Boukar et al. (2004)

(continued)

Table 10.1 (continued)

Trait	Cross	Population type	Marker system	LG	No. markers/QTLs	PV%	References
Seed size	524B × 219-01/Eight different populations.	RIL	SSR/SNP	LG1, LG10/LG2, LG5, LG6, LG7, LG8, LG10	6/10	9–19/47	Andargie et al. (2011), Lucas et al. (2013)
Seed weight	IT2246-4 × TVNu1963/524B × 219-01	F2/RIL	RFLP/SSR	LG2, LG6/LG1, LG2, LG3, LG10	2/6	37–53/8–19	Fatokun et al. (1992), Andargie et al. (2011)
Time of flower opening	524 B × 219-01	RIL	SSR	LG1	5	9–30	Andargie et al. (2013)
Days to flower	524 B × 219-01	RIL	SSR	LG1	3	6–19	Andargie et al. (2013)
Days to first flowering	ZN016 × ZJ282	RIL	SNP	LG11, LG10, LG3	3	10–32	Xu et al. (2013a, b)
Pod number per plant	ZN016 × ZJ282	RIL	SSR	LG3, LG2, LG4	3	11–20	Xu et al. (2013a, b)
Leaf senescence	ZN016 × ZJ282	RIL	SNP	LG11, LG3, LG7	2	11–29	Xu et al. (2013a, b)

In addition, a core germplasm of landraces collected from across cowpea-growing regions in Africa and other parts of the world has been characterized using SNP markers (Huynh et al. 2013). Using DNA markers, QTLs have been detected by several authors for key traits including drought tolerance (Muchero et al. 2010, 2013), seed quality traits (Lucas et al. 2013), resistance to root-knot nematodes (Huynh et al. 2016), root pathogens including *Macrophomina phaseolina* (Muchero et al. 2011) and fusarium wilt (Pottorff et al. 2012b, 2014), insects (Huynh et al. 2015; Lucas et al. 2012), and the parasitic weed Striga (Boukar et al. 2004; Ouedraogo et al. 2001, 2002a, b, 2012). Fatokun et al. (1993) reported an orthologous QTL for seed size in both cowpea and mungbean using RFLP generated linkage maps of both crops. Same RFLP markers spanned the regions associated with seed weight QTL in the two leguminous crops. In addition, an aphid resistance locus defined by an RFLP marker was reported by Myers et al. (1996). Muchero et al. (2009b) identified 12 QTLs for seedling drought tolerance and maturity using a RIL population based on the cross between IT93 K-503-1 and CB46. A few of these QTLs colocated with QTLs for recovery dry weight (rdw) and stem greenness (stg) under drought stress both under field and greenhouse conditions. A major QTL affecting cowpea leaf shape (associated with drought tolerance) was reported by Pottorff et al. (2012a). Recently, Muchero et al. (2013) utilized phenotypic data from multiple locations and identified seven SNP-trait associations with stay-green trait. Five of these loci also showed pleiotropic effects on biomass, grain yield, and delayed leaf senescence. These QTLs, particularly those identified in two RILs and diverse germplasm can be potential targets for marker-assisted breeding of cowpea varieties with improved drought tolerance. In another study, co-location of three *Macrophomina* resistance QTLs (*Mac-4, Mac-5 & Mac-9*) and three seedling drought response QTLs (*Dro-5, Dro-10 & Dro-7*) were identified from the RIL population IT93 K-503-1 × CB46 (Mucheroet al. 2009b 2011). Burridge et al. (2017) conducted a genome-wide association study and reported 32 significant QTLs for root architecture traits.

10.5 MAGIC Population in Cowpea

QTL mapping using bi-parental populations has limitations because of limited allelic diversity and genomic resolution. A multi-parent advanced generation inter-cross (MAGIC) population strategy has been proposed to integrate multiple alleles and provide increased recombination and mapping resolutions (Bandillo et al. 2013). The increased recombination in MAGIC populations can lead to rearrangements of alleles and greater genotypic diversity.

Cavanagh et al. (2008) proposed that MAGIC populations should provide state-of-the-art approach for developing plant population resources for genetic analysis and increased genetic variability in breeding. In creating a MAGIC population, multiple elite parents are inter-crossed for several cycles followed by single-seed descent, which results in RILs having a mosaic of genome blocks coming from all founders. Development of MAGIC populations have been carried out in a few crops including rice, wheat and chickpea (Huang et al. 2015). In cowpea, MAGIC population was established employing eight founder parents (SuVita 2, CB27, IT93K-503-1, IT89KD-288, IT84S-2049, IT82E-18, IT00K-1263, & IT84S-2246-4) that were not only genetically diverse but also carried genes for resistance to several biotic and abiotic stresses, seed quality and agronomic traits relevant to SSA. Phenotyping and genotyping of the MAGIC RILs at F_8 generation showed an average of 99.74% homozygosity and diversity in 38 agronomic traits (Huynh et al. 2017). Due to its wide genetic base, the cowpea MAGIC population has become an important genetic resource for high-resolution genetic mapping and for gene discoveries (Huynh et al. 2017).

10.6 Marker-Assisted Selection in Cowpea

Marker-assisted selection (MAS) offers great prospects for increasing genetic gain per crop cycle, by stacking favorable alleles at target loci and reducing the number of selection cycles. Markers identified in one population need to be validated in other populations or germplasm collections, and closely linked markers flanking the QTL should be used for indirect selection of the trait. Once potential markers, validated QTL are identified, they can be used in breeding. We describe below marker-assisted backcrossing (MABC), marker assisted recurrent selection (MARS) and genome wide association mapping (GWAM) schemes, which are currently being used in our cowpea breeding program to incorporate genes for resistance to some abiotic and biotic stresses (Fig. 10.1).

10.6.1 Marker-Assisted Backcrossing

Marker-assisted backcrossing (MABC) is a fast-track approach to increase the genetic gain of crops and is in use for variety development of several crops (Chamarthi et al. 2011; Varshney et al. 2014; Boukar et al. 2016; Cheng et al. 2017; Ouedraogo et al. 2017). MABC can be used to introgress major genes/QTL from one genetic background (donor parent) to another (recurrent parent) much more precisely than phenotypic selection. The outcome of MABC is a line containing only the major genes/QTL transferred from the donor parent, while retaining a vast proportion of the genome of the recurrent parent. Three types of selection can be done in MABC: foreground, recombinant and background. Foreground selection involves the selection of target genes/QTL on the carrier chromosome with the help of two QTL-linked flanking markers. It can be used to select for laborious or time-consuming traits and it allows selection of heterozygous plants at the seedling stage and therefore identifies plants desirable for backcrossing. Furthermore, identification and selection of recessive alleles can be done, which is otherwise difficult to achieve using conventional methods. Recombination events between the target locus and linked flanking mark-

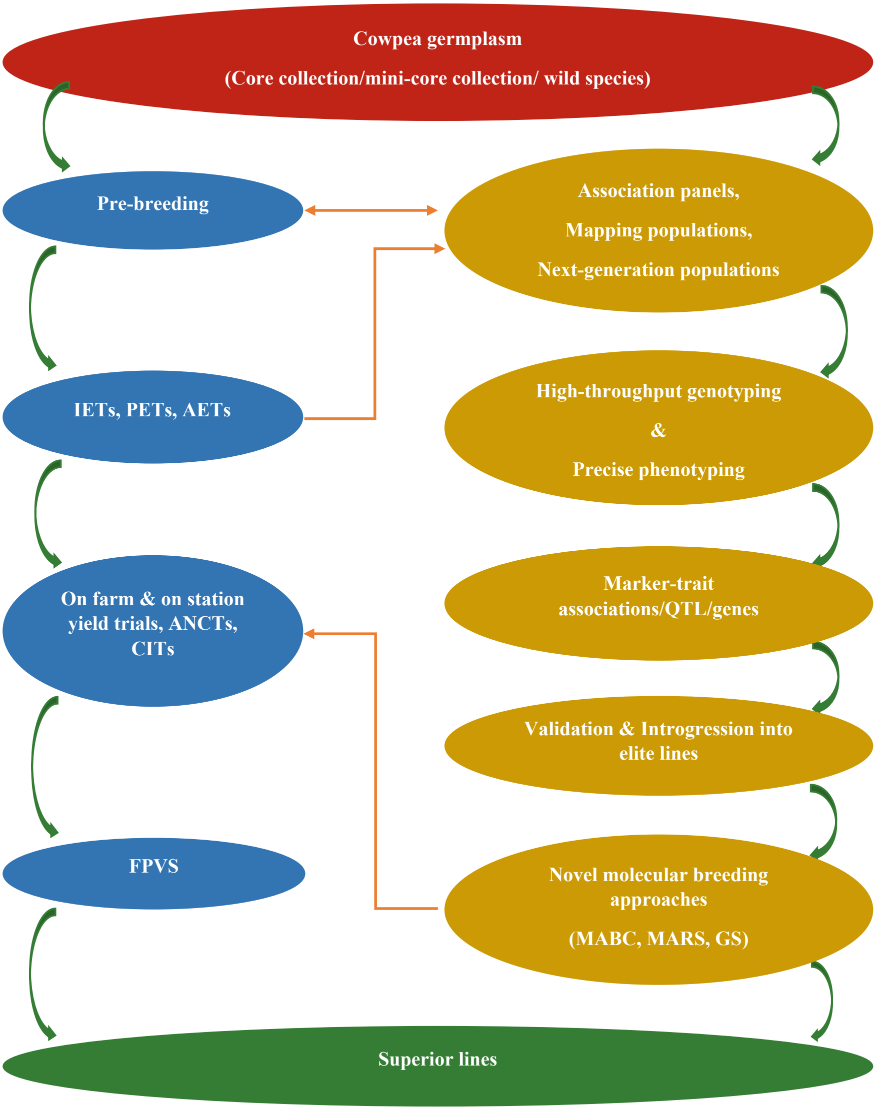

Fig. 10.1 Schematic diagram shows integrated molecular and conventional breeding approaches in cowpea. *MAGIC* Multi-parent Advanced Generation Inter-Cross populations, *QTL* Quantitative Trait Loci, *MABC* Marker Assisted Backcross, *MARS* Markers Assisted Recurrent Selection, *GS* Genomic Selection, *IETs* Initial Evaluation Trials, *PETs* Preliminary evaluation Trials, *AETs* Advance Evaluation Trials, *ANCTs* All Nigeria Co-ordinated Trials, *CITs* Cowpea International Trials, *FPVS* Farmer Preferred Varietal Selection

ers can also be identified in backcross progeny. This can be used to reduce linkage drag, which is difficult to overcome through the use of conventional backcrossing. Background selection involves selection of BC progeny with highest proportion of recurrent parent genome, using unlinked markers present on non-carrier chromosomes (Hospital and Charcosset 1997; Frisch et al. 1999; Chamarthi et al. 2011; Varshney et al. 2014; Batieno et al. 2016). Using tightly linked markers, a target gene can be transferred with minimum linkage drag in two backcross generations, which otherwise would take 8–10 generations by conventional backcrossing (Tanksley et al. 1989).

In cowpea, MABC has been implemented through the CGIAR-GCP-TLI (Consultative Group of International Agricultural Research-Generation Challenge Programme-Tropical Legumes I) project at IITA and NARS centers in collaboration with the University of California, Riverside (UCR). At IITA, using IT97K-499-35 as the donor, two released varieties, IT93K-452-1 and IT89KD-288, have been improved in Nigeria, for Striga resistance. At INERA, efforts are being made to improve Moussa and KVx745-11P for Striga resistance and seed size using IT93K-693-2 and KVx414-22, as donors for Striga resistance and seed size, respectively. At Eduardo Mondlane University (EMU), Mozambique, IT85F-3139 is being improved for grain quality (seed size) using CB27 as a donor.

10.6.2 Marker-Assisted Recurrent Selection

While MABC targets major large effect QTL that has been validated across different genetic backgrounds, MARS aims at accumulating a large number of QTLs in a given population using a subset of markers that are significantly associated with target traits (Bernardo 2008; Ribaut et al. 2010; Chamarthi et al. 2011; Xu et al. 2013a, b; Boukar et al. 2016). In brief, MARS is a modern breeding approach that enables breeders to increase the frequency of several beneficial alleles with small individual but additive effects in recurrent cycles. This involves multiple cycles of marker-based selection that include improvement of F_2 progeny by one cycle of MAS based on marker and phenotypic data, followed by three recombination cycles of the selected progenies based on marker data only and repetition of these cycles to develop the population for multi-location phenotyping (Tester and Langridge 2010). In MARS, a selection index is used that gives weights to markers according to the relative magnitude of their estimated effects on the trait (Lande and Thompson 1990). Several multinational companies, such as Syngenta and Monsanto, are routinely using MARS in their breeding programmes (Ribaut et al. 2010).

In cowpea, MARS has been implemented at IITA and NARS centers in collaboration with UCR by using genomic resources developed during the GCP-TL1 project. At IITA, Nigeria, to develop cowpea varieties with enhanced drought tolerance, two lines (IT84S-2246-4 and IT98K-1111-1) were crossed to develop a MARS population. After QTL mapping with 102 polymorphic SNPs, seven QTLs were identified for yield, drought tolerance and staygreen. One hundred and seventy seven plants were fixed for favorable alleles at these seven QTLs and advanced breeding lines are being generated. At Eduardo Mondlane University (EMU), Mozambique, the cross CB27 × IT97K-499-35 was used to initiate MARS for large seed, grain quality, and heat tolerance traits. Screening of lines fixed for favorable alleles is going on under drought and irrigated conditions. At ISRA, Senegal, the cross IT93K-503-1 × Mouride has been made for MARS for drought tolerance and resistance to Striga, nematodes and Macrophomina.

10.6.3 Genome-Wide Association Mapping Studies

The genome-wide association mapping (GWAM) approach provides opportunities to explore the tremendous allelic diversity existing in natural germplasm (Deshmukh et al. 2014). A GWAM or whole genome association mapping (WGAM) or linkage disequilibrium mapping (LDM) is used to evaluate associations between markers and trait(s) of interest scored across a large number of individuals. The advancements in genomic technologies have led to a better understanding of the genetic basis of traits using GWAM. This approach results in high-resolution mapping of genetic variability from germplasm sets that have undergone many rounds of recombination (Yu and Buckler 2006). However, to get the associations at a fine mapping resolution, large number of markers are required to screen the genome. Recently GWAM studies have been proven effective by identifying marker-trait associations in several legume crops such as cowpea (Lucas et al. 2013; Burridge et al. 2017; Qin et al. 2017), common bean (Villegas et al. 2017) and soybean (Dhanapal et al. 2015). As mentioned above, a GWAM study in cowpea identified 32 QTLs for root architecture traits. Further, comparisons of results from this study with others revealed QTL co-localizations between root traits and seed weight per plant, pod number and Striga tolerance (Burridge et al. 2017).

10.7 Using Wild Germplasm in Cowpea Breeding

Plant breeders mostly use existing germplasm and landraces to develop new varieties characterized by desirable agronomic traits. In many crops, yields have remained stagnant relatively because sufficient genetic diversity is missing for progress in some of the traits or due to genetic bottlenecks that occurred during the domestication process (Tanksley and McCouch 1997; Gur and Zamir 2004). It is well known that wild relatives provide important sources of genetic variation for crop improvement. However, their exploitation is limited by different sexual incongruity and linkage drag (Wang et al. 2017). Some wild cowpea relatives have been identified as potential sources of genes that confer resistance to a number of pests that have devastating effects on grain yield and stored seeds. *Vigna vexillata* is one such wild cowpea relative with resistance to pod sucking bugs (IITA 1988) and bruchids (Birch et al. 1986). Strong incompatibility barrier, however, exists between cowpea and *V. vexillata* (Fatokun 2002) which has prevented transferring the desirable genes from the latter to cultivated cowpea through conventional breeding. Some wild cowpea relatives are cross compatible with cultivated types but have hardly been used in developing improved varieties. With the advent of latest genomic tools, it is now feasible to transfer into elite germplasm the favorable alleles left behind by the domestication process more efficiently using genomics-assisted breeding strategies. These tools should facilitate overcoming linkage drag, which is one major reason for the non-interest in the use of wild cowpea lines by breeders. In this context, several methods such as construction of introgression libraries (ILs), advanced backcross-QTL (AB-QTL) analysis, have been suggested for transferring superior alleles from wild species to cultivated lines. AB-QTL analysis has been used in several legume species such as common bean (Blair et al. 2006), and soybean (Chaky et al. 2003) to develop ILs with seed weight, days to flowering and yield traits in common bean and yield traits in soybean. With an initiative of the Global Crop Diversity Trust project in cowpea, we initiated the use of wild cowpea accessions in cowpea breeding programme to introgress genes for drought tolerance into cultivated cowpea lines. In future, AB-QTL approach may be employed to introgress genes for drought tolerance in cowpea improvement efforts.

10.8 Summary

Cowpea [*Vigna unguiculata* (L.) Walp.] is a multipurpose African legume crop, which feeds millions of people and their livestock especially in West and Central Africa. Because of its ability to fix nitrogen, it improves soil fertility, and consequently helps to increase the yields of cereal crops when intercropped or grown in rotation and thus contributes to the sustainability of cropping systems (Singh and Ajeigbe 2007). Cowpea yields in farmer's fields are very low due to several constraints (abiotic and biotic), as well as limited access to quality seeds of improved varieties. Among abiotic constraints, drought is one of the most important factors that could affect all growth stages of the cowpea crop. In the last three decades, efforts of scientists at international and national cowpea research institutions have recorded good progress in variety development through conventional breeding. However, to meet the rising global demand for cowpea to feed the increasing human population, more efforts are required to speed up variety development. With the availability of latest genetic and genomic resources and the establishment of high-throughput SNP genotyping platforms, it is now possible to use modern molecular methods in cowpea to successfully and quickly develop and release improved varieties to farmers, which would help bridge the existing yield gap. However, high throughput plant phenotyping for precise and accurate agronomical, morphological and physiological data in large number of genotypes and segregating populations remains a bottleneck for modern breeding. Further, the shortage of qualified human resources and advanced research equipment and infrastructure in developing countries constitute other challenges. Although genomic resources for cowpea still lag behind as compared with similar crops, a number of cowpea genetic linkage maps and QTLs associated with desirable traits such as resistance/tolerance to Striga, drought, macrophomina, fusarium wilt, bacterial blight, root-knot nematodes, aphids, and foliar thrips have been reported. Several national and international cowpea breeding programs are exploiting the developed genomics resources to some extent to implement molecular breeding for abiotic and biotic traits, especially by MABC, MARS and GWAM to accelerate cowpea improvement. The recently available MAGIC RIL population and cowpea genome sequence (Amatriain et al. 2017) will further accelerate molecular breeding efficiently in cowpea improvement. The combination of conventional and molecular breeding strategies should result in the development of varieties with genetic gains that would boost cowpea production and productivity in SSA.

Acknowledgements Most of the cited work in this review were obtained from Tropical Legumes project (TL I, TLII and TLIII) of Bill and Melinda Gates Foundation and USAID Feed The Future projects (IITA project, Legume Innovation Lab and Climate Resilient Project for which the authors are grateful. We also would like to thank Prof. Tim J Close, Prof. Phil A Roberts, Dr. Lam Bao Huynh and Maria M Amatriain for their contributions to the development of cowpea genomic resources.

References

Agbicodo EM, Fatokun CA, Bandyopadhyay R, Wydra K, Diop NN, Muchero W, Ehlers JD, Roberts PA, Close TJ, Visser RGF, Linden CGV (2010) Identification of markers associated with bacterial blight resistance loci in cowpea [*Vigna unguiculata* (L.) Walp.]. Euphytica 175:215–226

Agbicodo EM, Fatokun CA, Muranaka S, Visser RGF, Linden CGV (2009) Breeding drought tolerant cowpea: constraints, accomplishments, and future prospects. Euphytica 167:353–370

Amatriain MM, Mirebrahim H, Xu P, Wanamaker SI, Luo MC, Alhakami H, Alpert M, Atokple I, Batieno BJ, Boukar O, Bozdag S, Cisse N, Drabo I, Ehlers JD, Farmer A, Fatokun C, Gu YQ, Guo YN, Huynh BL, Jackson SA, Kusi F, Lawley CT, Lucas MR, Ma Y, Timko MP, Wu J, You F, Barkley NA, Roberts PA, Lonardi S, Close TJ (2017) Genome resources for climate-resilient cowpea, an essential crop for food security. Plant J 89:1042–1054

Andargie M, Pasquet RS, Gowda BS, Muluvi GM, Timko MP (2011) Construction of a SSR-based genetic map and identification of QTL for domestication traits using recombinant inbred lines from a cross between wild and cultivated cowpea [*V. unguiculata* (L.) Walp.]. Mol Breed 28:413–420

Andargie M, Pasquet RS, Muluvi GM, Timko MP (2013) Quantitative trait loci analysis of flowering time related traits identified in recombinant inbred lines of cowpea (*Vigna unguiculata*). Genome 56:289–294

Bandillo N, Raghavan C, Muyco PA, Sevilla MAL, Lobina IT, Ermita CJD, Tung CW, McCouch S, Thomson M, Mauleon R, Singh RK, Gregorio G, Redona E, Leung H (2013) Multi-parent advanced generation inter-cross (MAGIC) populations in rice: progress and potential for genetics research and breeding. Rice 6:11

Batieno BJ, Danquah E, Tignegre JB, Huynh BL, Drabo I, Close TJ, Ofori K, Roberts P, Ouedraogo TJ (2016) Application of marker-assisted backcrossing to improve cowpea (*Vigna unguiculata* L. Walp) for drought tolerance. J Plant Breed Crop Sci 8:273–286

Belko N, Cisse N, Diop NN, Zombre G, Thiaw S, Muranaka S, Ehlers JD (2014) Selection for post-flowering drought resistance in short-and medium-duration cowpeas using stress tolerance indices. Crop Sci 54:25–33

Belko N, Zaman-Allah M, Cisse N, Diop NN, Zombre G, Ehlers JD, Vadez V (2012) Lower soil moisture threshold for transpiration decline under water deficit correlates with lower canopy conductance and higher transpiration efficiency in drought-tolerant cowpea. Funct Plant Biol 39:306–322

Belko N, Zaman-Allah M, Diop NN, Cisse N, Zombre G, Ehlers JD, Vadez V (2013) Restriction of transpiration rate under high vapour pressure deficit and non-limiting water conditions is important for terminal drought tolerance in cowpea. Plant Biol 15:304–316

Bernardo R (2008) Molecular markers and selection for complex traits in plants: Learning from the last 20 years. Crop Sci 48:1649–1664

Birch ANE, Fellows LE, Evans SV, Doherty K (1986) Para-aminophenylyalanine in *Vigna*: possible taxonomic and ecological significance as a seed defense against bruchids. Phytochemistry 25:2745–2749

Blair MW, Iriarte G, Beebe S (2006) QTL analysis of yield traits in an advanced backcross population derived from a cultivated Andean x wild common bean (*Phaseolus vulgaris* L) cross. Theor Appl Genet 112:1149–1163

Blum A (2017) Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant, Cell Environ 40:4–10

Boukar O, Bhattacharjee R, Fatokun C, Lava Kumar P, Gueye B (2013) Cowpea. In: Singh M, Upadhyaya HD, Bish IS (eds) Genetic and genomic resources of grain legume Improvement. Elsevier publisher, pp 137–150. http://dx.doi.org/10.1016/B978-0-12-397935-3.00006-2

Boukar O, Fatokun CA, Huynh BL, Roberts PA, Close TJ (2016) Genomic tools in cowpea breeding programs: status and perspectives. Front Plant Sci 7:757

Boukar O, Fatokun CA, Roberts PA, Abberton M, Huynh BL, Close TJ et al (2015) Cowpea. In: DeRon AM (ed) Grain Legumes (Hand book of plant breeding 10). Springer-Verlag, NewYork, NY, pp 219–250

Boukar O, Kong L, Singh BB, Murdock L, Ohm HW (2004) AFLP and AFLP-derived SCAR markers associated with *Striga gesnerioides* resistance in cowpea. Crop Sci 44:1259–1264

Boukar O, Massawe F, Muranaka S, Franco J, Mayiza-Dixon B, Singh B et al (2011) Evaluation of cowpea germplasm lines for protein and mineral concentrations in grains. Plant Genet Resour 9:515–522

Burridge JD, Jochua CN, Bucksch A, Lynch JP (2016) Legume shovelomics: High throughput phenotyping of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* subsp, *unguiculata*) root architecture in the field. Field Crops Res 192:21–32

Burridge JD, Schneider HM, Huynh BL, Roberts PA, Bucksch A, Lynch JP (2017) Genome-wide association mapping and agronomic impact of cowpea root architecture. Theor Appl Genet 130:419–431

Carvalho M, Lino-Neto T, Rosa E, Carnide V (2017) Cowpea: a legume crop for challenging environment. J Sci Food Agric https://doi.org/10.1002/jsfa.8250. (wileyonlinelibrary.com)

Cavanagh C, Morell M, Mackay I, Powell W (2008) From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants. Curr Opin Plant Biol 11:215–221

Ceccarelli S, Grando S, Baum M, Udupa SM (2004) Breeding for drought resistance in a changing climate. In: Rao S, Ryan J (eds) Challenges and strategies of dryland agriculture, CSSA Special Publication no. 32, CSSA and ASA, Madison, WI, pp 167–190

Chamarthi SK, Kumar A, Vuong T, Blair MW, Gaur PM, Nguyen HT, Varshney RK (2011) Trait mapping and molecular breeding in legumes: concepts and examples in soybean, common bean and chickpea. In: Pratap A, Kumar J (eds) Biology and breeding of food legumes. CABI International, Oxfordshire, UK, pp 296–313

Chen X, Laudeman TW, Rushton PJ, Spraggins TA, Timko MP (2007) CGKB: an annotation knowledge base for cowpea (*Vigna unguiculata* L.) methylation filtered genomic gene space sequences. BMC Bioinformatics 8:129

Cheng A, Ismail I, Osman M, Hashim H, Zainual NSM (2017) Rapid and targeted introgression of *fgr* gene through marker-assisted backcrossing in rice (*Oryza sativa* L.). Genome. https://doi.org/10.1139/gen-2017-0100

Chaky JM, Specht JE, Cregan, PB (2003) Advanced backcross QTL analysis in a mating between *Glycine max* and *Glycine soja*. In Abstracts of the conference on plant and animal genome, University of Nebraska, Lincoln, p 545

De Ronde JA, Spreeth MH (2007) Development and evaluation of drought resistant mutant germplasm of cowpea (*Vigna unguiculata* (L.) Walp.). ISSN 0378-4738 = Water SA. 33(3) (Special Edition) 2007. Available on website http://www.wrc.org.za

Deshmukh R, Sonah H, Patil G, Chen W, Prince S, Mutava R, Vuong T, Valliyodan B, Nguyen HT (2014) Integrating omic approaches for abiotic stress tolerance in soybean. Front Plant Sci 5:244

Dhanapal AP, Ray JD, Singh SK, Villegas VH, Smith JR, Purcell LC, Andy King C, Fritschi FB (2015) Genome-wide association analysis of diverse soybean genotypes reveals novel markers for nitrogen traits. Plant Genome 8:1–15

Dinesh HB, Ohithaswa HCHL, Iswanatha KPAV, Singh P, Mohanrao A (2016) Identification and marker-assisted introgression of QTL conferring resistance to bacterial leaf blight in cowpea (*Vigna unguiculata* (L.) Walp.). Plant Breed 135:506–512

Ehlers JD, Hall AE (1997) Cowpea (*Vigna unguiculata* L Walp). Field Crops Res 53:187–204

Ewansiha SU, Singh BB (2006) Relative drought tolerance of important herbaceous legumes and cereals in the moist and semi-arid regions of West Africa. J Food Agric Environ 4:188–190

Fatokun CA (2002) Breeding cowpea for resistance to insect pests: attempted crosses between cowpea and *Vigna vexillata*. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamo M. (eds) Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the world cowpea conference III held at the International institute of tropical agriculture (IITA), Ibadan, Nigeria, pp 52–61

Fatokun CA, Boukar O, Kamara A, Coulibaly O, Alene A, Boahen S et al (2012a) Enhancing cowpea productivity and production in drought-prone areas of Sub-Saharan Africa. In: Abate T (ed) Four seasons of learning and engaging small holder farmers: Progress of Phase 1. International Crops Research Institute for the Semi-arid Tropics, Nairobi, Kenya, pp 81–112

Fatokun CA, Boukar O, Muranaka S (2012b) Evaluation of cowpea [*Vigna unguiculata* (L.) Walp.] germplasm lines for tolerance to drought. Plant Genetic Resour 10:171–176

Fatokun CA, Dariush D, Menancio-Hautea DI, Young ND (1993) A linkage map for cowpea [*Vigna unguiculata* (L.) Walp.] based on DNA markers. In: Brien SJO (ed) Genetic Maps. Locus maps of complex genomes, 6th edn. Cold Spring Harbour Laboratory Press, NewYork, NY, pp 256–258

Fatokun CA, Menancio-Hautea DI, Danesh D, Young ND (1992) Evidence for orthologous seed weight genes in cowpea and mungbean based on RFLP mapping. Genetics 132:841–846

Ferry RL, Singh BB (1997) Cowpea genetics: a review of the recent literature. In Singh BB, Mohan-Raj DR, Dashiell KE, Jackai LEN (eds) advances in cowpea research, (Ibadan: Co-publication of International Institute of Tropical Agriculture (IITA); Japan International Research Center for Agricultural Sciences (JIRCAS), pp 13–29

Frisch M, Bohn M, Melchinger AE (1999) Minimum sample size and optimal positioning of flanking markers in marker assisted backcrossing for transfer of a target gene. Crop Sci 39:967–975

Gowda VR, Henry A, Vadez A, Shashidhar HE, Serraj R (2012) Water uptake dynamics under progressive drought stress in diverse accessions of Oryza SNP panel of rice (*Oryza sativa* L.). Funct Plant Biol 39:402–411

Gowda VR, Henry A, Yamauchi A, Shashidhar HE, Serraj R (2011) Root biology and genetic improvement for drought avoidance in rice. Field Crops Res 122:1–13

Gur A, Zamir D (2004) Unused natural variation can lift yield barriers in plant breeding. PLoS Biol 2:1610–1615

Hall AE (2012) Phenotyping cowpeas for adaptation to drought. Front Physiol 3:155

Hospital E, Charcosset A (1997) Marker assisted introgression of quantitative trait loci. Genetics 147:1469–1485

Huang BE, Verbyla KL, Verbyla AP, Raghavan C, Singh VK, Gaur P, Leung H, Varshney RK, Cavanagh CR (2015) MAGIC populations in crops: current status and future prospects. Theor Appl Genet 128:999–1017

Huynh BL, Ehlers JD, Amatriain MM, Lonardi S, Santos JRP, Ndeve A, Batieno BJ, Boukar O, Cisse N, Drabo I, Fatokun C, Kusi F, Agyare RY, Guo YN, Herniter I, Lo S, Wanamaker SI, Close TJ, Roberts PA (2017) A multi-parent advanced generation inter-cross population for genetic analysis of multiple traits in cowpea (*Vigna unguiculata* L. Walp.). bioRxiv preprint. https://doi.org/10.1101/149476

Huynh BL, Close TJ, Roberts PA, Hu Z, Wanamaker S, Lucas MR et al (2013) Gene pools and the genetic architecture of domesticated cowpea. Plant Genome 6:1–8

Huynh BL, Ehlers JD, Ndeye NN, Wanamaker S, Lucas MR, Close TJ et al (2015) Genetic mapping and legume synteny of aphid resistance in African cowpea (*Vigna unguiculata* L. Walp.) grown in California. Mol Breed 35:36

Huynh BL, Matthews W, Ehlers JD, Lucas M, Santos JP, Ndeve A et al (2016) A major QTL corresponding to the *Rk* locus for resistance to root-knot nematodes in cowpea (*Vigna unguiculata* L. Walp.). Theor Appl Genet 129:87–95

IITA (1988) Annual report and research highlights 1987/88. International Institute of Tropical Agriculture (www.iita.org)

Jin R, Wang Y, Liu R, Gou J, Chan Z (2016) Physiological and metabolic changes of purslane (*Portulaca oleracea* L.) in response to drought, heat, and combined stresses. Front Plant Sci 6:1123

Karapanos I, Papandreou A, Skouloudi M, Makrogianni D, Fernandez JA, Rosa E, Ntatsi G, Bebeli PJ, Savvas D (2017) Cowpea fresh pods- a new legume for the market: assessment of their quality and dietary characteristics of 37 cowpea accessions grown in southern Europe. J Sci Food Agric. https://doi.org/10.1002/jsfa.8418. (wileyonlinelibrary.com)

Khan MS, Kanwal B, Nazir S (2015) Metabolic engineering of the chloroplast genome reveals that the yeast *ArDH* gene confers enhanced tolerance to salinity and drought in plants. Front Plant Sci 6:725

Lande R, Thompson R (1990) Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743–756

Lucas MR, Diop NN, Wanamaker S, Ehlers JD, Roberts PA, Close TJ (2011) Cowpea-soybean synteny clarified through an improved genetic map. Plant Genome 4:218–224

Lucas MR, Ehlers JD, Roberts PA, Close TJ (2012) Markers for quantitative resistance to foliar thrips in cowpea. Crop Sci 52:2075–2081

Lucas MR, Huynh BL, da Silva Vinholes P, Cisse N, Drabo I, Ehlers JD et al (2013) Association studies and legume synteny reveal haplotypes determining seed size in *Vigna unguiculata*. Front Plant Sci 4:95

Lugan R, Niogret MF, Leport L, Guegan JP, Larher FR, Savoure A et al (2010) Metabolome and water homeostasis analysis of *Thellungiella salsuginea* suggests that dehydration tolerance is a key response to osmotic stress in this halophyte. Plant J 64:215–229

Lynch JP (2007) Roots of second green revolution. Aust J Bot 55:493–512

Mai-Kodomi Y, Singh BB, Myers O, Yopp JH, Gibson PJ, Terao T (1999a) Two mechanisms of drought tolerance in cowpea. Indian J Genet Plant Breed 59:309–316

Mai-Kodomi Y, Singh BB, Myers O, Yopp JH, Gibson PJ, Terao T (1999b) Inheritance of drought tolerance in cowpea. Indian J Genet Plant Breed 59:317–323

Matsui T, Singh BB (2003) Root characteristics in cowpea related to drought tolerance at the seedling stage. Exp Agric 39:29–38

Menendez CM, Hall AE, Gepts P (1997) A genetic linkage map of cowpea (*Vigna unguiculata*) developed from a cross between two inbred, domesticated lines. Theor Appl Genet 95:1210–1217

Muchero W, Diop NN, Bhat PR, Fenton RD, Wanamaker S, Pottorff M et al (2009a) A consensus genetic map of cowpea [*Vigna unguiculata* (L.) Walp.] and synteny based on EST-derived SNPs. Proc Natl Acad Sci USA 106:18159–18164

Muchero W, Ehlers JD, Close TJ, Roberts PA (2009b) Mapping QTL for drought stress-induced premature senescence and maturity in cowpea [*Vigna unguiculata* (L.) Walp.]. Theor Appl Genet 118:849–863

Muchero W, Ehlers JD, Close TJ, Roberts PA (2011) Genic SNP markers and legume synteny reveal candidate genes underlying QTL for *Macrophomina phaseolina* resistance and maturity in cowpea [*Vigna unguiculata* (L) Walp.]. BMC Genomics 12:8

Muchero W, Ehlers JD, Roberts PA (2008) Seedling stage drought-induced phenotypes and drought-responsive genes in diverse cowpea genotypes. Crop Sci 48:541–552

Muchero W, Ehlers JD, Roberts PA (2010) QTL analysis for resistance to foliar damage caused by *Thrips tabaci* and *Frankliniella schultzei* (Thysanoptera: Thripidae) feeding in cowpea [*Vigna unguiculata* (L.) Walp.]. Mol Breed 25:47–56

Muchero W, Roberts PA, Diop NN, Drabo I, Cisse N, Close TJ et al (2013) Genetic architecture of delayed senescence, biomass, and grain yield under drought stress in cowpea. PLoS ONE 8:e70041

Myers GO, Fatokun CA, Young ND (1996) RFLP mapping of an aphid resistance gene in cowpea (*Vigna unguiculata* L. Walp.) Euphytica 91:181–187

Onuh MO, Donald KM (2009) Effects of water stress on the rooting, nodulation potentials and growth of cowpea (*Vigna unguiculata* (L.) Walp.). Sci World J 4:31–34

Ouedraogo JT, Gowda BS, Jean M, Close TJ, Ehlers JD, Hall AE et al (2002a) An improved genetic linkage map for cowpea (*Vigna unguiculata* L.) combining AFLP, RFLP, RAPD, biochemical markers, and biological resistance traits. Genome 45:175–188

Ouedraogo JT, Maheshwari V, Berner D, St Pierre CA, Belzile F, Timko MP (2001) Identification of AFLP markers linked to resistance of cowpea (*Vigna unguiculata* L.) to parasitism by *Striga gesnerioides*. Theor Appl Genet 102:1029–1036

Ouedraogo JT, Ouedraogo M, Gowda BS, Timko MP (2012) Development of sequence characterized amplified region (SCAR) markers linked to race-specific resistance to *Striga gesnerioides* in cowpea (*Vigna unguiculata* L.). Afr J Biotechnol 11:12555–12562

Ouedraogo N, Sanou J, Gracen V, Tongoona P (2017) Incorporation of stay-green quantitative trait loci (QTL) in elite sorghum (*Sorghum bicolor* L. Moench) variety through marker-assisted selection at early generation. J Appl Biosci 111:10867–10876

Ouedraogo JT, Tignegre JB, Timko MP, Belzile FJ (2002b) AFLP markers linked to resistance against *Striga gesnerioides* race 1 in cowpea (*Vigna unguiculata*). Genome 45:787–793

Pottorff M, Ehlers JD, Fatokun C, Roberts PA, Close TJ (2012a) Leaf morphology in cowpea [*Vigna unguiculata* (L.) Walp.]: QTL analysis, physical mapping and identifying candidate gene using synteny with model legume species. BMC Genomics 13:234

Pottorff M, Roberts PA, Close TJ, Lonardi S, Wanamaker S, Ehlers JD (2014) Identification of candidate genes and molecular markers for heat-induced brown discoloration of seed coats in cowpea [*Vigna unguiculata* (L.) Walp]. BMC Genomics 15:328

Pottorff M, Wanamaker S, Ma YQ, Ehlers JD, Roberts PA, Close TJ (2012b) Genetic and physical mapping of candidate genes for resistance to *Fusarium oxysporum* f.sp. *tracheiphilum* race 3 in cowpea [*Vigna unguiculata* (L.) Walp]. PLoS ONE 7:e41600

Qin J, Shi A, Mou B, Bhattarai G, Yang W, Weng Y, Motes D (2017) Association mapping of aphid resistance in USDA cowpea (*Vigna unguiculata* L. Walp.) core collection using SNPs. Euphytica 213:36

Ribaut JM, de Vicente MC, Delannay X (2010) Molecular breeding in developing countries challenges and perspectives. Curr Opin Plant Biol 13:1–6

Rodrigues MA, Santos CAF, Santana JRF (2012) Mapping of AFLP loci linked to tolerance to cowpea golden mosaic virus. Genet Mol Res 11:3789–3797

Sanginga N, Lyasse O, Singh BB (2000) Phosphorus use efficiency and nitrogen balance of cowpea breeding lines in a low P soil of the derived savanna zone in West Africa. Plant Soil 220:119–128

Sicher RC, Timlin D, Bailey B (2012) Responses of growth and primary metabolism of water-stressed barley roots to rehydration. J Plant Physiol 169:686–695

Sinclair TR, Manandhar A, Belko N, Riar M, Vadez V, Roberts PA (2015) Variation among cowpea genotypes in sensitivity of transpiration rate and symbiotic nitrogen fixation to soil drying. Crop Sci 55:2270–2275

Singh BB (2002) Recent genetic studies in cowpea. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamo M (eds) Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the World cowpea research conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, 3–13

Singh BB, Ajeigbe H (2007) Improved cowpea-cereals-based cropping systems for household food security and poverty reduction in West Africa. J Crop Imp 19:157–172

Singh BB, Ajeigbe HA, Tarawali SA, Fernandez-Rivera S, Abubakar M (2003) Improving the production and utilization of cowpea as food and fodder. Field Crops Res 84:169–177

Singh BB, Mai-Kodomi Y, Terao T (1999a) A simple screening method for drought tolerance in cowpea. Indian J Genet 59:211–220

Singh BB, Mai-Kodomi Y, Terao T (1999b) Relative drought tolerance of major rainfed crops of the semi-arid topics. Indian J Genet 59:437–444

Singh BB, Matsui T (2002) Cowpea varieties for drought tolerance. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamo M (eds) Challenges and opportunities for enhancing sustainable cowpea production, Proceedings of the world cowpea research conference III held at the International Institute of Tropical Agriculture (IITA, Ibadan), pp 287–300

Singh SK, Raja Reddy K (2011) Regulation of photosynthesis, fluorescence, stomatal conductance and water-use efficiency of cowpea (*Vigna unguiculata* [L.] Walp.) under drought. J Photochem Photobiol 105:40–50

Singh VEJ, van Oosterom EJ, Jordan DR, Messina CD, Cooper M, Hammer GL (2010) Morphological and architectural development of root systems in sorghum and maize. Plant Soil 333:287–299

Slabbert R, Spreeth M, Kruger GHJ (2004) Drought tolerance, traditional crops and biotechnology: breeding towards sustainable development. S Afr J Bot 70:116–123

Tanksley S, McCouch S (1997) Seed banks and molecular maps unlocking genetic potential from the wild. Science 277:1063–1066

Tanksley SD, Young ND, Patterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. Nat Biotechnol 7:257–263

Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. Science 327:818–822

Tomar SMS, Kumar GT (2004) Seedling survivability as a selection criterion for drought tolerance in wheat. Plant Breed 123:392–394

Ubi BE, Mignouna H, Thottappilly G (2000) Construction of a genetic linkage map and QTL analysis using a recombinant inbred population derived from an inter-sub specific cross of a cowpea [*Vigna unguiculata* (L.) Walp.]. Breed Sci 50:161–173

Vadez V, Berger JD, Warkentin T, Asseng S, Ratnakumar P, Rao KPC, Gaur PM, Munier-Jolain N, Larmure A, Voisin AS, Sharma HC, Pande S, Sharma M, Krishnamurthy L, Zaman-Allah M (2012a) Adaptation of grain legumes to climatic change: a review. Agron Sustain Dev 32:31–44

Vadez V, Kholova J, Zaman-Allah M, Belko N (2013) Water: the most important ‘molecular’ component of water stress tolerance research. Funct Plant Biol 40:1310–1322

Vadez V, Rao S, Bhatnagar Mathur P, Sharma KK (2012b) DREB1A promotes root development in deep soil layers and increases water extraction under water stress in groundnut. Plant Biol 15:45–52

Vadez V, Rao S, Kholova J, Krishnamurthy L, Kashiwagi J, Ratnakumar P, Sharma KK, Bhatnagar-Mathur P, Basu PS (2008) Roots research for legume tolerance to drought: quo vadis? J Food Legumes 21:77–85

Vadez V, Rao S, Sharma KK, Bhatnagar-Mathur P, Devi JM (2007) DREB1A allows for more water uptake in groundnut by a large modification in the root/shoot ratio under water deficit. International Arachis Newsletter 27:27–31

Varshney RK, Mohan SM, Gaur PM, Chamarthi SK, Singh VK, Srinivasan S, Swapna N, Sharma M, Singh S, Kaur L, Pande S (2014) Marker-assisted backcrossing to introgress resistance to fusarium wilt race 1 and ascochyta blight in C 214, an elite cultivar of chickpea. Plant Genome 7:1–11

Vasconcelos EV, de Andrade Fonseca AF, Pedrosa-Harand A, de Andrade Bortoleti KC, Benko-Iseppon AM, da Costa AF, Brasileiro-Vidal AC (2015) Intra- and inter chromosomal rearrangements between cowpea *Vigna unguiculata* (L.) Walp. and common bean (*Phaseolus vulgaris* L.) revealed by BAC-FISH. Chromosome Res 23:253–266

Villegas VH, Song Q, Kelly JD (2017) Genome-wide association analysis for drought tolerance and associated traits in common bean. Plant genome 10:1–17

Wang C, Hu S, Gardner C, Lubberstedt T (2017) Emerging avenues for utilization of exotic germplasm. Trends Plant Sci 22:624–637

Warren CR, Aranda I, Cano FJ (2011) Responses to water stress of gas exchange and metabolites in *Eucalyptus* and *Acacia* spp. Plant, Cell Environ 34:1609–1629

Xu P, Wu X, Wang B, Hu T, Lu Z, Liu Y et al (2013a) QTL mapping and epistatic interaction analysis in asparagus bean for several characterized and novel horticulturally important traits. BMC Genet 14:4

Xu Y, Xie C, Wan J, He Z, Prasanna BM (2013) Marker-assisted selection in cereals: Platforms, strategies and examples. In: Gupta PK, Varshney RK (eds) Cereal Genomics II. https://doi.org/10.1007/978-94-007-6401-9_14

Yu J, Buckler ES (2006) Genetic association mapping and genome organization of maize. Curr Opin Biotechnol 17:155–160