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Review

Current status of molecular tools development for cowpea [*Vigna unguiculata* (L.) Walp.] improvement

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Cowpea [*Vigna unguiculata* (L.) Walp.] is a popular tropical grain legume which is widely produced and consumed in sub-Saharan Africa (SSA). The grains are rich in dietary protein for human while the haulm is high quality fodder for livestock particularly ruminants. Compared with many other crops cowpea is a laggard in development, evaluation and deployment of different molecular markers for use in genetics and breeding. Application of DNA-based markers is of considerable significance to crop improvement. Some DNA based markers have been used to study genetic diversity, linkage and quantitative traits loci (QTL) mapping in cowpea. Results from these studies have demonstrated the extent of genetic diversity in cowpea and its relationship with other members of the *Vigna* species. In addition, genetic linkage maps have been produced and used for detection of QTLs for some desirable traits. Some of these include QTLs for seed size, seed coat and eye color, leaf shape, pod length, resistance to macrophomina, domestication-related traits such as pod length, days to flowering, etc. In view of the potential benefits of DNA markers to the development of better performing improved cowpea varieties, concerted efforts are now being devoted to develop molecular tools for the crop. The developed consensus genetic linkage map and genome sequence for cowpea will boost the application of molecular tools for its genetic improvement. A panel of 17 SNP markers have been developed for use in quality assurance and control in cowpea breeding activities. This review aims at highlighting the molecular approaches that have been used and being pursued for genetic diversity, QTL mapping of some qualitative and quantitative traits as well as marker-assisted selection leading to the development of high performing new improved lines that meet the needs of farmers and consumers.

Key words: Cowpea, molecular tools, *Vigna unguiculata,* QTL mapping, marker-assisted selection.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important grain legume of sub-Saharan Africa (SSA) that is widely cultivated and consumed. It is especially well-suited to the SSA's dry savannah and sahel regions, where some other crops would fail or perform poorly due to water stress induced by unpredictable and short-duration

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rainfall, in addition to soil quality (De Ron, 2015). The crop's world production is estimated at over 8.9 million MT per year on about 14.4 million hectares. Sub-Saharan Africa accounts for 87% of the world's production. Nigeria is the largest cowpea producer, followed by Niger, Burkina Faso, Cameroon, and Mali (FAOSTAT, 2021).

Cowpea is important for diverse reasons. This crop has good nutritional value and is a valuable cash crop in semi-arid locations (Ehlers and Hall, 1997). In the food and feed business, it plays a crucial role in human nutrition due to the high dietary value of its grain, which contains 23 to 32% quality protein and substantial amounts of minerals and vitamins (Badiane et al., 2014). Cowpea is tolerant to low soil fertility because of its ability to fix nitrogen. It is a drought-tolerant crop that grows well in drought prone areas, making it particularly popular in semi-arid regions of the tropics where other food legumes do not perform well. Even in poor soils with organic matter less than 0.2%, pH range of 4.5-9.0 and sand content greater than 85%, cowpea has a remarkable ability to perform better than many other crops due to its ability to fix nitrogen (Xiong et al., 2016).

Low agricultural yields in SSA are largely due to poor soil fertility, high temperature, drought due to irregular rainfall and lack of irrigation, growing unimproved varieties, inadequate cultural practices, diseases and pests (Enete and Amusa, 2010). Drought, low soil fertility, and heat are abiotic constraints, while insects, bacteria, fungi, parasitic weeds, and nematodes are biotic constraints (Boukar et al., 2016). However, considerable scope exists to enhance cowpea productivity. Modern molecular genetics tools and techniques can complement conventional approaches to allow breeders effectively develop improved varieties that are well adapted and capable of producing high yields. Molecular marker-assisted breeding is now being used to improve efficiency of breeding programmes for many crops. Molecular markers have been found useful in different aspects of variety development starting from genetic diversity studies, confirmation of hybrids between parental lines up to selection of the final product, that is, the newly developed variety.

Conventional breeding is time-consuming, labour-intensive and has been linked to transfer of undesirable genes with desired ones (linkage drag) especially when wild relatives are crossed with cultivars. It is therefore imperative to integrate other breeding approaches which can enable higher levels of precision with respect to gene delivery leading to better performing improved varieties. Using molecular markers can help facilitate this process remarkably well. The integration of phenotypic and molecular markers in marker-assisted breeding has the potential to reduce the number of years required for cultivar development (Nkhoma et al., 2020).

Molecular markers are useful in genetics and plant breeding (Ganal et al., 2009) as they can be used for genetic diversity studies, genetic linkage mapping, gene cloning, and marker-assisted selection (Asare et al., 2010; Egbadzor et al., 2014; Xiong et al., 2016). Among the molecular markers available to plant breeders, the ones that have been used are Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs), Single Nucleotide Polymorphisms (SNPs), and Diversity Arrays Technology (DArT) SNPs. The latter two marker systems are now more commonly used due to their relatively low cost per data point and high throughput procedures. With these markers (SNPs), more robust data can be generated within a short period of time.

The objective of this paper is to review the efforts that are being made with molecular markers to improve cowpea and to point out research gaps that can be exploited.

CHALLENGES TO COWPEA PRODUCTION

Cowpea productivity in SSA is being constrained by several causes, including climate change-related stresses and socio-economic restrictions (Amusa et al., 2015). The most common biotic constraints of cowpea include insect pests and diseases that attack the foliage and stems. The most common fungal diseases include anthracnose (*Colletotrichum lindemuthianum*), fusarium wilt (*Fusarium oxysporum* f.sp. *tracheiphilum),* foot rot (*Fusarium solani* Matt. Scc), rust (*Uromyces phaseoli* Pers. Wint), and scab (*Elsinore phaseoli*) (Singh et al., 2003). Aphids (*Aphis craccivora* Koch) and leafhoppers are the insects that affect cowpea during each stage of development and growth while bud thrips (*Megalulothrips sjostedti* Trybom) attack the plant during the flowering stage, and pod borers (*Maruca vitrata*) attack the pods and young shoots (Boukar et al., 2016). A complex of pod sucking bugs damage seeds in the field while seed weevil attacks seeds in storage.

Numerous viruses infect cowpea such as *Cowpea aphid-borne mosaic virus* (CABMV, genus *Potyvirus*, family Potyviridae), *Bean common mosaic virus*-blackeye cowpea mosaic strain (BCMV-BlCM, genus *Potyvirus*, family Potyviridae), *Cowpea mosaic virus* (CPMV, genus *Comovirus,* family Secoviridae), Southern *bean mosaic virus* (SBMV, genus *Sobemovirus*), *Cowpea mottle virus* (CPMoV, genus *Carmovirus*, family Tombusviridae), *Cucumber mosaic virus* (CMV, genus *Cucumovirus*, family Bromoviridae), *Cowpea mild mottle virus* (CPMMV, genus *Carlavirus*, family *Betaflexiviridae)*, as well as *Cowpea golden mosaic virus* (CGMV, genus *Begomovirus*, family Geminiviridae) (Mbeyagala et al., 2014). Bacterial blight infections caused by *Xanthomonas campestris* PV. *vignicola* and *Xanthomonas campestris* pv. *vignaeuguiculatae* cause 71, 68, and 53% losses in pods per plant, seeds per pod, and fodder yield,

respectively (Singh et al., 2003). The parasitic weed *Striga gesnerioides ,* can cause significant damage to cowpea yield (Tignegre, 2010). The most common abiotic constraints such as drought, heat, and poor soil fertility are the major causes of low crop yields. Drought and heat hurt plant growth at all stages of development and high night temperatures in particular lead to flower abortion in cowpea which affect pod formation and grain yield (Fahad et al., 2017; Lamaoui et al., 2018). Boukar et al. (2018) reported that drought at the flowering phase caused yield loss of cowpea ranging from 360 to 1000 kg ha^{-1}. To increase cowpea yield in SSA, early maturing cowpea cultivars that can withstand extreme heat and escape drought are recommended.

MOLECULAR MARKERS IN GENETIC DIVERSITY STUDIES

Genetic diversity information, which is the foundation of breeding and genetic research, is particularly significant for cowpea breeding. Accurate assessment of genetic variability is important for the conservation and utilization of germplasm resources, and improvement of cowpea. Effective breeding and genetic conservation require a well-characterized agricultural genetic resource. Phenotypic traits and molecular markers are used to measure genetic diversity.

For example, Fatokun et al. (1993) employed 44 accessions of diverse species belonging to four subgenera of the genus *Vigna* to study the taxonomic relationship between the subgenus *Ceratotropis* and other subgenera using RFLP markers. The findings revealed that the genus *Vigna* has a lot of genetic variability, with *Vigna* species from Africa having a lot more variation than *Vigna* spp. from Asia.

RAPD markers were used to analyze genetic diversity and to group genotypes based on degree of genetic relatedness because they are easy and require minimal DNA (Oikeh et al., 2012). Zannou et al. (2008) showed that the RAPD method may be used to characterize genetic variation among cowpea cultivars. The RAPD markers were employed to assess the genetic diversity of 70 cowpea accessions collected across Benin Republic. The study indicated that the genetic diversity was very large. The fixation index revealed a considerable differentiation of cowpea cultivars in Benin based on molecular variance. Malviya et al. (2012) examined the genetic diversity of ten Indian cowpea cultivars using 18 sets of RAPD markers. A total of 181 bands with an average of 15 bands per primer were obtained. Out of 181 bands, 148 showed polymorphism (81.7%). Variation in genetic diversity among cowpea cultivars using different primers ranged from 0.1742 to 0.4054. Zinov'ev and Sole (2004) investigated 26 cultivated and 30 wild cowpea species from Western, Eastern, and Southern Africa. More polymorphisms were found in wild species from Eastern Africa, which supports the submission by Padulosi and Ng (1997) that part of Africa is where the greatest diversity among wild cowpea can be found. The authors further reported that wild cowpea lines from southern Africa in particular were characterized by small pod and seeds, dehiscent pods, hairiness of plant parts, perenniality, outbreeding and bearded stigma.

Nkongolo (2003) used RAPD markers to provide information about origins, taxonomy, domestication, and patterns of genetic variation of cowpea within cowpea populations from various agro-ecological zones of Malawi and discovered that there was a general lack of agreement between clustering based on the molecular markers and morphological traits. Nagalakshmi et al. (2017) discovered a high level of polymorphism among 30 genotypes characterized using RAPD markers. A total of 30 RAPD primers were chosen at random to examine the genetic diversity of 36 cowpea accessions. Five primers (OPC 14, OPB 1, OPA 10, OPG 13, and OPA 4) were determined to be more informative based on the polymorphism information content values (PIC) which ranged from 0.597 to 0.885, with OPC 14 having the highest PIC value. Based on the PCA plot, the first component explained 18.56% variation and the second and third components explained 16.85 and 12.77%, respectively among the 36 accessions of cowpea. The first three components explained 48.21% of the total variation (Nameirakpam and Khanna, 2018).

Pidigam et al. (2019) characterized genetic variation among 28 accessions of yard-long beans collected from different states of India using 48 random amplified polymorphic DNA markers and reported polymorphic information content value ranging from 0.23 to 0.93 among the genotypes. Inter Simple Sequence Repeat (ISSR) markers were utilized by Ajibade et al. (2000) to investigate the genetic links among 18 *Vigna* spp. They reported that closely related species within each subgenus clustered together. Simple sequence repeat (SSR)/Microsatellite marker-based diversity analysis revealed considerable genetic diversity among 141 cowpea accessions collected across Ghana's nine geographical regions. The accessions were clustered into five main branches loosely associated with the geographical regions. The average PIC was 0.38, with a range of 0.07 to 0.66 (Asare et al., 2010). Badiane et al. (2012) used SSR markers to assess the genetic diversity and phylogenetic relationships among 22 local cowpea varieties and lines collected across Senegal, and developed a set of 44 polymorphic marker combinations based on cowpea genomic or expressed sequence tags, with PIC values ranging from 0.08 to 0.33. Except for 53-3, 58-53, and 58-57, all of the local varieties were found in the same group, whereas Ndoute yellow pods, Ndoute violet pods, and Baye Ngagne were found in the second. In another study involving microsatellite markers, Chen et al. (2017) reported 155 alleles and 2.9 alleles per marker, and the average polymorphic information content (PIC)

value was 0.3615 using 105 selected genotypes from the National Genebank of China at the Institute of Crop Science (ICS) and found a low level of genetic diversity among the accessions. Sarr et al. (2021) conducted a similar study to analyze the genetic diversity of 671 accessions grown in eight regions of Senegal, as well as 66 wild relatives and intermediate forms (weedy). The findings revealed a narrow genetic variation between accessions from the different regions and cultivars with genetic similarity ranging from 0.861 to 0.965 with genetic differentiation indices between 0.018 and 0.100. The wild/weedy accessions showed more diversity than the cultivated with genetic diversity of 0.480 and 0.389, respectively. Haruna et al. (2020) characterized forty-six cowpea genotypes in Ghana for resistance to *Striga gesnerioides* using SSR primers. The findings showed that genetic diversity ranged from 0.04 to 0.49 with an average of 0.29; and average allele frequency of 0.78 genetic diversity and the polymorphism information content (PIC) varied from 0.08 to 1.00 with an average of 0.55. Ohlson and Timko (2020) screened seven cowpea lines against 58 unique *S. gesnerioides* populations collected across nine West African countries using SSR markers. Lioi et al. (2019) used a total of 19 SSR markers to identify genetic diversity of 13 cowpea landraces from a small geographical area in Apulia (southern Italy) using 12 of cultivar group *unguiculata* and 1 of cultivar group *sesquipedalis*. Gomes et al. (2020) assessed the genetic variation and gene flow in 59 *V. unguiculata* (cowpea) accessions from 10 landraces spanning across six agro-ecological zones of Mozambique using nuclear microsatellite markers (nSSRs). The results showed nine microsatellites that were highly polymorphic and revealed the existence of high genetic diversity between landraces from Mozambique (Ho: 0.222-0.426; He: 0.451-0.654). Also, AFLP markers were used to assess the genetic relationships among 117 cowpea accessions including 47 domesticated cowpea (ssp. *unguiculata* var. *unguiculata*), 52 wild and weedy annuals (ssp. *unguiculat*a var. *spontanea*), as well as 18 perennial accessions of the wild subspecies *pubescens*, *tenuis* and *alba.* The findings showed that domesticated cowpea was more diversified than wild annual cowpea (Coulibaly et al., 2002).

Fang et al. (2007) explored genetic links among 60 advanced breeding lines from six West African and American breeding programs, as well as 27 landraces from Africa, Asia, and South America using AFLP markers. The results showed that the 87 cowpea accessions shared a minimum of 86% genetic similarity and the percentage of polymorphic fragments per primer set ranged from 47.8 to 70.7%.

Egbadzor et al. (2014) characterized 113 cowpea accessions, 108 from Ghana and five from other countries, using SNP markers. Their study revealed that SNP markers were more effective than morphological, seed protein polymorphism, and SSR markers in differentiating across cowpea germplasm. Using genotyping by sequencing, Xiong et al. (2016) evaluated the genetic diversity and population structure of 768 cultivated cowpea genotypes from USDA GRIN cowpea germplasm collected from 56 countries. Based on PIC values, the accessions that originated in India and East Africa are most highly diversified (3.2 and 3.0), followed by Oceania and Europe with lowest PIC (0.17).

Muñoz-Amatriaín et al. (2017) conducted whole-genome sequencing of 37 cowpea accessions and developed a Cowpea iSelect Consortium Array (Illumina, Inc.) containing 51,128 SNPs. Carvalho et al. (2017) used these 51,128 SNPs to genotype 96 cowpea accessions comprising 43 landraces, and cultivars from the Iberian Peninsula and 53 landraces collected worldwide. Four sub-populations were identified with a lower genetic diversity level in the Iberian Peninsula accessions compared to worldwide accessions and average PIC and He values of 0.25 and 0.31, respectively, were found. A more comprehensive study by Muñoz-Amatriaín et al. (2021) using a high-density genotyping with 51,128 SNP to examine the genetic diversity of the University of California, Riverside (UCR) mini core, made up of 368 international accessions of cultivated cowpea, revealed six sub-populations distinguished by cultivar group and geographic origin. Based on SNP markers, Fatokun et al. (2018) used a sub-set of 298 lines from the loosely composed mini core collection of 370 landraces collected from 50 countries. The finding revealed three major clusters with a genetic distance ranging between 0.0096 and 0.462. A set of 40,089SNPs converted to the Kompetitive Allele-Specific PCR (KASP) SNPs was used to genotype 299 cowpea accessions. A pre-core pool of 434 SNPs and 50 informative core SNPs were selected and validated for use in future genetic diversity analyses of cowpea germplasm (Wu et al., 2021). Nkhoma et al. (2020) conducted a study with 100 cowpea genotypes using SNP markers and discovered that the SNP markers were fairly polymorphic, with a mean PIC value of 0.17 for the general population and 0.21 for mutant lines.

Diversity arrays technology (DArT), a new marker platform, was recently developed as a revolutionary method for whole-genome profiling without the need for sequence information. It is a high-throughput approach that can lead to discovery of hundreds of markers in a single experiment for a low price per data point (Huttner et al., 2005). Recently, Gbedevi et al. (2021) used DArT markers for genetic diversity and population structure study of 255 cowpea accessions collected from different regions and the Agricultural Research Institute of Togo Republic. The findings showed a range of 0.19 to 0.27 of polymorphic information content (PIC) with a mean value of 0.25 among the regions while the expected heterozygosity (He) varied from 0.22 to 0.34 with a mean value of 0.31 and observed heterozygosity (Ho) varied from 0.03 to 0.07 with an average of 0.05. The variation among accessions was higher (78%) within populations

and lower between populations (7%). The mean PIC value in this study was similar to the one obtained by Seo et al. (2020) where they reported a mean PIC value of 0.287 following assessment of 229 Korean germplasm lines based on SNP markers. Similarly, Sodedji et al. (2021) examined the genetic diversity and population structure of 274 cowpea accessions from different origins viz. western and central Africa, eastern Africa, and Asia using diversity array technology (DArT) showed 7% of the variance being among the populations with genetic distances ranging from 0.005 to 0.44. A genetic diversity of *Striga gesnerioides* were recently examined by Ohlson and Timko (2020). They reported that 58 different *S. gesnerioides* populations from nine different West African nations tested against seven cowpea lines revealed that none of the cowpea lines was resistant to all *S. gesnerioides* populations, and that no one S. *gesnerioides* population could overcome the resistance of all seven cowpea lines. The single sequence repeats used to genotype the *Striga* populations revealed significant divergence, showing that genetic relatedness is more commonly a result of geographic proximity than host compatibility. This study indicates that generating broad-spectrum and durable *S*. *gesnerioides* cowpea-resistant lines requires the stacking of multiple resistance genes. Adu et al. (2021) used 9,706 silicoDArT markers to reveal genetic variation among 16 cowpea accessions collection in Ghana based on agro-morphological traits.

Most findings revealed narrow PIC and low levels of heterozygotes within the germplasm characterized, which have been explained by the fact that cowpea is a highly self-pollinated crop with a low level of out-crossing. The self-pollinating nature of cowpea has been reported as the reason for the observed low genetic variation among cowpea landraces (Wamalwa et al., 2016; Carvalho et al., 2017).

QTL MAPPING FOR AGRONOMICALLY IMPORTANT TRAITS

The mapping and identification of major quantitative trait loci (QTLs) that harbor candidate gene(s) underlying beneficial traits, as well as related molecular and genetic studies, are key steps in deploying genomics-based breeding to improve crop varieties. Here, we describe some QTLs and genetic loci/genes with prospect for improving cowpea particularly breeding for yield and its components, grain quality traits, resistance to biotic and abiotic stresses. Quantitative Trait Loci are segments of the genome that contribute to variation in a trait of interest (Oikeh et al., 2012). QTL mapping is the foundation for the generation of markers for Marker-Assisted Selection (MAS). Marker-Assisted Selection enhances the breeding program and is successful in studying the genetic regulation of complex traits (Naidoo et al. 2012).

QTL ANALYSIS FOR YIELD AND ITS COMPONENTS

The basic goal of most plant breeding initiatives is to increase yield. Breeding for higher yield has been done using both conventional and marker-assisted methods. In conventional breeding, superior genotypes are chosen based on their phenotypic performance in a variety of situations (Acquaah, 2015).

Days to flowering and maturity, grain weight, pod number per plant, pod length, number of seeds per pod, 100-seed weight, number of pods per cluster, number of clusters per plant, number of primary branches per plant, days to 50% flowering and harvest index are among the traits targeted for improvement in cowpea variety development (Meena et al., 2015; Aliyu and Makinde, 2016).

Big seed size plays a major role in consumer preference. Several genes affect seed size, which is a significant component of grain yield (Song et al., 2007). Floral induction is the first step in seed development and it is influenced by a variety of elements such as the plant's age, environmental circumstances, and dry matter accumulation, among others. According to Fery and Singh (1997) genes that control seed size in cowpea have been reported by some authors.

Fatokun et al. (1992) published the first report on QTLs for seed weight in cowpea using 188 restriction fragment length polymorphism (RFLP) markers on 58 F_2 lines derived from a cross between cultivated and wild cowpea varieties. The authors identified two major QTLs with effects on this trait which explained 32 to 36% of the phenotypic variation and are orthologous to QTLs for seed weight in mung bean (*Vigna radiata*). In another study conducted by Ubi et al. (2000) using 94 F_8 RILs derived from the inter-subspecies cross involving an improved line and a wild relative, and 77 RAPD markers, five loci for seed weight were identified. These QTLs explained between 7 and 15% of the phenotypic variation. The relationship between the QTLs for seed weight in the study by Ubi et al. (2000) and those identified by Fatokun et al. (1992) was not clarified.

Flowering time is one of the most important traits that plays a key role in the adaptation of a variety to specific agro-ecological zone. Early maturing cultivars are referred to as climate wise cultivars since they can escape drought as well as insect and disease damages that generally occur later in the cropping season. On the other hand, earliness is associated with low yield due to the shortened vegetative and reproductive stages, which may result in reduced photosynthate accumulation and grain filling (Owusu et al., 2018). Timko et al. (2013) used bi-parental lines to conduct QTL analysis study under greenhouse settings for flowering period linked traits: time of flower opening and days to flowering. Five QTLs related to time of flower opening were discovered accounting for 8.8 to 29.8% of the phenotypic variance. Three QTLs for days to first flower were mapped using

SSR markers that explained 5.7 to 18.5% of the phenotypic variance. In the genetic map published by Xu et al. (2013), one major QTL which explained 31.9% of phenotypic variation for days to first flowering was mapped on chromosome 11 (Table 1).

Andargie et al. (2014) genotyped a population of 159 F_7 recombinant inbred lines derived from a cross involving asparagus bean with SSR markers and detected QTLs for seed, pod, and flower-related traits. For seed weight, seven QTLs were mainly detected on LG1, LG2, LG3 (two QTLs each on LG2 and LG3), LG7, and LG10 accounting for 9.2% of the phenotypic variance. Three QTLs were mapped onto LG1, LG2, and LG7 and explained 18.5% of the phenotypic variance for days to flowering. One major QTL for number of pods per plant which accounts for 20.1% of the phenotypic variation was detected on LG3. Following a genome-wide association study (GWAS) using diversity panel of 299 landraces and breeding lines, Xu et al. (2017) detected 72 SNPs for pod length. The phenotypic variation explained by any single SNP varied from 4.6 to 7.1%. Transcriptomic analysis in this study suggested the involvement of sugar, gibberellin, and nutritional signalling in the regulation of pod length. Lucas et al. (2013b) identified 10 QTLs for seed weight using eight bi-parental mapping populations and 1,536 SNPs. Pan et al. (2017) conducted a study using RAD sequencing (restriction-site associated DNA) technology to discover 34,868 SNPs in the cowpea genome using 170 $F_{2:3}$ biparental lines. Eleven QTLs for yield-related traits were mapped onto LGs (LG4, 5, 6, 7, 9, 10, and 11), four QTLs for pod length, four for thousand-grain weight, two QTLs for number of grains per pod, one QTL for carpopodium length accounting for 0.05 to 17.32% of phenotypic variation. A total of 215 recombinant inbred lines was used by Lo et al. (2018) to study domestication related traits of cowpea. Sixteen QTLs for nine traits located across the eleven chromosomes were detected. Two QTLs on days to first flower were detected, one each on chromosomes 5 and 9; three QTLs for seed weight were detected with one each on chromosomes 1, 6, and 8. Pod length was analyzed as a measure of the increase in organ size, and two QTLs were identified, one each on chromosomes 3 and 8. Two QTLs for leaf width were identified on chromosomes 1 and 8 while for number of seeds per pod, two QTLs were detected, one each on chromosomes 5 and 9. Two significant QTLs were detected for pod shattering, one each on chromosomes 3 and 5. These QTLs could serve as good candidates in MAS to improve cowpea for higher yield. Four of the QTLs affecting flowering time were mapped on chromosomes 1, 4, 5 and 9 using a set of 305 F_8 recombinants derived from multi-parent advanced generation inter-cross (MAGIC) population (Huynh et al., 2018). Lo et al. (2019) reported 17 QTLs for four traits, including seed weight, length, width, and density using 51,128 single nucleotide polymorphism markers spanning a large section of cowpea genome. This study used a mini-core collection of 368 accessions, and QTLs were mapped onto chromosomes 3, 4, 5, 6, 8, 10, and 11. This information could be valuable for developing cowpea varieties.

Muñoz-Amatriaín et al. (2021) conducted a GWAS using 51,128 SNPs markers including 368 worldwide cowpea accessions evaluated during the summers of 2016 and 2017 in California (USA) under long-days at the UCR Citrus Research Center and Agricultural Experiment Station in Riverside (CA) as well as under short days at the UCR Coachella Valley Agricultural Research Station in Thermal (CA) during the autumn of 2016 and 2017 at the International Institute of Tropical Agriculture (IITA) experimental fields of Malamadori and Minjibir, near Kano, Nigeria. Among 40 significant QTLs, 26 were associated with days to first flower under short days while 14 were associated with days to first flower under long days explaining between 5 and 9% of the phenotypic variation.

Garcia-Oliveira et al. (2020) reported a total of 30 QTLs accounting for 1.8 to 13.0% phenotypic variation for pod and seed traits using DArT markers (Table 1). Some major QTLs for number of peduncles per plant (qPeN2.2), pod length (qPoL3), seed breadth (qSB4), seed length (qSL7.2), and seed thickness (qST9) were discovered on chromosomes 2, 3, 4, 7, and 9 using a bi-parental $F_{2:3}$ population. Some QTLs for these traits were clustered especially on chromosomes 5, 7, 8, 9, and 10. More recently, Angira et al. (2020) reported one major QTL (qDTF9.1) for days to first flower and one major (qPH9.1) and a minor (qPH4.1) QTLs for plant height (PH) explaining 29.3 and 29.5% of the phenotypic variation (PVE), respectively using a dense SNP linkage map.

QTL MAPPING FOR GRAIN QUALITY TRAITS

The efforts of breeders are generally focused on improving yields, both by improving the resistance to biotic and abiotic stresses and increasing the maximum obtainable yields. However, cowpea is a consumer good bought and sold along a supply chain stretching from the original producers to the end-use consumers (Langyintuo et al., 2003). Consumers are generally unaware of the constraints on production and are thus focused on other traits of interest to them, usually visible ones termed consumer-related traits. This disparity between producers and consumers regarding the preferred characteristics can result in breeders, who mostly interact with producers, developing new varieties which do not meet the preferences of consumers. This can lead to new varieties not being accepted by the public, resulting in lower adoption rates. Breeders therefore need to understand the genetic control of consumer-related traits so that they can be taken into consideration from the stage of selection of parental lines in the breeding

Table 1. QTLs affecting some traits in cowpea.

Quantitative trait	Pedigree	Type	Marker type	Chromosome location	PV%	Nb QTL	Reference
Root-knot nematode (*Meloidogine* spp.) resistance	CB27 x 24- 125B-1	RIL	SNP	LG13, LG14, LG15, LG16	-	4	
	IT84S- 2049 x UCR779	$F_{2:3}$	SNP	LG19		1	Huynh et al. (2016)
	IT93K- 503- 1 x UCR779	$F_{2:3}$	SNP	LG14		1	
Root-knot nematode (*Meloidogyne* spp.)	524B x IT84S- 2049	RIL	SNP	LG9	-	1	Santos et al. (2018)
Fusarium wilt resistance (for race 4)	IT93K-503-1 × CB46,	RIL	SNP	LG8	19-47	1	Pottorff et al. (2014)
	CB27 × 24-125B-1	RIL	SNP	LG9	32-40	1	
	CB27 × IT82E-18	RIL	SNP	LG3	18-27	1	
Striga resistance	Gorom x Tvx 3236	F2	AFLP-markers	LG1, LG6	-	2	Ouédraogo et al. (2002a)
Heat tolerance	CB27 × IT82E-18	RIL	SNP	LG2, LG7, LG6, LG10, LG3	12-18	5	Lucas et al., 2013a
Number of pods per plant	TVu2185 x TVu6642.	F_2	SNP-markers (DArT)	LG8	7.4	1	Garcia-Oliveira et al. (2020)
Number of peduncles per plant	TVu2185 x TVu6642.	F_2	SNP-markers (DArT)	LG2, LG9	10	2	Garcia-Oliveira et al. (2020)
pod length	TVu2185 x TVu6642.	F_2	SNP-markers (DArT)	LG3, LG4, LG5, LG7, LG8, LG10.	1.8-12.2	6	Garcia-Oliveira et al. (2020)
Flower and seed coat color	ZN016 × Zhijiang 28-2	RIL	SNP and SSR	LG8	-	1 each	Xu et al. (2011)
Number of pods per plant	ZN016 × ZJ282	RIL	SSR	LG3, LG2, LG4	11-20	3	Xu et al, (2013)
Days to flowering	524 B × 219-01	RIL	SSR	LG1	6-19	3	Timko et al. (2013)
Days to first flowering	ZN016 × ZJ282	RIL	SNP	LG11, LG10, LG3	10-32	3	Xu et al. (2013)
Hilum-eye type	GEC'xIT98K-476-8	RIL	SNP	LG7, LG9, LG 10	-	3	Brijesh et al. (2022)
Seed coat	MAGIC	RIL	SNP	LG7, LG9, LG10	10.1-75.9	3	Herniter et al. (2019)
Domestication related trait	(JP81610 × JP89083) × JP81610	BC_1F_1	SSR	1–11 for most traits	LG3, LG7, LG8, LG11	3–57	Kongjaimun et al. (2012)
Seed weight	IT2246-4 × TVNuI963	F_2	RFLP	LG 2 LG6	37–53	2	Fatokun et al. (1992)
Peduncle length	TVu2185 x TVu6642.	F_2	SNP-markers (DArT)	LG1, LG7, LG10	3.8- 6.3	3	Garcia-Oliveira et al. (2020)
Seed weight	524B × 219-01	RIL	SSR	LG1, LG2, LG3, LG10	8–19	6	Andargie et al. (2011)
Pod length	(JP81610 × TVnu457) × JP81610	BC_1F_1	SSR	LG1, LG2, LG3, LG4, LG5, LG7, LG8, LG9, LG11	31	9	Kongjaimun et al., 2012
Number of seeds per pod	TVu2185 x TVu6642	F_2	SNP-markers (DArT)	LG8, LG9, LG10	10.4	4	Garcia-Oliveira et al. (2020)
Maturity	IT93K503–1 × CB46	RIL	AFLP	LG7, LG8	25–29	2	Muchero et al. (2010)
100-seed weight	TVu2185 x TVu6642	F_2	SNP-markers (DArT)	LG7, LG8, LG9	3.9- 9.1	3	Garcia-Oliveira et al. (2020)
Flowering time under long-day length	CB27 X IT82E-18	MAGIC RIL	SNP	LG4, LG5, LG9, LG11	31	4	Huynh et al. (2018.)
Seed size	IT82E-18 and IT00K-1263	MAGIC RIL	SNP	LG6, LG8	-	2	Huynh et al. (2018)
Seed size	Eight different population	RILs	SNP	LG5, LG7, LG2, LG6, LG8, LG10	47	10	Lucas et al. (2013b)
Flowering time under short day length	IT84S-2049, CB27, and IT82E-18	MAGIC RIL	SNP	LG1, LG4, LG5, LG9	9-10	4	Huynh et al. (2018)
Seed length	TVu2185 x TVu6642	F_2	SNP-markers (DArT)	LG3, LG5, LG7, LG8	10.3	6	Garcia-Oliveira et al. (2020)
Seed thickness	TVu2185 x TVu6642	F_2	SNP-markers (DArT)	LG5, LG8, LG9, LG10	10	4	Garcia-Oliveira et al. (2020)
Seed breadth,	TVu2185 x TVu6642	F_2	SNP-markers (DArT)	LG4, LG5, LG8, LG10	4.8-13	4	Garcia-Oliveira et al. (2020)
Seed size	524B × 219-01	RIL	SSR	LG1, LG10	-	6	Andargie et al. (2011)

AFLP = Amplified fragment length polymorphism; F_2 = second filial generation; F_3 = third filial generation; LG= linkage group; SNP= single nucleotide polymorphism; SSR= simple sequence repeat; RIL = recombinant inbred line; PV% = phenotypic variation explained by a given QTL; DArT = Diversity Array Technology; MAGIC = Multiparent Advanced Generation Inter Cross.
Source: Authors.

programmes. This would enable the development of new varieties with high yield, biotic and abiotic resistance/ tolerance characteristics and at the same time acceptable to consumers (Herniter et al., 2019).

The seed coat colour, which has been a subject of study for decades, with the genetic elements behind their expression established, is one of the most important qualities of cowpea impacting its attractiveness and introduction to markets (Herniter et al., 2019). In cowpea, the seed coat pattern is a significant consumer-related trait. Consumers make qualitative choices about a product's acceptability, quality, and taste depending on its appearance, according to previous studies (Kostyla et al., 1978; Jaeger et al., 2018). As a result, determining the genetic regulation of seed coat pattern traits would be beneficial to breeding programmes generating novel varieties with a good chance of commercial acceptability. Cowpea displays a variety of seed coat patterns, including varied eye shapes, Holstein, Watson, and full coat pigmentation, among others (Herniter et al., 2019). There are various patterns of seed coat displayed by cowpea and the preferred colours and pigmentations are region specific (Herniter et al., 2019). Colour Factor (C), Watson (W), Holstein-1 (H-1), and Holstein-2 (H-2) are four factors that regulate seed coat pattern as reported by Spillman (1911) and Harland (1919). In a bi-parental population, however, Harland (1919) discovered two H loci, which he dubbed "H-1" and "H-2". Aside individual population-based QTL mapping, few QTL analyses of seed coat patterns have been undertaken using numerous bi-parental populations. Seed coat pattern QTL study utilizing RIL populations revealed a total of 35 SNP loci, all of which were mapped on three chromosomes (Table 1), LG7 (C locus), LG9 (H locus), and Vu10 (L locus) (W locus) (Herniter et al., 2019).

Xu et al. (2011) conducted a study to map flower and seed coat colors using 209 F7:8 RILs with 184 SSRs and 191 SNPs. They identified one locus for each trait and both loci are tightly linked with a genetic distance of 0.4 cM. A similar study on QTL analysis using a bi-parental population for hilum-eye type has been carried out. In this study, three major genes controlling different seed hilum-eye types in cowpea and their three corresponding QTLs were successfully identified. The three genes were designated as W (Watson hilum-eye type), S (Small hilum-eye type), and R (Ring hilum-eye type) and mapped onto chromosomes 7, 9, and 10, respectively (Brijesh et al., 2022). Unfortunately, numerous issues impeded the implementation of QTL-based MAS in cowpea breeding, including the lack of consistent and substantial phenotypic impacts of QTLs in heterogeneous recipient genetic backgrounds (Zhao et al., 2021).

QTL MAPPING FOR RESISTANCE TO BIOTIC AND ABIOTIC STRESSES

During their growth cycles, plants are exposed to various favorable and unfavorable environmental conditions. Such conditions include biotic stresses like insect pest attacks and disease infections, as well as abiotic stresses such as heat, cold, drought, low soil fertility, increased salt levels, and toxic metals and metalloids in the soils. The principal and most frequently encountered climatic conditions that reduce agricultural crop yields are temperature (heat or frost), drought, flood and salt.

Efforts have been undertaken on linkage maps to identify QTLs for resistance to abiotic and biotic constraints in cowpea. For example Huynh et al. (2015) conducted a study on resistance to Aphis craccivora Koch and identified one minor and one major QTLs mapped on linkage groups 1 and 7, respectively using 1,536 SNPs and 92 recombinant inbred lines explaining 5 to 13% and 61 to 66% of phenotypic variations, respectively. More recently, a combination of SSR and SNP markers was used by Kusi et al. (2017) to identify QTL regions for aphid (*Aphis craccivora*) resistance in an F_2 population backcrossing and in a bi-parental recombinant inbred line population and reported a major QTL on chromosome 10 to a position of 11.5 cM. Huynh et al. (2016) reported a major QTL related to resistance to root-knot nematodes on linkage group 11 of distinct mapping populations. To map candidate genes for root-knot nematode resistance, Santos et al. (2018) used 84 F_{10} recombinant inbred lines population and transcriptome alterations in two cowpea near-isogenic lines (NILs). A major QTL, QRk-vu9.1 was discovered on chromosome 9 at position 13.37 cM. Pottorff et al. (2012a) reported a *Fusarium oxysporum* f. sp. *tracheiphilum* race 3 resistance locus (Fot3-1) to a 1.2 cM region and discovered SNP marker 1_1107 as co-segregating during their efforts to develop resistant cowpea varieties. Research conducted by Ouédraogo et al. (2002a) revealed that the genes for *Striga* races 1 and 3 were located on linkage groups one and six of the cowpea genome using AFLP markers (Table 1).

The numbering of cowpea linkage groups changed from 2009 based on the work done by Muchero et al. (2009). Linkage groups one and six became ten and nine, respectively. Ampadu (2017) used SNP markers distributed across the cowpea genome and detected QTLs on linkage group nine associated with *S. gesnerioides* resistance spanning the length of 19.89 cM. Omo-Ikerodah et al. (2008) employed 92 bi-parental recombinant inbred lines to map QTLs for resistance to flower bud thrips using AFLP and SSR markers. In another study, Muchero et al. (2010) reported 9 QTLs accounting for 6.1 to 40.0% of the phenotypic variance (R^2) for resistance to thrips damage. Resistance to blackeye cowpea mosaic potyvirus (B1CMV) and the southern bean mosaic virus (SBMV) were mapped to LG8 and LG6, respectively, whereas resistance to the cowpea mosaic virus (CPMV) and the cowpea severe mosaic virus (CPSMV) were mapped to the opposite ends of LG3 (Ouédraogo et al., 2002b).

Agbicodo et al. (2010) reported three QTLs for bacterial

blight resistance, CoBB-1, CoBB-2, and CoBB-3 on LG3, LG5 and LG9, respectively showing that potential resistance candidate genes co-segregated with CoBB resistance phenotypes. Two of the QTLs (CoBB-1 and CoBB-2) were confirmed in the two experiments explaining 22.1 and to 17.4% of phenotypic variation for the first and second experiments while CoBB-3 was discovered for the first experiment with less phenotypic variation explained of about 10%. Miesho et al. (2019) used a cowpea linkage map of 41,948 SNP markers to identify candidate genes associated with resistance to bruchid using a set of 217 mini-core cowpea accessions. Using plant mortality data from 3 years of field experiments and disease severity scores from two greenhouse trials, Muchero et al. (2011) reported QTL associated with *Macrophomina phaseolina* resistance as Mac-1 located on LG2, Mac-2, Mac-3, and Mac-4 on LG3, Mac-5 on LG11, Mac6 and Mac-7 on LG5, and Mac-8 and Mac-9 on LG6. Ohlson et al. (2018) also used a bi-parental F_2 population and genotyped with 99 newly created allele-specific polymerase chain reaction (AS-PCR) markers for QTL analysis and found one major and three minor QTLs for resistance to brown blotch on LG2, LG3, LG6, and LG8. Similarly, Ibié et al. (2021) used parents, F_1, F_2, and BC_1F_1 progenies and discovered QTLs linked to brown blotch resistance. Ten QTLs were found using a RIL population established from a hybrid between IT93K-503-1 (tolerant) and CB46 (sensitive) that differed in their tolerance to seedling-stage drought (Muchero et al., 2009). Some of these QTLs coincided with QTLs for stem greenness (stg) and recovery dry weight (rdw) after drought stress under greenhouse and field conditions. The 10 QTLs were located on LG1, 2, 3, 5, 6, 7, 9, and 10 and accounted for between 4.7 and 24.2% of the phenotypic variance.

Using RIL population, Pottorff et al. (2012b) discovered a significant QTL that affects cowpea leaf shape which may potentially influence drought tolerance. Muchero et al. (2013) used phenotypic data from 13 experiments carried out across four countries to conduct association mapping and identified QTLs for delayed senescence, biomass and grain yield in a panel of 383 diverse cowpea accessions and a recombinant inbred line population. Lucas et al. (2013a) reported five genomic regions in a RIL population that explained 11.5 to 18.1% of the phenotypic variation controlling heat tolerance in cowpea. Similarly, Pottorff et al. (2014) discovered three QTLs (Hbs-1, Hbs-2, and Hbs-3) that influence heat stress-induced seed coat browning in cowpea from two RIL populations. The underlying candidate genes encoding ACC oxidase 2 and ethylene-responsive element-binding factor 3 (ERF3) were revealed, and the QTLs explained 9.5 to 77.3% of the phenotypic variation.

CONCLUSION

This paper provides an overview of some of the recent advances in cowpea improvement attributable to molecular markers. Molecular marker applications to cowpea improvement include characterization of its germplasm and analysis of genetic diversity, population structure, as well as QTL analyses which identified genomic regions involved in an array of economically important traits. Some of the traits for which associated molecular markers have been identified following QTL analyses are resistance to abiotic constraints such as drought, heat, and biotic constraints such as bacterial blight, root-knot nematode, viruses, striga and alectra. QTLs with effects on yield and its components as well as grain quality and domestication related traits have been reported. Compared to many other crops, molecular marker assisted breeding has only recently begun in cowpea.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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