



Genetic Diversity of Cowpea: Implications for Crop Improvement and Food Security: A Review

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ABSTRACT

Cowpea is a major legume crop native to Africa and it provides food for man as well as livestock. Even though it is widely cultivated in Africa, a second domestication was probably occurred in Asia. Cowpea can thrive in a range of soil types, pH and temperatures. Besides, changing lifestyle and climate variability have brought in enough scope for its cultivation. Variability studies in cowpea are highly significant in order to identify superior genotypes for the further improvement of the crop. The genetic diversity analysis will help in the identification of potentially distinct genotypes with contrasting traits which can be used to operate effective selection of genetically diverse genotypes for yield improvement as well as nutritional enrichment and climate resilience. Hence, the present review is focused on the studies carried out in different parts of the world to characterize the genetic diversity in cowpea germplasm. This work was done at the Department of Botany, University of Calicut, Kerala. The study reviewed and systematically evaluated the various practices implied to assess the genetic diversity in cowpea. Literature search was done during March, 2023- July, 2024. About 150 Research papers were screened from various data bases like Google scholar, ResearchGate and a total of 79 research papers were used to write this review article. Present review article reveals the significance of cowpea as a food and fodder crop and the characterization of cowpea genotypes as an important genetic resource for developing climate resilient crops. The review also shed light on origin and distribution of cowpea, cowpea breeding, tools for genetic variability assessment among cowpea genotypes, new and improved molecular markers that are utilized in research and future aspects of cowpea improvement for maintaining sustainable nutrition to society.

Key words: Cowpea, Genetic diversity, Variability, Yield improvement.

A stable and secure food system is essential for sustainable development. Legume crop cultivation is a novel strategy for guaranteeing a sufficient supply of food and agricultural survival. They are abundant in dietary fibre, proteins, carbohydrates and micro-nutrients. An adequate daily intake of legumes promotes a healthier and well-balanced diet.

Cowpea [*Vigna unguiculata* (L.) Walp.] is a member of Dicotyledoneae belonging to the order *Fabales*, family *Fabaceae*, subfamily *Faboideae*, tribe *Phaseoleae*, subtribe *Phaseolinae*, genus *Vigna* and section *Catiang* (Verdcourt, 1970; Marechal, 1978). It is a diploid having somatic chromosome number 22 (2n=22). Morphologically, cowpea is a herbaceous plant which grows annually (Duraipandian *et al.*, 2022). They are typically climbing or trailing vines that bear compound leaves with three leaflets. The white, purple or pale-yellow flowers usually grow in pairs or threes at the ends of long stalks.

Cowpea is one of the most versatile and resilient legumes of cultivated crop. It is cultivated across the globe under varied types of soil and rainfall conditions mostly in the tropics and subtropic regions (Nalawade *et al.*, 2021) of Asia, Africa, central and southern America, parts of southern Europe and USA (Gupta *et al.*, 2023). In Asia, India alone accounts for almost half of the total Asian area (Kumar and Singh, 2004). The genus *Vigna* Savi which belongs to the tribe *Phaseoleae* of the family *Fabaceae*, includes over 100 species distributed in the tropical and subtropical areas of the world grouped under five subgenera: *Vigna*,

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Ceratotropis, *Plectotropis*, *Lasiosporon* and *Haydonia* (Panzeri *et al.*, 2022). The subgenus *Vigna* is distributed in Africa and includes about 40 wild and 2 domesticated species, namely *Vigna unguiculata* L. (popularly called cowpea, black-eyed peas, chawli and kunde) and *Vigna subterranea* L. (Bambara groundnut) while *Ceratotropis* (Piper) Verdc. distributed in Asia, contains 21 wild and 7 domesticated species used widely for food and forage, namely *Vigna radiata* L. Wilczek (mungbean or green gram), *Vigna mungo* L. Hepper (black gram), *Vigna aconitifolia* Jacq. Maréchal (moth bean), *Vigna umbellata* Thunb. Ohwi and Ohashi (rice bean), *Vigna angularis* L. Ohwi and Ohashi (adzuki bean), *Vigna reflexo-pilosa* Hayata (creole bean), *Vigna trilobata* L. Verdc. (jungli bean).

Cultivated species are categorized into four groups, namely *Biflora*, *Sesquipedalis*, *Textilis* and *Unguiculata* (Westphal, 1974; Maréchal, 1978; Ng and Marechal, 1985).

Yardlong bean belongs to the culti-group *Sesquipedalis*, while grain type falls under *Unguiculata* (Tripathi *et al.*, 2019). They are easily crossable and crosses produce fertile and viable progeny.

Origin and distribution of cowpea

The domestication and centre of origin of cowpea has been subjected to a significant discussion. Indian subcontinent as well as Africa having diverse and morphologically variant types of cowpeas and because of which it was considered as both India and Africa might be independent centres of origin and domestication of cowpea (Singh, 2020). The introduction of cowpea to Indian sub-continent happened more than 2000 years back (Tripathi *et al.*, 2019). India is believed to be the centre of cowpea diversity because of two important “cultigroups” viz., *Biflora* and *Sesquipedalis* which have evolved from *Unguiculata* (Ng and Marechal, 1985). Padulosi and Ng (1997) reported Southern Africa as centre of origin for wild cowpea. *Vigna unguiculata* var. *spontanea* (formerly var. *dekindtiana*) is believed to be the progenitor and it has been found in all low-lying areas of Sub-Saharan Africa (Boukar *et al.*, 2013).

Cowpea for human health

Cowpea has been used in the human diet, as well as in forage for animal feeding. For human consumption, its grain can be used after boiling, frying or steaming and can be made into salads or snacks. It can be eaten whole, tinned or frozen as well as mashed into flour for baking purposes. It is cultivated mainly for its grains which are rich in protein. Most of the improved varieties contain a protein content between 20 to 25 per cent on dry weight basis (Boukar *et al.*, 2019) which is almost equal to the protein content present in soybean. The whole grain protein content in cowpea is low in methionine and cysteine amino acids as against to proteins of livestock-origin, however, it is high in amino acids when compared with the cereals (Goncalves *et al.*, 2016). It also contains anti-nutritional elements that could be inconvenient to human and non-ruminant animal nutrition (Abebe and Alemayehu, 2022).

However, some anti-nutritional factors such as hemagglutinins and trypsin inhibitors are heat labile and can be easily inactivated by heating (Boukar *et al.*, 2015). Cowpea is rich in vitamin C and A and also has appreciable amount of thiamine, riboflavin, niacin, vitamin B5 and pantothenic acids as well as small amount of foliate (Asare *et al.*, 2013). With excellent nutritional and nutraceutical properties and several agronomic, environmental and economic advantages, cowpea is able to contribute to food security, maintenance of environment and promotion of health for populations (da Silva *et al.*, 2018).

Cowpea has been considered as a high-quality protein constituent of the daily diet for the economically weaker and depressed communities in developing countries and its consumption greatly contributes to decrease malnutrition (Jayathilake *et al.*, 2018). Cowpea has a major role in

providing nutritional security and livelihood for the farmers in African countries. Being an important orphan crop, cowpea is grown by farmers of Sub-Saharan Africa in large scale and it also contributes to the food and nutritional security of the region (Mekonnen, 2022).

Challenges and future prospects

From seedling stage to fruiting, insect pests damage cowpea. The most dangerous pests are those which attack during flowering and podding stages (Jackai and Adalla, 1997). The involvement of weeds are rivals for water, nutrient and light and can cause up to 76% yield loss. Cowpea production is challenged by lack of mechanized equipment needed for harvesting. Moreover, they need thorough labour for cultivation as well as harvesting which leads to high production cost (Osipitan *et al.*, 2021). Global food and nutritional security can be greatly affected by the climate change issues which in turn will lead to reduced productivity in almost all agricultural crops (Mekonnen *et al.*, 2022). Compared to other legumes, cowpeas are able to produce remarkable number of grains under worse climatic conditions (Timko and Singh, 2008). Cowpea is one of the crops which can tolerate drought (Carvalho *et al.*, 2017). Genetic improvement of cowpea by incorporating stress tolerance genes to the early maturing lines will be the best and effective mode of producing sustainable and enhanced crop yield with respect to varying climates (Agbicodo *et al.*, 2009).

Genetic diversity is important for natural evolution process as well as selection of populations. Because of genetic variation, various crops survive under diverse environmental conditions and adapt over adverse situation (Salgotra and Chauhan, 2023). By exploiting genetic diversity, breeders produce new cultivars which have high yield, biotic and abiotic stress tolerance and better nutritional quality (Swarup *et al.*, 2021). Thus evaluation of genetic diversity in cowpea is important for the conservation of germplasm and its application in hybridization programme (Panchta *et al.*, 2021) by which breeders could identify the better ones which can serve as parental lines in future breeding efforts. Since, cowpea is diploid and self-pollinated, chances of declining its genetic makeup is very high (Omomovo and Babalola, 2021). Therefore, concerted efforts are needed for exploring the genetic variability in cowpea and conserving them.

Cowpea genetic diversity: An overview

The major objective of plant breeding programmes is to increase the yield and improve other important traits, which depends on nature and magnitude of genetic variability present in that plant (Singh *et al.*, 2022). Cowpea genetic diversity is critical for crop improvement efforts that aim to increase productivity, resilience and nutritional quality. By harnessing this diversity, breeders may develop superior varieties that promote food security and sustainable agriculture, especially in areas where cowpea is a vital

component of the diet and economy. Efforts to retain and utilise genetic diversity in cowpea are critical to guaranteeing the crop's long-term survival and resilience. Breeders can choose desirable qualities for an improvement program through the collection, characterization and evaluation of the available cowpea germplasm, quantification of the diversity and grouping into homogenous clusters to make genetic variability identification easier (Gerrano *et al.*, 2015). Breeding efforts may be developed to improve cowpea varieties having high protein content, high fibre, low glycaemic index and high levels of antioxidants to tackle the potential threat of malnutrition and non-communicable diseases (Tripathi *et al.*, 2019). Genetic diversity can be evaluated using morphological traits, biochemical and molecular markers. Several studies have been done indicating the characterization of cowpea by morphological and quantitative traits (Pasquet, 1998; Stoilova and Pereira, 2013; Egbadzor *et al.*, 2014). Morphological markers were largely supplemented by biochemical markers, particularly isoenzymes that could be easily scored by electrophoresis (Schulman, 2007). Apart from the method of phenotype-based selection methods, breeders can use marker assisted selection (MAS) in order to improve crop varieties and select desirable traits within short time. Various molecular markers such as RAPD, SSR, ISSR, RFLP, SNP *etc.* are extensively used for plant genetic diversity studies and crop improvement (Hossain *et al.*, 2020). Molecular markers are more beneficial than traditional marker systems, they may be detectable in all of the plant tissues regardless of growth, development and any other physiological state of the plant and are usually not influenced by environmental factors (Waseem *et al.*, 2012). The present review provides an overview of various practices to analyse the genetic diversity of cowpea, with an emphasis on studies related to genetic diversity assessment.

Methods and approaches for assessing genetic diversity

Morphological diversity

Traditionally, genetic diversity is evaluated in crop species based on differences in morphological characters and qualitative traits. Characterization of different genotypes based on morphological traits is cost effective, easily recordable and does not require technical knowledge. Also, it is a powerful tool which can be utilised for the preliminary grouping of cultivars just before their characterization using molecular markers (Mazid *et al.*, 2013). Morphological characterization includes both qualitative and quantitative traits. Characters controlled by a few genes with major effects on the phenotype are said to be qualitative characters and their inheritance is referred to as qualitative inheritance. Characters controlled by a number of genes with small effects that may be measured in metric units are said to be quantitative characters and their inheritance is referred to as quantitative inheritance (Poehlman, 1987).

In order to reduce the dimensionality of the data and to examine the variation and the relative contribution of the traits for the total variability, principal component analysis (PCA) is carried out usually. Principal component analysis is used to identify a minimum number of components which accounts for maximum variability out of the total variability. Multivariate analytical technique, which simultaneously analyse multiple measurements on each individual under investigation, are widely used in analysis of genetic diversity (Mohammadi and Prasanna, 2003). Cluster analysis is commonly employed in analysis of genetic diversity in crop species. If the classification is successful, individuals within a cluster shall be closer when plotted geometrically and different clusters shall be farther apart (Hair *et al.*, 1995). However, morphological traits are often influenced by environmental factors. Temperature, rainfall and soil physiochemical properties are the major environmental factors that significantly affect plant height (Lemore *et al.*, 2022).

Onuminya *et al.* (2023) detected variations in raceme position, flower colour, seed shape and other distinctive qualitative features out of thirty seven traits among ten cowpea accessions collected from African countries. Gomes *et al.* (2021) reported an extensive morphological variability among cowpea genotypes grown in Mozambique. Walle *et al.* (2019) reported high genetic divergence among 324 cowpea genotypes collected from different areas of Ethiopia. Alvarez *et al.* (2021) observed that the traits that most contributed to genetic diversity were mass of one hundred grains and grain yield among the cowpea genotypes from Brazil.

The agro morphological characterization of selected cowpea varieties in the West African country Burkina Faso conducted by Barro *et al.* (2023) recommended the varieties Baguette, Baguette grimpat, Telma and IT83S-911 as potential parents for cowpea breeding. Analysis of morphological and agronomical variability of cowpea genotypes and their ethnobotanical nomenclature in Ethiopia interestingly revealed the presence of various names for cowpeas which are given according to the seed size, seed colour, seed taste *etc.* (Naoura *et al.*, 2020). However, a low level of genetic variability was observed among cowpea genotypes from two West African countries, Ghana and Mali as reported by Doumbia *et al.* (2013).

Many authors studied the genetic variability in cowpea employing multivariate analysis (Teodoro *et al.*, 2019; Vijayakumar *et al.*, 2020; Chaudhary *et al.*, 2021; Saidaiyah *et al.*, 2021). Honaizer *et al.* (2022) compared the grain protein profile of four Brazilian cowpea cultivars employing two-dimensional gel electrophoresis. 2-DE was enough to separate cowpea proteins which showed high homogeneity among grain protein profiles. Principal component analysis showed that there is a significant variation between the protein abundance among cowpea genotypes.

Biochemical diversity

Crop species have more overall allozyme diversity than other seed plant species (Hamrick and Godt, 1997). Several research on cowpea have been conducted throughout the years, employing biochemical markers. Biochemical genetics is the study of proteins, specifically enzymes, isozymes and allozymes (Silva, 2018). Biochemical diversity analysis allows each member in a group to express their own morphological and physiological individuality (Provenza *et al.*, 2003).

Vaillancourt *et al.* (1993) examined wild and cultivated cowpea accessions using 10 isoenzymes, while Panella and Gepts (1992) characterised cowpea accessions using 26 isoenzyme loci. Apart from isozyme markers, seed storage protein profiling is another strategy used to uncover genetic variation between cowpea cultivars (Carvalho *et al.*, 2017). Earlier researches aimed to provide a genetic tool for studying the genetic links among the taxa within the species *Vigna unguiculata* by examining the genetic regulation of the Vignin protein. There was a great deal of diversity among those wild taxa and the data provided no definitive response to the identity of the cowpea's progenitor or the centre of domestication, as reported by Panella *et al.* (1993). Gerrano *et al.* (2015) found a strong relationship between and within mineral elements and total protein concentration in cowpea leaves. The biometric study revealed that phenotypic variations were higher than genotypic variances indicating the influence of environment.

Alghamdi *et al.* (2019) performed biochemical and molecular characterization of cowpea landraces using seed storage proteins and SRAP marker proteins. They discovered a large number of bands common to various landraces, indicating their close relationships, as well as a few distinct bands, indicating varietal differences. Similarly, genetic relationship studies on Egyptian cowpea genotypes based on seed storage proteins and isozymes revealed 29 bands with molecular weights ranging from 7 to 265 kD, including one unique, 26 polymorphic and two monomorphic bands (Elghamery *et al.*, 2021). Ddamulira *et al.* (2015) discovered that cowpea grain yield and protein content were strongly influenced by genotypes (G), environments (E) and interactions (G×E).

Molecular diversity

Molecular markers indicate variations in the DNA. Several molecular markers are currently employed to determine genetic diversity. DNA markers have shown to be useful tools in a variety of studies, including phylogenetic analysis and positional cloning of genes. Plant breeders and geneticists now have access to a number of DNA markers and molecular breeding techniques, which enable them to overcome many of the obstacles associated with conventional breeding. Common DNA markers such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) have helped in mapping and association studies, resulting in the identification of genes of interest.

Random Amplified Polymorphic DNA (RAPD) uses primers that are 9 or 10 nucleotides long and after amplification, the amplicons are separated on agarose gels and visualised using ethidium bromide. Another useful tool for genome analysis is Inter-Simple Sequence Repeat amplification (ISSR) polymorphism. It is a PCR-based marker. Another option is Amplified Fragment Length Polymorphism Markers (AFLP), which can detect thousands of independent loci at low cost and time. Simple Sequence Repeats (SSR) have been especially useful for molecular genetic research due to their abundance, high level of polymorphism and ease of identification by automated techniques. Furthermore, SNP markers have been known as the greatest DNA markers due to their advantages such as high polymorphism, codominant inheritance, high density and high throughput.

RAPD markers differentiated 30 cowpea genotypes from various geographical locations of India into three clusters (Prasanthi *et al.*, 2012). Pidigam *et al.* (2019) found 100% polymorphism in a study of 28 yard long bean [*Vigna unguiculata* (L.) Walp. subsp. *Sesquipedalis* Verdc.] accessions collected from various Indian states using 31 RAPD markers. Saxena and Rukam (2020) revealed that ISSR markers can also be used to differentiate cowpea genotypes. Araujo *et al.* (2019) also used ISSR markers to detect the genetic variability in cowpea landraces collected mostly from small producers all over the states of Brazil and observed 76% polymorphism.

Vinay *et al.* (2022) examined 32 cowpea genotypes using 25 SSR markers and discovered a moderate level of genetic variability across the samples studied. The genetic diversity of semi-arid farmed cowpea genotypes in Sudan varied between 0.02 and 0.47, with an average of 0.25 (Elteib and Gasim, 2020). Sarr *et al.* (2020) concluded that *Vigna unguiculata* var. *spontanea* is the wild progenitor of cowpea. They evaluated the genetic diversity of cowpea genotypes in Senegal, including 671 accessions, 66 wild relatives and intermediate forms employing 15 SSR markers. Accessions from various regions revealed genetic similarity ranging from 0.861 to 0.965, with genetic differentiation indices ranging from 0.018 to 0.100. The wild/weedy forms remain more diverse than the cultivated, with genetic diversity values (He) of 0.389 and 0.480, respectively.

Iseghohi *et al.* (2019) observed a positive and significant association ($r = 0.377$, $p = 0.01$) between genetic and geographical distance of landrace collections. They employed SSR markers to evaluate the genetic diversity of eighteen cowpea genotypes collected from five agro-ecological zones in Nigeria. The observed heterozygosity and average polymorphism information content were 0.5721 and 0.2433, respectively. The landraces gathered from the humid woodland zone exhibited great genetic diversity and landraces from savannah zones had minimal genetic diversity.

Single Nucleotide Polymorphisms (SNPs) in the cowpea genome were found using Genotyping by Sequencing (GBS).

Cowpea genetic diversity was examined and population structure was calculated using a wide range of 768 cultivated genotypes gathered from 56 different nations. Polymorphism Information Content (PIC), heterozygosity, gene diversity, and major allele frequency were computed for each SNP. This study on the 768 global cowpea genotypes based on genome-wide SNPs suggested three well-differentiated genetic populations known as structured populations or clusters (Xiong *et al.*, 2016). Gumede *et al.* (2022); Edema *et al.* (2023) also used SNP markers to assess the genetic diversity and population stratification of cowpea genotypes.

In the same way, by using Single Nucleotide Polymorphic (SNP) markers, the genetic diversity and population stratification of cowpea genotypes were estimated. To analyse 62 cowpea genotypes that varied in their key characteristics, a total of 2,746 SNP markers were used. Out of the 62 genotypes, only 55% of the SNP markers were polymorphic. Polymorphic Information Content (PIC) varied from 0.46 to 0.51 with an average of 0.49 and gene diversity ranged from 0.46 to 0.51 with an average of 0.55, which indicates that cowpea parental lines were genetically diverse (Edema *et al.*, 2023).

Seo *et al.* (2020) investigated the genetic diversity and population structure of global and Korean cowpea germplasms, analysing 384 cowpea accessions from 21 countries with 51,128 Single-Nucleotide Polymorphisms (SNPs). Their findings suggested that the Korean cowpea accessions might have come directly from West Africa, rather than Asia or Europe. Wu *et al.* (2021) performed an SNP test on 299 cowpea accessions, processing 40,089 Kompetitive Allele Specific PCR (KASP) markers and identifying a core set of 50 SNP markers that are highly informative, representative and evenly distributed. In order to examine the phenotypic and genetic variation in 97 cowpea accessions from Portugal and Mozambique, Guimaraes *et al.* (2023) used SSR, SilicoDArT and SNP markers. They noticed that farmers in Portugal and Mozambique have managed to preserve the genetic variety of cowpea regionally.

CONCLUSION

Cowpea is known as “poor man’s meat” due to its high protein content and ease of cultivation by farmers. It is mostly cultivated for grains, but immature pods and leaves are often consumed owing to their nutritional value. Cowpea exhibits morphological, biochemical and even genome-wide diversity. Genetic diversity is required for an effective breeding programme. By identifying and characterising distinct germplasm collections, breeders can have access to important genetic resources for producing improved varieties with desirable characteristics. This includes features like crop potential, drought tolerance, pest resistance and nutritional value. The use of different genetic material also minimises the danger of genetic sensitivity to diseases, pests and environmental changes.

Furthermore, genetic diversity allows for hybridization and the introduction of desirable features from wild or landrace relatives into cultivated cowpea varieties. This genetic mixing can help cowpea adapt to shifting environmental circumstances and emerging threats. Crop enhancement improves cowpea yield and resilience, which benefits food security on multiple fronts. Improved cultivars with higher yields and enhanced nutritional profiles can help boost food availability, particularly in areas where cowpea is a main crop. Furthermore, improved resistance to climatic stresses like heat and drought might help protect food production from the effects of climate change. Moreover, cowpea’s genetic diversity conservation guarantees the crop’s long-term viability. Diverse genetic resources should be preserved because they protect against genetic degradation and offer alternatives for future breeding projects aimed at addressing changing agricultural concerns.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any indirect losses resulting from the use of this content.

Conflict of interest

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