Review Article

Ekin
Journal of Crop Breeding and Genetics

7(1):1-20, 2021

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Genetic Divergence in Cowpea (*Vigna unguiculata* L. Walp) - an Overview

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Citation:
Panchta R., Arya RK., Vu NN., Behl RK., 2021. Genetic Divergence in Cowpea (*Vigna unguiculata* L. Walp) - an Overview. Ekin J. 7(1):1-20, 2021.

Received: 13.10.2020 ***Accepted: 14.11.2020*** ***Published Online: 29.01.2021*** ***Printed: 30.01.2021***

ABSTRACT

Cowpea [*Vigna unguiculata* L.Walp] is an important summer/rainy season legume crop which is tolerant to drought as well as water logging conditions. It provides nutritious grain and an inexpensive source of protein. In cowpea breeding programmes, the major emphasis has been on the collection and conservation of genetic pools. The knowledge on the nature and extent of genetic variability present in any crop species plays an important role in designing a suitable breeding method. Genetic diversity is the foremost basic requirement for a successful breeding programme. Heritability is a biostatistic commonly used in plant breeding and genetics works that estimates how much variation in a phenotypic trait in a population is due to genetic variation among individual plants in that population. Genetic advance is the improvement in the mean genotypic value of selected plant families over that of base population. It depends upon phenotypic variability, heritability and intensity of selection. The evaluation of cowpea germplasm, quantification of the magnitude of variability existing for different characters and classification into groups help in identifying potential distinct genotypes which are having contrasting characters, can be used to operate effective selection of genetically diverse genotypes for the improvement of yield. In this review paper, the research work carried on different aspects of genetic divergence is discussed under the sub-heads, i.e. variation of qualitative and quantitative traits, genetic variability, heritability and genetic advance, correlation and path analysis, genetic divergence using D^2 analysis and genetic diversity by using molecular markers.

***Keywords*:** Cowpea, genetic variability and divergence, heritability, genetic advance.

Introduction

Cowpea [*Vigna unguiculata* L.Walp] (2n=22) is an important summer/rainy season legume crop which is tolerant to drought as well as rain water stagnation conditions. Like other legumes, it also has unique ability to fix atmospheric nitrogen into nitrate, thus improve fertility status of soils (Kumar et al. 2015). Cowpea is a dicotyledonous crop plant in the order Fabaceae, subfamily Faboideae (Papillionoideae), tribe Phaseoleae, subtribe Phaseolinae, genus *Vigna*. The genus *Vigna* is pantropical and has high variability. In addition to cowpea, other members include mungbean (*V. radiata*), adzuki bean (*V. angularis*), blackgram (*V. mugo*) and the bambara groundnut (*V. subterranea*). Cowpea, *V. unguiculata* subspecies *unguiculata* includes four cultigroups: *unguiculata*, *biflora* (or *cylindrica*), *sesquipedalis* and *textilis* (Ng and Maréchal 1985). Moreover, *V. unguiculata* subspecies *dekindiana*, *stenophylla* and *tenuis* are the immediate wild progenitors of cultivated cowpea. Based on the distribution of diverse wild cowpeas along the entire length of eastern Africa, east and southern Africa was proposed to be the primary region of diversity and west and central Africa to be the secondary center of diversity (Steele 1972; Singh et al. 1997; Timko and Singh 2008; Lal and Vashisht 2008; Rana, 2011). These authors also suggested Asia as a third center of diversity, specific for two cultigroups *cylindrical* (catjang bean) and *sesquipedalis* (yardlong bean).

Cowpea provides nutritious grain and an inexpensive source of protein for both rural poor and urban consumers and it is also called as vegetable meat (Lal and Vashisht 2008; Ram 2014). Cowpea grain contains about 23-28% protein and 64% carbohydrate and therefore, has an enormous potential to contribute to the alleviation of malnutrition among resource-poor farmers (Bressani 1985; Singh et al. 1997; Lal and Vashisht 2008). Besides being used as pulse in form of dry seed, cowpea's immature pod and green leaf and growing twig can be utilized as vegetable. However, it is more important as the source of green as well as dry fodder. Among fodder legumes, cowpea is grown for both grain and fodder in all tropical and sub-tropical climates. Area, production and yield under cowpea cultivation for dry grain over the world were 12.6 million hectares, 5.6 million tonnes and 443 kg/ha, respectively. In which Niger, Nigeria, Burkina Faso are the first rankings in harvested area and production also (FAOSTAT, 2014). In India, cowpea is grown as sole, inter-crop, mix-crop and in agro-forestry combinations. Its area is estimated to be cultivated in almost half of 1.3 m ha of area occupied by Asian region. Cowpea is a minor pulse in Indian cultivated mainly in arid and semi-arid tracts of Rajasthan, Karnataka, Kerala, Tamilnadu, Maharashtra and Gujarat. In northern India, it is mainly grown for fodder during *kharif* as well as summer in pockets of Punjab, Haryana, Delhi and West UP along with considerable area in Rajasthan (Tiwari and Shivhare 2016).

In cowpea breeding programmes, the major emphasis has been on the collection and conservation of genetic pools. Cowpea germplasm is maintained in collections around the world with varying levels of accessibility and documentation. The largest collections are held by the IITA over 16000 cultivated varieties and almost 2000 wild accessions of cowpea and some other collection from the United States Department of Agriculture (USDA), University of California-Riverside, India, Brazil, these collections that cover a wide spectrum of growth habits, environmental responses and varying pest and disease susceptibilities (Singh et al. 1997; Timko and Singh 2008; IITA 2017). The knowledge on the nature and extent of genetic variability present in any crop species plays an important role in designing a suitable breeding method. Genetic diversity is the foremost basic requirement for a successful breeding programme. A quantitative assessment of the genetic divergence among the collection of germplasm and their relative contribution of different traits towards the genetic divergence provide essential and effective information to breeder in his hybridization programme and thereby genetic improvement of yield (Nagalakshmi et al. 2010).

It is difficult to judge what proportion of observed variability is heritable and non-heritable *i.e.* environmental. The breeding in cowpea is mainly depends on magnitude and nature of interactions of genotypic and environmental variations. It is imperative to partition the observed variability into its heritable and non-heritable components and to have an understanding of parameters like genetic co-efficient of variation, heritability and genetic advance (Thakur et al. 2011). Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) are measured to study the variability presenting in the germplasms. The high GCV and PCV encompass the possibility of large variation. Therefore, its correct understanding is very important for efficient utilization in crop improvement programme. Sivasubramanian and Madhavamenon (1973) classified GCV, PCV into three groups as: 0-10% = low; 10-20% = moderate; >20% = high.

Knowledge of heritability influences the choice of selection procedures, to predict gain from selection and to determine the relative importance of genetic effects. Evaluations of the components of variation and heritability are, therefore, among traits that will facilitate improvement of crops such as cowpea. Genetic advance is the improvement in the mean genotypic value of selected plants over the base population. It depends upon phenotypic variability, heritability and intensity of selection (Ngoc et al. 2019). When, genetic advance as percent of mean (GAM) was high for the trait, effective progress in improvement through selection could be achieved in cowpea (Ajayi et al. 2014). Correlation among traits also helps to simultaneously select for more than one trait of importance at a time. Yield is a complex character determined by several component characters. Therefore, selection for yield should take into account related traits as well. Path coefficient analysis provides an effective means of partitioning the genotypic correlation coefficients into direct and indirect effects of the component traits on yield on the basis of which crop improvement programmes can be logically devised (Vidya and Oommen 2002).

In cowpea, quantification of the magnitude of variability in characters and classification into groups help in identifying potential distinct genotypes having contrasting characters can be used for effective selection of genetically diverse genotypes for yield improvement. Keeping these aspects in view, the proposed review was undertaken the following sub-heads:

Variation of qualitative and quantitative traits

Traditionally, genetic diversity evaluated in crop species are based on differences in morphological characters and qualitative traits (Kumar et al. 2015), probably due to the fact that the assay of qualitative traits does not need any sophisticated equipment or complex experiments, they are generally simple, rapid and inexpensive to score. It has been used as a powerful tool in the classification of cultivars and also to study taxonomic status. It is this precious source of material that serves as the essential foundation for the breeding of new improved varieties. Such characteristics are often controlled by multiple genes and are subject to varying degrees of environmental modification and interaction. Qualitative traits, such as yield performance and quality characters are of major importance in breeding and consequently, these traits are usually focused on during the evaluation of accessions. However, these traits express high environmental effects and often also genotype with environment interaction. Tanksley et al. (1989) emphasised the fact that many of the morphological traits are also difficult to analyse because they do not have the simple genetic control assumed by many in genetic models. Magloire (2005) studied 20 cowpea accessions from Cameroon, South Africa, Kenya to assess agro-morphologic diversity among them. Based on 15 qualitative traits (growth pattern, twinning tendency, stem pigmentation,...) and 12 quantitative traits (plant height, pod length, 100 seed weight,...) which resulted in high genetic variability in almost these traits.

Muhammad et al. (2010) compared four local fodder cowpea genotypes namely CP-1, CP-8, CP-31 and CP-801 at Peshawar. CP-1 required minimum days to emergence (4.7 days), flowering (87 days) and pod formation (94.7 days) and the shortest plant length (235 cm). CP-8 produced the longest plants (382 cm) though statistically not different than CP-31 and CP-801. CP-8 gave the maximum grain yield (969l kg/ha), seeds/pod (11.3) and 100 seed weight (11.4 g). Generally, the fresh fodder yield in all germplasm gradually increased from 14 ton/ha, 50 days after emergence to 64.2 ton/ha, 125 days after emergence. They concluded that maximum fresh fodder can be obtained 125 days after emergence and CP-8 gave the maximum grains as well as fresh fodder yield among the germplasms tested.

Kumar et al. (2015) carried out the characterization of 20 forage cowpea genotypes. All genotypes showed indeterminate type of main stem and raceme type of layer in canopy; whereas genotypes *viz.*, KBC 2, IC 249141, HC 46, CO 4, EC 3941-1, EC 101980, Kohinoor and CO 5 showed unique state of expression among nine characters *viz.*, incidence of cowpea mosaic virus, seed crowding in pod, text a structure, eye colour, pod shape, pod attachment to peduncle, terminal leaflet shape, twining tendency and flower pigmentation, respectively. While two genotypes HC 46 and EC 3941-1 had wrinkled text a structure with pigmented wings and tan (brown) eye colour with curved pod shape, respectively. Remaining genotypes showed various states of expression in a group of two or more genotypes together. Animasaun et al. (2015) investigated ten cultivars of cowpea in the screen house in Nigeria. The results showed considerable variations in growth and yield characters evaluated. Cultivars NGB/06/047, IFE BROWN 2012 and IT98K-133-1-1 had optimal growth performance with respect to fruiting and seed yield parameters. Proximate results indicated that crude protein varied from 23.42-26.78%. Ash content ranged between 3.60-4.21%, crude fibre varied from 2.10-2.98% and carbohydrates 56.10-59.59%.

Nguyen et al. (2016) carried out an experiment on 30 genotypes of cowpea. Both within and between group variations was studied. The group low fodder-medium grain yield had maximum genotypes *i.e.* RC-101, GC-13-1, TC-142, TC-141, PGCP-11, KBC-5, GC-5, Pant lobia-3, while CP-05040, GPCP-28, PGCP-24 and GC-1105 having considerable value for grain yield. However, in low fodder-high grain yield group only one genotype *i.e.* PGCP-2-3 was placed. However, only three genotypes, *viz.* KBC-5, Goa Cowpea-3 and GC-901 were included in the high fodder-low grain yield production and could be utilized for cowpea fodder production after testing over time and space. The genotype TPTC-29 was included in medium fodder-medium grain yield and one genotype (GC-1106) was included in medium fodder-high grain yield, which had excellent grain production as well as good potential for fodder production. It could be utilized for dual purpose after extensive testing over time and space.

Vu et al. (2017) recorded the maximum seed yield per plant by genotype PL-3 (49.63g), followed by CPD 240 (46.66), RC 101 (42.78), KBC 10 (36.11) and PGCP 28 (35.56) which will be most promising to exploit and utilize for incorporation of yield potential. Some other genotypes having high mean value for other characters can be used as a donor. The highest biological yield per plant was observed in genotype UCP 12-007 (443.89g), followed by KBC-6 (426.67), KBC-8 (392.89), GC 901 (377.22) and VCP 09-019 (332.78). Being utility of cowpea as fodder crop high biological yield is needed. With respect to seed protein content, the mean seed protein content was exhibited highest (26.69%) in the genotype TPTC-29. Besides, the genotypes PCP-07-272, Goa Cowpea-3, UCP 12-007 and VCP 09-019 also indicated high seed protein content (26.40, 25.81, 25.38 and 25.08%), respectively. More or less similar findings were also supported by (Lazaridi et al. 2017).

Genetic variability, heritability and genetic advance

The presence of considerable amount of variability in the basic genetic material ensures better chances of evolving desired plant types. The estimates of heritability, PCV, GCV and genetic advance are helpful to determine the method of selection to improve a particular plant population for a specific trait. The magnitude and type of genetic variability help the breeder to determine the selection criteria and breeding schemes to be used for improvement purposes (Omoigui et al. 2006). Ariyo (1995) evaluated twenty-six varieties of cowpea to study genetic variability, inter-character relationships as well as the path analysis of components of seed yield. Seed yield was the most variable character with GCV and PCV of 22.11 and 35.25%, respectively. Weight of 100 seeds had the highest heritability estimate of 92.33% while number of peduncles per plant exhibited the lowest heritability estimate of 30.11%.

Manonmani et al. (2000) studied ten genetically diverse genotypes of cowpea recorded highest GCV (29.12), PCV (31.13) and GA (56.15) values with 87.5% h^2 for FY, whereas the highest h^2 (96.9%) was observes in dry fodder. Vidya et al. (2002) evaluated 50 varieties of yard-long bean were for yield and a few related characters. High genotypic coefficient of variation, heritability in broad sense and genetic advance estimated for the characters *viz*., yield of vegetable pods per plant, number of pods per plant and pod weight indicated the scope for improvement of these characters through selection. Chauhan et al. (2003) studied the genetic parameters in 18 forage cowpea genotypes. Genotypic and phenotypic coefficients of variation, broad sense heritability and genetic advance of yield components, *i.e.* days to maturity, plant height, pods per plant, pod length, seeds per pod, 100-seed weight, plant stand, seed yield per plant and seed yield per plot. The additive gene effects were significant for plant height, pods per plant, plant stand and 100-seed weight.

Lesly (2005) carried out evaluation 169 genotypes which showed the high PCV and GCV values were recorded by seed yield per plant, hundred seed weight, harvest index, number of pods per plant and germination percentage. Both GCV and PCV values showed similar pattern of changing over the characters. All the characters showed high heritability except seeds per pod, pod length and number of branches per plant. The highest heritability was recorded by hundred seed weight. High genetic advance was observed for germination percentage, plant height, number of cluster per plant, number of pods per plant, hundred seed weight, harvest index and seed yield per plant. Malarvizhi et al. (2005) studied 60 genotypes for genetic variability, heritability and genetic advance of fodder cowpea. All the 60 genotypes varied significantly for all the 13 characters. They reported high heritability and genetic advance for number of branches per plant, number of leaves per plant, dry weight of leaves, dry weight of stem, dry matter yield and plant height, indicating that these traits were controlled by additive genetic effects, providing better source population for developing high-yielding fodder cowpea cultivars.

Omoigui et al. (2006) carried out a screen house experiment at Samaru, Nigeria in 1999 and 2000 dry seasons to estimate the genotypic variability of some reproductive traits and their heritability in nine cowpea varieties. Results of the study showed that there was considerable variation among cultivars for duration of reproductive phase and rate of photosynthate partitioning. Genotypic coefficients of variation were also high for days to first flower, 100-seed weight, plant height and harvest index. Broad-sense heritability estimate (h^2) was 98.9% for 100-seed weight, 94% for duration of reproductive phase, 84.5% for days to first flower, 83.9% for days to maturity and 77.3% for harvest index. This information showed that there is sufficient genetic variance to warrant selection for improvement in the cowpea genotypes studied.

In a study, 14 promising vegetable purpose cowpea genotypes were evaluated for fodder yield, green pod yield and other component traits. Results showed that there was considerable variation among cultivars for all the traits. Genotypic and phenotypic coefficients of variation were high for green pod yield and green forage yield. High heritability coupled with high genetic advance as per cent of mean was noticed for characters plant height, number of branches, number of pods per plant, green pod yield and green forage yield. They concluded that there was sufficient genetic variance to warrant selection for improvement in these cowpea genotypes and that considerable progress in forage cowpea breeding could be achieved by exploiting these traits (Lohithaswa et al. 2009). Idahosa et al. (2010) studied genotypic variability of eight parent line cowpeas to evaluate some genetic parameters namely coefficient of variation, genetic variance and heritability estimates in the broad-sense. Per se mean performance was variable among the genotypes for all characters investigated which indicated the superiority of some parent lines. Highly significant heritability effects were observed for all characters except for 100-seed weight (42.2%) which expressed moderate heritability estimate. Days to 50% flowering, pod length, pod weight and grain yield characters showed that some levels of genetic variability existed.

Two genetically diverse parents belonging to determinate and indeterminate were crossed (V-1188 x Goa local) and advanced to F_2 and F_3 generations. These were evaluation for estimation genetic variability in various characters *viz*. plant height, branches per plant, canopy spread, clusters/plant, pods/plant, seed per pod, pod length and 100 seed weight, seed yield. There were differences in GCV and PCV values in both F_2 and F_3 population and GCV, PCV were moderate to high in F_2 comparing low to moderate in F_3. Similar trend was also observed with heritability and genetic advance as per cent mean that have declined in F_3. Plant height, pods/plant, seed yield could be considered for selection as they had higher PCV and GCV values coupled with appreciable heritability and genetic advance as per cent mean (Shashidhar et al. 2010).

Crosses were made between five cultivated cowpea varieties and a wild relative var. *pubescens* (as pollen parent) to study the inheritance of hairiness, pod shattering, as well as heritability and correlations among yield and yield related traits. F_1 plants exhibited dominance for both hairiness and pod shattering traits. Broadsense heritability estimates showed that days to pod maturity averaged 77.93%, 100 seed weight 68.45%, seeds per pod 69.76% and number of branches per plant had 62.54% (all high). Days to first flowering and pod length were moderate (57.31 and 54.29%, respectively). Number of pods per plant had low heritability estimates with an average of 39.0%. High broad sense heritability averaged 77.93% for days to pod maturity, 68.45% for 100 seed weight, 69.76% for seeds per pod and 62.54% for number of branches per plant. Days to flowering and pod length per plant had moderate estimates of 57.31 and 54.29%, respectively (Mohammed et al. 2010).

Singh et al. (2010) observed significant mean square for all the traits indicating adequate variability among the genotypes. High phenotypic and genotypic coefficient of variation coupled with high heritability and genetic advance as per cent of mean were reported for plant height, stem weight, leaves weight, biological yield, dry matter yield and green fodder productivity indicating predominance of additive gene effects in controlling these characters. Green fodder yield was significantly and positively correlated with green fodder productivity, dry matter yield, biological yield, leaves weight and stem weight. Path coefficient analysis revealed that the stem weight had maximum and positive direct effect on green fodder yield at phenotypic and genotypic level followed by leaves weight and dry matter yield. Biological yield and dry matter per cent had negative direct effect on green fodder yield.

In a study of 10 cowpea accessions, (Manggoel et al. 2012) revealed PCV and GCV were high for the trait's days to 50% flowering, number of flowers/plant, number of peduncles/plant, number of pods/plant, 100-seed weight and grain yield (26.54-41.46), except pod length and seeds per pod. High broad-sense heritability was high (63.16 - 96.74%) for all these characters. Nwofia et al. (2012) investigated twelve genotypes of cowpea in two seasons 2010 and 2011. It was found that plant height, number of branches, leaves, pods and dry matter per plant, number of seeds/pod and seed weight/pod, pod length gave moderate to high at both GCV and PCV. Heritability and genetic variance of all these characters were also high 54.0-94.95 and 12.89-99.01, respectively.

Costa et al. (2013) studied 57 African cowpea lines from the Cowpea Germplasm Bank of Embrapa Meio-Norte, Brazil to estimate the genetic divergence between cowpea lines. Genotypic coefficient of variation was highest for the traits yield (19.77%) and 100 seed weight (16.33%) and lowest in grain length (8.95) and grain width (7.48). The heritability estimates ranged between 37.38 and 95.92% for NPP and W100G, respectively. The heritability values of the traits PL, NGP, GRL and W100G were all higher than 70%, indicating the possibility of genetic progress with selection.

Ten genotypes of cowpea were studied for the interrelationship among quantitative traits. Estimates of phenotypic and genotypic coefficients of variation, broad sense heritability and genetic advance as percent of mean and correlations were performed on 20 quantitative traits. Genotypes differed significantly for all traits studied which showed the existence of sufficient genetic variability among the tested genotypes. High broad sense heritability values for all traits studied except for plant height (moderate) shows that these traits are less influenced by environmental effects. The high positive genotypic and phenotypic correlations between numbers of pods per plant, number of seeds per pod, number of seeds per plant and seed weight indicates that selection for these will result in increase in yield (Ajayi et al. 2014).

In a study, 17 genotypes of vegetable cowpea (*V. unguiculata* cv-gr. *sesquipedalis*) collected from different places in the country were evaluated for different horticultural traits. Genetic variation, character association, cause-effect analysis and genetic diversity was measured among the genotypes. Chattopadhyay et al. (2014) found out all the eight characters under study differed significantly among the genotypes. High to moderate GCV and PCV values were found for number of pods per plant, pod yield per plant, pod weight, number of seeds per pod and pod length. The PCV agreed closely with GCV for all the characters except pod width and pod yield per plant (the GCV ranged from 10.29% to 32.35%, while PCV ranged from 10.40% to 32.93%). Higher estimates of broad sense heritability coupled with higher genetic advance for number of pods per plant, pod yield per plant, pod weight, number of seeds per pod and pod length.

Santos et al. (2014) studied 20 genotypes which also revealed coefficients of heritability based on the mean of families showed magnitudes from moderate to high for the character of days to flowering, green pod length, number of beans per pod, number of pods per plant and hundred seed weight, confirming that most of the phenotype is attributed to genetic causes. The low value of heritability and the high value of the phenotypic coefficient of variation observed for grain yield. Gerrano et al. (2015) estimated the level of phenotypic variability among a collection of 25 cowpea genotypes in South Africa. Sixteen phenotypic markers were recorded. the differences among genotypes were highly significant for all traits. This indicated the high level of genetic variability among the cowpea genotypes studied. Genetic and phenotypic coefficient of variation and broad-sense heritability were estimated for all phenotypic traits.

Khanpara et al. (2015) observed significant differences among the genotypes for all the 12 characters studied. The highest range of variation was observed for green pod yield per plant followed by ten pod weight, number of pods per plant and plant height. Higher values of PCV than GCV were observed for green pod yield per plant, number of pods per plant, pod length and number of seeds per pod which indicates partly interaction of the genotypes with the environment or other environmental factors influencing the expression of these characters. The value of PCV and GCV more or less equal were observed in the remaining characters which indicated that these characters were less influenced by the environment. High heritability along with high genetic advance was observed for green pod yield per plant, plant height, pod length, pod width, number of seeds per pod, number of pods per plant, ten pod weight, number of pods per cluster and hundred fresh seed weight indicating that these traits were mainly governed by additive gene action and responsive for further improvement of these traits.

The investigation was carried out on 15 genotypes to study genetic parameters for ten quantitative characters of bush type cowpea. The differences among all the genotypes were statistically significant for all the ten quantative characters. High estimates of genotypic coefficient variation, heritability (broad sense) and genetic advance were observed for the characters such as plant height, number of pods per plant, edible pod yield per plant as well as edible pod yield per hectare, suggesting additive gene action for expression of these characters (Tudu et al. 2015). Genetic variance components indicate high genetic contributions over non-genetic to plant phenotypic variability with high heritability values (0.75-0.91) by investigation of Aliyu and Makinde (2016).

Chandrakar et al. (2016) studied 21 genotypes of vegetable cowpea. They found highly significant differences among the genotypes for all the characters except pod width. It indicates that sufficient variability existed among the genotype. The GCV value was higher for plant height, pod yield per plant indicated that these characters exhibited variability among the genotypes. The highest heritability was recorded for the characters plant height (60 DAS), days of maturity, fruiting duration, 50 per cent flowering,pod setting per cent per plant, 100 dry seed weight, 100-green seed weight. Khandait et al. (2016) reported the highest PCV and GCV for characters *viz*., number of flower cluster per plant, number of pods per plant, number of pods cluster, number of branches at 30 DAS, pod weight and pod length. Heritability estimates were observed very high for pod length, number of pods per plant, pod weight, number of flower cluster per plant and pod width. The highest estimate of genetic advance as percentage of mean was recorded for number of flower cluster plant followed by number of pods per plant, pod length, number of pods per cluster, pod weight, pod width, number of flowers per cluster, number of branches at 30 DAS, pod yield plot, pod yield per ha and pod yield per plant.

Sixty-six bush type advance breeding lines of vegetable cowpea developed at Varanasi were evaluated for different horticultural traits for genetic variability, character association, cause effect analysis and genetic diversity among the genotypes through D^2 statistics. High values of genotypic and phenotypic coefficient of variations, heritability (h^2B) and genetic advance were recorded for pod yield per plant, number of peduncles and pods per plant, peduncle length, number of primary branches per plant, pod length, pod weight and number of seeds per pod (Lal et al. 2017). Magashi et al. (2017) studied genetic variability for growth and yield in cowpea varieties. They found highly significant difference in the plant height, number of days to 50% flowering, number of days to maturity, number of pods per plant, pod length, number of seeds per plant, 100 seed weight ash and protein content. While significant difference was found seedling height, number of branches per plant and fibre content. No significant difference was found in the remaining.

Genetic variability, heritability, genetic advance and genetic advance as per cent of mean for twelve characters were assessed by field evaluation of 22 genotypes at Vellanikkara. The high degree of variability was observed for all the characters. High magnitude of the PCV, GCV, heritability and genetic advance was observed for plant height, grain yield per plant and length of the pods. Seeds per pod and protein content exhibited low PCV and GCV, but high irritability and low genetic gain. The difference between the phenotype coefficient of variation and genotype coefficient of variation were found maximum in pod weight (30.15%), followed by number of pods per plant (18.12%) and test weight (16.27%), (Sarath and Reshma, 2017).

The magnitude of phenotypic coefficient of variance was slightly higher than genotypic coefficient of variance for all the characters, which revealed the less sensibility of characters to environmental factors under field conditions. The high estimates of GCV and PCV was observed for biological yield per plant, followed by plant height, harvest index, number of clusters per plant, pod yield per plant, seed yield per plant, number of pods per plant, number of branches per plant, pod weight and days to 50% flowering (Ngoc et al. 2019).

The estimates of heritability (broad sense) were observed high in biological yield per plant, followed by plant height, number of clusters per plant, days to 50% flowering, days to maturity, pod weight, harvest index, seed yield per plant etc. Further, genetic advance as per cent of mean observed high for characters like biological yield per plant, followed by plant height, harvest index, number of clusters per plan, seed yield per plant, pod yield per plant etc., while the estimates of genetic advance as per cent of mean was moderate for pod breadth and number of pods per cluster indicating that the improvement of these through selection as well as their exploitation through combination breeding. The estimates of high heritability coupled with high genetic advance, suggesting that simple selection could be done for the improvement of these traits (Ngoc et al. 2019).

High heritability values in yield contributing the characters are useful while making selection, it indicated that the variation in these characters was mainly under genetic control and was less influenced by environment but selection based on this factor alone may limit the progress, as the same very prone to environmental changes (Maurya et al. 2014). It is apparent that the improvement of various characters, individually or simultaneously, different selection intensities are to be exercised depending on estimates of genetic components of variability in view of their heritability. High genetic variations combined with high heritability could provide effective selection of phenotypic trait for further improvement in cowpea through hybridization. The results in genetic analysis of triple test cross by Mittal et al. (2010) also indicated the additive genetic variance was presented for most of characters and suggested simple selection to get improvement in cowpea. The evaluation of cowpea genotypes may be reliable based on study of heritability; however, still more solid base may be formed by estimating the performance through genetic advance. Johnson et al. (1955) stated that heritability estimated coupled with genetic advance were more helpful than heritability alone in predicting the progress from the selected better genotypes. However, there are limitations of using broad sense heritability as it includes both additive and non-additive gene effects. Therefore, it is necessary to estimate broad sense heritability in conjunction with the genetic advance.

Correlation and path analysis

The success of cowpea as a pulse crop largely depends on the extent of improvement made for seed yield. Selection is based on the performance of grain yield a polygenically controlled complex trait is usually not very efficient. But the one based on its component character could be more efficient. The study of association of seed yield with other component characters helps the breeder to select for the characters and to construct a suitable plant type leading to higher seed yield. The importance of correlation studies in selection programmes is appreciable when highly heritable characters are associated with the important character like yield. Path coefficient is an excellent means of studying direct and indirect effects of interrelated components of a complex trait particularly if the high correlation between two traits is a consequence of the indirect effect of other traits. Path-coefficient analysis measures the direct influence of one variable on another. By determining the inter-relationships among grain yield components, a better understanding of both the direct and indirect effects of the specific components can be attained.

The experiment by Ariyo (1995) showed that number of peduncles per plant, number of branches per plant showed significant and positive genotypic and phenotypic correlation with seed yield. The number of pod per peduncle only showed positive genotypic correlation with seed yield. Path coefficient analysis revealed that seed yield was affected by weight of 100 seeds, number of seeds per pod, number of branches per plant, number of peduncles per plant and number of pods per peduncle. Kumar et al. (2001) studied 72 diverse genotypes of cowpea to find out correlations and path analysis for grain yield and its related eight traits. Genotypic correlations were higher than their corresponding phenotypic correlations. The grain yield per plant was positively correlated with all the traits except days to maturity and seeds per pod. 100-seed weight had the maximum direct and indirect effect on grain yield of cowpea. Most of the component traits had positive association among themselves and with grain yield of cowpea.

A total of 600 cowpea germplasm lines were evaluated in augmented randomized block design. A wide spectrum of variability was observed for all the characteristics except for days to maturity and number of seeds per pod which exhibited moderate variability. Seed yield showed positive correlation with 100-seed weight and pod length. While positive association of 100-seed weight with pod length and plant height revealed that taller plants, in general, had longer pods with bold seeds resulting in higher seed yield (Singh and Verma 2002).

In a study, eight cowpea genotypes and cultivars from different locations in Turkey were evaluated association analysis. Pod length and 100 seed weight showed a positive and highly significant correlation with seed yield per plant. Path analysis results revealed that pod length had the highest direct positive effect on seed yield per plant, followed by 100 seed weight and pods number per plant. On the other hand, seed yield per plant was directly and negative affected by first pod height and branches number per plant (Peksen and Artik 2004).

Lesly's evaluation (2005) resulted in number of clusters per plant, number of pods per plant, harvest index and hundred seed weight were significantly correlated with seed yield both at genotypic and phenotypic levels. Number of clusters per plant and pods per plant significantly associated with harvest index both at genotypic and phenotypic levels. The highest direct effect recorded by harvest index and higher indirect effect of number of clusters per plant and number of pods per plant through harvest index was exhibited at genotypic and phenotypic level. Kumari et al. (2005) evaluated 50 genotypes of cowpea grown during *kharif* 2001 at Hisar for tannin content, crude protein and in vitro protein digestibility. Crude protein ranged from 27.57-21.15%. The in vitro protein digestibility was recorded a range 61.40-45.00%. Substantial amount of variability for tannin content was present in the existing material, while it was meagre for protein and IVPD. Tannin and IVPD had negative direct effect and crude protein had positive direct effect on seed yield.

Mittal et al. (2006) observed that forage yield in cowpea can be improved by selecting plants with greater stem diameter and length in the segregating populations of the cross at PAU, Ludhiana. Bhandri and Verma (2007) revealed that plant height, number of leaves per plant, leaf: stem ratio, crude protein and dry matter digestibility had major contribution in determining quantity and quality of green and dry forage yield. Ajeigbe et al. (2008) reported that variations in protein content among cowpea varieties. High positive correlations (0.86) were observed between the content of fat and crude fibre, ash and protein (0.78), carbohydrate and viscosity of cowpea flour (0.76) and between ash and tannin (0.61) content of cowpea seed, negative correlations were observed between the content of crude protein and carbohydrate (-0.98) in cowpea seed. The physicochemical properties evaluated generally had high broad sense heritability (56-99%).

In study of Mohammed et al. (2010), correlations among number of pods per plant, seeds per pod, seeds per plant and seed yield per plant (0.485–0.568) were significantly positive. Correlations between seed per pod and seed per plant with number of pods per plant were 0.909 and 0.996, respectively. Umar et al. (2010) studied the morphotypic variations of eight local varieties of cowpea in relation to their yield in 2004 rainy season. The magnitude of the genotypic correlation coefficients was in most cases higher than their corresponding phenotypic (rp) and environmental (re) correlation coefficients. High and positive rg exists between days to 50% flowering and plant height (rg=0.9113), days to maturity and fodder weight (rg=0.9301), days to maturity and 100 seed weight (rg =0.6958) and number of leaves per plant and fodder weight (rg=0.8096). On the other hand, high but negative rg exists between plant height and pod per plant (rg=-0.6011). Also, the relationship between days to maturity and number of seed per pod were all negative and moderate.

The experiment by Ushakumari et al. (2010) using fourteen genotypes of cowpea in India to study the nature of genotypic association between the eight yield contributing characters and direct and indirect effects of the different characters on yield. The study revealed that the days to maturity, number of branches per plant and number of pods per plant showed positive significant correlation with seed yield. Path coefficient analysis exhibited days to fifty per cent flowering, plant height, pods per plant showed positive direct effect on yield except days to maturity, branches per plant, seeds per pod and hundred seed weight which showed negative direct effects. The characters plant stands at harvest and pods per plant exhibited moderate direct effects on yield. The implications of the results revealed that selection can be done for days to maturity, number of branches per plant and number of pods per plant for improving the grain yield in cowpea.

Carvalho et al. (2012) carried out evaluation on eight parent cowpea lines/cultivars and twenty eight combinations (F_2) derived from these parents to find out the association between quantitative traits and yield. Results have shown that genotypes with high pod weight and pod length, 100-grain weight and number of beans per pod should be used to improve grain yield in cowpea. In the study of Manggoel et al. (2012) found out positive correlation between grain yield and number of peduncles per plant, flowers per plant, pods per plant and 100 seed weight. Path analysis showed high positive direct effects of number of peduncles plant, flowers per plant and 100 seed weight. Seed yield was found positively and significantly correlated to number of leaves per plant, seed weight per pod, dry matter yield per plant in 2010 and number of pods per plant in 2011. The consistent positive direct effect of number of leaves on yield in both years and high magnitude direct effect of seed weight in 2010 and number of pods per plant in 2011 suggests that the traits have high direct positive influence on seed yield (Nwofia et al. 2012).

Results trial on 4 local varieties by Udensi et al. (2012) revealed that significant relationships between yield and yield-contributing traits existed which could be indices for selection. Genotypic correlations coefficients were high and more significant than the phenotypic and environmental correlation coefficients. Path coefficient analysis shows that number of pod per plant had the highest direct effects to cowpea yield. This was followed by number of flowers, number of seeds per pod, leaf area at 5 weeks and pods length and 100 seed weight, respectively. Other morphological traits had negative direct effects on seed yield such as vain length at 10 weeks, number of leaves at 5 weeks, number of leaves at 10 weeks, leaf area at 10 weeks, days to 50% flowering and days to maturity.

Sahai et al. (2013) evaluated 168 exotic and indigenous cowpea germplasm lines and four checks/controls in augmented design to study estimates of the correlation coefficients and path analysis of morphological as well as fodder and grain yield attributes. The present study showed a high impact of direct effects of correlation and suggested that going for plant types with higher biomass per plant, dry weight per plant, stem girth, number of secondary branches, leaves per plant, pods per plant and pod clusters per plant would be effective for improving both fodder and seed yield in cowpea. Trial outcome of Ajayi et al. (2014) has given the interrelationship among quantitative traits. The high positive genotypic and phenotypic correlations between numbers of pods per plant, number of seeds per pod, number of seeds per plant and seed weight indicates that selection for these will result in increase in yield.

Association studies revealed that genotypic correlation coefficients were higher than their phenotypic correlation coefficients in most of the cases. Positive and significant phenotypic correlations were observed for number of pods per plant ($r = 0.577$) and number of seeds per pod ($r = 0.575$) with pod yield per plant. On the other hand, significantly negative correlations were exhibited for days to 1st flowering ($r = -0.695$) and days to 50% flowering ($r = -0.660$) with pod yield per plant imply that a lot of breeding programmes are needed to improve such traits. The number of pods per plant followed by pod weight showed highly positive direct effects on pod yield per plant. From the correlation and path analysis, it can be concluded that emphasis should be given on number of pods per plant and pod weight for selecting high yielding genotypes (Chattopadhyay et al. 2014).

A field trial was conducted in 2011 rainy season to evaluate the performance of five cowpea genotypes and a local cultivar at Mubi in the Northern Guinea Savanna ecological zone of Nigeria. The path coefficient analysis of grain yield and yield attributes showed that number of pods per plant gave the highest percentage yield contribution of 31.85%. This was followed by plant height at 6 WAS which contributed 5.37%. The highest combined contribution of 8.66% came from pod number and 100-grain weight. Residual percentage contribution was 49.24%. This showed that yield attributes in this study explained 50.76% of the variability in grain yield in the experimental material Furthermore, the investigation suggests that number of pods per plant, plant height and 100 seeds weight can be considered as selection criteria in cowpea (Kwaga 2014).

Investigation's result of Santos et al. (2014) also found that the greatest positive correlations between the character pairs; days to flowering x days to maturity; days to maturity x pod weight; days to maturity x number of beans per pod; pod weight x number of beans per pod; green pod length x pod weight; and number of pods per plant x grain yield. Only the variables of days to maturity and number of beans per pod correlated in a negative and significant way with grain yield. Considering the direct positive effects on grain yield, green pod length was the variable that showed the greatest effect (1.8128). Nevertheless, total correlation of green pod length exhibited a low value (0.0847) due to the indirect negative effect of the other variables. Total positive correlation of 0.7982 was observed on the variable of number of pods per plant and grain yield, but its direct effect was negative (-0.7521), while for this variable, the indirect effect of the other variables, mainly of the number of pods per plant (1.8946), is important since it indicates the direction of correlation. The days to maturity presented direct positive effect; nevertheless, total correlation was negative (-0.3952). The days to flowering obtained high direct effect on grain yield, while the indirect effects of the other variables ratified the total correlation, showing low association with grain yield (0.1959). The variable of number of beans per pod obtained direct negative effect (-3.5249) with the variable of grain yield. The result also gave a low residual effect (0.2081).

In another study, Sapara and Javia (2014) estimated genotypic and phenotypic correlations of green pod yield with different components from 40 genotypes of vegetable cowpea. The genotypic and phenotypic correlations agreed closely with each other. Yield contributing character number of pods per plant had positive and highly significant association with green pod yield per plant at phenotypic level. Phenotypic interrelationship between days to 50% flowering and days to 1st pod picking was negatively significant with green pod yield. The genotypic and phenotypic path analysis revealed the high to moderate direct effect of green pod yield per plant with number of pods per plant and pod length. Therefore, number of pods per plant and pod length was important component for improving green pod yield in vegetable cowpea.

Seed yield of 49 accessions was studied by Shanko et al. (2014) exhibited positive and significant correlation with number of primary branches per plant, number of secondary branches per plant, number of pods per plant, number of seeds per pod and plant height. Path analysis revealed that, yield per plant had the maximum positive direct effect on seed yield followed by number of pods per plant, while number of secondary branches per plant, days to flowering, days to maturity and number of seed per pod exhibited negative direct effect phenotypically. In addition, genotypic path analysis revealed that, maximum direct effect on seed yield was exerted by number of pods per plant and yield per plant. However, days to 50% flowering, days to maturity, number of secondary branches per plant and number of seed per pod exerted negative direct effect on seed yield.

Meena et al. (2015) evaluated 72 cowpea germplasms which resulted in seed yield per plant had positive significant correlation with days to 50% flowering, plant height, primary branches per plant, pods per plant, pod length, seeds per pod and 100-seed weight at both genotypic and phenotypic levels. Path coefficient analysis revealed that primary branches per plant and 100-seed weight had high direct positive effect on seed yield per plant at both genotypic and phenotypic levels. Pod length, days to maturity, 100 seed weight and pod wall proportion had high positive direct effects on seed yield per plant at only genotypic level. From the correlation studies on 15 genotypes, Tudu et al. (2015) found that there was a good deal of association among yield and its various attributes. Number of branches per plant, edible pod yield per plant and number of seeds per plant are important correlated characters contributing towards pod yield in bush type cow pea.

Relationships between flowering; pod maturity and seed size were positive and significant. By contrast, pods per plant, seeds per plant and total seed yield recorded negative correlations with pod maturity. However, seeds per plant and pods per plant are the most contributory components to seed-yield with correlation coefficients of $r=0.95$, 0.89, respectively. Although seed size had negative correlation with seeds per pod, but strong linkage between seed number (seeds per pod, seeds per plant) and seed yield (Aliyu, and Makinde 2016). The investigation of Patel et al. (2016) was carried out on 32 diverse genotypes of cowpea to study the correlations and path coefficient for different traits. Association analysis between green pod yield per plant and other eleven quantitative characters revealed that green pod yield per plant was highly significant and positively correlated with pod length and sugar content. Path coefficient analysis indicate the highest positive direct effect on green pod yield per plant by pod length followed by days to 50 per cent flowering, shelling %, number of pods per plant, sugar content and plant height at final harvest. Lal et al. (2017) investigated sixty-six bush type advance breeding lines of vegetable cowpea to find the association among characters. Results revealed that pod yield per plant showed strong positive correlation with number of peduncles and pods per plant, pod weight, pod length, number of seeds per pod and number of primary branches per plant, while negative correlation with days to 50% flowering at genotypic and phenotypic levels. The maximum direct positive effect on pod yield per plant was found contributed by number of pods per plant followed by pod weight.

No significant correlations were observed among traits related to seed yield and nutrient and protein content in an investigation on one commercial variety and twenty-three cowpea local populations collected from Greece (Lazaridi et al. 2017). However, plant height was negatively correlated with days to first flower, flowering duration, days to first mature pod, Ca and Mg concentrations, while was positively correlated with height to first pod, pod length, number of seeds per plant and seed weight per plant. Days to first flower were positively correlated with flowering duration, days to first mature pod and Ca seed concentration with a negative correlation to height to first pod and pod length. The number of pods per plant was significantly correlated with number of seeds per plant and seed weight per plant. Number of seeds per plant was correlated with seed weight per plant and hundred seed weight. While, seed crude protein content was positively correlated with number of branches and days to first mature pod. Magashi et al. (2017) indicated a positive relationship between the number of days to 50% flowering and number of days to maturity, similar relationship exist between number of days to maturity and number of pod per plant and also between number of days to 50% flowering and number of pod per plant, pod length and number of days to 50% flowering, seed per pod and number of days to 50% flowering, seed per pod and number of days to maturity, pod per plant and pod length a positive relationship also exist. Similarly, in the result indicated that positive relationship exist between 100 seed weight and number of days to flowering, days to maturity, pod per plant, pod length and seed per pod. A positive relationship was also found between leaf area and seedling height, pod per plant and 100 seed weight. However negative relationship was between plant and other parameters.

An investigation of Srinivas et al. (2017) on 30 genotypes of cowpea were carried out during summer season in the year 2014-2015. The result on phenotypic and genotypic correlation coefficient revealed that pod yield per plot was significantly and positively correlated with number of branches per plant (0.7659), number of nodes (0.5523), pod length (0.3960), number of seeds per pod (0.2815), number of cluster per plant (0.550), number of pods per plant (0.547), number of pods per cluster (0.524), plant height (0.437) and protein content (0.2871). However, days for 50% flowering (-0.2081) showed significantly and negatively correlated with pod yield per plot. Other characters *viz*., days taken for first flowering (0.1946), pod diameter (-0.1035) showed negative non significantly correlated with pod yield per plot. Path coefficient analysis of different yield and yield contributing traits on number of branches per plant, number of nodes per plant, number of clusters per plant, number green pods per plant, number of pods per plant, number of seeds per pod, pod weight (g), pod yield per plot and percentage of protein content exhibited positive direct effects on pod yield per plot these characters play a major role in recombination breeding and suggested that direct selection based on these traits will be rewarded for crop improvement of cowpea.

In a study, Ngoc et al. (2019) reported the seed yield per plant highest and positive significant correlation with number of pods per plant, pod yield per plant, number of clusters per plant, number of branches per plant, number of pods per cluster, pod weight, number of seeds per pod and harvest index. If the selection is made any of the component traits, simultaneous selection of the all the traits could be achieved. The estimates of correlation coefficient revealed that the biological yield per plant was significantly and positively associated with plant height, days to 50% flowering, days to maturity, pod length, pod weight and number of seeds per pod. Among all the traits under study, harvest index, biological yield per plant, pod yield per plant, number of seeds per pod and number of pods per plant reflected high direct and positive effect of on seed yield per plant. This suggested that direct selection based on these traits would result in higher breeding efficiency for improving seed yield per plant. Thus, these traits might be estimated as the most important component traits for seed yield per plant. Number of seeds per pod had shown negative indirect effect through pod length but positive indirect effect via biological yield per plant. Plant height and biological yield per plant had high negative indirect effects *via* harvest index on seed yield per plant. Similarly, harvest index revealed this type effect on seed yield per plant *via* biological yield per plant.

Ngoc et al. (2019) had led to improve the understanding many interrelated processes involved the genetic control of variation and seed yield thus would provide some guidelines in selection, in the prediction of possible advantages of genetic recombination and for selection in segregating generations.

Genetic divergence using D^2 analysis

Borah and Fazlullah Khan (2001) studied sixty genotypes of fodder cowpea (*Vigna unguiculata* (L.) Walp) for the genetic diversity. They were grouped into 10 clusters indicating high genetic divergence among cowpea genotypes. The clustering pattern showed that geographic diversity is not an index of genetic diversity. Intra cluster distance exhibited a range of 13.59 to 16.01. The maximum inter-cluster distance (D) was noticed between cluster I and X (41.60). It was least between cluster V and VI (17.52). Based on the intercluster distance and cluster mean for various characters, it could be seen that the cluster I, II, IX and X were the most divergent from the other clusters. The genotypes from these clusters may possibly be utilized for hybridization programme. Dry matter yield, green fodder yield and plant height contributed highly towards the genetic divergence among the types studied.

The D^2 analysis of 155 cowpea lines revealed that genotypes exhibited considerable diversity and were grouped in 10 clusters. Cluster I exhibited maximum number of germplasm lines. Inter-cluster distance was maximum between clusters IV and X followed by clusters IX and X. Cluster IX recorded highest mean seed yield per plant, 100-seed weight and also the intra-cluster distance. The genotypes belonging to these clusters *viz*., GC 3 and DCP 10 may be useful in multiple breeding programme to recover transgressive segregants with the desirable combinations of yield components (Saini et al. 2004). Cowpea 127 germplasm lines were grouped into 10 clusters on the basis of D^2 analysis. Cluster I exhibited maximum number of genotypes. Inter-cluster distance was maximum between clusters VIII and X followed by clusters IV and VIII. Intra-cluster distance was maximum in cluster VIII. The clustering was useful to identify the diverse genotypes. Cluster IX registered the highest mean value for plant height (88.74 cm), seed yield per plant (35.69 g) and 100-seed weight (14.93 g) (Jain et al. 2006).

Girish et al. (2006) evaluated 100 cowpea genotypes to quantify the genetic diversity existed among them. The genotypes fell into 11 clusters. Among the 11 quantitative characters studied, fodder yield contributed highest (75.73%) towards the divergence followed by plant height (8.28%) and seed yield (6.3%). Cluster VI had minimum days to first flower opening and days to maturity and also had maximum number of pods per plant, pod length, number of seeds per pod and seed yield. Cluster IX exhibited lowest means for seed yield, fodder yield, pod length, number of seeds per pod, primary branches and plant height. The genotypes from clusters VI and IX, which have high and low cluster means for majority of the characters are suggested as promising parents for hybridization. Bhandri and Verma (2007) carried out divergence analysis on 22 genotypes and grouped into seven clusters. The maximum inter-cluster distance found between cluster V and VII. Crude protein, dry matter digestibility, dry matter yield and number of leaves per plant were major contributors towards genetic divergence.

Dalsaniya et al. (2009) studied diversity among 60 genotypes of cowpea which were grouped in to 12 clusters revealing the presence of considerable diversity in the material. The clustering pattern of the varieties usually did not confirm to geographical distribution. The intra cluster distances of 12 groups were ranged from 0.0 to 15.0. The maximum inter cluster distance (D) was noticed between cluster IX and X (D = 55.42) followed by that between cluster X and XII (D = 51.83), while the closest proximity was observed between cluster VII and XI (D = 9.99) followed by the cluster VIII and XII (D = 10.86). It was also noted that genotypes of cluster-X which had higher cluster mean values for yield and other desired characters like leaf area, ten pods weight, number of pods per plant and green pod yield per plant etc. could be directly tested in multilocation trials for their suitability or could be used as a donor parent in breeding programme. The characters like plant height, green pod yield per plant, protein content and leaf area were found to contribute much to the total genetic divergence in cowpea.

The study was done on cowpea genotypes with upright growth and early maturity using multivariate techniques to estimate the relative contribution of the response variables to genetic divergence studied. Twenty-eight lines of the Genbank of the Universidade Federal do Ceará and Embrapa Meio Norte were used. For the analysis of genetic divergence, the canonical variables, Mahalanobis' distance and the Tocher cluster method were used. Most of the maximum distances were observed when combined with CE-46 genotypes. The 28 genotypes were separated into 11 groups. There was a wide distribution of genotypes in different groups, indicating a wide diversity among the genotypes. The greatest distances were observed between the groups VIII and XI (343.2) and VII and VIII (323.2). The lowest D^2 distance values were observed in the intercross between the groups VII and XI (22.5) and VI and IX (36.4). The variables beginning of flowering and crop cycle contributed most to the genetic divergence among the genotypes (Dias et al. 2009).

Sixty-six genotypes of cowpea were investigated to understand the extent of genetic diversity through twelve quantitative traits. Mahalanobis's D^2 analysis established the presence of wide genetic diversity among these genotypes by the formation of 23 clusters. Cluster I had the maximum number of genotypes *i.e.* 22 and cluster 23 had only one genotype. The minimum intra cluster distance was observed in the cluster II. The inter-cluster distance (D) was found to be the maximum between the clusters XXII and XXIII and the same was minimum between clusters II and V. The results indicated that grain yield per plant contributed maximum to the total divergence followed by 100 seed weight and days to 50% flowering. Number of branches per plant had least contribution to the total divergence followed by petiole length. The existence of wide genetic diversity among the types chosen from the same geographical location was obviously seen. In this study, the variety Vellayani local had the maximum value for plant height and pod length and thereby distinguished from other varieties and it is present singly in the cluster XXIII. The clustering pattern of the varieties in the present study clearly indicated that there was no parallelism between genetic and geographic diversities (Nagalakshmi et al. 2010). According to (Costa et al. 2013), the dissimilarity measures were ranged from highest to shortest in the line pairs IT82D-889 and IT89KD-245 (221.35) and IT98K-128-4 and IT97K-1042-3 (1.21), respectively. Based on the UPGMA analysis, the lines were distributed into four distinct groups, which consisted of few lines, with the exception of the fourth group that contained 71% of the genotypes studied.

Nancee et al. (2013) carried out experiment at Research Farm of the Department of Vegetable Science, CCS Haryana Agriculture University, Hisar during spring/summer season of the year 2011. Based on D^2 values, forty-six genotypes were grouped into eight clusters containing two to fifteen genotypes. These clusters consisted of genotypes with different geographical origins and indicated no correlation between genetic and geographical divergence. The genotypes of cluster VIII showed maximum genetic divergence with cluster V followed by Cluster II, hence, the genotypes belonging to cluster VIII and cluster V may be selected for generating genetic variability and hybridization. Cluster II having two genotypes was found to be the best performing for agronomic characters followed by cluster I with three genotypes and cluster V with three genotypes. Thus, to generate desirable genetic variability, the crossing between cluster II, I and V genotypes would be useful. The number of seeds per pod contributed highest towards divergence followed by plant height at final harvest (cm).

The present study, multivariate analysis was carried out by Ahamed et al. (2014) to assess the genetic diversity among eleven cowpea germplasm. Mahalanobis generalized distance (D^2) analysis was used to group the cowpea geno-types. Considering the mean values, the germplasm was grouped into four clusters. Maximum numbers (4) genotypes were included in cluster III and a minimum number (2) genotypes were included in both cluster II and IV. Among the clusters, the highest inter-cluster distance was obtained between the cluster IV and II (23.952) and the lowest one was obtained between II and I (6.753). The maximum value of inter-cluster distance indicated that genotypes belonging to cluster IV were far diverged from those of cluster II. The first female flower initiation was earlier in BD-8344 (97 days) than the other germplasm. BD-8348 produced maximum number of pods per plant (41.20). The highest grain yield per plant (66.65g) was recorded from entry BD-8344 and the lowest grain yield per plant (28.80g) was also obtained in BD-1604.

Brahmaiah et al. (2014) evaluated forty cowpea genotypes for 18 quantitative characters to estimate the genetic diversity existing among them by using Mahalanobis D^2 statistics. The genotypes were grouped into six clusters. The cluster strength varied from single genotype (Clusters III, IV and V) to 25 genotypes (Cluster I). Clusters IV and VI had high inter cluster distance. Clusters II, III and I had maximum 100-seed weight, number of seeds per pod and seed yield respectively. Cluster IV had maximum seedling vigour index, germination per cent, peduncle length, number of clusters per plant and number of primary branches. The genotypes from clusters IV and IV may be inter crossed to obtain high variation. Chattopadhyay et al. (2014), grouped into cowpea genotypes in seven clusters, cluster I had maximum of 7 genotypes, Cluster II, III, IV and V comprised of 2 genotypes each, while Cluster VI and VII had one genotype each. The absence of relationship between genetic diversity and geographical distance indicates that forces other than geographical origin such as exchange of genetic stock, genetic drift, spontaneous mutation, natural and artificial selection are responsible for genetic diversity. The maximum inter-cluster value was observed between cluster I and VI (85.245) followed by 84.974 between Cluster III and VI which indicated that the genotypes included in these clusters had the maximum divergence. Cluster II had genotypes with high pod productivity, while the genotypes in Cluster III flowered much earlier. Crossing between genotypes within these groups could produce highly productive and early maturing cowpea genotypes.

The investigation on genetic diversity studies for seed yield in cowpea was conducted by using 44 genotypes of cowpea. Nath and Tajane (2014) have reported that there was substantial genetic diversity among the genotypes studied. 44 genotypes were grouped into 6 clusters to study the genetic divergence for seed yield per plant. There was no parallelism between genetic diversity and geographical distribution. For seed yield, the pair of genotypes *viz*., Phule CP 05001 and UPC-5286 were most divergent from one another (D^2=1225.35). On the basis of inter-cluster distance, cluster means and per se performance observed in the present studies, following genotypes are suggested for hybridization to improve seed yield in cowpea. 1.UPC-5286, 2.Phule Pandhari, 3.NBPGR- 05-66, 4. Pusa-do-fasali, 5.Shweta, 6.NBPGR-05-67, 7.CP-23-GPM, 8.NBPGR 05-71.

Sandeep et al. (2014) carried out genetic divergence using D^2 analysis in 50 diverse genotypes of cowpea. All the 50 genotypes were grouped into twelve clusters. Cluster I was largest comprising of twenty seven genotypes followed by Cluster II with twelve genotypes, cluster IV with three genotypes and cluster III, V, VI, VII and VIII, IX, X, XI, XII were represented each by single genotype. Intra-cluster D^2 values ranged from 0 to 38.06. The inter-cluster D^2 values ranged from 44.08 to 276.55. The maximum inter cluster distance was observed between VII and XII clusters followed by clusters IV and XII and cluster X and VII. The maximum contribution towards genetic divergence is by days to 50% flowering (25.22%) followed by plant height (12.24%) and biological yield per plant.

Forty-four genotypes of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verd.) were investigated to understand the extent of genetic diversity through twelve quantitative traits. Mahalanobis's D^2 analysis established the presence of wide genetic diversity among these genotypes by the formation of 3 clusters. Cluster I had the maximum number of genotypes *i.e.*34 and cluster III had only four genotypes. The inter-cluster distance (D) was found to be the maximum between the clusters II and III and the same was minimum between clusters I and II. The results indicated that 100 seed weight contributed maximum to the total divergence followed by pod yield per plant. Intercrossing among the genotypes belonging to cluster II, V and IV was suggested to develop high yielding varieties with other desirable characters or may be used as potential donors for future hybridization programme to develop superior yard long bean variety with good consumer preference and high pod yield (Vavilapalli et al. 2014a).

In another study, Vavilapalli et al. (2014b) in order to assess the divergence among 22 cowpea genotypes, Mahalanobis D^2 statistics was applied. The 22 genotypes were grouped into 6 clusters, where clusters I was the largest, containing eleven genotypes followed by the clusters III (5 genotypes) and cluster II with three genotypes. The inter cluster distance was maximum between cluster III and VI followed by cluster III and V. Based on inter cluster distance and per se performance of genotypes, the entries *viz*., VU 1, VU 2, VU 6, VU 8 and VU 21 were selected, which could be intercrossed to recover good recombinants and desirable segregants. The pod yield per plant contributed maximum divergence (66.23%) which was followed by pod weight (20.78%) and plant height (8.23%). In another study, ten cultivars were divided into two broad genetic groups (A&B) based on squared Euclidean distances. Group A consist of two clusters which members showed low performance in terms of economic traits and group B comprised two clusters of superior cultivars for most of the traits studied (Animasaun et al. 2015).

Results of Chandrakar et al. (2016) on twenty-one characters showed low quantum of divergence among 21 genotypes of vegetable cowpea. These cowpea genotypes were grouped into five clusters on the basis of D^2 analysis. Maximum number of genotypes (9) was accommodated in cluster-II. The average inter and intra cluster divergence (D) values had also been calculated. The maximum inter cluster distance was observed in between cluster I and V (5.04). The cluster III showed maximum value for mean green pod yield (146.820g.) followed by cluster IV (146.156g). Crossing between the genotypes of maximum two clusters appeared to be most promising to combine the desirable characters. Srinivas et al. (2016) grouped 30 genotypes of cowpea into six clusters. Maximum inter cluster D^2 value was observed between VI (6987.85) and III (4806.87), indicating that the genotypes included in these clusters had maximum divergence. The diversity among the genotypes measured by inter-cluster distance was adequate for improvement of cowpea by hybridization and selection. Asoontha and Mareen, (2017) grouped 12 genotypes of yard long bean into five clusters. The inter cluster distance was maximum for cluster I and cluster II (10.93) followed by cluster II and cluster V (8.91) and cluster II and cluster III (8.87). The least intercluster distance was recorded between cluster I and cluster IV. The intracluster distance was highest for cluster III followed by cluster II and cluster I. The intracluster distances was least and zero for cluster IV and cluster V as they are the solitary ones.

Study on 66 advance breeding lines of vegetable cowpea, Lal et al. (2017) proposed divergent occurrence among these genotypes. Based on degree of divergence the genotypes get grouped into seventeen clusters. The top three characters which contributed most towards the genetic divergence were number of peduncles per plant, peduncle length and pod length. The genotypes of cluster VII showed maximum genetic divergence with genotypes of cluster VIII. Further, cluster VII had the high yielding genotypes, while cluster XVII had early flowering genotypes. (Lovely et al. 2017) conducted a study on nature and magnitude of genetic divergence among 50 genotypes of vegetable cowpea collected from different agro climatic regions of South India. All the genotypes were grouped into four clusters with genotypes from different geographic locations being grouped in the same clusters. The cluster I had the highest number of genotypes. The genotype VS41 remained in a solitary cluster as a divergent genotype that cannot be accommodated in any of the clusters. Pod yield per plant contributed the maximum towards divergence. Patel et al. (2017) evaluated 32 cowpea genotypes by using D^2 statistics. The genotypes were grouped into eight clusters. The maximum inter-cluster distance (D^2=35.43) was observed between cluster-VI and VIII. Clusters II, III and I had maximum 100-seed weight, number of seeds per pod and seed yield respectively. Therefore, it was concluded that the genotypes belonging to these cluster should be intercrossed in order to generate more variability. Vu et al. (2017) studied cluster pattern in cowpea and revealed that highest inter-cluster average D^2 values among genotypes existed between clusters III and VI (52.08). Therefore, the crossing between the genotypes of most divergence clusters i.e. III (KBC-8, GC 901, VCP 09-019, KBC-6, PTB-1, UCP 12-007) and VI (TC 150) could able to produce high seed yielding transgressive segregants. It would be logical to attempt crosses between the diverse genotypes belonging to clusters separated by large inter-cluster average D^2 values. This clearly showed presence of wide variation from one cluster to another in respect of cluster mean for six clusters.

In a meterolygraph study of 30 cowpea genotypes, Arya et al. (2019) placed KBC 9 and DC 7-15 in the medium fodder-medium grain yield producing group and could be utilized for both, fodder as well as grain production. Moreover, the genotypes (UPC 12-007, KBC-6, KBC-8 and GC 901) had good potential of grain production as well as excellent potential of fodder production. Likewise, the genotypes (PL-3 Sel., CPD 240 and RC 101) were included in medium fodder-high grain yield, which had good potential of grain production as well as excellent potential of fodder production. The hybridization among the diverse genotypes viz., UCP 12-007 (high fodder yield) with PL-3 Sel. (high grain yield) may be suggested for developing dual purpose genotypes through the transgressive segregation.

Genetic diversity in cowpea by using molecular markers

Assessment of genetic variability within cowpea is fundamental for the conservation of genetic resources and its utilization in hybridization programme. The use of new tools of molecular biology like diversity studies can overcome some of the breeding program limitations and speed up selection time of new varieties for crosses. Different molecular techniques were used for diversity study of wild and cultivated cowpea which included Amplified Fragment Length Polymorphisms (AFLP); Chloroplast DNA Polymorphisms; Random Amplified Polymorphic DNA (RAPD) Restriction Fragment Length Polymorphisms (RFLP); DNA Amplification Fingerprinting (DAF); and analysis of Simple Sequence Repeats (SSRs) or Sequence Tagged Microsatellite Sites. Of these techniques, analysis of SSRs has proven to be particularly useful since these sequences are abundant and distributed throughout eukaryotic genomes (Doumbia et al. 2014).

In a study, Ba et al. (2004) used RAPD analysis to characterize genetic variation in domesticated cowpea and its wild progenitor, as well as their relationships from West, East and southern Africa. A total of 28 primers generated 202 RAPD bands. 108 bands were polymorphic among the domesticated compared to 181 among wild/weedy cowpea accessions. Wild accessions were more diverse in East Africa, which is the likely area of origin of *V. unguiculata* var. *spontanea*. var. *spontanea* is supposed to have spread westward and southward, with a loss of variability, loss counter-balanced in southern Africa by introgressions with local perennial subspecies. Although the variability of domesticated cowpea was the highest ever recorded, cultivar-groups were poorly resolved and several results obtained with isozyme data were not confirmed here. However primitive cultivars were more diverse than evolved cultivars, which still suggest two consecutive bottlenecks within domesticated cowpea evolution. As isozymes and AFLP markers, although with a larger number of markers, RAPD data confirmed the single domestication hypothesis, the gap between wild and domesticated cowpea and the widespread introgression phenomena between wild and domesticated cowpea.

Genetic relationships among elite lines of cowpea were assessed using four enzyme systems and 10 random oligonucleotide primers. Multimeric isozyme profile and polymorphic RAPD markers reflected presence of considerable amount of genetic variability among the genotypes. Keeping in view the need for developing varieties with early maturity, determinate growth habit and high yield and root rot disease resistant and susceptible lines were used in the present investigation to assess their genetic relationships. Except superoxide dismutase (SOD), other three-isozyme systems, peroxidase (PRX), esterase (ESt) and polyphenol oxidase (PPO) yielded polymorphic bands. The cluster analysis showed that genotypes could be grouped into three main clusters. The level of polymorphism observed with both RAPD and isozyme indicated diversity in genotypes for root rot resistance. RAPD analysis indicated that amongst the released varieties, Bundel lobia-1 was most distinct. Its similarity coefficients with Bundel lobia-2, Kohinoor and UPC-5286 were 79, 78 and 75 per cent, respectively (Sahay et al. 2008).

RAPD markers were used to assess the genetic diversity among selected genotypes of *Vigna unguiculata*. Out of 35 RAPD primers tested, 14 allowed amplifications of random polymorphic (RAPD) loci. A total of 113 amplified products were obtained out of which 16 were monomorphic and 97 were polymorphic. Average polymorphism across 24 genotypes was found to be 79.87 per cent. For the genotypes tested, 2 to 14 bands were obtained, with an average of 8.007 bands per primer. The 24 genotypes were grouped into two major clusters at a similarity coefficient of 0.50. Genetic similarity matrices of the genotypes ranged from 0.3462 to 0.8681, indicating a high genetic variability among the genotypes. Similarity value for all other genotypes varied between the two extreme values. The results indicated that RAPD markers were efficient for the identification of genotypes and for determination of genetic relationships among them (Malhotra et al. 2009). Patil et al. (2013) estimated the genetic diversity of thirty genotypes of cowpea in a study by using RAPD markers. RAPD profiles for 30 genotypes were generated with 20 random decamer primers. Out of 20 primers screened 17 primers gave scorable DNA fragments and each of the 17 primers revealed various levels of polymorphism. These primers generated 1238 DNA fragments in the average range of 381.94 bp to 1131.71 bp, of which 908 were polymorphic. The level of polymorphism among the genotypes was found to be very high (71.20%). The overall range of similarity among 30 genotypes was found to be very wide, ranging from 0.321 to 0.800 which indicates there was high variability among the cowpea cultivars under study.

The genetic diversity of cowpea in Ethiopia was analyzed using 19 uniform accessions, 62 variable accessions (yielding 185 sub-types) and two mung bean accessions (four subtypes) as out-group. A set of 23 polymorphic SSR markers was identified and polymorphism was scored. A total of 75 allelic variants was defined, with the average number of alleles per locus calculated to be three. The average genetic diversity (D) was 0.47 and PIC was 0.4. The accessions showed no clustering by geographical origins. Three well-characterized molecular markers (SSR1, C42-2B and 61RM2) for race specific resistance to Striga gesnerioides in the cowpea cultivar B301 were used to evaluate the accessions for their potential for use in genetic improvement against this pest. Only two accessions, 222890–2 from Gambela and 286–2 from the Southern Nations, Nationalities and Peoples (SNNP) region, were found to cluster with B301 and contain the SSR1 resistance allele (Desalegne et al. 2016).

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