

## Chapter 2

# Genetic Improvement and Variability

**Abstract** Clusterbean has been reported with vast variability including branched or unbranched plant types, hairy or smooth stems, straight or sickle-shaped pods, pubescent or glabrous leaves, determinate or indeterminate growth, regular or irregular pod bearing habits. Various methods used for genetic improvement of the crop and for assessment of variability are discussed in this chapter.

### 2.1 Introduction

The use of diverse germplasms in crop improvement is one of the most sustainable methods to conserve valuable genetic resources and simultaneously to increase agricultural production and food security (Ogwu et al. 2014). The genetic resources have been used as foundations to broaden adaptation, to create resistance against disease, insect, stress, to improve quality, stature and to increase yield potential of the crop. The wild relatives are the major source of genetic support and help the crops in maintaining its valuable status. The selections made through local land races have resulted in the release of superior genotypes of clusterbean (Henry et al. 1992; Bharodia et al. 1993; Mishra et al. 2009).

### 2.2 Genetic Variability

The genetic variability within the crop helps in its maximum utilization and becomes the basis of species conservation. Similarly, the knowledge of genetic divergence among the varieties has immense importance for plant breeders while use of diverse germplasm has a significant contribution towards high yield and quality characteristics (Maniee et al. 2009; Singh et al. 2014). Clusterbean germplasm has been reported with vast variability including branched or unbranched plant types, hairy or smooth stems, straight or sickle-shaped pods, pubescent or

glabrous leaves, determinate or indeterminate growth, regular or irregular pod bearing habits (Saini et al. 1981). Variability for different characters in clusterbean has been summed by Mishra et al. (2009), the ranges described are: days to 50 % flowering (25–76 days), days to 50 % maturity (66–128 days), plant height (55–238 cm), branches/plant (0–29), clusters/plant (2–86), pod length (1.6–17 cm), pods/plant (4–412), seeds/pod (2–15), seed yield/plant (1.2–71 g) and 100-seed weight (1.5–5.3 g). Pathak et al. (2011c) reported wide range of variability for different morphological traits of clusterbean, viz., plant height (31.8–42.8 cm), number of primary branches (7.7–13.1), number of secondary branches (10.8–29.8), number of pods/plant (21.1–44.9), number of seeds/pod (6.9–9.4), 100-seed weight (2.57–3.06 g), seed yield/plant (5.5–11 g) and days to 50 % flowering (30.3–35.8 days). Similarly, wide variation in different biochemical parameters of clusterbean seed, viz., endosperm (30.4–46.3 %), gum content (23.5–33.5 %), crude fibre (4.1–8 %), fat content (1.8–5.2 %), crude protein (28.3–35 %), ash content (3.5–6 %) and carbohydrate content (38.8–59.1 %) has also been reported (Pathak et al. 2011a).

The inheritance studies indicates that the foliage colour, branching and pollen fertility are controlled by a single gene with the dominance of the dark green foliage colour over the light green colour, unbranching over branching and pollen fertility over pollen sterility, whereas, pod length was controlled by several gene pairs (Dabas 1975). Singh et al. (1990a) reported dominance of serrated leaf margin over the smooth margins and pubescent leaf surface over the glabrous surfaces. Saharan et al. (2004) observed the dominance of hairy types over non-hairy, branched over unbranched, purple colour flower over white colours, broad leaf over narrow leaves, curved pods over non-curved pods and plucked leaf surface over non-plucked leaf surface. The inheritance of branching pattern and leaf serration in clusterbean was also studied and it was observed that single dominant gene was responsible for branching pattern and leaf serration (Saharan et al. 2004).

Understanding of the inheritance of gum content is a key for successful genetic improvement of clusterbean for quality traits. The inheritance of gum content in the seed of clusterbean is quite complex in nature and both additive and non-additive types of gene action are operating in the expression of gum content (Dabas 1975). The gum content expression is reported to be controlled by additive, dominant and epistatic effects and modified by the environment. Additive gene effects are more important in most of the crosses, therefore, it is suggested that selective intermating in  $F_2$  or  $F_3$  generation should be resorted to exploit additive type of genetic components and plant or lines may be selected in later generations for genetic improvement for higher gum content (Singh et al. 1990b). The operation of both the additive and non-additive gene actions in the expression of gum content, its positive association with seed yield and negative correlation with seed weight (Pathak et al. 2011a) have hampered the breeding of clusterbean for gum content, therefore, some concrete efforts are required to be taken up for improvement of gum and protein content.

Burton and De Vane (1953) suggested that Genotypic Coefficient of Variation (GCV) together with heritability estimates would give reliable indication about

the expected improvement of desired trait. Clusterbean has been reported with moderate-to-high estimates of GCV for seed yield and yield attributing traits (Pathak et al. 2011a; Kapoor and Bajaj 2014). The seed yield/plant is a primary trait which depends on number of variables, but high GCV values reported for a particular trait indicate that direct selection for seed yield is one of the best approaches for its genetic improvement. Higher estimates of variability observed in the characters show that there is ample scope for selection to improve the traits in clusterbean (Arora and Gupta 1981).

In quantitative genetic analysis of clusterbean, Chaudhary et al. (2003) found higher estimates of GCV for number of clusters/plant, primary branches/plant, pods/plant, seed yield/plant, biological yield/plant and days to 50 % flowering. They found similar trends for Phenotypic Coefficient of Variation (PCV). Reflecting the susceptibility to environmental fluctuation they observed wide differences between PCV and GCV for number of clusters/plant, number of pods/plant, seed yield/plant, biological yield/plant, harvest index and seeds/pod.

The study of heritability in conjunction with genetic advance is more useful than heritability alone in prediction of resultant effect of selecting the best individual (Singh et al. 2010). Traits having high heritability and genetic advance are supposed to be under control of additive genes, hence the characters with higher heritability and genetic advance may be improved by selection based on phenotypic performance (Shekhawat and Singhania 2005). The high estimates of heritability and genetic advance for various traits of clusterbean indicate that the seed yield and its components are governed by non-additive gene action (Pathak et al. 2011c), whereas days to flower initiation, plant height, number of branches/plant, number of pods/plant, number of seeds/pod are governed by additive gene action (Arora et al. 1999). Studies revealed that both the additive and non-additive genes have important role in the expression of almost all the traits in clusterbean. Heritability was high for plant height and number of clusters/plant and moderate in 100-seed yield and number of clusters/plant. Comparatively, high value of heritability coupled with genetic advance was observed for number of branches, plant height and number of clusters/plant. Similarly, moderate-to-high genetic variability and heritability estimates for number of traits among the varieties of clusterbean has been reported (Anandhi and Oommen 2007; Weixin et al. 2009).

The characters, viz., number of branches/plant, leaf area index, number of pods/plant, seed yield/plant, number of clusters/plant, harvest index, number of pods/cluster and plant height showed higher estimates of GCV and PCV (Raghu Prakash et al. 2008). Stafford and Barker (1989) reported heritability and interrelationships of pod length and seed weight in clusterbean and suggested that the selection for gum content can expect progress, but not as quickly as other traits, such as pod length and seed weight. Rai et al. (2012) observed maximum range of variability for number of branches, plant height, clusters/plant, pod length and pod yield/plant. High heritability coupled with high genetic gain in percentage was observed for pod yield/plant, number of pods/plant, days to 50 % flowering, number of branches and plant height. High heritability coupled with low genetic advance was recorded for pods/cluster, number of seeds/pod and pod width. Girish et al. (2013) reported

broad genetic base among the genotypes on the basis of higher value of GCV and PCV for stem girth, vegetable pod yield/plot, dry pod yield/plot, seed yield/plant, endosperm and gum content. They reported high heritability coupled with high genetic advance for stem girth, cluster length, pod length, endosperm, protein and gum content. Similarly, Kapoor and Bajaj (2014) also recorded high heritability along with high genetic advance for number of leaves/plant, number of branches, dry matter yield and green fodder yield. Vir and Singh (2015) estimated the genetic variability, intercharacters associations in the germplasm of clusterbean and observed high degree of genetic variability for seed yield, 100-seed weight, number of seeds/pod, number of pods/plant, number of pods/cluster, number of branches/plant, number of clusters/plant, plant height, days to 50 % flowering and days to maturity during both summer and rainy seasons.

### 2.3 Karyotype Analysis

Determination of chromosomes numbers and karyotype analysis is an important method for the assessment of the genomic status of morphologically diverse populations. Meagre information is available on these aspects in clusterbean as there are difficulties in getting well spread mitotic metaphase chromosome preparations, staining and resolution resulting problems for identification of correct position of centromere(s) (Purohit et al. 2011). The somatic chromosome counts reported by many authors for clusterbean is  $2n = 14$  (Jahan et al. 1994; Patil 2004). Purohit et al. (2011) reported the  $2n$  number of chromosome as 14 in the root-tip cells without any indication of existence of polyploidy/aneuploidy or any numerical variation.

Patil (2004) has guessed that the karyotypes of *C. senegalensis* and *C. serrata* could be resolved into asymmetrical and symmetrical types, respectively. *C. serrata* has slightly longer chromosomes and represents comparatively primitive status in relation to the rest of the species on the basis of karyotypic studies. Arora et al. (1985) suggested the presence of unequal translocations in chromosomes that plays a role in change of karyotypes. Arora et al. (1985) found 7.16–9.261  $\mu$ , 7.33–11.52  $\mu$  and 5.5–9.6  $\mu$  chromosome length in *C. tetragonoloba*, *C. serrata* and *C. senegalensis*, respectively and 44, 45 and 45 % total form (TF) values, respectively.

Polyploidy is a common technique to overcome the sterility of a hybrid species in plant breeding and is induced by treating seeds with the chemical, viz., colchicine. Vig (1963) induced polyploidy in clusterbean and observed a mixoploid plant resulting from aqueous colchicine treatment and obtained true autotetraploids. Bewal et al. (2009) induced autotetraploidy in *C. tetragonoloba* and found that the tetraploids plants has reduced plant height, length of rachis, length and breadth of leaflet and number of seeds/pod from the corresponding diploids plants along with considerable increase in internodal length, length and breadth of standard petal, width of wing petal and pod length. The tetraploid plants have normal meiotic behaviour and may lead to a good seed set.

## 2.4 Hybridization

Hybridization is the process of interbreeding between individuals of different species or genetically divergent individuals from the same species and is one of the important tools for creation of genetic variability in any crop (Harrison and Larson 2014). The manual hybridization in clusterbean is difficult and laborious due to highly self-pollination, small flower, its structures and flower drop. The earliness may be transferred from wild species (*C. serrata* and *C. senegalensis*) to cultivated species of clusterbean with the help of hybridization. Besides this, locally collected landraces can be used in breeding programme for incorporation of desirable traits in clusterbean. Multiple crossing, back cross, pedigree and bulk pedigree are the major varietal improvement programmes in a number of self-pollinated species for different inherited characters such as seed size, disease resistance, seed colour and duration of maturity (Knauff and Ozias-Akins 1995). But these methods are not found to be efficient for the improvement of quantitative traits like seed yield. Various physical and chemical mutagens have been reported for induction of variability in this crop.

### 2.4.1 Conventional Method of Hybridization

The interspecific hybridization between *C. tetragonoloba* and *C. serrata* through conventional methods was reported as complete failure probably due to the rejection of pollen by the stigma (Sandhu 1988). Other approaches like bud pollination, amputation of stigma and style, use of organic solvents also failed to overcome the stigmatic incompatibility barriers. Scope for interspecific hybridization is, therefore, limited in clusterbean. Ahlawat et al. (2013) studied three species of *Cyamopsis* to find out barriers of interspecific crosses between *C. tetragonoloba* × *C. serrata* and *C. tetragonoloba* × *C. senegalensis* for crop improvement. They reported interspecific hybridization between *C. tetragonoloba* × *C. serrata* with least (10.43 %) pod setting.

## 2.5 Heterosis and Combining Ability

Heterosis and combining ability have been used as an important breeding approach in crop improvement for selection of parents and utilization of hybrid vigour and is a good method to assess the nature of gene action involvement in the inheritance of character (Vasal 1998). The knowledge of gene action and combining ability analysis gives an insight for identifying the better combiners which may be hybridized to exploit heterosis for selection of better crosses in further breeding work and also elucidate the nature and magnitude of various types of

gene effects involved in the expression of quantitative traits (Nigussie and Zelleke 2001).

Several workers found the existence of wide range of heterosis in clusterbean (Chaudhary et al. 1981) but heterosis breeding has its own limitations in this crop due to absence of stable source of male sterility. Different gene effects were observed in different crosses and it was noticed that additive, dominance and epistatic effects were operating for both endosperm and gum content in clusterbean. Heterosis and combining ability studies for seed yield and component characters in clusterbean have been reported by number of workers (Saini et al. 1990; Arora et al. 1998). Hooda et al. (1999) found high specific combining ability (SCA) effects for number of characters with the crosses like Durgajay  $\times$  AG 111, HFG 516  $\times$  HFG 590 and AG 111  $\times$  HFG 516 and suggested that the genic interactions for best crosses were accountable to additive  $\times$  additive or additive  $\times$  dominance or dominance  $\times$  dominance type of gene effects. The cross HFG 516  $\times$  HFG 590 was recorded with high heterosis, low inbreeding depression and high SCA effect for seed yield, component characters and disease resistance.

The extent of heterosis and combining ability in this crop gave substantial support for developing hybrids but its possibility is ruled out in the absence of techniques for economic production of large quantities of hybrid seed.

## 2.6 Mutation

Mutation is a sudden heritable change and is caused by alteration in the base gene sequence. It can be induced either spontaneously or artificially both in seed and vegetatively propagated crops (Bhosle and Kothekar 2010). The utilization of mutagenesis has been established as one of the best preference in the crops improvement (Dubinin 1961). Mutagenesis involves screening of genetic material and the selection of individual mutants having improved traits and their incorporation into breeding programmes. The efficient mutagenesis should be free from association with undesirable changes for creation of desirable changes. Mutations occur unexpectedly and sudden in nature and the frequency of such mutations is very low so breeder cannot rely on this for plant breeding. Therefore, artificial means using physical and chemical mutagens were discovered to induce mutations in the plant. A number of physical and chemical mutagens known for their mutagenesis competences have been used in different crops (Mullainathan et al. 2014). Physical mutagen includes seed treatment with X-ray and gamma rays, whereas chemical mutagen includes ethyl methane sulphonate (EMS), diethyl sulphonate (DES), sodium azide (SA), methyl methane sulphonate (MMS), nitroso guandine (NG), nitroso methyl urea (NMU), etc.

### ***2.6.1 Natural Mutation***

Naturally occurring genetic mutations may be caused by breaks, chimaeras, etc., and can change the appearance of the foliage, flowers, fruit or stem of any plant and may lead to abnormal plants (Tariq et al. 2008). A mutant of clusterbean having rosette-type inflorescence (raceme) with reduced fertility was reported in South Africa (Stafford 1989). The first report of the male sterility and partial male sterility in clusterbean was published from India (Mittal et al. 1968). Semi-sterile plants reported by Mittal et al. (1968) and Vig (1965) were shown to be caused by reciprocal translocations and produced fewer seeds/pod and more racemes/plant, grew taller and remained vegetative for longer time as compared to fertile plants. The practical utility of male sterility, partial male sterility and rosette raceme mutants has not been demonstrated in clusterbean (Arora and Pahuja 2008).

### ***2.6.2 Mutations Induced Through Physical Factors***

Physical mutation has been a successful tool in bringing improvement in self-pollinated crops and provides beneficial variation for practical plant breeding purpose (Tariq et al. 2008). It can be induced by irradiation with ionizing or non-ionizing rays. In the beginning, X-rays were used for this purpose but later gamma rays, viz.,  $^{60}\text{Co}$  and  $^{137}\text{Cs}$  became more popular (Auerbach and Robson 1946). Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cell and tissue (Gunckel and Sparrow 1961). The first successful attempt of mutation induced through physical mutagens in clusterbean was carried with gamma rays using  $^{60}\text{Co}$  as source of radiation (Vig 1965). The effects of irradiation on morphology and cytology of clusterbean using gamma rays were studied (Lather and Chaudhary 1972) and decreased germination percentage, seedling survival and pollen fertility was recorded with increased dosage. The dose of 100–200 kR was proved to be lethal with no germination. By contrast, Chaudhary et al. (1973) observed increased yield, protein and gum contents in an  $M_2$  population generated through irradiation with low doses of gamma rays.

The effects of irradiation ranging from 10 to 250 kR gamma rays on clusterbean for quantitative characters assessed in the  $M_2$  generation (Chowdhury et al. 1975) showed that the number of branches and seed yield/plant were less in the  $M_2$  generation than the control but the peduncle length and plant height were greater. Similarly,  $M_2$  progenies of clusterbean obtained through gamma irradiation was recorded with increased plant height, number of clusters/plant, number of pods/cluster, number of pods/plant and seed yield/plant but the induced variation reduced the number of seeds/pod and pod length (Amrita and Jain 2003). The yield potential of  $M_3$  progenies derived from certain high yielding  $M_2$  progenies of the clusterbean variety exposed to various doses of gamma rays was recorded



with more number of pods/plant and seed yield (Yadav et al. 2004). Patil and Rane (2015) studied the frequency and spectrum of chlorophyll mutations in clusterbean using different doses of gamma rays and reported increased frequency of chlorophyll mutations with increasing doses of gamma rays.

The effect of 0–60 kR doses of X-rays was studied on seeds of clusterbean variety Pusa Navbahar, the low doses were found to be beneficial, while high doses were inhibitory, which were generally less in the second generation (Rao and Rao 1982). Early flowering and determinate type are the remarkable mutants derived with gamma irradiation. An early flowering mutant with more number of pods was generated from the 10 kR treatment of X-rays. The determinate plants have reduced plant height, non-branching habit, synchronous and early maturity, increased cluster size and the main shoot either terminated into a leaf or an inflorescence (Singh et al. 1981).

Among physical mutagens, ultraviolet (UV) rays are non-ionizing and are effective in producing purine or pyrimidine dimers, resulting in point mutations. Studies suggest that UV rays can be effectively used to irradiate pollen in the late or early uninucleate stages (Toker et al. 2007). Lingakumar and Kalandaivelu (1998) subjected clusterbean variety Pusa Navbahar seedlings to continuous UV-B radiation for 18 h, post-irradiated with white light and UV-A enhanced fluorescent radiations and found that UV-B alone reduced plant growth, pigment content and photosynthetic activities. While, supplementation of UV-A promoted overall seedling growth and enhanced the synthesis of chlorophyll and carotenoids with a relatively high photosynthesis. Joshi et al. (2007) reported decline in the amount of photosynthetic pigments,  $O_2$  evolution with induced UV-B radiation and modification in the absorption spectra of chloroplasts, whereas the combination of UV-A and UV-B irradiation partially reversed these changes.

Arora et al. (1997a) carried out the genetic analysis of seed mass following hybridization and irradiation in clusterbean and observed that the additive component of genetic variance for the inheritance of 100-seed weight was more important in irradiated generations ( $F_1M_1$  and  $F_2M_2$ ), whereas non-additive gene action was more important in case of non-irradiated populations ( $F_1$  and  $F_2$ ). Vig (1969) observed that the seed of clusterbean had initially higher germination with the dose of 10 kR gamma rays but in the later hours 30 kR treated material gave higher germination percentage. Radiation speeds up the growth of roots, but there is no correlation between rate of root elongation and size of dose, however, a direct correlation between the radiation dose and the rate of germination was observed (Vig 1969). The comparison of nature and magnitude of variability among unirradiated and irradiated populations of clusterbean revealed that the irradiated populations had higher seed weights, early flowering and maturity (Arora and Lodhi 1995). The physical mutagens often result in the larger scale deletion of DNA and changes in chromosome structure. Therefore, presently they have been shifted to chemical mutagens.



### ***2.6.3 Mutations Induced Through Chemical Factors***

As ionizing radiation results in high rates of chromosome aberrations and is accompanied with detrimental effects, it was obvious to look for alternate sources and as a result chemical mutagens have been discovered. The chemical-induced mutations have significant importance in evaluation and crop improvement and are considered as more viable mutants (Heslot et al. 1961). The most widely used chemical mutagens are alkylating agents, among these EMS being the most popular because of its effectiveness and ease of handling, especially its detoxification through hydrolysis for disposal (Hajra 1979). EMS usually creates point mutations in plants (Okagaki et al. 1991), whereas SA creates marginal mutagenic in different organisms (Arenaz et al. 1989). SA is inexpensive, relatively safe to handle and has no carcinogenic effect and has also been reported to induce high frequency of point mutation without detectable chromosomal aberrations (Nilan et al. 1973) and due to the efficiency of mutagenic effects of SA, no new chemical mutagens of widespread use in plant breeding have been discovered (Sikora et al. 2011).

The  $M_1$  plants of clusterbean developed from EMS-treated seeds were chlorophyll deficient and showed profuse vegetative growth (Gohal et al. 1972). Similarly, mutants with changed leaf texture or shape, growth habit and pod size were obtained by treating seeds with EMS or hydrazine hydrate and unbranched and regular pod bearing mutants were obtained by treating the seeds with hydroxyl amine (Swamy and Hashim 1980). Some mutants showed changes in seed colour from normal violet to light grey or light brown. Rao et al. (1982) observed determinate and spreading variants in the  $M_1$  and  $M_2$  generations of Pusa Navbahar, when soaked in 200, 400 and 600 ppm kitazin and 1000, 2000 and 3000 ppm Saturn for 12 and 24 h. Velu et al. (2008) induced high frequency of viable mutations in  $M_2$  generation of clusterbean by both EMS and SA. They further reported increased mutation frequency with increased dose of SA, while in case of EMS the mutation frequency decreased with increasing doses. Unbranched and regular pod bearing mutants (Swamy and Hashim 1980), chlorophyll deficient and mutants with profuse vegetative growth (Gohal et al. 1972) has been observed and isolated by treating the seeds with EMS and hydroxyl amine. Further, the seeds treated with EMS or hydrazine hydrate resulted into changed leaf structure, growth habit and pod size in the populations of the clusterbean, some of the mutants were recorded with extensive branching and late flowering and some were recorded with changes in seed colour (Swamy and Hashim 1980).

### ***2.6.4 Mutations Induced Through Physical and Chemical Factors***

The successfulness of mutagen depends on its effectiveness and efficiency. The effectiveness generally means the rate of point mutations relative to dose, whereas

efficiency refers to the rate of point mutations relative to other biological effects induced by the mutagen and is considered a measure of damage (Konzak et al. 1965). Mutation induction using both physical and chemical mutagens suggest that the lower doses of irradiation coupled with chemical mutagens are popular for in vitro mutation (Medina et al. 2004). In clusterbean the mutagenic efficiency and effectiveness were decreased with an increase in the dose of gamma rays or concentration of EMS in alone or gamma rays followed by EMS in combination treatments. Gamma rays in alone induced more mutagenic efficiency due to their high penetrating power as compared to EMS in alone or gamma rays followed by EMS in combination treatments (Dube et al. 2011).

Velu et al. (2007a) estimated the mutagenic effectiveness and efficiency of gamma rays and EMS in the clusterbean and reported that the frequency and efficiency of mutation was more in EMS as compared to gamma rays. Their finding suggests that EMS induced more number of mutants effectively and efficiently than gamma rays. Further, Velu et al. (2007b) reported that increasing dose/concentration of gamma rays and EMS decreased the values of morphological and yield parameters in  $M_1$  generation. Similarly Mahla et al. (2010) studies the effectiveness and efficiency of gamma rays and EMS in clusterbean and observed steady reduction in germination and subsequent survival of the treated population, seedling height and pollen fertility with increasing doses/concentration of mutagens. Babariya et al. (2008) treated the seeds of clusterbean with gamma rays and EMS and studied the effect of mutagenic treatments on the character association in  $M_2$  generation. The results revealed that besides generating variability, mutagenic treatments can alter the mode of association between any two characters and selection for improved plant architecture.

The reduction of germination, survival, growth of seedlings, plant height, number of leaves/plant, number of branches/plant, number of pods/plant, number of clusters/plant, pod length, pod breadth, fresh and dry weight of matured plant were decreased with increasing doses and concentration of gamma rays and EMS in clusterbean, whereas days to first flowering increased with increasing doses and concentration of gamma rays and EMS (Velu et al. 2012). The mutation frequency was increased with the increase in the mutagen dose of gamma rays, whereas in case of EMS it decreased with the increase in doses. Yadava and Chowdhury (1974) studied the cytological effects of different doses of gamma rays and sodium nitrate and found that 100 and 150 kR doses of gamma rays were lethal and caused the highest abnormalities in different parts of the plant. High yielding mutants were induced by treating the clusterbean seeds with gamma rays and aqueous solutions of EMS and NMU either alone or in various combinations, the  $M_2$  mutants thus obtained had long pods, increased number of pods and early maturity. These mutants also showed increased yields and gum contents (Singh and Aggarwal 1986). Basha and Rao (1988) studied combined mutagenic effects of gamma rays and SA in clusterbean varieties and found SA as the most efficient mutagen.

Mullainathan et al. (2014) reported higher percentage of mitotic aberrations with higher dosage of gamma radiation, EMS, SA and colchicine treated plants.

Along with broken metaphases, anaphasic laggards/bridges, clumping up of chromosomes, unequal anaphase and precocious movements of chromosomes during metaphases have also been observed in the plants treated with mutagenic agents. The mitotic aberrations are more in colchicine treated plants than the plants treated with gamma rays, EMS and SA.

Use of physical mutagens coupled with chemical mutagens has opened new outlook in the field of hybridization of this crop and there is requirement of more planned studies to obtain more desirable and fruitful mutagens of clusterbean. The lower doses of gamma rays (up to 50 kR) and lower concentrations of EMS (below 0.2 %) may be used in future breeding programme for inducing broad spectrum and high frequency of viable mutations in this crop.

## 2.7 Genotype $\times$ Environment Interaction and Stability

Genotype  $\times$  environment interaction ( $G \times E$ ) is the change in the relative performance of a character of two or more genotypes studied in two or more environments and involves changes in rank order for genotypes between environments and changes in the absolute and relative magnitude of the genetic, environmental and phenotypic variances between environments (Haldane 1946). The studies of  $G \times E$  and adaptation involve relative observation of genotypic responses in terms of yield under target environmental conditions (Allard 1960). A variety may achieve stability through individual or population buffering and the yield is demonstrated as their effects (Allard and Bradshaw (1964). Similarly, when the varieties are compared over a series of environments, their relative rankings generally differ and this causes difficulty in demonstrating the significant superiority of any variety (Eberhart and Russell 1966). Thus, to evaluate the stability of a genotype for their yield a precise knowledge of  $G \times E$  interaction is of vital importance and phenotypically stable lines are of great importance for the crops like clusterbean.

Stable varieties/genotypes of clusterbean over the wide range of environmental conditions have been screened over the years by different workers (Pathak et al. 2010) and all the workers emphasized the importance of genotype over environment, the linear regression of the genotype over environmental index and the deviation from regression coefficient for determination of stability and adaptability of the genotype for seed yield and other important yield influencing traits in clusterbean. Chaudhary et al. (2005) evaluated clusterbean for seed yield and its components over three environments for stability analysis. They observed that genotype, environment and  $G \times E$  interaction were significant for all the traits indicating presence of variability for genotypes environments and non-linear response of genotypes over the environments. D'almeida and Tikka (2003) carried out stability analysis for seed yield and quality traits, viz., seed size, protein and gum content and reported significant  $G \times E$  interaction for seed yield, protein and gum content while it was non-significant for seed size. Linear and non-linear components of

G  $\times$  E interaction were also significant for seed yield, protein and gum content, whereas both were non-significant for seed size.

Studies in clusterbean for green fodder yield (Paroda and Mehrotra 1976) and dry matter yield (Jhorar et al. 1980) indicated that different genotypes behave differently under varying environmental conditions. Henry and Mathur (2005) studied three different environments for different quantitative and qualitative characters and found that the gum content was better under late sown environment, whereas protein content exhibited higher value under the crop sown with onset of monsoon. In the same study they found that the high gum content and low value of protein content was exhibited by early maturing genotypes. Jain and Patel (2012) reported stable varieties, viz., GAUG-0309, GAUG-0416, GAUG-0513 and GAUG-0522 for earliness; GAUG-0416, GAUG-0308, GAUG-0004 and GAUG-0309 for plant height; GAUG-0411 for pods/plant and GAUG-0309 and GAUG-0411 for seed yield and suitable for cultivation in North Gujarat.

## 2.8 Correlation and Path Analysis

Selection of a variety is mainly based on phenotypic characters in the breeding programme but the response to selection depends on many factors including the information on association of characters, direct and indirect effects contributed by each character (Mohammadi et al. 2003). Correlation and path analysis establish the magnitude and direct and indirect effects of relationship between yield and its components. Correlation estimates the degree of association between the variables whereas, path coefficient analysis provides an indication that which variables exert an influence on other variables (Akanda and Mundit 1996). The correlation coefficient between the predictor and response variable is partitioned using path coefficient analysis, which provides a method of separating direct and indirect effects and measuring the relative importance of the causal factors involved to develop selection criteria for complex traits in several crop species (Milligan et al. 1990).

The correlation studies in clusterbean suggest that a plant having few branches, more number of clusters and pods, bolder seeds, long peduncles is expected to have higher seed yields (Vijay 1988). Shekhawat and Singhania (2005) observed that pods/plant, branches/plant, clusters/plant, pods/cluster, 100-seed weight and plant height had direct and positive effect on seed yield. Seed yield was significantly and positively correlated with plant height, seeds/pod, pods/plant, primary and secondary branches/plant. Pods/plant, seeds/pod and 100-seed weight had the maximum positive effect on seed yield/plant. Also, plant height, seeds/pod, gum content, primary and secondary branches had sizeable indirect effect via pods/plant. 100-seed weight had a positive direct effect on yield and positive indirect effects via clusters/plant. It is desirable to improve both seed weight and clusters/plant for better seed yield (Arora et al. 1997b). Thus, pods/plant, branch numbers, plant height and 100-seed weight may be considered as effective parameters of selection to increase seed yield in clusterbean

(Pathak et al. 2011b). Studies revealed that grain yield and gum content has positive correlation while seed weight has negative correlation with gum content, whereas endosperm always has a positive correlation with gum content. Similarly, seed yield and gum content were positively correlated with height, branch number and pod number whereas it had negative association with pod length and 100-seed weight, similarly endosperm had negative association with seed size and pod length (Pathak et al. 2011b).

Seed yield/plot was significantly and positively correlated with biological yield/plot, number of clusters and pods/plant and plant height. Stafford and Seiler (1986) recorded high and positive correlations between seeds/pod and seed yield and pods/plant and seed yield. The correlation of 100-seed weight and seed yield was low and positive. Grain yield/plant was found to be positively and significantly associated with all characters except gum content and pod length. Number of seeds/pod, number of pods/plant and pod length was the most important component characters which directly contributed to seed yield (Patel and Chaudhari 2001). Vir and Singh (2015) reported positive and significant correlations of number of seeds/pod, number of pods/plant, number of pods/cluster, number of clusters/plant, days to 50 % flowering and days to maturity with seed yield/plant. The positive correlation between number of pods/plant and seed yield was mainly due to positive direct effect of pods/plant (Singh et al. 2004). Weixin et al. (2009) reported negative association of quality related characters to seed yield. There are positive and significant correlation of seed yield with dry pod yield, number of pods/cluster, 100-seed weight, number of clusters/plant, branches/plant, seed recovery, germination, number of seeds/pod and dry biomass/plant. Whereas negative and significant correlation was recorded in days to flower initiation, plant height, days to maturity and days to 50 % flowering. Plant height, number of seeds/pod, days to 50 % flowering, number of clusters/plant, dry pod yield/plant and dry biomass/plant had direct positive effects on seed yield. Kumar and Ram (2015) suggested that the number of clusters/plant, number of pods/plant, pod yield/plant, plant height and days to maturity are the important traits for selection to yield improvement in clusterbean.

Anandhi and Oommen (2010) reported positive association of number of pods/plant, number of seeds/pod, pod weight and number of pod clusters/plant with vegetable pod yield and suggested that selection based on number of pods/plant, number of seeds/pod, pod weight and number of pod clusters/plant may bring out desired improvement towards enhancing the vegetable pod yield in clusterbean besides this selection of dwarf and early flowering genotypes would result in better yielding types. Girish et al. (2012) reported positive association of vegetable pod yield with pod breadth whereas Shabarish and Dharmatti (2014) observed positive and significant correlation of pods/plant with vegetable pod yield/plant in clusterbean. Malaghan et al. (2014) studied correlation among the vegetable pod yield components and their direct and indirect effects on the vegetable pod yield of clusterbean. The study revealed that the pod yield/plant was significantly and positively associated with pod length, pod breadth, pods weight and pods/plant. The pods/plant, pod length, pod weight and pod breadth had positive direct effect

on vegetable pod yield/plant. They suggested that the parameters, viz., pods/plant, pod length, ten fresh pod weight and pod breadth may be the potential traits for selection of higher yielding vegetable pod genotypes.

Genetic path coefficient analyses showed that pods/plant and 100-seed weight were important factors in determining seed yield whereas, seeds/pod was least important indicating that most of the yield components contributes via number of clusters/plant and had maximum direct effect as well as positive correlations with yield (Arora and Gupta 1981) and revealed positive association of number of seeds/pod, number of branches/plant and number of seeds/pod with the seed yield. Number of clusters on branches had highest direct effect on seed yield/plant followed by the clusters on main stem and pods on branches. The total number of branches on the plant, days to maturity and total number of pods contributed towards grain yield/plant via number of clusters on branches followed by the number of pods on the branches. Number of clusters on the branches in turn contributed to the yield directly as well as via number of pods on the branches and total number of pod. Thus, number of clusters and pods on the branches are the most cordial component of seed yield in clusterbean.

## 2.9 Genetic Resources

Development of newer materials in the form of varieties is an essential requirement for increased and sustainable production of the crop. The low productive clusterbean varieties and the varieties inclined to one or the other diseases and pests, needs to be replaced with the newer ones. Increase of genetic resources through germplasm collection both from indigenous and exotic sources is the most important criterion for any crop breeding and improvement programme.

National Bureau of Plant Genetics Resources, New Delhi have collected about 5000 accessions of clusterbean from dry habitats of northern India including two wild species viz., *C. serrata* and *C. senegalensis*. 4878 accessions with indigenous origin have also been conserved in medium term storages and 3714 accessions have been put for *ex situ* conservation (Mishra et al. 2009). Effective evaluation of more than 375 accessions against important diseases have resulted in promising resistance lines against bacterial leaf blight (GAUG-9406, GG-1, RGC-1027), Alternaria leaf blight (GAUG-9406, GAUG-9005, GG-1, GAUG-9003) and root rot (GG-1, HGS-844, GAUG-9406) (Kumar 2008). Certain lines of clusterbean, viz, Sona, Suvidha, IC-09229/P3, Naveen, PLG-85 and RGC-471 for seed type, and others like, Pusa Mausmi, Pusa Sadabahar, Pusa Navbahar, IC-11388, PLG-850 and Sharad Bahar were released as promising varieties for vegetable purposes.



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