GENETIC ANALYSIS OF MUTANTS FROM IRRADIATED COWPEA (*Vigna unguiculata* [L.] Walp.) SEEDS AND POLLEN

By

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A Thesis in the Department of Crop Protection and Environmental Biology submitted to the Faculty of Agriculture and Forestry in partial fulfillment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN, IBADAN.

Cocoa Research Institute of Nigeria

Idi-Ayunre, Ibadan. **December, 2016**

ABSTRACT

Cowpea, *Vigna unguiculata*, is an important human and livestock protein source in Nigeria, but its production is constrained by ravages of pests and climate change. Conventional breeding efforts used to fortify it against these constraints had resulted into its narrow genetic base. In order to overcome this challenge, other mutation procedures such as physical and chemical mutagens could be used. Nevertheless, information on gamma and ultra-violet (UV) irradiated cowpea seeds and pollen has not been adequately documented. Therefore, genetic analysis of cowpea mutants from gamma and UV irradiated seeds and pollen, respectively was investigated.

Cowpea accessions seeds: IB, IB-Y1, IB-CR and IB-BPC from the University of Ibadan and IT86D-719, IT86D-1010, IT89KD-347-57 and IT90K-284-2 from International Institute of Tropical Agriculture were irradiated at 100, 200, 300, 400 and 500 Gy doses at the rate of 202 Gy/min using ^{60}Co gamma. Pollen were irradiated for 60, 120, 180, 240, 300 and 360 minutes at 30,000µWs/cm^2 UV prior to hand self-pollination using standard procedures. Radio-sensitivity of irradiated accessions were determined using seed germination (SG), seedling survival (SV), lethal dosage 50% (LD_{50}) for SG and SV, primary leaf area (PLA) and seed set (SS) at M_1 and M_2 generations. The M_1 of gamma irradiation (GI) and M_2 of UV irradiation (UVI) treatments were advanced to M_2GI and M_3UV for phenotyping on field and their genetic stability confirmed at M_3GI and M_4UV, respectively. Genetic diversity of all mutants was determined using microsatellites. Ribulose-bisphosphate carboxylase primers were used for sequence analysis and classification of the mutants. Inheritance pattern was evaluated at M_5 of gamma induced mutants (GIM) for erect-tall (ER), yellow flush (YF), four-primary leaf (FP), crinkled leaf, lettuce leaf (LL), twisted-pale leaf (TP) and burnt leaf (BL) traits. Data were analysed using descriptive statistics and Chi-square at $\alpha_{0.05}$.

The M_1 generation of IT90K-284-2 had 74.0% SG, while each of IB, IB-Y-1, IB-CR and IB-BPC had 20.0% SG at 500 Gy of GI. The IB, IB-Y-1, IB-CR and IB-BPC had

0.0% SV each, while 50.0% was observed in IT86D-1010 and IT90K-284-2 at 400 and 500 Gy, respectively. The LD_{50} for SG and SV were lowest (326 and 149 Gy, respectively) in IB-Y-1 and highest (1053 and 620 Gy, respectively) in IT90K-284-2. The PLA of M_1 ranged from 2.17±0.26cm^2 to 5.98±0.85cm^2. Low GI (100 Gy) and UVI (60min) increased SS of M_1 plants. Mutant phenotypes and frequencies varied across the cowpea accessions and did not correspond to GI treatments. Ten GIM were stable at M_3, whereas all UV induced mutants reverted to normal at M_4. Polymorphic information content (0.51) obtained from microsatellites showed wide genetic diversity among the mutants and parental lines. The main mutant classes were insertion-deletions and point mutations. Inheritance of ER, TP, YF, FP and BN followed monogenic recessive pattern. Genetic interaction of crinkled and TP in homozygous recessive (*crl crl tp tp*) conditioned LL phenotype.

Radio-sensitivity of cowpea to gamma irradiation varied among the accessions. Ultra-violet radiation was less potent for cowpea pollen mutagenesis and might not be effective for mutation breeding.

Keywords: Cowpea radio-sensitivity, Gamma irradiated cowpea seed, Ultra-violet irradiated pollen, Mutant phenotypes, Pollen mutagenesis.

Word count: 499

ACKNOWLEDGEMENTS

First and foremost, I am greatly indebted to my supervisor, Dr. C.O. Ilori for his numerous suggestions, goodwill and above all for his patience and guidance throughout the period of this work. Special thanks to the Head of Department Prof. A.A. Omoloye for the role he played at a critical period during my Ph.D. studentship. I am grateful to the members of my supervisory committee; Dr. M.O. Balogun and Dr. O.A. Dada as well as the Postgraduate Coordinator, Dr. O.Y. Alabi for their contributions, thorough review and words of encouragement.

I am grateful to Prof. B. Fawole, Prof. G.I. Atiri, Prof. A.O. Togun, Prof. R.O. Awodoyin, Dr. Biodun Claudius-Cole, Dr. S.A. Adejumo, Dr. F. Aduramigba-Modupe, Dr. Fayinminu, Dr. V. Dania, Dr. O.S. Olubode and Dr. Anna Muyiwa for their concern and constant encouragement. I am grateful to the Genetic Resources Unit of the International Institute of Tropical Agriculture, Ibadan for the provision of seeds of cowpea accessions used in this study. My sincere appreciation also goes to the International Atomic Energy Agency, Seibersdorf, Austria for the provision of gamma irradiation facility used for this study and Dr. Souleymane Bado for the assistance during mutagenesis and critical review of radio-sensitivity aspect with Brian Forster. To Mrs. Oluwakemi Stanley, Mrs. Tope Owoeye, Mr. Olaniyi Oyatomi, David Igwe and Deji Owatti, I say a very big thank you for being there at the right time to assist me at the critical stage of getting the right protocol and primers for the molecular analysis. Amos Akisanya, I am very grateful for sharing those tough periods with me.

I appreciate the support and help received from other members of staff of the Department of Crop Protection and Environmental Biology of the University of Ibadan notably Mr. O.O. Oyelade, Mr. D.O. Omobusuyi, Mrs. O.K. Odeh, Mr. E. Nnodim, Mr. Bakare, Mrs. C.T. Omosehin, Mrs. S.O. Ayodele, Mr. J.O. Folorunsho and Mr. D.O. Adewoyin. To my friends and colleagues: Br. Amos Olajide, Yinka Saibu, Wale, Felicia

Aigbokhan, Mrs. Omotanwa Tanimola and Kemi Adejumo, thank you for the goodwill and encouragement you gave me throughout this period.

To my family, I am grateful to my late father for the love and strong support he gave me in the course of my education pursuit. It would have been your most fulfilling and joyful moment if you are alive to witness the completion of my Ph.D. degree. I deeply acknowledge my mother and mother-in-law for their encouragement and prayer support. To my brothers and sisters, I say thank you for the interest you have in my progress. I greatly appreciate my sister-in-law, Mrs. Bukola Makinde for her encouragement and assistance at the roof top garden of CPEB Department and on the field at the Teaching and Research Farm of the University of Ibadan. May God bless and reward you all.

I am greatly indebted to "My Sweet Heart", Adesola for your love and invaluable supports you gave me in the course of this work. Thanks for standing by me all through. And to my sons: Excellence, Israel and Enoch, thanks for your cooperation.

Finally, I wholeheartedly thank the source all wisdom, knowledge and understanding, the Almighty God, who has been so merciful to me, gave me the gift of life and supported me in the course of my academic pursuit in spite of all odds. For making it possible for me to achieve this goal, I give you all the glory.

DEDICATION

This work is dedicated to the Most High God the source of all wisdom, knowledge and understanding.

CERTIFICATION

I certify that this work was carried out by Festus Olakunle Olasupo of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan.

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CHAPTER 1

INTRODUCTION

Cowpea (*Vigna unguiculata*) is one of the most valuable tropical and sub-tropical leguminous crops to serve as a protein source for human nutrition, animal feed and is used for sustainable farming in the 21st Century (Singh, 2012). It is a native of southern Africa, and it has spread to cover over 65 countries worldwide (Singh, 2012). Cowpea is a cheap source of readily available protein in Nigerian diets as well as a good source of carbohydrate, potassium, phosphorus, calcium, iron, copper, zinc, boron, vitamins and carotene (Oyenuga, 1967; Amjad *et al*., 2006). The growing world human population is estimated to reach 9.6 and 10.9 billion by the end of 2050 and 2100, respectively (Gerland *et al*., 2014). Cowpea merits consideration as a strategic crop species in addressing the complex challenges of hunger, malnutrition, farming sustainability, changing climatic conditions and increasing food prices. All these challenges will confront the world agriculture and the global community in the coming decades (Widders, 2012; Fedoroff, 2015).

Cowpea has an estimated global production area of over 14.5 million hectares (FAOSTAT, 2015), and its annual grain production has increased to over 6.3 million tonnes in 2008 (Singh, 2012). Outside Africa, other production areas are the Central and South America and Asia with several smaller areas spread over southern Europe, Southern USA., and Oceania. Among countries, Nigeria is ranked number one world producer of cowpea with an output of 2,950,000MT in 2013 (FAOSTAT, 2015). In spite of this fact, cowpea yield and production in Nigeria has major constraints. These include ravages of diseases and insect pests. These barriers are closely linked with the genetic potential of the crop, inappropriate cultural practices and a low product quality (Rachie, 1985). Abiotic stresses such as low pH, low fertility, excessively high temperatures, drought and inadequate crop protection practices also limit production. Some recent achievements in yield increase, product quality improvement and amelioration of these

challenges in cowpea have been attributed to combined efforts of scientists affiliated with the International Institute of Tropical Agriculture (IITA), the Dry Grain Pulses Collaborative Research Support Program (CRSP), Purdue Improved Cowpea Storage (PICS) and national programmes (Widders, 2012).

The primary objective of a plant breeder is to develop crops that perform better than the existing ones in terms of yield and quality in some traits of interest. This depends to a large extent on the availability of genetic variation in the plant population. Therefore, creating genetic diversity is central to plant breeding programmes. Although the germplasm of naturally occurring genetic variability of a crop species is kept and maintained by plant breeders and genebanks, efforts are still being made to select for new variability through spontaneous mutations or to create novel variability through induced mutation. Induced mutation is also an important tool for research aimed at investigating basic biological problems like metabolism, development and regulation.

Stadler (1928a), was the first worker to report on mutagenesis in plants, using chemicals and radiation treatments. In most cases seed and vegetative meristems are used as the starting materials for mutagenesis and among the mutagenic agents used for seed treatment, gamma irradiation is known to be very effective in inducing a wide range of mutations (Bado *et al.*, 2015). However, seed and vegetative meristem treatments may lead to chimerism of resulting tissues and the need for greater screening to identify the resultant mutations. Many of these problems can be overcome by using pollen as the starting material for mutagenesis (Yang *et al.*, 2004). Mutation induction has become an established tool in plant breeding to supplement existing germplasm and to improve cultivars in certain specific traits. The first mutant cultivar was produced in tobacco in Indonesia in 1936 and since then, 3220 cultivars in over 220 crop species have been developed and released to farmers all over the world (Bado *et al*., 2015). However, little work has been reported on mutation breeding in cowpea (Osanyinpeju and Odeigah, 1998), which has been widely used among other legumes. The exception is India where 10 cowpea mutants out of 343 mutant cultivars from different crops have been released to farmers (Kharkwal and Shu, 2009).

When compared to seed mutagenesis, pollen mutagenesis is of greater advantage. This is because pollen mutagenesis involves mutagenic treatment, usually in the form of irradiation, to the pollen prior to hand pollination, while the female tissue remains free of somatic damage. The M_1 plant arising from pollination with mutated pollen is non-chimeric and will be hemizygous for any uniquely induced mutation (Yang *et al*., 2004). The dominant mutations in this case will be expressed in the M_1 while recessive mutations will be expressed in the M_2. Because of the absence of chimera, fewer M_2 seeds are needed per plant for screening than for an equivalent seed mutagenesis experiment. Further limitations of seed treatment are the possible occurrence of separated female and male germ cell primordial as well as somatic selection against mutant cells during plant development (Gavazzi and Sanguineti, 1983). In addition to these, Chin and Gordon (1989a), considered the use of irradiated pollen for limited gene transfer as a self-contained "DNA injection" system in which the irradiated pollen is both a source of donor DNA fragment as well as a vector for delivering the genetic fragments to the embryo sac. They proposed this technique, as a natural and rapid means of transferring a few or single genes into plants without resorting to the use of recombinant DNA technology. Moreover, it has been suggested that pollination with irradiated pollen may nevertheless be useful in practical plant breeding by causing a shift in the segregation ratio towards the maternal phenotype in the second (M_2) generation (Chin and Gordon, 1989b). They further suggested that, this could be a quicker method to produce pure breeding maternal progeny with a few specific paternal characteristics when compared to backcrossing. However, the use of irradiated pollen for mutation induction has not been given much attention in cowpea breeding.

Although ionizing radiations can cause deletions as well as other types of chromosomal rearrangements, Shirley *et al*. (1992) suggested that mutations that delete several kilobases at the locus of interest are particularly useful as mapping tools or for the identification and isolation of genes by positional cloning or genomic subtraction. The first step in mutation breeding is to select the genotypes for improvement. These comprise the current best performing cultivars and elite genotypes. The next step is to determine the optimal mutation treatment, this is done by radio-sensitivity testing (Mba *et*

al., 2012), and various responses between species and genotypes. Therefore, the objectives of this study were to:

1. assess the radio-sensitivity of cowpea varieties to seed treatment by gamma radiation
2. evaluate the radio-sensitivity of cowpea varieties to pollen treatment by UV radiation
3. select for novel induced mutations in cowpea
4. characterize some cowpea mutants lines genetically and morphologically
5. determine the inheritance pattern of some cowpea mutants

CHAPTER 2

LITERATURE REVIEW

2.1 Origin and Domestication of cowpea

Cowpea, *Vigna unguiculata* is an important annual grain legume crop cultivated in the tropical and subtropical regions of the world. It is grown between latitude 35°N and 30°S of the equator in Africa, Asia, Southern Europe, Central and southern America and the United state of America (Singh *et al.*, 1997). Cowpea is adapted to warm conditions and sensitive to chilling (Hall *et al.*, 1997).

Speculations of the origin and domestication of cowpea had been based on botanical and cytological evidence, information on its geographical distribution and cultural practices, and historical records (Faris, 1965; Steele and Mehra, 1980; Ng and Marechal, 1985; Ng, 1995). Several authors have different suggestions as regards the origin of cowpea. Vavilov (1951) and Steele (1972), suggested Euthopia as the origin of the crop, while others favoured West Africa (Piper, 1913; Rachie and Roberts, 1974., Rawal, 1975). Ng and Padulosi (1991), recently suggested the Natal-Transvaal region of South Africa as the probable origin of the species, from where they radiated outwards to the coastal regions in the Southern part of Africa, while its domestication might have taken place in West Africa where the centre of maximum diversity of cultivated varieties is found.

Ng and Marechal (1985) suggested Asia where probable wild progenitors are absent as the ancient origin of cowpea due to its wide distribution and early cultivation in the region. It is believed that cowpea reached south western Asia about 2300 BC and India more than 2000 years ago from East Africa along with other crops such as sorghum and finger millet (Steele, 1976).

Ng (1995), postulated that during the process of evolution of *Vigna unguiculata*, there was a change of growth habit, from perennial to annual form and from predominantly outbreeding to inbreeding, while cultivated cowpea (subsp. *unguiculata*)

evolved through domestication and selection of the annual wild cowpea (var. *dekindtiana).* The wide geographical distribution of var. *dekindtiana* throughout sub-saharan Africa suggests that the species could have been brought under cultivation in any part of the region (Padulosi and Ng, 1997). However, Ng and Marechal (1985) and Ng (1995), established West Africa as the centre of maximum diversity of cultivated cowpea, an area encompassing the Savanna region of Nigeria, Southern Niger, part of Burkina Faso, Northern Benin, Togo and the North Western part of Cameroon. The oldest archeological evidence of cowpea (from carbon dating of wild cowpea) found in Africa shows the existence of gathering (if not cultivation) of cowpea by African hunters or food gatherers as early as ca.1500 BC (Padulosi and Ng, 1997). Ng (1995), earlier postulated that cowpea cultigroup *unguiculata* was, domesticated in West Africa through the process of selection ca. 2000 BC. Cowpea was later brought to Europe probably through northeastern Africa around 300BC and to India about 200 BC (Padulosi and Ng, 1997).

2.2 Importance and production of cowpea

Cowpea is of major importance to the livelihood of millions of relatively poor people in less developed countries of the tropics (Quin, 1997). It has many important uses. For example, it is utilized for human consumption in the form of dry seeds, green pods, green seeds and tender green leaves. Its haulms are also used to feed livestock.

Cowpea has relatively high lysine content and is thus an excellent improver of cereal grains (Bressani, 1985). It is not only the cheapest source of readily available protein in Nigeria diets, it is also a good source of carbohydrate, potassium, phosphorus, calcium, iron, copper, zinc, boron, vitamin and carotene (Oyenuga, 1967; Amjad *et al.*, 2006). Farmers also enjoy its spillover benefits to their farmlands through, for example, in-situ decay of root residues and ground cover from cowpea's spreading and low growth habit. In addition, cowpea in a crop mixture helps to maintain soil fertility by fixing atmospheric nitrogen through symbiosis with nodule bacteria-*Bradyrhizobium spp* (Quin, 1997).

The world cowpea production was estimated at over 6.3 million tonnes and 95.1% of it is from Africa (Singh, 2012; FAOSTAT, 2015). West Africa is the major production

region for cowpea in the world. It is produced mainly in the dry savannah and semiarid zone of which Nigeria, Niger, Senegal, Ghana, Mali and Burkina Faso are the principal producing countries. Nigeria was reported as the largest producer and consumer of cowpea with a production of 2,950,000 MT in 2013 (FAOSTAT, 2015). However, cowpea yield and production in Nigeria has major constraints. These include ravages of diseases and insect pests, the barriers closely linked with the genetic potential of the crop, inappropriate cultural practices and a low product quality (Rachie, 1985). Recently, much research attention has been given to cowpea and considerable improvement strategies have been focused on the elimination of some of these problems that reduce yield in the crop.

2.3 Taxonomy of cowpea

Cowpea belongs to the order *Fabales*, family *Fabaceae*, subfamily *Faboideae*, tribe *Phaseoleae*, subtribe *Phaseolinae*, genus *Vigna*, and section *Catiang* (Verdcourt, 1970; Marechal *et al*., 1978, Padulosi and Ng, 1997). The genus Vigna is now divided into 5 sub genera: *Vigna*, *Haydonia*, *Plectotropis*, *Lasiospron* and *Ceratotropis* (Pasquet and Padulosi, 2012). Furthermore, the subgenus *Vigna* is divided into 6 Sections: *Catiang*, *Comosae*, *Liebrechtsia*, *Macrodontae*, *Reticulatae* and *Vigna*. The genus *Vigna* is made up of 80 species (Pasquet and Padulosi, 2012). Cowpea belongs to the Section *Catiang* which contains only two distinct species: *Vigna unguiculata* (L.) Walp. and *Vigna nervosa* Markotter (Marechal *et al*., 1978).

Padulosi (1993) divided the species *Vigna unguiculata* into six subspecies which constitutes the primary genepool of cowpea:

1. Subspeices *unguiculata* which contains the four cultivated groups of cowpea namely cultigroup: *unguiculata*, *biflora*, *sesquipedalis* and *textilis.*

(a) unguiculata – which is the major group

(b) biflora or catiang – which is differentiated mainly by its small erect pods and is grown in Southeast Asia

(c) sesquipedalis (the yard-long bean) – which is differentiated mainly by its very long pods and climbing growth habit, and is grown for its fresh pods in Asia

(d) textilis – which was grown in west Africa for the textile fibres obtained from its long peduncles.

2. Subspeicies *dekindtiana* which contains five groups namely *dekindtiana*, *huliensis*, *congolensis*, *grandiflora* and *ciliolata*,
3. Subspecies *protracta* which contains three, groups: *protracta*, *kgalagadensis* and *rhomboidea*,
4. Subspecies *pubenscens*,
5. Subspecies *stenophylla* and
6. Subspeicies *tenuis* which contains three groups: *tenuis*, *oblonga* and *parviflora*.

2.5 Cowpea morphology and growth habit

There exists a great variability in the morphology of cultivated varieties and wild relatives of cowpea. These variations were observed in protein and molecular marker electrophoretic band patterns (Vaillancourt and Weeden, 1992; Vaillancourt *et al*., 1993; Panella *et al.*, 1993, Pasquet, 1993). Padulosi and Ng (1997) and Pasquet and Padulosi (2012), reported the variation of some vegetative and reproductive organs of wild cowpea, and plant growth habit. These traits are useful to discriminate the various subspecies and varieties of the species and they are of great importance to plant breeder.

Cultivated cowpeas are glabrous or glabrate annual legumes with strong primary (tap) roots and much-branched, secondary and higher-order lateral roots (Summerfield and Roberts, 1985), which are characterized by the presence of nodules. Growth habit of cultivated varieties of cowpea could be herbaceous, viny or semiviny, erect or semi-erect, climbing or trailing, determinate or indeterminate. The leaves are trifoliolate varying in shape from globose, subglobose, hastate to subhastate (Porter *et al*., 1974). Its flowers are papilionaceous with large showy standard, truncate keel, diadelphous, uniform anthers and bent bearded style on a short raceme (Mishra *et al.*, 1982). Both gynoecium and androecium are well enclosed within the keel in a normal flower (Rachie *et al*, 1975). As a result of the flower morphology cowpea is self-pollinated although, sometimes cross pollination may occur.

The flower colour varies between white and purple in cultivated varieties. The flowers of cultivated varieties are smaller in size than most wild relatives (Harland, 1920). The inflorescence, rachis and peduncle length also vary depending on the variety. Pods of cowpea are coiled, round, crescent or linear in shapes. The pods are usually green in colour with or without purple pigmentation. The seed coat may be rough or smooth in texture with great varieties of colour spectra from white to brown, red, tan and black.

2.5 Flowering and pollination in cowpea

There is variation among the cultivated species in the time it takes to flower. Some cultivars may come into flower as early as 22 to 30 days from planting and matures 30 days later. Others take more than 100 days to flower and mature 210 to 240 days after planting (Summerfield and Roberts, 1985). Steel and Mehra (1980), reported that genotypes which come into flower early have shorter blooming periods (the number of days for which new flowers continue to open) than later flowering ones although flowering patterns vary appreciably in different climates and among accessions within large collections of germplasm. Many authors have reported that, (1.) Most cowpea genotypes respond to photoperiod in a manner typical of quantitative short day plants; (2.) Some genotypes are insensitive to a wide range of photoperiod; and (3.) warmer temperatures can hasten the appearance of the flowers in both day length-sensitive and insensitive genotypes (Doku,1970; Ojomo,1972, Doto and Whittington, 1981; Hadley *et al*., 1983).

Cowpea inflorescences are compound racemes of several modified simple racemes borne on penduncles usually between 5cm and 60m long (up to 1m long in var. *textilis*). Each simple raceme has several (6 to12) buds but only the oldest pair develops; they open into typical papilionaceous flowers several hours after anthers have dehisced. Anthers mature and dehisce about 7 to 8 hours before the time of opening of the corolla. Cowpea flowers are therefore cleistogamous (Steele *et al*., 1985). High temperature and increased duration of sunshine hastens dehiscence of cowpea anthers, while higher humidity delays the process (Kumar *et al.*, 1976).

Summerfield and Roberts (1985), reported that most flowers are inevitably self pollinated. However there is probably always a small proportion of outcrossing especially in humid climates. Although cowpeas are generally self-fertile, sub species *mensensis* is known to set fruits poorly in the absence of a pollinator. This is due to the orientation of the style and stamens in the flower; the style being very long and the stigma projected beyond reach of anthers. The flowers are also large and fragrant than most others suggesting that the subspecies *mensensis* is an obligate outcrosser (Lush, 1979). The cowpea flower is generally large and can be emasculated manually and pollinated with relative ease.

2.6 Genetics of cowpea

The review of early studies on cowpea genetics was conducted by Fery (1980, 1985a) who reported all the pertinent areas of cytogenetics, quantitative and qualitative genetics as well as updated list of genes. A set of rules for the gene nomenclature of *Vigna***,** and supplementary literature on cowpea genetics were added by Fery and Singh (1997) and Singh (2002). Cowpea is diploid and has 2n = 2x = 22 chromosomes (Faris, 1964). Karyotype analysis in cowpea is difficult due to its extremely small chromosome size (Hall *et al*, 1997). Conventional techniques, C- and H-banding, and an image analysis system have been used for cowpea characterization using pachytene and mitotic prometaphase and metaphase chromosomes. (Saccardo *et al*., 1992).

Several inheritance studies have been reported to reveal the knowledge of the growth habits, flower traits and earliness parameters, pigmentation, nitrogen fixation, seedling vigor traits, pod traits, seed traits, grain quality, yield component, root traits and heritability estimates for cowpea. Fawole (1988, 1990, 1997, 2001) among many others, reported the most prominent inheritance studies on the genetics of leaf mutant traits, which showed two independent non-allelic recessive genes *pt* and *pt-*$_2$ which condition the non-petiolate leaf phenotype the relationship between these two genes and the *un* gene conditioning the unifoliolate leaf mutant. Studies of various aspects of grain quality in cowpea had revealed genetic variability for protein, fat, ash, carbohydrate, and cooking

time. The heritability (Hb) estimate were 76% for cooking time, 95% for protein, 72% for fat, 83% for ash and 79% for carbohydrate (Nielsen *et al*., 1993).

Many studies have been conducted on the genetics of cowpea resistance to diseases. Few among them are the report on a recessive gene which confers resistance to bacterial blight (Prakash and Shivashankar, 1984); recessive gene *roc* which governs resistance to brown blotch (Abadassi *et al*, 1987), inheritance of resistance to fusarium wilt (Rigert and Foster, 1987), resistance to stem and root-knot (Bateman *et al*, 1989) and two genes *Uv-1* and *Uv-z* responsible for the rust (*Uromyces vignae* Barclay) resistance exhibited by the cultivar Dixie Cream (Chen and Health, 1993). Vale *et al*.(1995) studied the inheritance of resistance to cowpea severe mosaic comovirus (CpSMV) using cowpea variety Macaibo as the resistant parent and Pitiuba as the susceptible parent.

2.7 Cowpea breeding and improvement

The history of cowpea breeding dates back to the late 1800s and early 1900s in the southeastern United State and Texas (Fery, 1985_b; Miller, 1988) and Califomia (Mackie, 1946). It was later initiated in Asia (Mishra *et al*., 1985), Africa (Singh and Ntare, 1985), and Latin America (Watt *et al*., 1985; Arujo, 1988). The reasons for which limited breeding research were devoted to cowpea are that it is mainly grown by poor people for subsistence or sales within local regions and commercial breeding companies have shown little interest in this crop (Hall *et al*, 1997). The development of cultivars with resistance to diseases incited by bacterial and fungal pathogens has been a major goal of most cowpea breeding programs since the early part of the 19^{th} century (Fery and Singh, 1997). Consequently, Singh *et al*., (1997) reported that a range of varieties have been developed, combining diverse plant type and maturity with resistance to several diseases, insect pests and parasitic weeds, using available genetic resources.

The use of traditional breeding approaches in the development of improved varieties of crop plant typically takes more than a decade to complete, due to the need to employ sequential and repeated phenotypic evaluations, and performance trials. Molecular marker-assisted selection (MAS) is a new breeding strategy that is based on

selection with markers that are linked to traits or to estimates of genotypic effects (quantitative trait loci or QTL) and have the potential to expedite delivery of improved crop varieties (Ehlers *et al*., 2012). However, little progress will be achieved in the improvement of a crop species having narrow genetic background even with the application of modern breeding technology. Therefore, widening the genetic base of the germplasm is a key factor to achieving progress in cowpea improvement.

2.8 Mutation breeding

Genetic variation is the fuel for adaptation under artificial or natural selection, and ultimately this has its origin in mutation (Keightley, 2004). Although the germplasm of naturally occurring genetic variability of a crop species is kept and maintained by plant breeders, efforts are still being made to obtain new variability through spontaneous mutations or create novel variability through induced mutations by physical or chemical agents (Hadjichristodoulou, 1991). According to Sigurbjornsson (1972), recent experience and indeed success in plant breeding has clearly shown that the mastering of mutation breeding techniques may become crucial to further success in the breeding of many crop species. Furthermore, Sigurbjornsson (1972) and Muthusamy (2005) outlined three reasons for which plant breeders are now paying more attention to mutation induction and breeding. First, for some crops, especially the cereals, the breeding intensity has been so great that for some ecological regions it will be increasingly difficult to achieve further progress only from germplasm already existing and readily available. The second reason is the rapid and alarming erosion of our genetic resources. These resources are vital if sustained progress in plant breeding is to be expected. Third, the method does not seem to be promising in theory but has in the last few years already given rise to a number of agronomically significant crop varieties.

Hall *et al.*, (1997), further stressed that when incorporating recessive traits, backcross breeding can be slow or require considerable effort. For these traits, mutation breeding can be effective, especially if the trait is easy to screen. The greatest significance of mutations lies in the fact that it can create something new that did not exist before (Gaul, 1964). It also provides alleles that are required for various types of

genetic studies of populations (Goodenough, 1964; Gardner *et al*., 1991). Fawole (1997), among many other authors, stressed the possibility of using the mutants in cultivar improvement, physiological studies and the development of a genetic linkage map for cowpea. In addition to these vital roles in plant breeding, a new role of induced mutation in releasing of gene silencing in transgenic plants has been reported (Bhatia, 1999).

2.9 Induced mutation techniques

From the time of Stadler (1928a, 1928b), artificial agents such as chemicals and radiation mutagens have been reported to effect new genetic changes in plants. Examples of such chemical mutagens include mustard gas, hydroxyl amine, epoxide, urea and alkylating agents such as ethyl methane sulphonate (EMS), while radiations used as mutagens include X-rays, Gamma-rays, Beta-rays, ultraviolet-rays and Neutrons.

In the beginning, mutation breeding was based primarily upon X- rays, but now gamma rays, thermal neutrons (Micke *et al*., 1990) and other forms of ionizing radiations are used. However the, fear of detrimental effects of human exposure to ionizing radiation made a number of researchers put their hope upon chemical mutagens. Of all the known chemical mutagens, EMS has been reported to be more potent when compared with other mutagens (Chopra and Swaminathan, 1966; Strickberger, 1976) and is capable of inducing high density of point mutations (Greene *et al.*, 2003). However, several practical problems with chemical mutagens have been identified by Micke *et al*. (1990), which include soaking of seeds, penetration of the relevant target cells, safety of handling and disposal, poor reproducibility, and persistence of the mutagen or its metabolites. Various authors have however reported high frequencies of chromosomal aberrations following high dosage of radiations such as X-rays (Ojomo and Chheda, 1975) and fast neutrons. Micke *et al.*, (1990) further stressed that ionizing radiations have the advantage of good penetration, precise dosimetry and a wide random spectrum of mutations.

Generally, irradiation of seed at dormancy is used for mutation induction in annual seed propagated crop plants. However, this leads to chimerism of resulting tissues and the need for greater screening to identify the resulting mutations (Yang *et al*., 2004). Further limitations of the seed treatment are represented by the possible occurrence of

separated female and male germ cell primordia and somatic selection against mutant cells during plant development (Gavazzi and Sanguineti, 1983; Yang *et al.*, 2004).

A new approach to induce mutation in plant breeding is the use of irradiated pollen as the starting material for mutagenesis. This technique was initiated by Pandey (1975, 1978). Grant *et al.*, (1980) further proposed injection into the egg of pulverized donor DNA from the irradiated pollen tube, a phenomenon called "egg transformation" (Pandey, 1980a, 1980b). In addition, irradiated pollen may be used for limited gene transfer as a self-contained "DNA injection" system in which the irradiated pollen is both a source of donor DNA fragment as well as a vector for delivering the genetic fragment to the embryo sac (Chin and Gordon, 1989a).

Chin and Gordon (1989b) also suggested that pollination with irradiated pollen can cause a shift in the segregation ratio towards the maternal phenotype in the second (M_2) generation, thereby making the technique useful in practical plant breeding.

Mutation induction in higher plants through irradiated pollen have been reported in many crops; by the use of U.V. radiation in maize (Gavazzi and Sanguineti, 1983) and gamma radiation in *Nicotiana* (Pandey, 1975; 1978; 1980a, 1980b), maize (Pandey, 1983; Sanford *et al*., 1984a), in rice (Chin and Gordon 1989a; 1989b; Sanford *et al*., 1984b) and Arabidopsis (Yang *et al*., 2004). However, the use of irradiated pollen for mutation induction has not been given much attention in cowpea breeding.

2.10 Mutagenesis by gamma radiation and mode of action

Among the radiation-based methods, gamma-ray and fast neutron bombardment now supersedes X-ray in most applications. Of these, gamma irradiation is known to be the most effective in inducing a wide range of mutations (Bado *et al*., 2015). Gamma-rays penetrate deeply into target tissues than other radiations (Mba *et al*., 2010) and it is less destructive, whereas fast neutron bombardment causes translocations, chromosome losses and large deletions (Sikora *et al*., 2011). It is an electromagnetic radiation with short wavelength which has the tendency to dislodge an electron from its orbit around the nucleus, thereby producing an ion as the corresponding proton becomes positively charged and release energy (ionization or ion pairs) as its passes through a tissue. The

principal effect of ionizing radiation is the ionization of water, which forms highly reactive hydroxyl radicals (Microbiology on-line, 2009). These radical react with cellular components, especially DNA to cause mutations.

2.11 Mutagenesis by UV radiation and mode of action

Application of ultraviolet radiation has been less reported among the radiation mutagens used for pollen treatment. UV is an electromagnetic radiation that does not carry enough energy per quantum to ionize atoms or molecules. It has longer wavelength (100–400 nm) with low penetration power into plant tissues when compared to ionizing radiations. According to Mba, et al. (2012), UV radiation is classified based on their wavelengths into three forms, ultraviolet A (UVA) 315-400 nm, ultraviolet B (UVB) 280-315 nm and ultraviolet C (UVC) 100-280 nm. UVC has been implied to be the most energetic and biologically damaging among the three. The mutagenic effect of UV is due to its ability to react with DNA and other biological molecules such as bases in DNA molecules and other aromatic amino acids of proteins. UVB and UVC produce pyrimidine dimers on reacting with DNA, while UVA produces very few of these. The pyrimidine dimers produced form lesions that interfere with transcription and DNA replication, lead to mutations, chromosomal rearrangements and lethality.

2.12 Molecular consequences of mutations

Mutations may occur in any stage in the cell life cycle. According to Gardner *et al*. (1991) the immediate consequence of the mutation and its ability to produce a phenotypic change are determined by its dominance, the type of cell in which it occurs and when it happens relative to the life cycle of the organism. However from the molecular point of view, the effects of any gene mutation on an organism will vary depending upon the functional region of the gene (either promoter, intron, coding, or 3' untranslated regions) where the mutation occurs, and whether the function of an essential protein has been altered (Lee *et al*., 2012). The effect of mutations on the function of a gene will be determined by the type of changes that has occurred in the nucleotide sequence.

2.13 Classes of gene mutations

Generally, mutations are classified based on the type and the extent of molecular changes in the nucleic acid affected by mutational event. Gene mutations occurs when there is a small-scale change involving one or a few nucleotides, while chromosomal aberration involves a large-scale mutation that affects the structure of the chromosome. Gene mutations may be further classified into three types as illustrated (below) by Lee *et al*., (2012):

Point mutations (substitution mutations)

Transition: A→G, G→A, C→T and T→C

Transversion: A→C, A→T, C→A, C→G, T→A, T→C, G→C and G→T

Silent mutation: GTC(Val)→GTA(Val)

Missense mutation: GTC(Val)→TTC(Phe)

Non-sense mutation: AAG(Lys)→TAG(Stop)

Insertion and Deletion (Indels)

Insertion: ATATGTATAAAG→ATATGTCTGATAAAG

Deletion: ATATGTATAAAG→ATAATAAAG

Frameshift mutation: GTC CTG TTA A…TAA(Stop)→TCC TGT TAA(Stop)

GTC CTG TTA A…TAA(Stop)→GAG TCC TGT TAA(Stop)

Inversion

5’AGGTTTGCCTACTGG 3’→5’ AGCGTTTGCCTACTGG 3’

CHAPTER 3

MATERIALS AND METHODS

3.1 Selection of cowpea accessions and seed multiplication

Eight cowpea accessions were used as parents in this study. These are the cultivar Ife Brown (IB) and 3 mutants derived from it (Ife Brown Yellow1 (IB-Y-1), Ife Branched Peduncle (IB-BPC) and Ife Brown Crinkled (IB-CR)) and four elite cultivars (IT86D-719, IT86D-1010, IT894KD-374-57 and IT90K-284-2). The morphological characteristics of these accessions are shown in Table 3.1. Ife Brown and its mutant derivatives were collected from the Genetics unit of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, while the four elite cultivars were obtained from the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA), Ibadan.

All the plants used for this study were raised at the roof top garden of CPEB, University of Ibadan. Four seeds of the cowpea lines were planted in each of the pots filled with top soil. Plant seedlings were thinned to one stand per pot two weeks after planting to avoid competition among the seedlings. The plants were watered daily and weeding was carried out as soon as weeds were observed. Insect pests were controlled by spraying with Cyper DiForce^{R} (Cypermethrin + Dimethoate) at the rate of 1 litre per hectare two weeks after emergence and every ten days afterwards, while 2 kg per hectare dose of Z – Force^{R} (Mancozeb) was used as fungicide when necessary. At maturity, dry pods were harvested from each of the accessions into separate envelopes and stored till further use.

Table 3.1. Morphological characteristics of cowpea accessions used in the study

Cowpea accession	Source	Plant type	Terminal leaflet shape	Leaf colour	Peduncle type	Leaf texture	Flower pigmentation	Pod anthocyanin pigmentation	Seed colour	Seed texture	Pedigree
IB (Ife Brown)	UI, Nigeria	Semi-erect	Subglobose	Green	Not branched	Smooth	2	None	Brown	Rough	Cultivar
IB-Y-1	UI, Nigeria	Semi-erect	Subglobose	Yellow	Not branched	Smooth	2	None	Brown	Rough	Mutant of IB
IB-BPC	UI, Nigeria	Semi-erect	Subglobose	Green	Branched	Smooth	2	None	Brown	Rough	Mutant of IB / Variety
IB-CR	UI, Nigeria	Semi-erect	Subglobose	Green	Not branched	Crinkle	2	None	Brown	Rough	Mutant of IB
IT86D-719	IITA, Nigeria	Semi-erect	Subglobose	Green	Not branched	Smooth	1	Whole pod	White	Rough	Elite cultivar
IT86D-1010	IITA, Nigeria	Semi-erect	Hastate	Green	Not branched	Smooth	2	Tip only	White	Smooth	Elite cultivar
IT89KD-374-57	IITA, Nigeria	Semi-spreading	Subglobose	Green	Not branched	Smooth	3	None	Cream	Rough	Elite cultivar
IT90K-284-2	IITA, Nigeria	Semi-erect	Subglobose	Green	Not branched	Smooth	4	None	Tan	Smooth	Elite cultivar

UI = University of Ibadan; IITA = International Institute of Tropical Agriculture

Flower Pigmentation Code:
1 = None
2 = Wing petal is pigmented
3 = Wing petal is pigmented, standard petal is partially pigmented with light base
4 = Wing petal is deeply pigmented, standard is pigmented with V-shaped light base
5 = Completely pigmented

3.2 Experiment 1: Assessment of radio-sensitivity of cowpea to gamma-irradiation by seed treatments

The cowpea accessions IB, IB-Y-1, IB-BPC, IB-CR, IT86D-719, IT86D-1010, IT89KD-374-57 and IT90K-284-2 were used for this experiment. Cowpea mutagenesis by seed irradiation was carried out at the Plant Breeding and Genetics Laboratory of International Atomic Energy Agency (IAEA), Seibersdorf, Austria. Cobalt-60 (^{60}Co) gamma source (Plates 3.1) was used for radiation treatments. The seeds were kept in the desiccator for 3 days. This was to equilibrate the seeds to 8% moisture content prior to ionization with gamma rays. Two hundred seeds each of the 8 cowpea accessions were irradiated with ^{60}Co gamma rays at the treatment dosages of 100, 200, 300, 400, 500 and 0 Gy (control treatment) making a total of 48 treatments. The seeds from each treatment (Table 3.2) were used to raise M_1 plants at the roof-top garden of CPEB, University of Ibadan. The seeds were planted in plastic pots and polyethylene bags filled with top soil. Plants from each treatment were arranged together in rows. The plants were watered daily and weeding was carried out as soon as weeds were observed. Insect pests were controlled by spraying with cypermethrin + dimethoate at the rate of 1 litre/ha two weeks after emergence and every ten days afterwards, while 2 kg/ha of Mancozeb was used as fungicide when necessary. At maturity, all the pods produced from each treatment were harvested together in an envelope dried, and the seeds were shelled for advancement to M_2 generation.

Data were collected on seedling emergence percentage and number of survived plants. Observations were made on changes in morphology among the M_1 generation. Data were also collected from 10 plants selected randomly from each treatment on the following: primary leaf area, seedling height at 3 weeks, terminal leaflet area and plant height at 6 weeks. Data were analysed using descriptive statistics. Gamma radiation lethal dosage 50% (LD_{50}) in cowpea was estimated by using the following procedure:

$$\%\ Seedling\ emergence = \frac{No\ of\ emerged\ seedlings}{No\ of\ irradiated\ seeds\ planted}\ X\ 100$$

$$\%\ Seedling\ survival = \frac{No\ of\ survived\ seedlings}{No\ of\ emerged\ seedlings}\ X\ 100$$

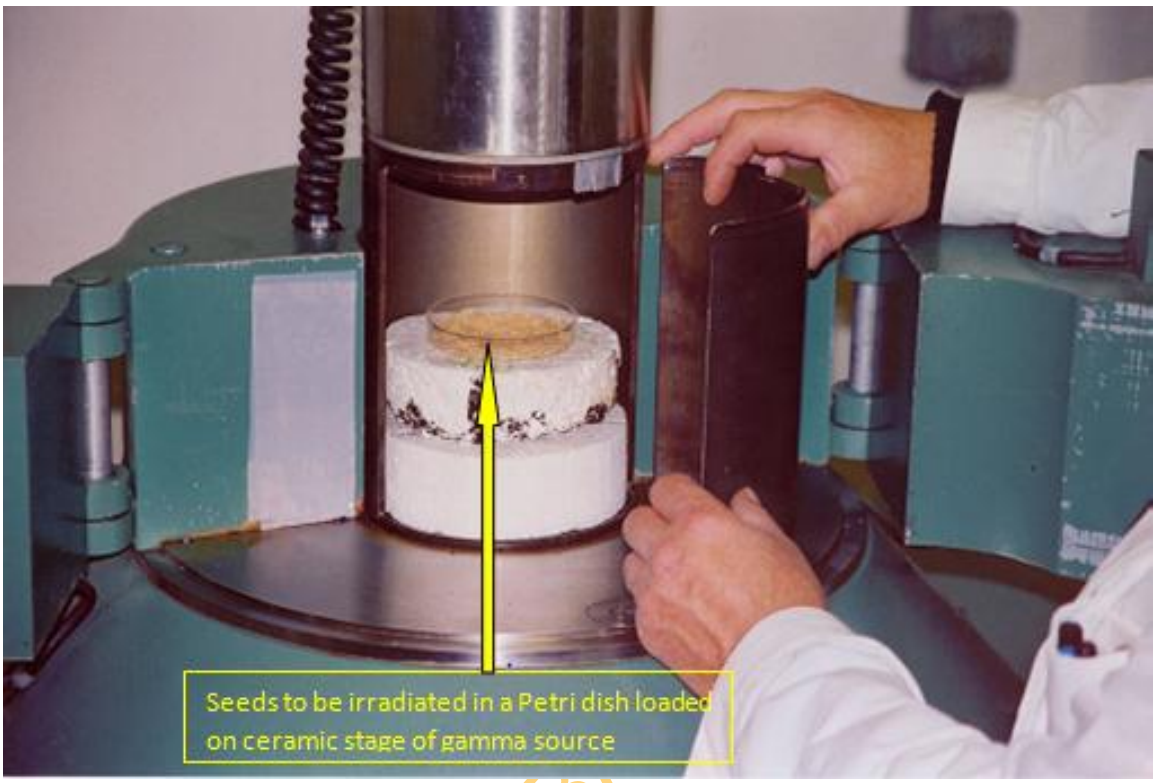

Plate 3.1. Close-up of the raised loading stage of Cobalt-60 gamma source showing seeds to be irradiated at IAEA

Table 3.2. Quantity of gamma irradiated cowpea seeds planted at M_1 in each accession

Accession	Number of seeds planted in each treatment					
	0 Gy	100 Gy	200 Gy	300 Gy	400 Gy	500 Gy
IB	150	170	178	175	182	181
IB-Y-1	150	200	186	190	190	190
IB-BPC	150	194	194	193	194	193
IB-CR	150	165	165	167	167	167
IT86D-719	200	175	190	182	181	182
IT86D-1010	200	200	200	200	200	200
IT89KD-374-57	200	200	200	200	200	200
IT90K-284-2	200	191	200	200	200	200

The difference in seedling survival was expressed as percentages of control using the equation:

$$S = \frac{[Seedling\ survival]_{Tr}}{[Seedling\ survival]_{Co}}\ X\ 100$$

Where: *S* = % difference in seedling survival, *Tr* = Treatment and *Co* = Control

A graph of the absorbed doses was plotted against the percentage differences (Dosage Effect Curve) for each accession to show the damage due to mutagenic treatment. By inserting the 'line of best fit' and reading off the dose corresponding to 50% reduction, the LD_{50} was calculated using the straight line equation: $y = mx + c$.

3.3 Experiment 2: Cowpea mutagenesis by pollen irradiation with UV rays

The cowpea accessions IB, IB-Y-1, IB-BPC, IB-CR, IT86D-719, IT86D-1010, IT89KD-374-57 and IT90K-284-2 were used for this experiment. These cowpea lines were raised at the roof-top garden of CPEB, University of Ibadan to produce flowers. Matured (opened) flowers of each of these cowpea accessions were separately harvested into labeled air-filled transparent nylon bags in the morning (07:00 – 08:00 hour). The flowers were stored in the refrigerator at 10°C until afternoon when their pollen was collected for UV irradiation. Pollen from these flowers was carefully collected from dehisced anthers with the aid of sterile forceps into cell-wells separately. The cell-wells were sealed with paper tape immediately to avoid pollen contaminations. The pollen from each of the cowpea accessions (in cell-wells) were exposed to 30,000 µWs/cm^2 ultraviolet (UV) radiation for 0, 60, 120, 180, 240, 300 and 360 minutes. For each radiation treatment, 20 freshly emasculated pre-anthesis flower buds on the cowpea plant were self pollinated with irradiated pollen grains in the evening time (18:00 – 19:00 hour) and labeled appropriately. All the flowers pollinated on these parent plants were tagged with appropriate labels. At maturity, all dry pods in each treatment were harvested into labeled envelopes and data were collected on the number of seed set in each treatment at M_0 generation. The seeds were prepared for advancement to M_1 generation.

The ultra-violet lethal dosage 50% (LD_{50}) in cowpea was estimated using the following procedure. Percentage seed set at M_0 generation following hand pollination

with UV irradiated pollen was calculated for each treatment. The difference in percentage seed setting between each treatment and control was calculated and expressed as percentages of control. A graph of the absorbed doses was plotted against the percentage differences (Dosage Effect Curve) for each accession to show the damage due to mutagenic treatment by UV on cowpea pollen. By inserting the 'line of best fit' and reading off the dose corresponding to 50% reduction, the LD_{50} was calculated using the straight line equation $y = mx + c$.

The M_1 plants were raised at the roof-top garden of CPEB, University of Ibadan. All the seeds harvested from M_0 generation per treatment (Table 3.3) were planted in plastic pot and polyethylene bags filled with top soil. Plants from each treatment were arranged together in rows. The M_1 plants were observed for any change in morphology when compared with the control treatments. At maturity, all the pods produced by M_1 plants from each treatment were harvested together in labeled envelopes, dried and the seeds were prepared for advancement to M_2 generation.

In the M_1 generation, data were taken on percentage germination and number of surviving plants from each treatment. Screening for mutant (dominant or pseudo-mutant) was carried out by scoring the M_1 plants for any change in phenotype observed when compared with the parent plants (control treatments). Data were also collected from 10 plants selected randomly from each treatment on the following quantitative parameters: primary leaf area, seedling height at 3 weeks, terminal leaflet area and plant height at 6 weeks. Data were analysed using descriptive statistics.

3.4 Experiment 3: Mutant screening and phenotyping

Screening for gamma and UV induced mutants of cowpea in the M_2 generation was carried out on the field at the Teaching and Research Farm of University of Ibadan. The number of seeds planted to each treatment was determined by seed availability as shown in Tables 3.4 and 3.5. For all the treatments, field plantings were made at spacing of 60 cm between rows and 30 cm within the rows. Screening for mutant in each treatment was carried out by scoring the plants from germination to maturity for any change in phenotype observed when compared with the parent plants

Table 3.3. Quantity of seeds planted at M_1 following 30,000 µWs/cm^2 UV radiation treatment of cowpea pollen

Accession	Number of seeds planted in each treatment						
	0 (min)	60 (min)	120 (min)	180 (min)	240 (min)	300 (min)	360 (min)
IB	149	153	122	60	49	29	6
IB-Y-1	56	40	34	22	0	0	0
IB-BPC	98	107	83	55	26	15	0
IB-CR	122	126	120	65	36	26	6
IT86D-719	116	121	87	41	11	3	0
IT86D-1010	96	104	90	29	6	0	0
IT89KD-374-57	120	129	118	44	14	6	0
IT90K-284-2	102	103	84	50	45	14	18

Table 3.4. Quantity of cowpea seeds planted for gamma induced mutation screening at M_2 generation

Gamma Radiation (Gy)	No of seeds planted to each accession							
	IB	IB-Y-1	IB-CR	IB-BPC	IT86D-719	IT-86D-1010	IT89KD -374-57	IT90KD -284-2
0	500	500	500	500	500	500	500	500
100	3000	1500	3000	2000	2000	2000	1750	1500
200	1000	250	250	750	2000	1500	1750	1300
300	0	0	100	300	1700	1000	1500	1300
400	0	0	0	0	750	750	750	750
500	0	0	0	0	500	750	750	500

Table 3.5. Quantity seeds planted for UV induced mutation screening at M_2 generation

Accession	Number of seeds planted to each treatment						
	0 min	60 min	120 min	180 min	240 min	300 min	360 min
IB	500	800	800	580	470	400	300
IB-Y-1	500	500	460	350	0	0	0
IB-BPC	500	1000	1000	570	500	500	500
IB-CR	500	600	450	300	300	250	0
IT86D-719	500	555	435	345	300	250	200
IT86D-1010	500	550	550	300	250	0	0
IT89KD-374-57	500	750	700	500	400	250	0
IT90K-284-2	500	700	550	480	450	300	250

(control treatments). Putative mutants observed at seedling / early growth stage were carefully uprooted with the aid of a hand-trowel, transplanted into plastic pots filled with sterilized soil and transferred to the screen house at the roof-top garden of CPEB, University of Ibadan for adequate care. Selections of putative mutants were made progressively from seedling stage to the maturity stage of the plants on field. Seeds obtained from these putative mutants were later advanced to M_4 generation in the screen house to confirm their mutant phenotypes when compared with their parents. Mutation frequency (rate) per radiation level was calculated as the total number of mutants occurring in 100 plants (Gaul, 1964).

3.5 Experiment 4: Evaluation of cowpea mutants for some morpho-agronomic characters

The cowpea mutants that were selected at M_3 and M_4 in this study were evaluated at M_5 generation with their parents for their distinguishing characteristics. The evaluation study was carried out in the first and second planting seasons of 2013 at the Teaching and Research Farm, University of Ibadan on a 100 m x 75 m plot. A spacing of 45 cm within row and 75 cm between rows was used for all the lines. A Randomized Complete Block Design with four replicates was used for both trials. Weeding operation was done manually when necessary. Insect pests were controlled by spraying with cypermethrin + dimethoate at the rate of 1 litre/ha two weeks after emergence and every ten days afterwards, while 2 kg/ha of mancozeb were used as fungicide when necessary.

Each of the mutant lines was comparatively characterized with their parents. Data were collected from ten randomly selected plants per line on the following parameters:

1. Length of primary leaf (cm)
2. Width of primary leaf (cm)
3. Plant height at 6 weeks (cm)
4. Terminal leaflet area (cm^2)
5. Number of branches per plant
6. Length of peduncle (cm)
7. Number of peduncles per plant

8. Length of branches per plant (cm)
9. Number of pods per peduncle
10. Number of seeds per pod
11. Seed length (cm)
12. Seed width (cm)
13. 100- seed weight (g)

The length of primary leaf was measured from the base of the primary leaf (end of petiole) to the leaf apex with the aid of a ruler, while its width was measured by taking the distance between the widest opposite margins of the primary leaf using a ruler. Plant height was measured from the base of the stem to the meristem region using metre rule. The conversion factors for primary leaf area (0.43) and for terminal leaflet area (0.35) were determined using graphical method. Data on length and breadth of primary leaf and terminal leaflet with their respective conversion factor were used to estimate the primary leaf area and terminal leaflet area. Seed length and width were measured with the aid of vernier caliper, while the 100-seed weight was measured with the aid of weighing balance.

Analysis of variance (ANOVA) was carried out using the Statistical Analysis Systems (SAS). Where significant differences were observed, the means were separated using Least Significant Difference (LSD) at 5% level of probability.

3.6 Experiment 5: Molecular analysis of cowpea mutants

3.6.1 Genomic DNA extraction and quantification

The 32 cowpea genotypes used in this investigation are listed in Table 3.6, twenty four of which were induced mutant lines selected in this study. Seeds of cowpea lines were planted in plastic pots filled with top soil at the roof-top garden of CPEB, University of Ibadan. Young leaf samples of these lines were harvested at four weeks after planting. Harvested leaf samples were stored in freezer at -80^{o}C and later transferred into a lyophilizer for 72 hours to remove the moisture content from the leaf and present the leaf in a dried form for easy grinding. Steel balls were placed in the extraction tubes and samples were punched to bits into each extraction tube. The samples were ground in a

Table 3.6. Cowpea genotypes used for molecular characterization

Genotype	Code	Pedigree	Mutation Source
IB	01	Parent	-
IB-ER	02	Mutant of 01	Gamma ray
IB-ER-2	03	Mutant of 01	Gamma ray
IB-BPC	04	Mutant of 01	Spontaneous mutation
IB-CR	05	Mutant of 01	Spontaneous mutation
IB-LT	06	Mutant of 05	Gamma ray
IB-CR100HT	07	Mutant of 05	Gamma ray
IB-Y-1	08	Mutant of 01	Spontaneous mutation
IB-Y-2	09	Mutant of 01	Ethyl Methane Sulphonate
IT86D-719	10	Parent	-
IT-719Y	11	Mutant of 10	Gamma ray
IT-719BN-1	12	Mutant of 10	Gamma ray
IT-719BN-2	13	Mutant of 10	Gamma ray
IT-719SLY	14	Mutant of 10	Gamma ray
IT-719G200BT	15	Mutant of 10	Gamma ray
IT-719G400MS	16	Mutant of 10	Gamma ray
IT-719FPL	17	Mutant of 10	Gamma ray
IT-719G100DW	18	Mutant of 10	Gamma ray
IT89KD-374-57	19	Parent	-
IT89KD-NL	20	Mutant of 19	Gamma ray
IT89KD-G400UF	21	Mutant of 19	Gamma ray
IT89KD-G400HT	22	Mutant of 19	Gamma ray
IT90K-284-2	23	Parent	-
IT90K-284FPL-2	24	Mutant of 23	Gamma ray
IT90K-284TRV	25	Mutant of 23	Gamma ray
IT90K-UVFPL-REV	26	Mutant of 23	UV ray
IT90K-BS-1	27	Mutant of 23	Gamma ray
IT90K-BS-2	28	Mutant of 23	Gamma ray
IT90K-BS-3	29	Mutant of 23	Gamma ray
IT90K-BS-4	30	Mutant of 23	Gamma ray
IT90K-284SP	31	Mutant of 23	Gamma ray
IT90K500-EM	32	Mutant of 23	Gamma ray

2000 geno-grinder machine for 2 minutes at 150 strokes per minute.

Genomic DNA was extracted from the thirty two leaf samples using modified method of Cetyl trimethyl ammonium bromide (CTAB) and sodium dodecyl sulphate (SDS) extraction protocol in a mini prep format (Dellaporta *et al*., 1983). This technique was used in order to accommodate more plant tissues for higher DNA yield. Extraction buffer was added to the ground sample, vortexed and placed on ice for 30 min. 20% SDS was added to each tube and were incubated in water bath at 65°C for 10 min with continuous agitation. 5M sodium chloride and CTAB buffer was added to each sample and incubated at 65°C for 10 min. The samples were removed from the water bath and 400 µL (24:1) chloroform: isoamyl alcohol added. The sample was then centrifuged at 12,000 rpm for 10 min and the supernatant transferred into new 1.2 mL extraction tube and equal volume of isopropanol was added to it. This was stored at -20°C for 1 hour for DNA precipitation. Samples were removed and centrifuged at 12,000 rpm for 10 min. The supernatant was decanted and the DNA pellet was washed with 70% cold ethanol and air dried. Pellet was re-suspended in 100 µl distilled water and 1.7 µl RNAse was added. DNA concentration and purity was determined using Nanodrop spectrophotometer at absorbance values of 260 nm and 280 nm. DNA quality was checked on 1 % agarose gel electrophoresis.

3.6.2 Simple sequence repeats (SSR) genotyping

DNA sample from the 28 lines and 4 parental lines was genotyped using SSR markers. Sixteen SSR markers were run on the 32 samples and only markers that showed polymorphism among the lines were used (Table 3.7). Markers that were monomorphic among lines were not informative hence were not used.

PCR was carried out in a total volume of 15 µl containing 20 ng of genomic DNA, 1.5 µl of 10X PCR buffer, 1 µl of 25 mM $MgCl_2$, 1 µl of 2.5 mM dNTPs, 0.02 µl Taq polymerase (Inaqaba), 1µl of tween20, 1µl each of forward and reverse primer and 5.32 µl of sterile distilled H_2O. Amplification conditions were: an initial denaturation step of 2 mins at 94°C, followed by 28 cycles each consisting of a denaturation step of 1 min at 94°C, annealing step of 1 min, and a final extension step of 72°C for 5 mins.

Table 3.7. List of SSR markers used in the study

Marker	Primer Sequence 5' - 3'
VM34_F	AGCTCCCCTAACCTGAAT
VM34_R	TAACCCAATAATAAGACACAT
VM37_F	TGTCCGCGTTCTATAAAT
VM37_R	CGAGGATGAAGTAACAGA
VM54_F	CACACACACACATAGATA
VM54_R	TCCATCACTGATCACCTGTT
VM57_F	GGAAGGGGTAGAGGAAAAGTGAA
VM57_R	TGATGATGATGGGTGAATGAGTTG

All amplification reactions were performed using PTC-200 Peltier thermal cycler (MJ Research Inc., Watertown, MA).

Mini acrylamide gel electrophoresis was used for better resolution. The plate were treated by wiping both long and short plates with ethanol. Amplicons (amplified DNA fragments) were run on 6% acrylamide gel electrophoresis (Acrylamide, 10XTBE, 10% Ammonium persulfate and Temmed). The gel was allowed to polymerize and run with 0.5XTBE buffer (45 mM Tris-acetate, 5 mM Boric acid, and 1 mM EDTA, pH 8.0) at 100V for 1 hour. 100 bp ladder was used as a molecular size standard. Gels were visualized by staining with ethidium bromide solution (0.5 µg/ml) and banding patterns was photographed over UV light using UVP-computerized gel photo documentation system.

Microsatellite markers were scored as follows:

- 1 for present alleles
- 0 for absent alleles
- 9 for missing data.

The polymorphic information content (PIC); that is the level of polymorphism shown by each SSR marker for distinguishing cowpea lines, was determined following the procedure of Weir (1996).

PIC = 1 - ΣPi2

Where, Pi is the frequency of the ith allele.

Each SSR fragment was treated as binary matrix in which band presence was coded as 1 for present and 0 for absent. Based on the binary matrix, Euclidean dissimilarity index was computed. Subsequently, using neighbor joining clustering algorithm, a dendogram was generated with the unweighted pair group method using arithmetic average (UPGMA) algorithm of DARwin5.0.158 software (Perrier et al., 2003; Perrier and Jacquemoud-Collet, 2006).

3.6.3 Sequencing reaction and analysis

The SSR markers used in the diversity study were all polymorphic and could not be used for sequencing. Therefore, universal marker; RBCL (ribulose 1,5-bisphosphate

carboxylase) primers were used for this analysis. For the sequencing reactions, the following primers were used:

H535 - 5'CTTTCCAAGGCCCGCCTCA3' for forward sequence

C705 - 5'CATCATCTTTGGTAAAATCAAGTCCA3' for reverse sequence

Genomic DNA was subjected to the following cocktail mix: 1.0 µL of 10X PCR buffer, 1.0 µL of 25 mM $MgCl_2$, 0.5 µL of 5 pMol forward primer (H535), 0.5µL of 5 pMol reverse primer (C705), 1.0 µL DMSO (Dimethyl sulfoxide), 0.8 µL of 2.5 mM DNTPs, 0.1 µL of 5 ug/µL Taq polymerase (Thermoscientific), 2.0 µL of 10 ng/µL of genomic DNA and 3.1 µL of H_2O. PCR was carried out in a total volume of 10 µL containing 20 ng

of genomic DNA. Amplification reactions were performed with Veriti 96 well thermal cycler (AppliedBiosystems) using the following conditions: an initial denaturation step of 5 mins at 94°C, followed by 36 cycles each consisting of a denaturation step of 30 secs at 94°C, annealing step of 30 secs at 56°C, extension temperature of 72°C for 45 secs, a final extension step of 72°C for 7 mins and a hold temperature of 10°C. The PCR product (amplicon) was loaded on 1.5% Agarose to check the amplification, after which the amplicon was purified.

The PCR product was purified by adding 2vol (20 µL) of absolute ethanol. It was incubated at room temperature for 15 minutes, spun down at 10,000rpm for 15 minutes and the supernatant was decanted. Then 100 µL of 70% ethanol was added, the mixture was votexed and spun down at 10,000rpm for 15 minutes. The supernatant was decanted and PCR product was air dried. 20 µL of DNAse and RNAse free H_2O was added and the product was checked again on 1.5% agarose.

The reactions for 96 – well reaction plates (microcentrifuge tubes) was prepared by adding the following reagents to a separate tube: 4.0 µL of 2.5X terminator ready reaction mix, 2.0 µL of 5X Bigdye Sequencing Buffer (BigDye Terminator v3.1), 3.2 pmol primer, 20 ng PCR product and deionized water was added to make a total volume of 20 µL. It was mixed well and spun down briefly. The sample was loaded on the Veriti 96 well thermal cycler (Applied Biosystems). The tubes were placed in a thermal cycler and set to the correct volume. Initial denaturation step of rapid thermal

ramp to 96°C for 1 min was performed. This was followed by 25 cycles each consisting of a denaturation step of 10secs at 96°C, annealing step of 5 secs at 50°C, extension temperature of 60°C for 4 min, a hold temperature of 4°C and the contents of the tubes in a microcentrifuge were spin down.

To the PCR product in the 96-well reaction plate, 5 µL 125 mM EDTA was added. This was followed by the addition of 60 µL of 100% ethanol to each well to each well. The plate was sealed with plate septa, mixed by inverting 4 times and incubated at room temperature for 15 minutes. The plate was spin down at 3000 rpm for 45 minutes at 4°C. The plate was inverted and it was spin up to 900rpm. To each well, 60 µL of 70% ethanol was added and centrifuge at 3000rpm for 15 min. The plate was inverted and it was spin up to 900rpm for 1 minute and the samples were re-suspended in injection buffer i.e. HID Formamide (Applied Biosystems).

The samples were loaded on 3130xl Genetic Analyzer in order to generate sequence data. DNA sequences were edited and analyzed using BioEdit and MEGA softwares. The phylogenetic reconstruction of the mutant lines including the parents was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsentein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown above the branches (Felsentein, 1985). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 0.0000% sites). Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. Evolutionary analyses were conducted in MEGA5 (Tamura *et al*., 2011).

3.7 Experiment 6: Inheritance studies of cowpea mutants

3.7.1 Inheritance of lettuce-leaf (IB-LT) cowpea mutant

The lettuce-leaf cowpea mutant arose from M_2 generation following seed irradiation of IB-CR (Ife Brown Crinkled) with ^{60}Co gamma at 100 Gy (Appendix 17). The origin and description of normal parent, IB-CR was reported by Fawole (1997). The parent (IB-CR) has deep green crinkled leaves but the lettuce-leaf (IB-LT) mutant has a characteristic pale (slivery-white) and twisted leaves.

Inheritance studies of lettuce-leaf were conducted at the rooftop garden of CPEB Department in crosses between crinkled line and the mutant as well as between the normal lines and the mutant. The following crosses were made:

IB-LT x IB-CR

IB-LT x Ife Brown

IB-LT x IT86D-719

Hybridization was made according to the method described by Rachie *et al*., (1975). In the first method, crosses to produce F_1s were achieved by utilizing open flowers of the pollen parent (male) and one-day pre-anthesis buds of the seed parent (female). The female flower was emasculated in evening (18:00 hr) by using thumb finger nail to cut at the middle part of standard petals, wing petals and keel petal in order to expose the stamens and stigma. The stamens were then removed with fingertips. Flowers from the male parent were picked early in the morning (07:00 hr) of anthesis. The keel petals were carefully removed to expose the pollen grains. Pollen of the male parents was carefully dusted on the stigmatic surface of the emasculated flower. The flower was then tagged with appropriate label. The second method used involves collecting flowers from the male parent into labeled envelope early in the morning (07:00 hr) of anthesis. The envelopes were then kept in air-filled transparent nylon and stored in the refrigerator until evening (18:00 hr). Pollen from these flowers was then used to pollinate freshly emasculated pre-anthesis buds as previously described. The two methods were used so as to maximize efficient utilization of flowers. At maturity, seeds set from the crosses were harvested into labeled envelopes. A portion of the F_1 seeds were sown in plastic pots

filled with top soil. Seeds from each cross were planted in 10 pots at the roof-top garden of CPEB, University of Ibadan. Cultural practices were carried out as earlier described. The seeds harvested from F_1 plants fumigated in containers with Phostoxin^R against storage pest, till they were planted for F_2 generation.

The following generations were produced for each cross P_1, P_2, F_1, F_2, BC_1 and BC_2. These generations were evaluated at the Teaching and Research Farm of University of Ibadan in the early planting season of 2013. A randomized complete block design with three replicates was used. Cultural practices were carried out as previously explained. Six weeks after plating, individual plants were scored for the presence or absence of the following leaf traits: smooth, crinkled, twisted, non-twisted, green or pale colour as well as combination of the traits (joint segregation). The observed F_2 and back cross data were tested for goodness-of-fit to the appropriate genetic ratios by the chi-square test. The formula for the chi-square test is

$$X^2 = \sum \frac{(Oi - Ei)^2}{Ei} = \frac{(O_1 - E_1)^2}{E_1} + \frac{(O_2 - E_2)^2}{E_2} + \cdots \frac{(O_n - E_n)^2}{E_n}$$

Where O = number of observations within a class,

E = expected number in the class according to the hypothesis under test,

n = number of classes (Gomez and Gomez, 1984)

3.7.2 Inheritance of yellow leaf trait in cowpea mutant (IT86D-719Y)

The yellow leaf mutant was selected in the M_2 generation from the seed of IT86D-719 cowpea accession irradiated with ^{60}Co gamma at 200 Gy in this study. The parent (IT86D-719) has normal green leaves but the yellow leaf mutant plant is characterized by yellow folia colouration which is more pronounced at the flush. This mutant is also different from IB-Y-1, a brightly yellow cowpea mutant described by Porbeni (2009).

The inheritance study of yellow leaf mutation was carried out at the roof-top garden of CPEB, University of Ibadan in crosses between the normal lines and the mutant in the following crosses:

IT86D-719Y x IT86D-719

IT86D-719Y x IB

Crosses were made to produce the hybrid seeds following the methods previously described in section 3.6.2. The seeds harvested from the crosses were planted in plastic pots filled with top soil to produce F_1 plants. F_2s and backcrosses to both parents were produced for each of the crosses. Six families viz: P_1, P_2, F_1, BC_1, BC_2 and F_2 were produced for evaluation in each of the crosses. These families were evaluated in the early and late planting seasons of 2013 at the Teaching and Research Farm of the University of Ibadan. Cultural practices were carried out as earlier explained. Data on foliage colours were classified into distinct phenotypic classes and tested for goodness of fit to appropriate genetic ratios using the Chi-square test, as previously described.

IT86D-719Y was crossed with two yellow foliage mutant derivatives of Ife Brown, IB-Y-1 and IB-Y-2 (Fawole, 2003). The seeds produced from the crosses were planted at the rooftop garden of CPEB, University of Ibadan. Individual plants were scored for the presence of yellow or green foliage colour at six weeks after planting. All the seeds harvested from F_1 generation were preserved as previously explained for further observation at F_2. The F_2 and the BCs were planted at the Teaching and Research Farm of the University of Ibadan in the late planting seasons of 2013. Data on foliage colour were taken at six weeks after planting. The observed F_2 and BC data were tested for goodness-of-fit to the appropriate genetic ratios by the chi-square test.

3.7.3 Inheritance of four-primary leaves, fasciated stem and double-standard petals flower mutants

(i) The four-primary leaf mutants

IT-719FPL-2Fas and IT-284-FPL-2 were the mutants that arose from M_2 generation following seed irradiation of IT86D-719 and IT90K-284-2 respectively with ^{60}Co gamma at 300 Gy radiation level in this study. The two parents are characterized by production of two-primary leaves, but the mutants produce four-primary leaves at germination.

(ii) The four primary leaf double-standard petal and fasciated stem mutant

IT-719FPL-2Fas mutant (i above) combines four-primary leaves trait with double standard petal flowers and fasciated stem. This mutant produced flowers with four standard petals which are fused in pairs thus referred to as 'double standard petal trait', whereas the parent produced flowers with one pair of fused standard petals. In addition to these, the mutant stem was flattened with the production more than one leaf at opposite nodes in contrast to alternate nodes with only one leaf per node produced by the parent.

Inheritance studies of four-primary leaf mutants were conducted in crosses between normal lines and the mutants. For the study of four primary leaf trait in the first mutant, the following crosses were made:

(i) IT86D-719 x IT-719FPL-2Fas

IB x IT-719FPL-2Fas

In a similar way, the following crosses were made for the study of four-primary leaf trait in the second mutant:

(ii) IT-284-FPL-2 x IT90K-284-2

IT-284-FPL-2 x IB-CR

In the crosses involving the first four primary-leaves mutant, F_1 seeds were produced using the mutants as pollen donor. This is due to difficulty encountered in the production of hybrids on the mutant as female parent. However, in the crosses involving the second four-primary leaves mutant, hybrid seeds were easily obtained. F_1 generations were raised at the roof-top garden of CPEB, University of Ibadan to produce the F_2 seeds. Six families viz: P_1, P_2, F_1, F_2 and backcrosses were produced for evaluation in each of the crosses. These families were planted for assessment in the early and late planting seasons of 2013 at the Teaching and Research Farm of the University of Ibadan. Cultural practices were carried out as earlier explained. Data were collected on the number of primary leaves per plant at a week after germination. The segregation pattern of this trait was tested for goodness-of-fit to an appropriate Mendelian segregation ratio using Chi-square method as previously stated.

The two four-primary leaves mutants IT-719FPL-2Fas and IT-284-FPL-2 were crossed using IT-284-FPL-2 as the female parent to test their allelic relationship. The resulting F_1 seeds were planted at the roof-top garden of CPEB, University of Ibadan. Six families viz: P_1, P_2, F_1, F_2 and Backcrosses were generated for evaluation in the early and late planting seasons of 2013 at the Teaching and Research Farm of the University. Individual plants in each generation were scored for number of primary leaves at one week after planting. Chi-square was used to test for goodness of fit to an appropriate genetic ratio.

Studies on the inheritance of fasciated stem and four standard petals were conducted at the roof-top garden of CPEB, University of Ibadan in crosses between the normal cowpea lines and the mutant. The following crosses were made:

IT86D-719 x IT-719FPL-2Fas

IB x IT-719FPL-2Fas

In all the crosses, F_1 seeds were produced using the mutant under study as pollen donor. This is due to difficulty encountered in the production of hybrids on the mutant as female parent. The resulting F_1 seeds were planted at the roof-top garden of CPEB, University of Ibadan. Six families viz: P_1, P_2, F_1, F_2 BC_1 and BC_2 were generated for evaluation in the early and late planting seasons of 2013 at the Teaching and Research Farm of the University of Ibadan.

Data on fasciated stem and number of standard petals were classified into distinct phenotypic classes and tested for goodness of fit to appropriate genetic ratios using the Chi-square test, as previously described.

3.7.4 Inheritance of burnt leaf cowpea mutant

Two burnt leaf mutants; IT-719BN-1 and IT-719BN-2 were selected in the M_2 generation from the seed of IT86D-719 cowpea accession irradiated with ^{60}Co gamma at 100 and 300 Gy respectively in this study. The parent (IT86D-719) has normal green leaves whereas, the burnt leaf mutant plants are characterized by pale green leaves which look like fresh leaves exposed to naked flame.

The inheritance study of burnt leaf mutation was carried out at the roof-top garden of CPEB, University of Ibadan in crosses between the normal lines and the two burnt leaf mutants in the following crosses:

(i) IT86D-719 x IT-719BN-1

IT90K-284-2 x IT-719BN-1

(ii) IT-719BN-2 x IT86D-719

IT-719BN-2 x IT90K-284-2

In the crosses involving IT-719BN-1, F_1 seeds were produced using the normal parents, this was due to the difficulty encountered in the production of hybrid seeds on the mutants as female parents. However, for crosses involving the IT-719BN-2, hybrids and backcrosses were easily obtained using the mutant as female parent. In the crosses involving IT-719BN-1, the following six families were produced: P_1, P_2, F_1, BC_1, F_2 and F_3. But for the crosses involving IT-719BN-2 the following seven families were produced: P_1, P_2, F_1, BC_1, BC_2 and F_2. These families were evaluated in the early and late planting seasons of 2013 at the Teaching and Research Farm of the University of Ibadan. Data were collected on the type of leaves produced by individual plants at a week after germination. The segregation pattern of this trait was tested for goodness-of-fit to an appropriate Mendelian segregation ratio using Chi-square method as previously stated.

The allelic relationship of the two mutants IT-719BN-1 and IT-719BN-2 was studied by producing hybrid seeds from the cross; IT-719BN-2 x IT-719BN-1. The resulting F_1 and F_2 seeds were evaluated at the Teaching and Research Farm of the University of Ibadan respectively. Individual plants were scored for leaf traits at the onset of flowering.

3.7.5 Inheritance of tall-erect cowpea mutant (IB-ER)

The tall-erect cowpea mutant arose from M_2 generation following seed irradiation of Ife Brown (IB) with ^{60}Co gamma at 100 Gy. The parent (IB) produced many creeping branches with average size peduncles. But the tall-erect mutant has a characteristic tall, perfectly erect and non-branching stem with long peduncles. The mutant produces very short branches or none at all.

Inheritance studies of tall-erect mutant were conducted at the roof-top garden of CPEB, University of Ibadan in crosses between the parent (Ife Brown) and the mutant (IB-ER) as well as between IT89KD-374-57 (a semi-spreading cowpea) and the mutant. The following crosses were made:

IB-ER x IB

IB-ER x IT89KD-374-57

Crosses were made to produce the hybrid seeds following the methods previously described in section 3.6.2. The seeds harvested from the crosses were planted in plastic pots filled with top soil to produce F_1 plants. F_2s and backcrosses to both parents were produced for each of the crosses. Six families viz: P_1, P_2, F_1, BC_1, BC_2 and F_2 were produced for evaluation in each of the crosses. These families were planted for evaluation in the early and late planting seasons of 2013 at the Teaching and Research Farm of the University of Ibadan. Cultural practices were carried out as earlier explained.

Data on plant architecture were classified into distinct phenotypic classes and tested for goodness of fit to appropriate genetic ratios using the Chi-square test, as previously described.

CHAPTER 4

RESULTS

4.1 Radio-sensitivity of cowpea to gamma-irradiation treatments

4.1.1 Effect of gamma irradiation on cowpea seedling emergence at M_1 generation

Observations on seedling emergence at M_1 generation of all the cowpea accessions studied are presented in Figure 4.1. Gamma-irradiation above 200 Gy reduced seedling emergence of all cowpea accessions and was lethal to Ife Brown and its derivatives at 500 Gy with very low seedling emergence (<20%). However, three of the elite cultivars (IT86D-719, IT86D-1010 and IT89KD-374-57) were moderately tolerant to gamma irradiation; having a range of 35% - 54% emergence at 500 Gy and 400 Gy. The accession IT90K-284-2 was very tolerant to gamma irradiation which recorded high emergence (74% - 94%) across all radiation treatments. In all the cowpea accessions, 65% - 80% emergence was observed at 300 Gy treatments. The seedling emergence at 200 Gy was lower than the control treatment in all the accessions except in Ife Brown, IB-BPC and IT90K-284-2. The seedling emergence at 100 Gy was very high (88% - 98%) in all the cowpea accessions, whereas lower values (82%) and (85%) were observed in IT86D-719 and IT89KD-374-57 (Figure 4.1).

4.1.2 Effect of gamma-irradiation on cowpea seedling survival at M_1 generation

The results of cowpea seedling survival at M_1 generation following cowpea seed irradiation with ^{60}Co gamma ray are presented in Figure 4.2. No seedling survival was recorded at 500 Gy, 400 Gy and 300 Gy in IB, IB-Y1, IB-CR and IB-BPC except in IB-CR and IB-BPC where <5% seedlings survived. Percentage seedling survival at 500 Gy, 400 Gy and 300 Gy (<65%) was lower than the control treatment in IT86D-719, IT86D-1010, IT89KD-374-57 and IT90K-284-2 except in IT90K-284-2 where 80% survival was observed at 300 Gy.

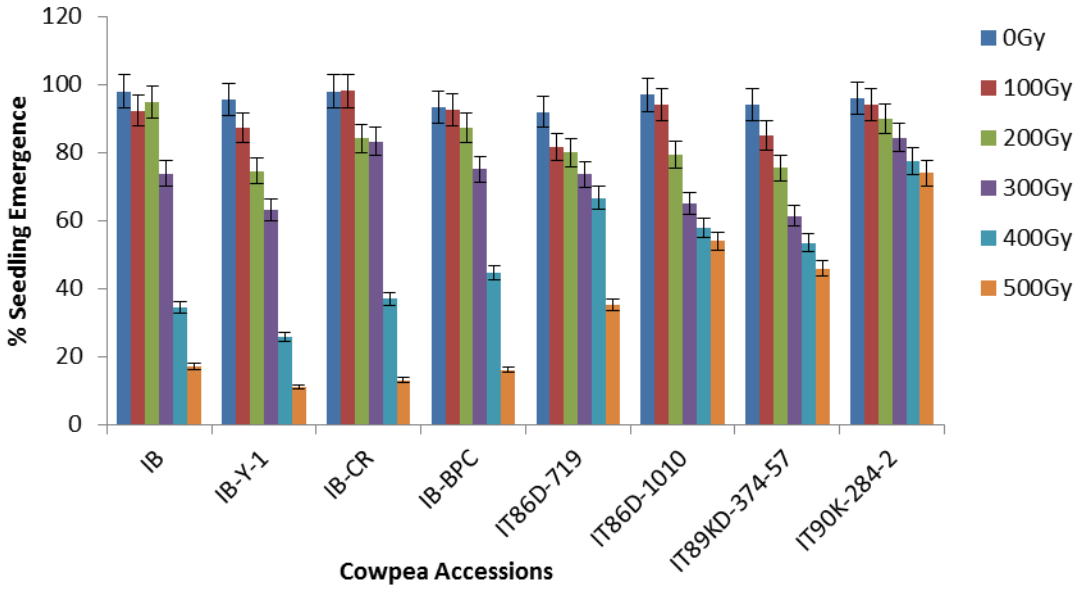

Figure 4.1. Effects of gamma rays on cowpea seedling emergence at M_1 generation

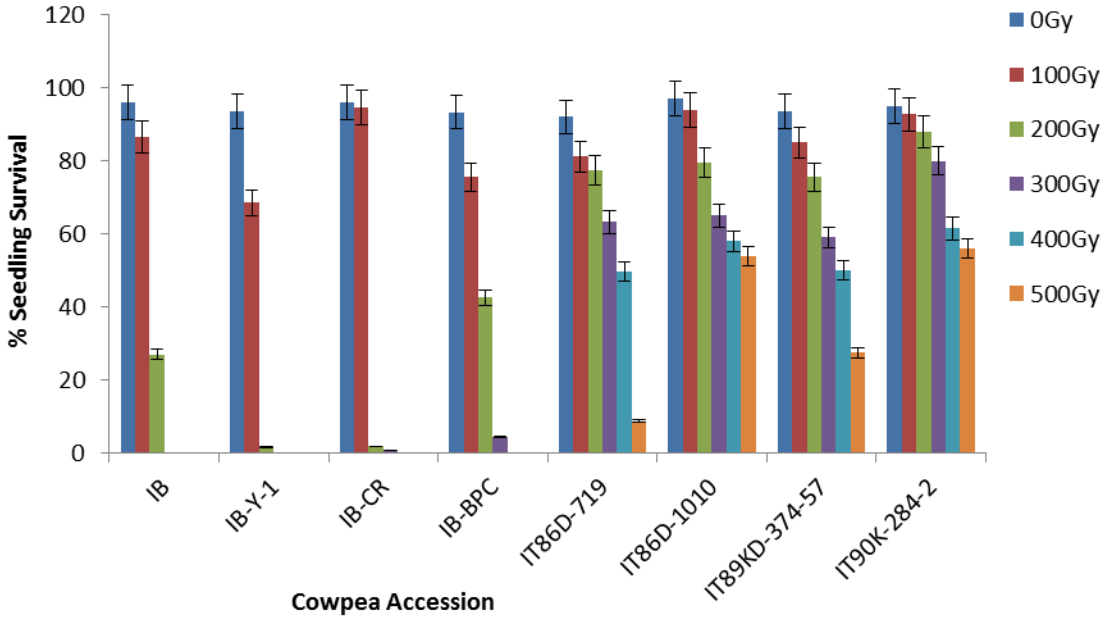

Figure 4.2. Effects of gamma rays on cowpea seedling survival at M_1 generation

Highest value of seedling survival (88%) was observed in IT90K-284-2 at 200Gy when compared with other accessions which ranged from 2% to 79%. The percentage seedling survival recorded for 5 of the 8 cowpea accessions at 100Gy (69% - 87%) were lower than the control treatments (92% - 97%), however high survival value was observed in IB-CR (95%), IT86D-1010 (94%) and IT90K-284-2 (93%).

4.1.3 Gamma irradiation dosage effect curve and lethal dosage 50% (LD_{50}) in cowpea

The gamma irradiation response curves for seedling emergence and seedling survival of the 8 cowpea accessions used in this study are presented in Figures 4.3 and 4.4, respectively, while the LD_{50} for seedling emergence and seedling survival rates are presented in Table 4.1. The rates of seedling emergence and seedling survival reduced as gamma radiation dosage increased in IB and its derivatives. A similar trend was also observed among the 4 elite cowpea cultivars, but more gradually. The dosage effect curves for the cowpea accessions are shown in Appendixes 1 to 16.

A wide variation was observed in the gamma radiation LD_{50} among the 8 cowpea accession studied (Table 4.1). The lowest and the highest LD_{50} of 148.8Gy and 620.2Gy were observed in IB-Y-1 and IT90K-284-2, respectively. IB and its 3 mutant derivatives recorded lower values of gamma radiation LD_{50} which ranged from 148.8Gy and 198.8Gy. The results showed that the optimum gamma radiation dosage for mutation induction varied with cowpea genotypes. It also demonstrates that the lethal effect of high radiation doses would limit the number of M_2 plants available for mutant screening in certain genotypes of cowpea.

4.1.4 Effect of seed characteristics on cowpea sensitivity to gamma rays

The relationship between seed characteristics and relative susceptibility of cowpea cultivars to gamma irradiation is presented in Table 4.1. Cowpea cultivars with a smooth seed coat (IT86D-1010 and IT90K-284-2) recorded higher values of seedling emergence LD_{50} (520.5Gy and 1053.6Gy) and seedling survival LD_{50} (449.4Gy and

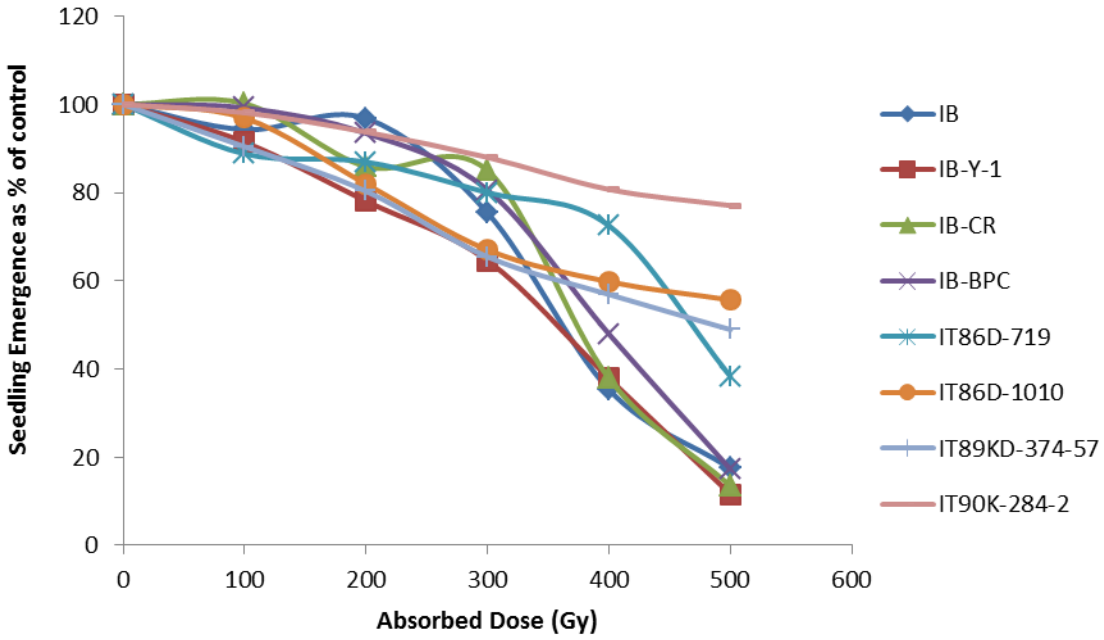

Figure 4.3. Effects of gamma irradiation on emergence rate of cowpea seedlings

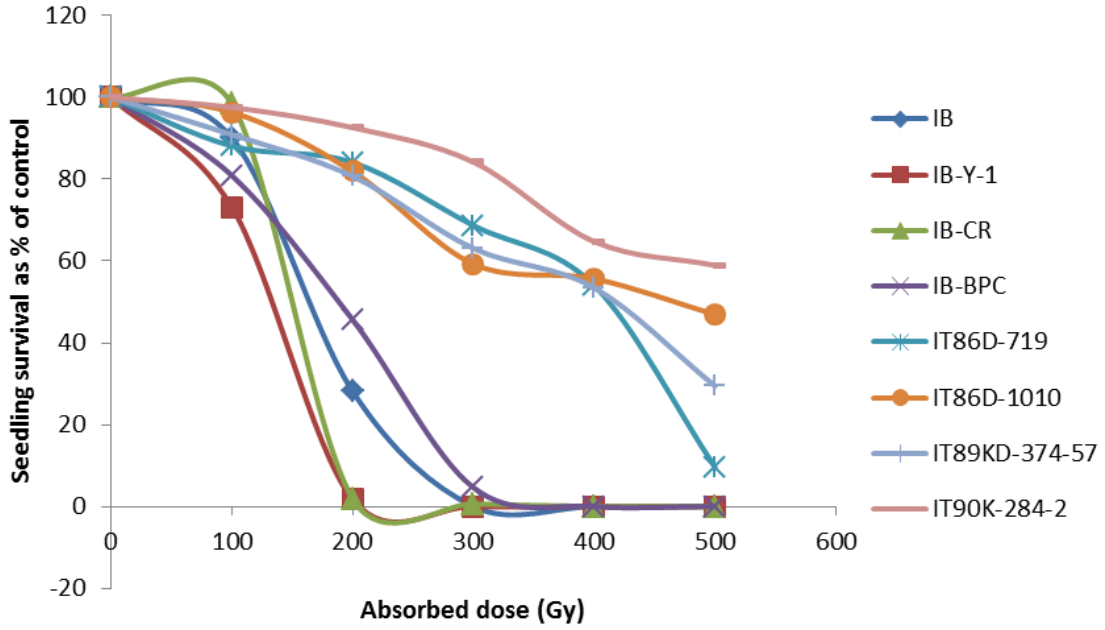

Figure 4.4. Effects of gamma irradiation on cowpea seedling survival rate after seed treatments

Table 4.1. Effect of seed characteristics on cowpea sensitivity to gamma radiation

Cowpea accession	Seed coat texture	Mean testa thickness (mm)	Mean seed weight (g)	$SGLD_{50}$ (Gy)	$SSLD_{50}$ (Gy)
IB	Rough	$0.12 \pm 6.8718 \times 10^{-4}$	$0.15 \pm 3.7884 \times 10^{-4}$	363.9	190.3
IB-Y-1	Rough	$0.11 \pm 6.3246 \times 10^{-4}$	$0.11 \pm 3.2945 \times 10^{-4}$	329.0	148.8
IB-CR	Rough	$0.11 \pm 9.6667 \times 10^{-4}$	$0.14 \pm 5.2872 \times 10^{-3}$	365.1	177.5
IB-BPC	Rough	$0.12 \pm 7.6085 \times 10^{-4}$	$0.15 \pm 2.1492 \times 10^{-4}$	389.1	198.8
IT86D-719	Rough	$0.15 \pm 9.4281 \times 10^{-4}$	$0.15 \pm 2.5382 \times 10^{-4}$	516.2	357.1
IT86D-1010	Smooth	$0.22 \pm 6.5320 \times 10^{-4}$	$0.16 \pm 7.4559 \times 10^{-4}$	520.5	449.4
IT89KD-374-57	Rough	$0.18 \pm 6.3596 \times 10^{-4}$	$0.17 \pm 5.1953 \times 10^{-4}$	473.6	392.0
IT90K-284-2	Smooth	$0.32 \pm 7.4907 \times 10^{-4}$	$0.19 \pm 8.4539 \times 10^{-4}$	1053.6	620.2

$SGLD_{50}$ = Seedling emergence LD_{50}; $SSLD_{50}$ = Seedling survival LD_{50}

620.2Gy) than cultivars with rough seed coat (IB, IB-Y-1, IB-CR, IB-BPC, IT86D-719 and IT89KD-374-57).

The highest mean testa thickness (0.32mm) corresponded to the highest LD_{50}s (1053.6 Gy and 620.2 Gy) for seedling emergence and survival (respectively) were observed in IT90K-284-2, whereas IB-Y-1 which has the thinnest testa (0.11mm) had the lowest LD_{50}s (329 Gy and 148.8 Gy). Although IT90K-284-2 had the highest seed weight (0.19 g) with corresponding highest LD_{50}s (1053.6 Gy and 620.2 Gy), the second highest seed weight observed in IT89KD-374-57 (0.17 g) did not correspond with low LD_{50}s (473.6 Gy and 392 Gy). Similarly, IB, IB-BPC and IT86D-719 had relatively equal seed weights (0.15g), but varied in observed LD_{50}s. From the results, cowpea accessions with rough testa surface and thin testa were more sensitive to gamma irradiation. In general, the testa thickness of cowpea appeared to affect the responses of cowpea seedling emergence and seedling survival to gamma irradiation than cowpea seed weight.

A wide variation in the estimated LD_{50} was observed among the 8 cowpea accession. The lowest LD_{50} for seedling emergence (326 Gy) and seedling survival (148.8 Gy) were recorded for IB-Y-1, while IT90K-284-2 recorded the highest LD_{50} for seedling emergence (1053.6 Gy) and seedling survival (620.2 Gy). Generally, lower values of LD_{50} were observed for IB and its derivatives, which range from 363.9 Gy and 389.1 Gy for seedling emergence and 148.8 Gy to 198.8 Gy for seedling survival.

4.1.5 Effect of gamma irradiation on some growth habits and yield component of cowpea at M_1 generation

Observations on the effects of gamma radiation treatments on some growth habits and yield component of cowpea in the M_1 generation are presented in Tables 4.2 to 4.6. Higher value of primary leaf area was observed in cowpea accessions IB-Y1, IB-BPC, IT86D-719 and IT89KD-374-57 at 100 Gy when compared with their control treatments. In all the cowpea accessions, primary leaf area reduced progressively with increasing radiation treatment (from 200 Gy upward) with the lowest value of primary leaf area observed at 500 Gy in all the accessions studied.

Table 4.2. Effect of gamma radiation on primary leaf area of cowpea in the M_1 generation

Cowpea accession	Primary leaf area (cm^2) at different gamma radiation dosage					
	0 Gy	100 Gy	200 Gy	300 Gy	400 Gy	500 Gy
IB	25.90 ± 1.18	20.56 ± 1.03	6.08 ± 0.48	2.45 ± 0.24	NS	NS
IB-Y-1	20.13 ± 1.50	22.40 ± 1.04	5.63 ± 0.53	NS	NS	NS
IB-CR	16.46 ± 0.77	14.42 ± 0.72	4.48 ± 0.35	2.45 ± 0.36	NS	NS
IB-BPC	24.98 ± 0.94	25.98 ± 0.85	7.67 ± 0.60	2.17 ± 0.26	NS	NS
IT86D-719	19.55 ± 0.71	19.68 ± 0.86	18.51 ± 1.03	11.83 ± 1.06	6.63 ± 0.99	2.37 ± 0.56
IT86D-1010	22.06 ± 1.06	21.91 ± 0.72	15.76 ± 1.13	11.18 ± 0.82	6.61 ± 1.22	4.91 ± 0.72
IT89KD-374-57	26.69 ± 1.24	26.81 ± 1.06	19.79 ± 0.87	12.68 ± 0.85	6.90 ± 0.80	5.13 ± 0.54
IT90K-284-2	25.79 ± 0.78	24.35 ± 2.17	17.42 ± 1.22	9.99 ± 1.55	6.70 ± 0.83	4.24 ± 0.96
SD	3.72	3.96	6.52	4.91	0.13	1.25
CV	16.4	18	54.7	65.2	1.9	30

NS = No survived plant

Table 4.3. Effect of gamma radiation on terminal leaflet area of cowpea in the M_1 generation

Cowpea accession	Terminal leaflet area (cm^2) at different gamma radiation dosage					
	0 Gy	100 Gy	200 Gy	300 Gy	400 Gy	500 Gy
IB	50.16 ± 3.62	62.58 ± 2.55	58.35 ± 3.23	51.08 ± 2.49	NS	NS
IB-Y-1	59.86 ± 3.46	66.02 ± 2.41	64.25 ± 8.60	NS	NS	NS
IB-CR	33.75 ± 3.45	34.79 ± 3.16	34.70 ± 7.57	31.07 ± 0.00	NS	NS
IB-BPC	56.43 ± 3.26	55.12 ± 2.99	57.84 ± 4.09	56.54 ± 6.61	NS	NS
IT86D-719	45.72 ± 1.88	45.87 ± 1.57	45.19 ± 1.74	42.52 ± 1.74	41.97 ± 1.53	44.29 ± 1.55
IT86D-1010	26.26 ± 1.88	27.58 ± 1.57	26.88 ± 1.63	26.52 ± 1.41	25.64 ± 1.84	27.14 ± 1.44
IT89KD-374-57	36.66 ± 2.92	38.44 ± 2.74	37.66 ± 3.15	38.61 ± 2.68	36.94 ± 2.41	36.99 ± 2.92
IT90K-284-2	48.48 ± 3.92	51.03 ± 3.06	48.62 ± 3.98	48.95 ± 2.20	42.55 ± 3.10	47.99 ± 3.09
SD	11.57	13.54	13.05	10.9	7.84	9.19
CV	25.9	28.4	28	25.8	21.3	23.5

NS = No survived plant

Table 4.4. Effect of gamma radiation on seedling height of cowpea in the M_1 generation

Cowpea accession	Seedling height (cm) at different gamma radiation dosage					
	0 Gy	100 Gy	200 Gy	300 Gy	400 Gy	500 Gy
IB	13.04 ± 0.53	13.24 ± 0.49	7.03 ± 0.65	NS	NS	NS
IB-Y-1	9.40 ± 0.35	10.08 ± 0.18	5.73 ± 0.23	NS	NS	NS
IB-CR	13.37 ± 0.46	13.96 ± 0.44	6.47 ± 0.55	5.3 ± 0.00	NS	NS
IB-BPC	11.89 ± 0.43	11.81 ± 0.31	7.00 ± 0.53	6.15 ± 0.14	NS	NS
IT86D-719	11.22 ± 0.21	11.29 ± 0.25	10.23 ± 0.22	6.93 ± 0.40	5.28 ± 0.27	3.53 ± 0.13
IT86D-1010	8.51 ± 0.23	8.66 ± 0.17	8.18 ± 0.20	6.26 ± 0.25	5.94 ± 0.21	3.78 ± 0.13
IT89KD-374-57	7.86 ± 0.19	7.88 ± 0.22	7.45 ± 0.16	6.96 ± 0.17	5.91 ± 0.19	4.23 ± 0.21
IT90K-284-2	8.38 ± 0.14	8.58 ± 0.21	7.93 ± 0.14	7.02 ± 0.11	5.76 ± 0.17	4.23 ± 0.19
SD	2.2	2.26	1.35	0.67	0.31	0.35
CV	21	21.1	18	10.4	5.4	8.9

NS = No survived plant

Table 4.5. Effect of gamma radiation on plant height at six weeks of cowpea in the M_1 generation

Cowpea accession	Plant height at six weeks (cm) at different gamma radiation dosage					
	0 Gy	100 Gy	200 Gy	300 Gy	400 Gy	500 Gy
IB	18.84 ± 0.60	21.94 ± 0.46	14.21 ± 1.42	NS	NS	NS
IB-Y-1	12.41 ± 0.42	16.57 ± 0.71	8.87 ± 0.33	NS	NS	NS
IB-CR	18.85 ± 0.73	20.11 ± 0.58	10.53 ± 0.77	9.50 ± 0.00	NS	NS
IB-BPC	19.10 ± 0.54	20.79 ± 0.58	12.52 ± 0.27	10.83 ± 0.18	NS	NS
IT86D-719	19.59 ± 0.10	18.87 ± 0.21	17.46 ± 0.37	16.27 ± 0.29	14.00 ± 0.27	11.14 ± 0.71
IT86D-1010	20.66 ± 0.06	19.44 ± 0.27	17.86 ± 0.28	16.27 ± 0.29	14.00 ± 0.27	11.14 ± 0.71
IT89KD-374-57	15.93 ± 0.10	16.03 ± 0.15	15.28 ± 0.20	14.49 ± 0.29	12.28 ± 0.49	10.27 ± 0.46
IT90K-284-2	19.77 ± 0.32	20.58 ± 0.32	19.00 ± 0.28	16.60 ± 0.36	14.28 ± 0.39	13.03 ± 0.36
SD	2.69	2.06	3.63	3.08	0.92	1.16
CV	14.8	10.7	25.1	22.1	6.7	10.2

NS = No survived plant

Table 4.6. Effect of gamma radiation on seed setting of cowpea in the M_1 generation

Cowpea accession	Seed setting per plant at different gamma radiation dosage					
	0 Gy	100 Gy	200 Gy	300 Gy	400 Gy	500 Gy
IB	43 ± 4	46 ± 2	18 ± 5	NS	NS	NS
IB-Y-1	36 ± 5	38 ± 3	15 ± 6	NS	NS	NS
IB-CR	44 ± 7	47 ± 4	17 ± 5	13 ± 6	NS	NS
IB-BPC	41 ± 5	43 ± 2	22 ± 4	14 ± 6	NS	NS
IT86D-719	35 ± 6	37 ± 7	27 ± 2	20 ± 5	16 ± 6	16 ± 3
IT86D-1010	38 ± 6	40 ± 2	25 ± 5	17 ± 8	13 ± 4	12 ± 6
IT89KD-374-57	33 ± 3	35 ± 5	26 ± 3	23 ± 6	18 ± 2	16 ± 5
IT90K-284-2	32 ± 7	35 ± 6	21 ± 6	17 ± 3	14 ± 5	12 ± 4
SD	4.53	4.73	4.44	3.72	2.22	2.31
CV	12	11.8	20.8	21.5	14.6	16.5

NS = No survived plant

The terminal leaflet area at 100 Gy and 300 Gy was larger than the control treatments in all the cowpea accessions except in IB-CR (at 300 Gy), IB-BPC (at 100 Gy) and IT86D-719 (at 200 Gy and 300 Gy). The enlargement of terminal leaflet area due to gamma irradiation was also observed in IT89KD-374-57 from 100 Gy to 500 Gy. In IT86D-719 and IT90K-284-2 gamma irradiation reduced terminal leaflet area at 400 Gy and 500 Gy when compared with un-irradiated plants.

The effect of gamma radiation on seedling height of cowpea in the M_1 generation is presented in Table 4.4. Cowpea seedling height at 100 Gy was higher than control treatments in all the accessions except in IB-BPC (11.81cm). Generally, a progressive reduction in the seedling height was recorded at radiation treatments 200 Gy, 300 Gy, 400 Gy and 500 Gy in all the cowpea accessions.

A trend similar to the radiation effect on seedling height was observed in plant height at 6 weeks (Table 4.5). At 100 Gy, plant height was greater than the control treatment in all cowpea accessions except in IT86D-1010 and IT89KD-374-57. From 200 Gy to 500 Gy, plant height reduced progressively with the shortest plants observed at 500 Gy in all the accessions.

Results of the effect of gamma radiation treatments on seed setting of cowpea in the M_1 generation (Table 4.6) revealed a general increase in the seed set per plant at 100 Gy in all the accessions. Further exposure of cowpea seeds to gamma radiation above 100 Gy reduced seed setting of M_1 plants progressively with increasing radiation treatment.

4.2 Radio-sensitivity of cowpea to pollen treatment with UV rays

4.2.1 Effect of UV irradiated pollen on the M_0 generation of cowpea

The mutagenic effect of the treatments as observed in the seed setting at M_0 generation, are presented in Table 4.7. The UV radiation dosage effect curves showed that treatment of fresh pollen grains with 30,000 µWs/cm^2 UV rays for up 60 minutes before pollination increased seed setting in all the cowpea accessions used in this study except IB-Y-1 where it was reduced by 28.6%. Percentage increase in seed setting was highest (9.2%) in IB-BPC at UV irradiation of pollen for 60 minutes, followed by IT86D-1010 (8.3%). Further pollen irradiation with 30,000 µWs/cm^2 UV above 60 minutes prior

Table 4.7. Effect of UV irradiated pollen on cowpea seed setting in the M_0 generation

Cowpea accession	Percentage increase in seed setting at different duration of UV irradiation						LD_{50} (min)
	60min	120min	180min	240min	300min	360min	
IB	2.7	-18.1	-59.7	-67.1	-80.5	-96.0	194.9
IB-Y1	-28.6	-39.3	-60.7	-100.0	-100.0	-100.0	142.6
IB-CR	3.3	-1.6	-46.7	-70.5	-78.7	-95.1	208.1
IB-BPC	9.2	-15.3	-43.9	-52.7	-84.7	-100.0	208.6
IT86D-719	4.3	-25.0	-64.7	-90.5	-97.4	-100.0	170.2
IT86D-1010	8.3	-9.2	-69.8	-93.7	-100.0	-100.0	174.3
IT89KD-374-57	7.5	-1.7	-63.3	-88.3	-95.0	-100.0	183.8
IT90K-284-2	1.0	-17.6	-51.0	-55.9	-86.3	-82.4	210.1

to hand-pollination reduced seed setting of all the cowpea accessions used in this study. Irradiation of cowpea pollen with UV for 60 minutes reduced seed setting of IB-Y1 by 28.6% (Table 4.7). Generally, a close range of LD_{50} values (between 142.6min and 210.1min) were observed for pollen treated with 30,000 µWs/cm^2 UV rays among the 8 cowpea accessions studied as shown in the UV radiation dosage effect curves (Appendixes 17 – 24).

4.2.2 Effects of UV irradiated pollen on seedling emergence and plant survival in the M_1 generation

The results of seedling emergence and survival of M_1 plants are presented in Figures 4.5 and 4.6 respectively. A range of 90% - 100% seedling emergence was observed in all treatments across the eight cowpea accessions studied except radiation treatment 120 min in IT90K-284-2 where a reduction in seedling emergence (83%) was observed. Similarly, a low survival range (90% - 100%) was observed across all treatments in all the cowpea accessions studied except in IB-BPC (87%), IT86D-719 84%) and IT89KD-374-57 (85%) at 300 min. Lower percentage seedling survival (83%) was also observed at 240 min in IT86D-1010.

4.3 Screening and selection for mutant phenotypes in the M_2 and M_3 generations

Based on the observed phenotypic changes and deviation from the phenotypes of each of the parents, diverse mutation spectra were selected in the M_2 plant population in all the gamma induced treatments and M_3 generation of UV induced treatments. The mutation spectra and frequencies observed at M_2 plant generation are presented in Table 4.8. Diverse spectra of mutants were observed and selected in the M_2 plant populations across different treatments applied.

4.3.1 Yellow and white seedling albino mutants

Yellow or white seedling (albino) mutants were observed in most treatments where plants survived at M_1 generation except in all the control treatments. The albino seedlings were chlorophyll deficient lethal mutants that could not survive but died a week after seedling emergence (Plate 4.1).

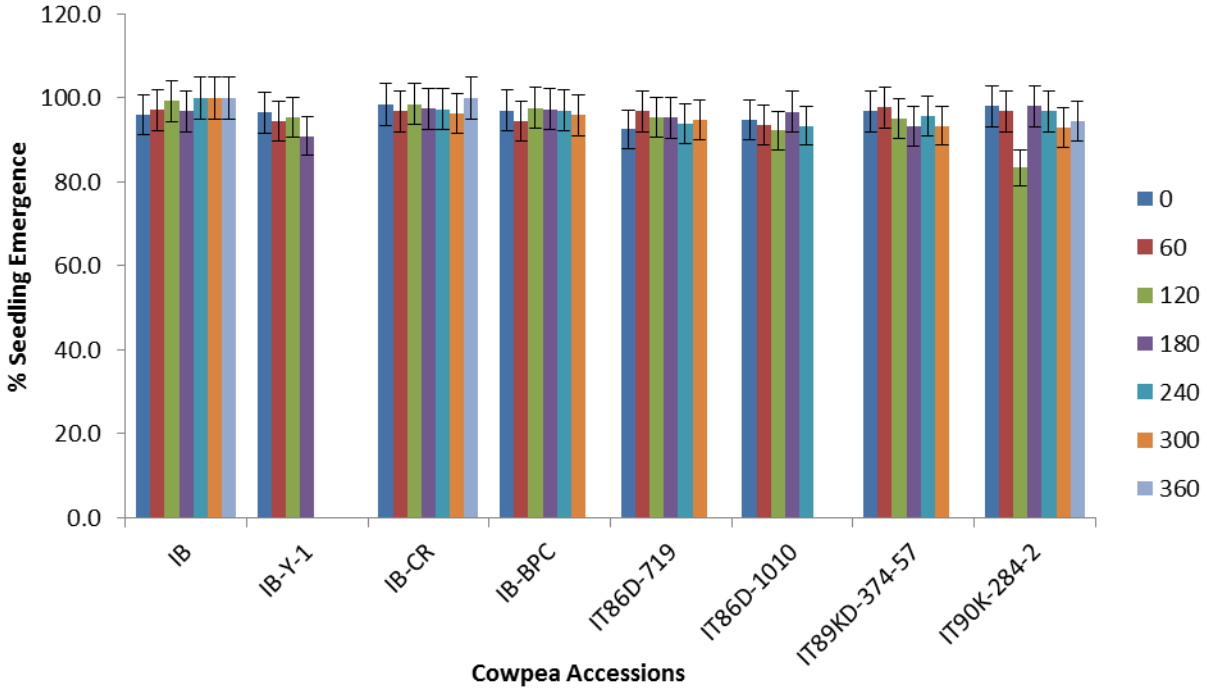

Figure 4.5. Effects of UV rays on the emergence of cowpea seeds at M_1 generation

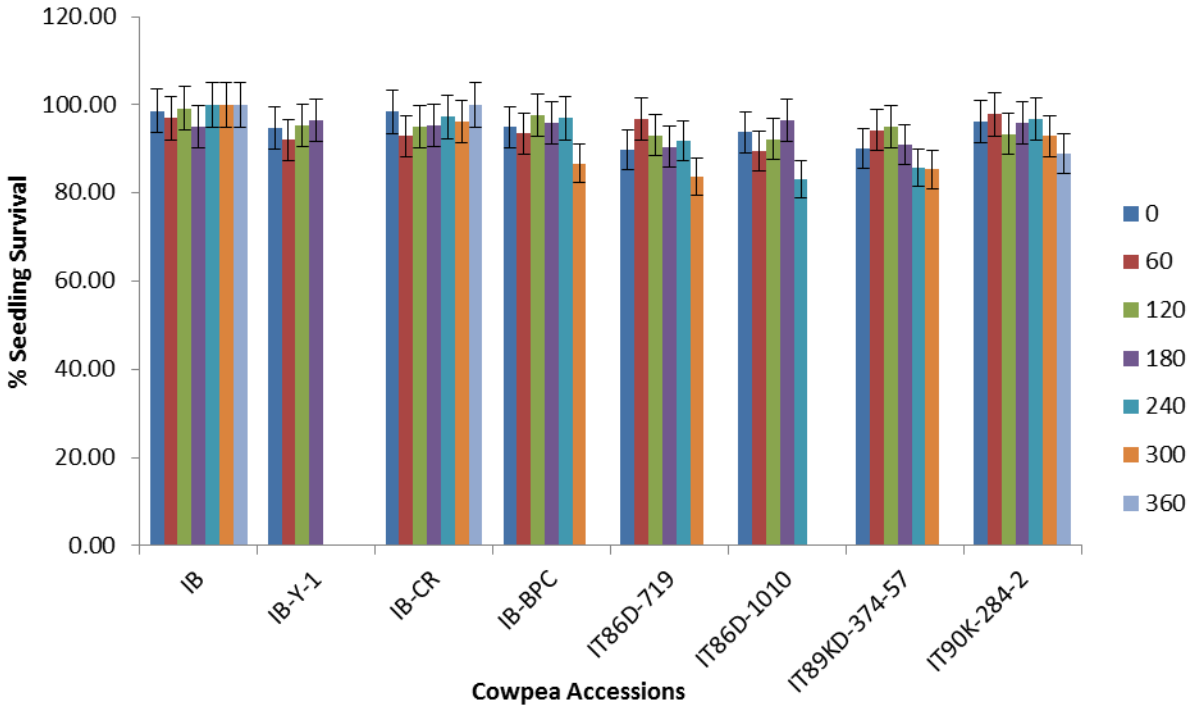

Figure 4.6. Effects of UV rays on the seedling survival of cowpea at M_1 generation

Table 4.8. Spectra and frequencies of mutation in the M_2 generation following ^{60}Co gamma irradiation of cowpea seeds at different dosage levels

Cowpea Accession	Gamma Radiation Dosage (Gy)	Total Number of Plants	Mutation Spectra																	Frequency (%)
			YS	BL	FPL	TPL	YL	VL	SP	NPU	NL	ER	BS	LL	MS	SL	SY	YD	NT	DC	
IB	0	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0000
	100	2990	10	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0.4013
	200	1000	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1000
	300	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	400	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	500	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IB-Y-1	0	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0000
	100	1495	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3344
	200	250	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4000
	300	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	400	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	500	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IB-CR	0	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0000
	100	2995	4	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0.2003
	200	250	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4000
	300	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0000
	400	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	500	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IB-BPC	0	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0000
	100	2000	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1000
	200	748	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1337
	300	300	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3333
	400	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	500	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

YS = Yellow / White Seedling, BL = Burnt Leaf, FPL= Four Primary Leaves, TPL = Three Primary Leaves, YL = Yellow Leaf, VL = Variegated Leaf, SP = Short Pod, NPU = Non-petiolate Unifoliolate, NL = Narrow Leaf seedling, ER = Erect Tall, BS = Big seed, LL = Lettuce Leaf, MS = Male Sterile, SL = Serrated Leaf, SY = Small Leaf Yellow, YD = Yellow Dwarf, NT = Non-petiolate terminal leaflet, DC = Dwarf Crinkled, NS = No plant survived at M_1

Table 4.8. Continued. Spectra and frequencies of mutation in the M_2 generation following ^{60}Co gamma irradiation of cowpea seeds at different dosage levels

Cowpea Accession	Gamma Radiation Dosage (Gy)	Total Number of Plants	Mutation Spectra																	Frequency (%)
			YS	BL	FPL	TPL	YL	VL	SP	NPU	NL	ER	BS	LL	MS	SL	SY	YD	NT	DC	
IT86D-719	0	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0000
	100	2000	8	1	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0.7000
	200	1996	3	0	0	7	2	0	0	0	0	0	0	0	0	2	2	0	0	0	0.8016
	300	1700	9	1	2	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0588
	400	745	8	0	0	4	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1.7450
	500	500	0	0	0	1	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0.8000
IT86D-1010	0	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0000
	100	2000	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2000
	200	1490	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2013
	300	1000	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3000
	400	740	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2703
	500	750	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1333
IT89KD-374-57	0	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0000
	100	1750	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0057
	200	1746	7	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4582
	300	1500	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0.2667
	400	750	7	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1.2000
	500	740	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5405
IT90K-284-2	0	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0000
	100	1500	3	0	0	15	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1.3333
	200	1290	5	0	0	7	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1.0078
	300	1300	0	0	1	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0.3846
	400	748	3	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.9358
	500	500	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0.4000

YS = Yellow / White Seedling, BL = Burnt Leaf, FPL= Four Primary Leaves, TPL = Three Primary Leaves, YL = Yellow Leaf, VL = Variegated Leaf, SP = Short Pod, NPU = Non-petiolate Unifoliolate, NL = Narrow Leaf seedling, ET = Erect Tall, BS = Big seed, LL = Lettuce Leaf, MS = Male Sterile, SL = Serrated Leaf, SY = Small Leaf Yellow, YD = Yellow Dwarf, NT = Non-petiolate terminal leaflet, DC = Dwarf Crinkled, NS = No plant survived at M_1

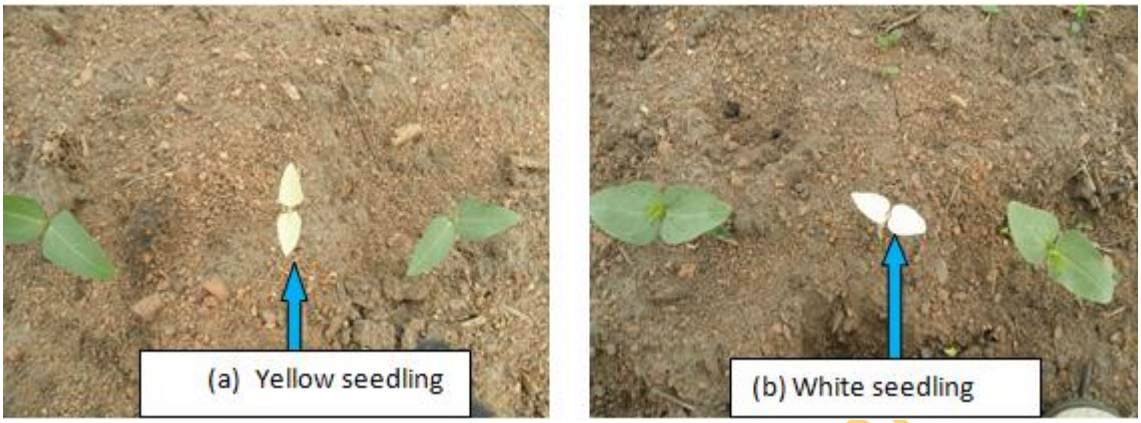

Plate 4.1. Abino seedling (lethal) mutants; (a) Yellow seedling mutant, (b) White seedling mutant

4.3.2 Burnt leaf mutants

Burnt leaf mutants were observed in the radiation treatments 100Gy and 300Gy in IT86D-719. The mutants were characterized by leaves that appear as if they have been partially burnt in a flame of fire. The burnt leaf mutation affects the upper surface feature and colour of the leaves which is partially folded at the margin and pale green in contrast to the straight margin and normal green colour of the parent (Plate 4.2). These mutants bred true at M_3 generation.

4.3.3 Four-primary-leaf, fasciated stem and double standard petals mutant

Three seedlings which produced Four-primary-leaf mutants were observed in the treatment 300Gy of IT86D-719 and IT90K-284-2 (Plate 4.3). The three mutants (IT-719FPL-1, IT-719FPL-2Fas and IT-284-FPL) were selected at M_2 generation on the field and bred true at M_3. The secondary leaves subsequently produced by these mutants were normal trifoliate (Plate 4.3).

In addition to the four-primary leaves produced by IT-719FPL-2Fas, the mutant also produced fasciated stem (Plate 4.4). The fasciated stems of this mutant were characterized with opposite nodes as opposed to alternate nodes produced by the parent and between one and two leaves were produced at each node of the mutant. The mutant grew with more vigor and biomass when compared with the parent. A cross between this mutant and IT90K-284-2 produced fasciated stem F_2 plant with multiple leaves at opposite nodes (Plate 4.5) and another with fasciated peduncle which yielded up to 12 pods on the peduncle (Plate 4.6).

The four-primary leaves mutant (IT-719FPL-2Fas) also produced flowers with double standard petals (Plate 4.7). Dissected flowers of these mutants revealed the presence of extra stamens and carpel (Plates 4.8 and 4.9). Observation on sampled mutants showed five types of flowers that possessed varied number of floral parts on the same plant (Table 4.9). All the mutant flowers possess 12 stamens, whereas the parent flowers produced 10 stamens. In the mutant flowers, the staminate were between 1 and 3 (Plate 4.8). In addition to these, the mutant flowers were observed with one, two and

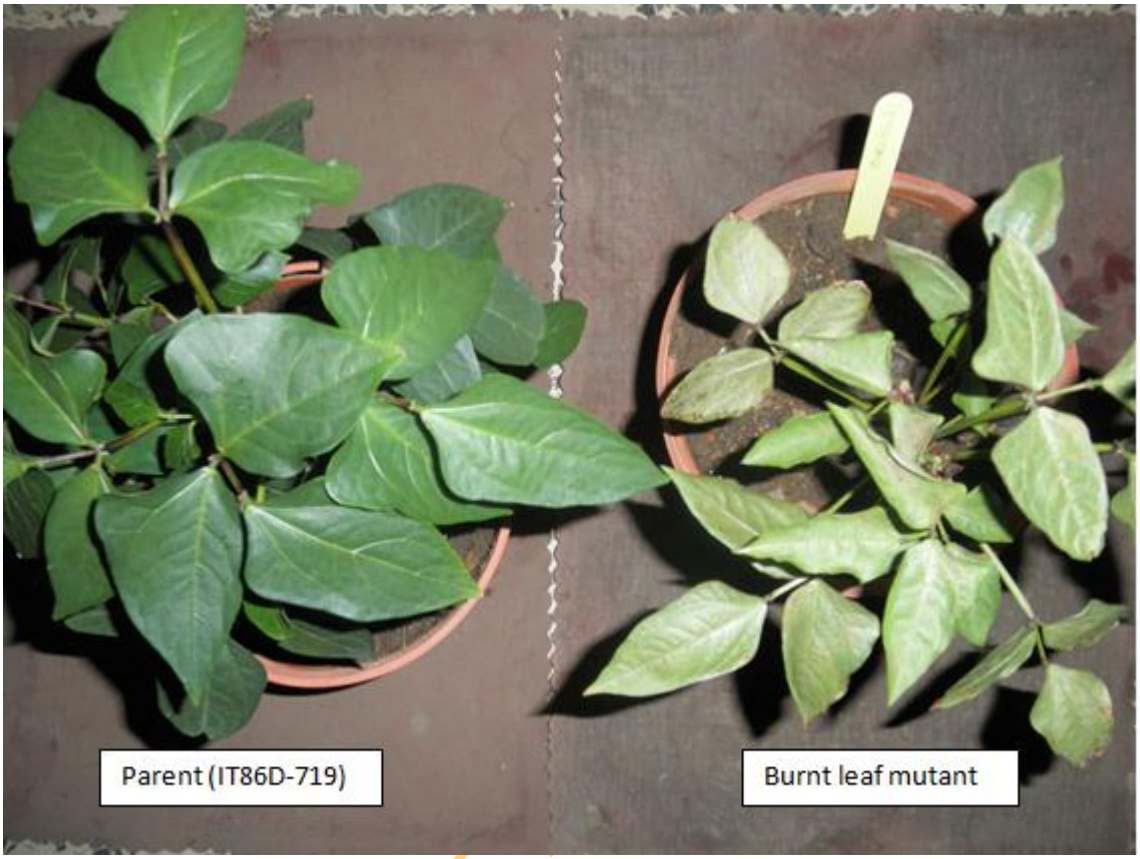

Plate 4.2. The Burnt leaf cowpea mutant (IT-719BN-1) induced by gamma-irradiation

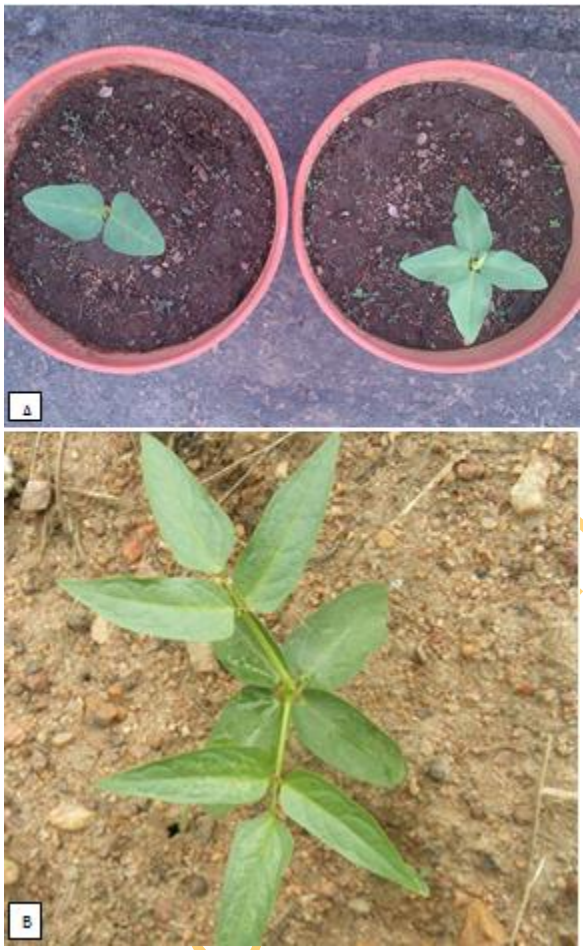

Plate 4.3. Four-primary-leaf mutant (IT-719FPL-1) induced by gamma ray;
(A) Parent (IT86D-719) and the mutant seedlings at emergence
(B) The mutant seedling producing leaves at opposite nodes

Plate 4.4. IT-719FPL-2Fas mutant showing fasciated stem as compared to the parent

Plate 4.5. Fasciated stem mutant producing multiple leaves at opposite nodes

Plate 4.6. Fasciated stem mutant producing fasciated peduncle with many pods

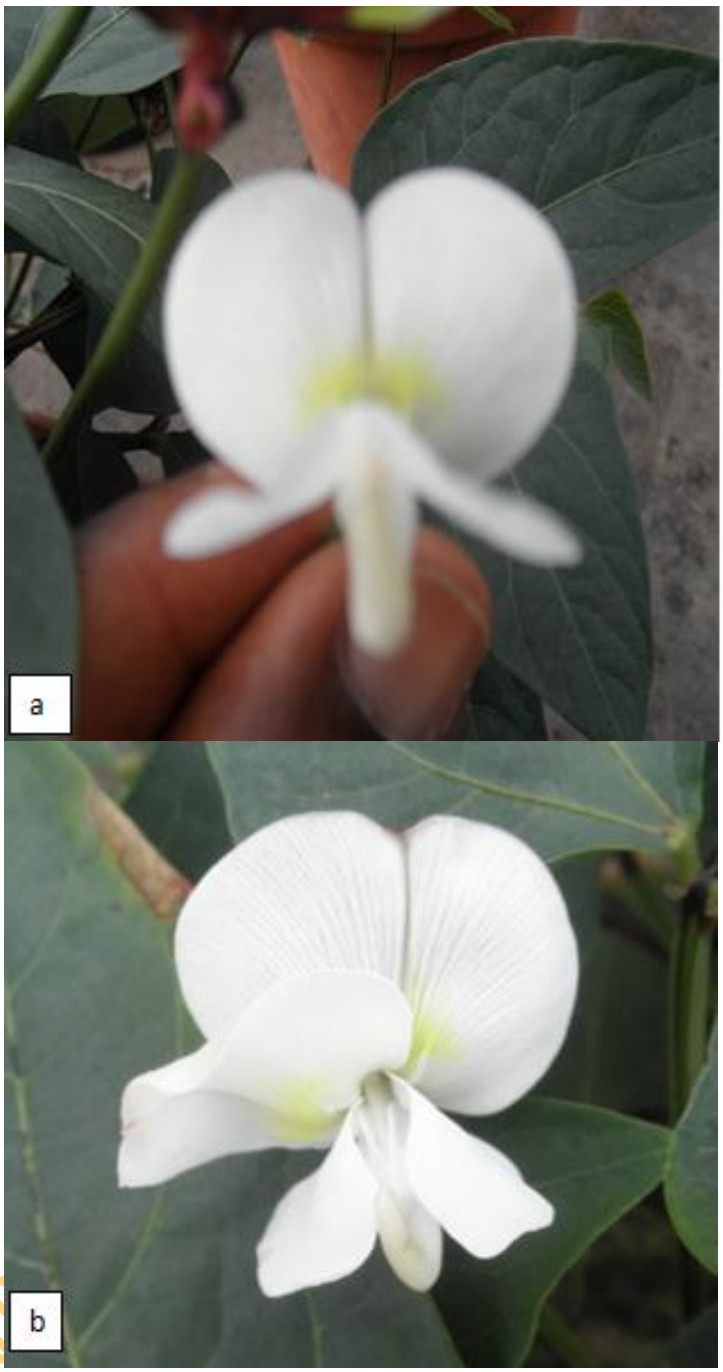

Plate 4.7. Double standard petal flower of the mutant (IT90K-FPL-2Fas) induced by gamma rays; (a) Parent normal cowpea flower showing one standard petal, (b) Mutant flower showing two standard petals

Plate 4.8. Dissected mutant flowers of IT90K-FPL-2Fas producing one and two staminates

Plate 4.9. Dissected mutant flowers of IT90K-FPL-2Fas showing one, two and three carpel

Table 4.9. Types and frequencies of the floral parts produced by four-primary leaf cowpea mutant

Genotype	Mutant Type	Number of floral parts							Frequency of mutant type in the population
		Petal			Stamen			Carpel	
		Standard	Wing	Keel	Fused	Staminate	Total		
IT86D-719 (Parent)	Parent	1	2	2	9	1	10	1	1
IT-719FPL-2	A	2	2	2	11	1	12	1	0.5
(Mutant)	B	2	2	2	10	2	12	1	0.3
	C	2	2	2	10	2	12	2	0.15
	D	2	2	2	9	3	12	1	0.03
	E	2	2	2	9	3	12	3	0.02

three carpel (Plate 4.9). The variation observed in the number of petals, stamens and carpel was such that an increase in the number of one flora part complemented a reduction of the other flora part (Table 4.9).

4.3.4 Three primary leaf mutant

The mutants with three primary leaves at germination (Plate 4.10) were observed at M_2 generation in IB-BPC, IT86D-719, IT86D-1010, IT89KD-374-57 and IT90K-284-2 treated with gamma radiation (Table 4.8) and IT86D-719 and IT90K-284-2 treated with UV radiation. However none of these mutants bred true but they all reverted back to normal seedlings at M_3.

4.3.5 Yellow leaf mutant (yellow-flush)

Yellow leaf mutants were observed and selected from gamma irradiated cowpea IT86D-719 and IT89KD-374-57 at M_2 generation (Table 4.8). However, only one of the yellow mutants selected from IT86D-719 treated with 200Gy radiation (IT86D-719Y) bred true at M_3 generation. This mutant produced yellow leaves which are more significant at flushing and as the leaves grow older, the yellow foliage colour tends to faint (Plate 4.11).

4.3.6 Variegated leaf mutant

Variegated leaf mutant (Plate 4.12) was only observed and selected in IB-CR at radiation level 300Gy. However, the leaf variegation in this mutant was not spread over but limited to a branch of the plant. A variegated pod produced from this branch (Plate 4.13) generated seeds which were planted out at M_3. However, some of the seedlings generated from the variegated pod were devoid of chlorophyll (Plate 4.14) and did not survive while others seeds harvested from the mutant produced M_3 plants that lack variegated leaf trait.

Plate 4.10. Three primary leaf mutant induced by UV radiation

Plate 4.11. Yellow leaf mutant (IT86D-719Y) induced by gamma radiation

Plate 4.12. Variegated leaf mutant of IB-CR induced by gamma ray

Plate 4.13. Variegated pod produced by the variegated leaf mutant of IB-CR

Plate 4.14. Chlorophyll deficient abino seedlings produced by variegated leaf mutant

4.3.7 Non-petiolate unifoliolate mutant

The non-petiolate unifoliolate mutant was selected in IT89KD-374-57 at the treatment level 400Gy. This mutant produced only non-petiolate single leaves (Plate 4.15) instead of the normal trifoliate leaves of cowpea. However, the flower buds produced by this mutant were abnormal (Plate 4.16). The buds failed to produce normal flower and consequently no seed was produce from this mutant.

4.3.8 Narrow leaf seedling mutant

A narrow leaf seedling mutant selected in IT89KD-374-57 at the treatment level 400Gy is presented in Plate 4.17. Apart from the narrow leaf trait of the seedling's primary leaves, the plant produced petiolate leaves with unstable leaflet number which ranged between unifoliolate and trifoliolate (Plate 4.18).This plant bred true for these traits at M_3 generation.

4.3.9 Erect-tall mutant

One erect tall mutant cowpea was selected from the M_2 population of IB at the treatment level 100Gy. This mutant was erect, tall, non-branching with raised peduncles (Plate 4.19) as opposed to the parent which is semi-erect with many spreading branches. The mutant bred true for these traits at M_3 generation.

4.3.10 Big seed mutants

Four big seed mutants were selected from IT90K-284-2 at the treatment levels 100Gy, 200Gy and 300Gy. Only three of these mutants bred true for bigger seeds (Plate 4.20) when compared to the parent seeds at M_3 generation.

4.3.11 Lettuce leaf mutants

The lettuce leaf mutant selected from M_2 population of IB-CR at radiation level 100Gy is presented in Plate 4.21. This mutant (IB-LT) has pale green twisted leaves traits that make it appear like lettuce plant in contrast to the crinkled leaf of the parent.

Plate 4.15. Non-petiolate unifoliolate mutant of IT89KD-347-57 induced by gamma ray

Plate 4.16. Non-petiolate unifoliolate mutant from IT89KD-347-57 producing abnormal flower buds

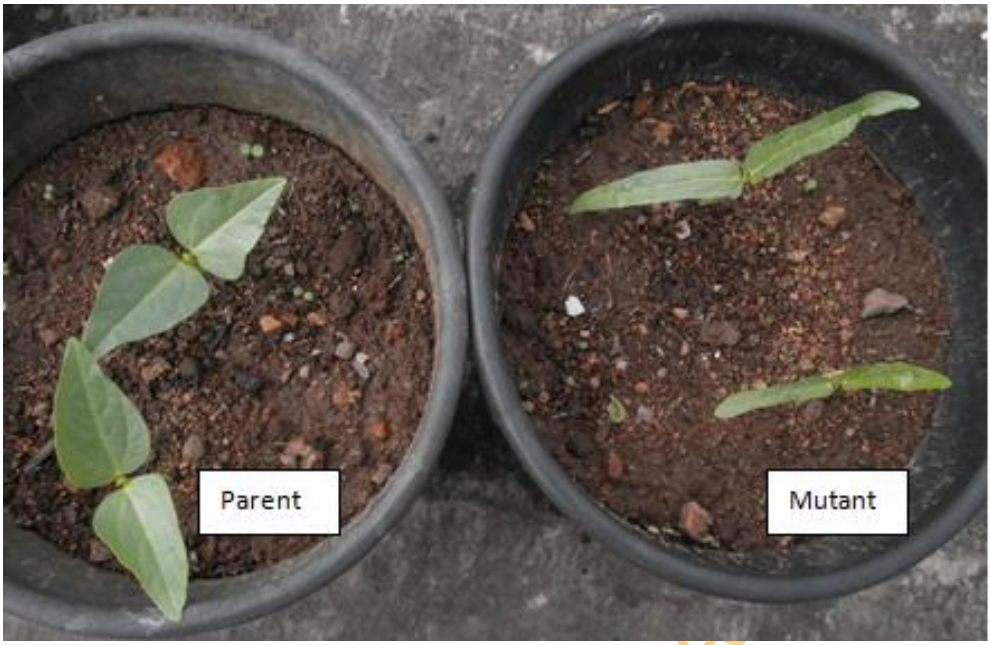

Plate 4.17. Narrow leaf seedling mutant (IT89KD-NL) induced by gamma ray

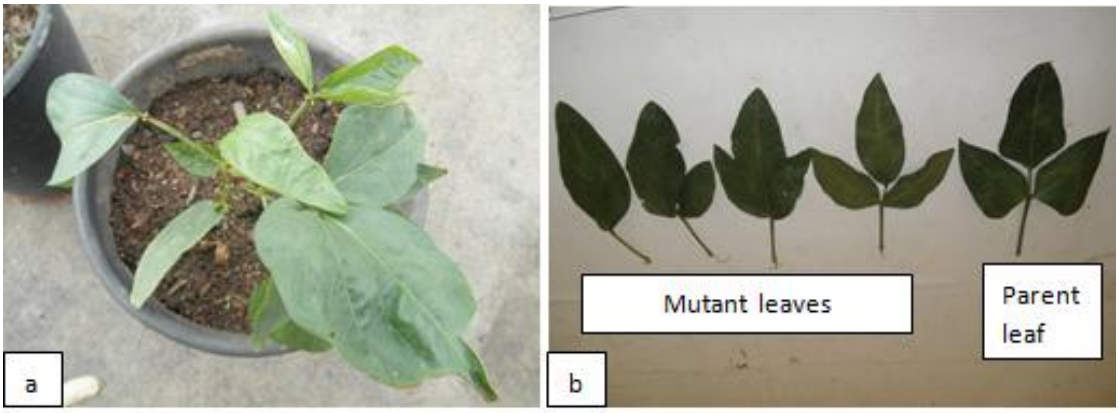

Plate 4.18. Narrow leaf mutant (IT89KD-NL); (a) The mutant plant producing petiolated unifoliolate, difoliolate and trifoliolate leaves, (b) Leaves of mutant compared to parent

Plate 4.19. Erect tall mutant (IB-ER) of Ife Brown cowpea induced by gamma radiation

Plate 4.20. Big seed mutants: (A) Parent seed. (B) Big seed mutant IT90K-BS-1. (C) Big seed mutant IT90K-BS-3. (D) Big seed mutant IT90K-BS-4.

Plate 4.21. Lettuce leaf mutant (IB-LT) of IB-CR induced by gamma radiation

4.3.12 Small leaf yellow mutant

Two small leaf yellow mutants cowpea were selected at M_2 generation from IT86D-719 at treatment level 200Gy. One of these plants (IT-719G200SLY) produced small yellow trifoliate leaves (Plate 4.22), flowers with short style (Plate 4.23) and set seeds at maturity, while the other mutant plant produced small deformed flowers and did not set seed.

4.3.13 Other cowpea mutants

Other mutants observed in this study include male sterile mutants, serrated leaf mutant, yellow dwarf mutant, non-petiolate terminal leaflet mutant (Plate 4.24) and dwarf crinkled mutant. All these mutants could not be advanced to M_3 because they did not produce seed.

4.3.14 Frequencies of gamma-induced morphological mutants of cowpea

The record on frequencies of morphological mutants of cowpea is presented in Table 4.10. No definite trend was observed in the mutation frequencies with respect to radiation treatments in this study. However, higher mutation frequencies were recorded in IT86D-719 and IT90K-284-2 than other cowpea accessions. Accession IB and its derivatives generally had low frequencies of morphological mutants at the dosage sub-ministered.

4.3.15 UV induced mutations spectra and frequencies in the M_2 generation

The spectra and frequencies of observed mutations at all UV radiation durations are presented in Table 4.10. Based on the observed phenotypic changes, only three spectra of mutants were selected across the treatments in the M_2 plant population (Table 4.11). Yellow abino mutant seedlings which died after a week of germination were observed in IB-Y-1 at 60, 120 and 180 min durations. The four-primary leaf and three-primary leaf mutants selected produced the normal trifoliate secondary leaves. However, these mutants were not stable, but reverted back to two-primary leaf plants. The three-primary leaf mutant reverted to two-primary leaf in M_4 generation, while the four-primary leaf

Plate 4.22. Small leaf yellow mutant IT-719G200SLY induced by gamma radiation

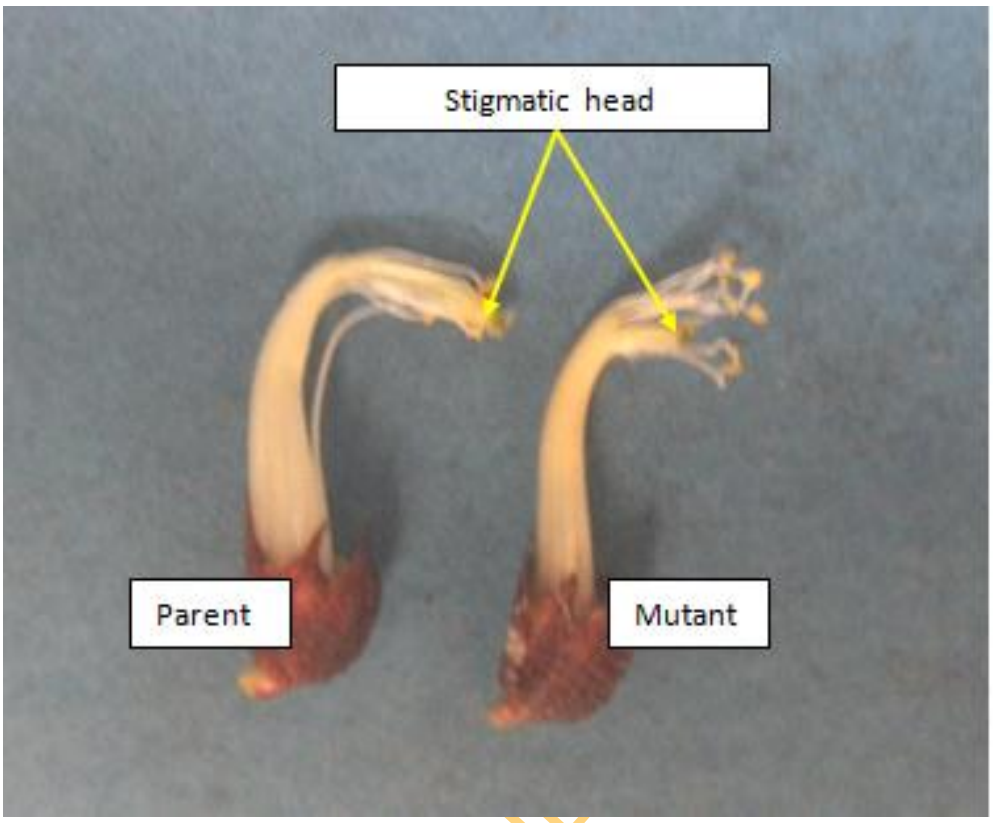

Plate 4.23. Small leaf yellow mutant flower of IT-719G200SLY with short style

Plate 4.24. Non-petiolate terminal leaflet mutant

Table 4.10. Frequencies of gamma induced mutants of cowpea at five radiation levels in the M_2 generation

Cowpea Accession	Mutation Frequencies (%) at Different levels of Gamma rays (%)					
	0Gy	100Gy	200Gy	300Gy	400Gy	500Gy
IB	0.0000	0.4013	0.1000	NS	NS	NS
IB-Y-1	0.0000	0.3344	0.4000	NS	NS	NS
IB-CR	0.0000	0.2003	0.4000	0.0000	NS	NS
IB-BPC	0.0000	0.1000	0.1337	0.3333	NS	NS
IT86D-719	0.0000	0.7000	0.8016	1.0588	1.7450	0.8000
IT86D-1010	0.0000	0.2000	0.2013	0.3000	0.2730	0.1333
IT89KD-374-57	0.0000	0.0057	0.4582	0.2667	1.2000	0.5405
IT90K-284-2	0.0000	1.3333	1.0078	0.3846	0.9358	0.4000

NS = No survival at M_1 generation

Table 4.11. Spectra and frequencies of mutation in the M_2 generation following UV irradiation of cowpea pollen for different treatment duration

Cowpea accession	Duration of UV treatment (min)	Number of M_2 Plants	Mutation Spectra			Mutation Frequency (%)
			Yellow / White Seedling	Four Primary Leaves	Three Primary Leaves	
IB	0	500	0	0	0	0
	60	800	0	0	0	0
	120	800	0	0	0	0
	180	580	0	0	0	0
	240	470	0	0	0	0
	300	400	0	0	0	0
	360	300	0	0	0	0
IB-Y-1	0	500	0	0	0	0
	60	500	2	0	0	0.004
	120	460	2	0	0	0.0043
	180	350	2	0	0	0.0057
	240	NS	0	0	0	0
	300	NS	0	0	0	0
	360	NS	0	0	0	0
IB-CR	0	500	0	0	0	0
	60	1000	0	0	0	0
	120	1000	0	0	0	0
	180	570	0	0	0	0
	240	500	0	0	0	0
	300	500	0	0	0	0
	360	500	0	0	0	0
IB-BPC	0	500	0	0	0	0
	60	600	0	0	0	0
	120	450	0	0	0	0
	180	300	0	0	0	0
	240	300	0	0	0	0
	300	250	0	0	0	0
	360	NS	0	0	0	0

NS = No survival

Table 4.11. continued. . Spectra and frequencies of mutation in the M_2 generation following UV irradiation of cowpea pollen for different treatment duration

Cowpea accession	Duration of UV treatment (min)	Number of M_2 Plants	Mutation Spectra			Mutation Frequency (%)
			Yellow / White Seedling	Four Primary Leaves	Three Primary Leaves	
IT86D-719	0	500	0	0	0	0
	60	550	0	0	0	0
	120	435	0	0	0	0
	180	345	0	0	1	0.0029
	240	300	0	0	0	0
	300	250	0	0	0	0
	360	200	0	0	0	0
IT86D-1010	0	500	0	0	0	0
	60	550	0	0	1	0.0018
	120	550	0	0	0	0
	180	300	0	0	0	0
	240	250	0	0	0	0
	300	NS	0	0	0	0
	360	NS	0	0	0	0
IT89KD-374-57	0	500	0	0	0	0
	60	750	0	0	0	0
	120	700	0	0	0	0
	180	500	0	0	0	0
	240	400	0	0	0	0
	300	250	0	0	0	0
	360	NS	0	0	0	0
IT90K-284-2	0	500	0	0	0	0
	60	700	0	0	2	0.0029
	120	550	0	0	5	0.009
	180	480	0	0	6	0.0125
	240	450	0	0	2	0.0044
	300	300	0	0	1	0.0033
	360	250	0	1	4	0.02

NS = No survival

mutant reverted back to three-primary leaf in the M_4 and finally to two-primary leaf plat in the M_5 generation. The mutation frequencies in the M_3 generation were very low with no mutation observed in most treatments and the highest frequency (0.02%) observed in IT90K-284-2 at 60 min of UV radiation treatment. This suggests that UV radiation is not effective for induced mutation in cowpea.

4.4 Morphological and agronomic evaluation of mutant lines of cowpea and their parents

4.4.1 Variation in growth habit traits and yield components of Ife Brown cowpea and its mutant derivatives

The mean values of the growth habits and yield component traits observed among Ife brown cowpea and its mutant derivatives are presented in Tables 4.12 and 4.13. In the partitioning of phenotypic variance for all the traits studied, observed genotypic variance (V_g) were greater than environmental variance (V_e) except in pod length where the two components were equal.

IB-ER was significantly taller than all other lines with the mean height of 121.36 cm, while IB-Y1 was significantly the shortest with the mean height of 25.41 cm at 6 weeks after planting. IB-LT was significantly taller than IB-CR but was not significantly taller than IB and IB-BPC. The highest values of phenotypic variance (V_P), 1232.74 and V_g (930.62) were recorded for plant height at 6 weeks and the trait (plant height) is highly heritable (H = 0.75) among IB and its derivatives.

The largest mean terminal leaflet area (64.6 cm^2) was observed in IB-ER which was significantly larger than other lines but not significantly different from IB-Y1. Mean terminal leaflet area of IB-LT was significantly larger than 34.42 cm^2 observed in IB-CR but not significantly different from IB. However, the terminal leaflet area (53.62 cm^2) observed in IB was not significantly different from IB-BPC.

Table 4.12. Mean values and variance components of five growth habits of Ife Brown cowpea line and its mutant derivatives

Genotype	PLHSW	TLA	NB	LBPPL	PEDL
IB	66.36cd	53.62bc	4.08ab	58.85a	26.77c
IB-ER	121.36a	64.60a	0.73c	2.17d	60.11a
IB-Y1	25.41e	62.65a	3.90b	33.48c	26.95c
IB-CR	64.99d	34.42d	4.00b	44.56b	23.25d
IB-LT	73.36bc	51.27c	4.43a	44.73b	26.34c
IB-BPC	74.57b	56.71b	3.85b	37.11c	37.54b
Mean	71.01	53.88	3.50	36.82	33.49
SD	32.83	13.86	1.53	19.89	14.40
CV	46.24	25.73	43.79	54.03	20.32
V_p	1232.74	211.36	2.65	454.78	239.07
V_g	930.62	114.66	1.86	361.51	192.66
V_e	302.12	96.70	0.79	93.27	46.41
H	0.75	0.54	0.70	0.79	0.81

PLHSW = Plant height at six weeks after planting, TLA = Terminal leaflet area, NB = Number of branches, LBPPL = Length of branches per plant, PEDL = Length of peduncle

Means with the same letter are not significantly different at 5% level of probability by the Least Significant Difference (LSD)

Table 4.13. Mean values and variance components of five yield related traits of Ife Brown cowpea mutant lines and its mutant derivatives

Genotype	PEDPPL	PODPPED	PODL	SEDPPOD	HSEDW
IB	25.15a	2.93c	13.77ab	12.23b	14.78a
IB-ER	13.50c	3.35b	13.24cd	11.60c	12.87c
IB-Y1	24.48a	2.93c	13.01d	12.13bc	11.40d
IB-CR	21.08b	2.90c	14.05a	13.28a	13.70b
IB-LT	25.55a	2.75c	11.59e	4.00e	12.79c
IB-BPC	25.78a	5.80a	13.50bc	9.05d	14.75a
Mean	22.59	3.44	13.19	10.38	13.38
SD	5.99	1.35	1.17	3.38	1.22
CV	26.53	39.30	8.87	32.54	9.13
V_p	39.69	2.05	1.49	13.32	1.77
V_g	22.35	1.36	0.74	11.72	1.69
V_e	17.33	0.69	0.75	1.60	0.08
H	0.56	0.66	0.49	0.88	0.96

PEDPPL = Number of peduncle per plant, PODPPED = Number of pods per peduncle, PODL = Pod length, SEDPPOD = Number of seeds per pod, HSEDW = Hundred seed weight

Means with the same letter are not significantly different at 5% level of probability by the Least Significant Difference (LSD)

IB-ER had the least number of branches (<1.00) which was significantly different from other lines. IB-LT produced highest number of branches (4.43) which was not significantly different from 4.08 recorded in IB. However, the number of branches recorded in IB, IB-Y1, IB-CR and IB-BPC were not significantly different from each other. High heritability value (0.70) was recorded in this trait.

High value of coefficient of variation (CV), 54.03% was recorded for average length of branches. IB produced 58.85 cm long branches which were significantly longer than the braches of all its mutant derivatives. The 2.17 cm recorded in IB-ER was significantly the shortest among all the lines. The length of branches observed in IB-CR was not significantly different from 44.73 cm recorded in IB-LT. Similarly, average length of branches in IB-Y1 was not significantly different from 33.11 cm observed in IB-BPC. This trait is highly heritable (H = 0.79).

IB-ER had the longest peduncle of 60.11cm which was significantly different from other lines (Table 4.12). However, the 23.25 cm long peduncle produced by IB-CR was significantly the shortest among other cowpea lines. The 26.34 cm long peduncle observed in IB-LT was not significantly different from the peduncle length of IB and IB-Y1. The high value of heritability (0.81) observed among the genotypes showed that the trait is highly heritable. The number of peduncles observed in IB-BPC (25.78) was the highest but not significantly different from IB, IB-Y1 and IB-LT. IB-ER produced the lowest number of peduncle among other lines.

Low values of CV for number of pods per peduncle was generally observed among the cowpea lines in the yield indexes evaluated (the highest being 39.3). IB-BPC had the number of pods per peduncle (5.8) followed by IB-ER (3.35) which were significantly different from others lines. The number of pods per peduncle produced by IB, IB-Y1, IB-CR and IB-LT were not significantly different from one another.

The genotypic variance (V_g) and environmental variance (V_e) observed in pod length were 0.74 and 0.75 respectively. IB-CR had the longest pods (14.05 cm) which was not significantly different from 13.77 cm observed in IB. IB-LT produced the shortest pods (11.59 cm) which was significantly different from other lines. The pod

length observed in IB was not significantly different from that of IB-BPC (13.5 cm) and a similar trend was recorded between IB-BPC and IB-ER (13.24 cm).

IB-CR was observed to have the largest number of seeds per pod (13.28), while the least value (4) was recorded in IB-LT. In both cases, the observed values were significantly different from other lines. Average number of seeds observed in the pods of IB (12.23) was not significantly different from 12.13 seeds recorded in IB-Y1. IB-ER had 11.6 seeds per pod which was not significantly different from IB-Y1. High heritability estimate (0.88) was recorded for this trait among the lines.

No significant difference was recorded between IB and IB-BPC with the mean weights of 14.78 g and 14.75 g respectively. IB-CR produced a mean weight of 13.7 g for 100 seeds which was significantly different from other lines. The mean weights observed in IB-ER and IB-LT was not significantly different from each other but different from other lines. However, IB-Y1 was significantly different from other lines and had 100 seed weight of 11.4 g. This trait is highly heritable (H = 0.96) among the six cowpea lines.

4.4.2 Variation in growth habit traits and yield components of IT86D-719 cowpea and its mutant derivatives

The mean values of the growth habits and yield component traits observed among IT86D-719 cowpea line and its mutant derivatives are presented in Tables 4.14 and 4.15 respectively. Induced mutants derived from IT86D-719 in this study recorded a wide genetic variability for all growth parameters evaluated. This was demonstrated in high coefficients of variation and genotypic variance (V_g) which were greater than environmental (V_e) except in the length of peduncle, pod length and number of seeds per pod where observed V_e of 73.34, 1.54 and 4.23, respectively were higher than V_g. The fasciated stem mutant (IT-719FPL-2Fas) was more vigorous than the parent, IT86D-719 and other lines with respect to plant height at six weeks (although not significant at the 5% level), number of branches and length of branches.

The tallest plants of 84.91 cm and 82.02 cm were observed in IT-719FPL-2Fas and IT86D-719, respectively which were not significantly different from each other but different from other lines. The height of IT-719BN (20.84 cm) was significantly

Table 4.14. Mean values and variance components of five growth habits of IT86D-719 cowpea line and its mutant derivatives

Genotype	PLHSW	TLA	NB	LBPPL	PEDL
IT86D-719	82.02ab	43.95a	3.78c	46.00b	31.76a
IT-719Y	76.70b	32.45b	4.53b	65.35a	31.33a
IT-719FPL-1	77.22b	27.28c	5.30a	67.10a	28.37a
IT-719FPL-2Fas	84.91a	27.33c	5.53a	69.56a	29.05a
IT-719BN	20.84c	13.97d	3.05d	13.40c	23.80b
Mean	68.34	29.00	4.44	52.28	28.86
SD	28.55	10.78	1.24	24.26	8.92
CV	22.96	16.47	18.89	22.81	29.67
V_p	956.73	138.91	1.76	698.69	81.59
V_g	710.63	116.12	1.06	556.46	8.26
V_e	246.10	22.80	0.70	142.23	73.34
H	0.74	0.84	0.60	0.80	0.10

PLHSW = Plant height at six weeks after planting, TLA = Terminal leaflet area, NB = Number of branches, LBPPL = Length of branches per plant, PEDL = Length of peduncle
Means with the same letter are not significantly different at 5% level of probability by the Least Significant Difference (LSD)

Table 4.15. Mean values and variance components of five yield related traits of IT86D-719 cowpea line and its mutant derivatives

Genotype	PEDPPL	PODPPED	PODL	SEDPPOD	HSEDW
IT86D-719	12.05c	3.08c	13.94a	11.25a	14.96a
IT-719Y	16.98b	3.35c	13.41ab	11.13a	14.10b
IT-719FPL-1	63.65a	3.75b	13.11b	9.30b	14.06b
IT-719FPL-2Fas	63.85a	4.53a	13.07b	9.33b	14.10b
IT-719BN	10.58c	2.03d	12.49c	9.08b	15.04a
Mean	33.42	3.35	13.21	10.02	14.45
SD	25.60	1.08	1.31	2.24	0.61
CV	17.83	21.01	9.40	20.55	2.89
V_p	806.85	1.32	1.78	5.29	0.42
V_g	771.32	0.83	0.24	1.05	0.25
V_e	35.53	0.49	1.54	4.23	0.17
H	0.96	0.63	0.14	0.20	0.59

PEDPPL = Number of peduncle per plant, PODPPED = Number of pods per peduncle, PODL = Pod length, SEDPPOD = Number of seeds per pod, HSEDW = Hundred seed weight
Means with the same letter are not significantly different at 5% level of probability by the Least Significant Difference (LSD)

different from other lines and it was recorded as the shortest plant at six weeks. IT86D-719, IT-719Y and IT-719FPL-1 were not significantly different from one another in height at six weeks. High value of heritability (0.74) was observed for this trait.

IT86D-719 had the largest terminal leaflet area (43.95 cm^2) which was significantly different from other lines. IT-719Y recorded 32.45 cm^2 which was significantly different from other lines. The terminal leaflet area of IT-719FPL-1 and IT-719FPL-2Fas were not significantly different from each other. However, the terminal leaflet area of IT-719BN (13.97 cm^2) was the least recorded among all the lines. High value of heritability (0.84) was recorded for terminal leaflet area among IT86D-719 and its four mutants.

The highest mean number of branches 5.53 and 5.3 were observed in IT-719FPL-2Fas and IT-719FPL-1 respectively which were significantly not different from each other but different from other lines. The least mean number of branches (3.05) was observed in IT-719BN which was significantly different from other lines. The trait is highly heritable (H = 0.80) among five cowpea genotypes evaluated.

The mean length of branches (69.56cm, 67.1 cm and 65.35 cm) observed in IT-719FPL-2Fas, IT-719FPL-1 and IT-719Y respectively were significantly not different from one another but longer than other lines. IT-719BN had the shortest length of branches (13.4 cm) which was significantly different from IT86D-719.

The least length of peduncles (23.80 cm) recorded in IT-719BN was significantly different from other lines. However, the lengths of peduncles observed in the parent and other lines were not significantly different from one another.

IT-719FPL-2Fas and IT-719FPL-1 had 63.85 and 63.65 number of peduncles respectively which was significantly higher than other lines. The least (10.58) was observed in IT-719BN which was significantly not different from IT86D-719 but different from IT-719Y. The V_g component of variance recorded (771.32) constituted more than 95% variation observed in this trait. This trait is highly heritable (H = 0.96) among the five cowpea lines.

The highest number of pods per plant (4.53) was observed in IT-719FPL-2Fas followed by IT-719FPL-1 (3.75) and each of them was significantly different from other lines. IT-719BN produced the smallest number of pods per peduncle (2.03) which was significantly different from other lines. IT86D-719 and IT-719Y were not significantly different from each other.

The pod length (13.94cm) recorded in IT86D-719 was significantly different from other lines but not different from IT-719Y. However, the shortest pod length (12.49cm) was observed in IT-719BN. IT-719FPL-2Fas, IT-719FPL-1 and IT86D-719 were not significantly different from one another. The environmental variance component (V_e) contributed 1.54 out of the V_p (1.78) obtained in this trait. Very low heritability estimate (0.14) observed in this trait showed that it is non-heritable among the five genotypes.

Highest number of seeds per pod (11.25 and 11.13) observed in IT86D-719 and IT-719Y respectively was significantly different from other lines but not different from each other. IT-719FPL-1, IT-719FPL-2Fas and IT-719BN were not significantly different from one another. The V_e component (4.23) constituted larger proportion of the V_P (5.29) recorded in this trait. Low value of heritability (0.20) was observed in this trait.

The highest mean 100 – seed weight (15.04 and 14.96) observed in IT-719BN and IT86D-719 respectively was significantly not different from each other but different from other lines. IT-719Y, IT-719FPL-1 and IT-719FPL-2Fas were not significantly different from one another.

4.4.3 Variation in growth habit traits and yield components of IT86KD-237-57 cowpea and its mutant derivative

The mean values of the growth habits and yield component traits observed among IT86KD-237-57 cowpea line and its mutant derivative are presented in Tables 4.16 and 4.17 respectively. Among all the traits studied, observed V_g was greater than V_e except in the length of peduncle, number of pods per peduncle, pod length and number of seeds per pod where 3.9, 0.43, 1.41 and 3.05 respectively observed as V_e were higher than V_g.

Table 4.16. Mean values and variance components of eight growth habits of IT86KD-237-57 cowpea lines and its mutant derivatives

Genotype	PLL	PLB	PLA	PLHSW	TLA	NB	LBPPL	PEDL
IT86KD-237-57	5.35b	3.36a	15.39a	86.79a	37.34b	4.88a	90.43a	24.20a
IT86KD-G400NL	7.51a	1.43b	8.50b	43.84b	72.13a	3.90b	51.06b	22.51a
Mean	6.43	2.39	11.95	65.32	54.73	4.39	70.74	23.36
SD	1.11	0.98	3.59	28.00	25.61	0.82	21.39	5.51
CV	4.14	6.30	8.16	27.64	34.91	15.24	11.65	23.80
V_p	2.39	1.89	24.63	1240.08	961.02	0.91	840.88	31.55
V_g	2.32	1.87	23.68	914.20	595.93	0.46	772.99	0.65
V_e	0.07	0.23	0.95	325.88	365.09	0.45	67.90	30.90
H	0.97	0.99	0.96	0.74	0.62	0.51	0.92	0.02

PLL = Primary leaf length, PLB = Primary leaf breadth, PLA = Primary leaf area, PLHSW = Plant height at six weeks after planting, TLA = Terminal leaflet area, NB = Number of branches, LBPPL = Length of branches per plant, PEDL = Length of peduncle

Means with the same letter are not significantly different at 5% level of probability by the Least Significant Difference (LSD)

Table 4.17. Mean values and variance components of five yield related traits of IT86KD-237-57 cowpea line and its mutant derivatives

Genotype	PEDPPL	PODPPED	PODL	SEDPPOD	HSEDW
IT86KD-237-57	29.88a	3.08a	13.17a	8.90a	17.51a
IT86KD-G400NL	27.70a	3.05a	11.71b	8.53a	15.14b
Mean	28.79	3.06	12.44	8.71	16.32
SD	5.31	0.66	1.39	1.73	1.40
CV	18.22	21.74	9.55	20.08	4.62
V_p	29.20	0.43	2.43	3.05	3.35
V_g	1.68	0.00	1.02	0.00	2.79
V_e	27.52	0.43	1.41	3.05	0.57
H	0.06	0.00	0.42	0.00	0.83

PEDPPL = Number of peduncle per plant, PODPPED = Number of pods per peduncle, PODL = Pod length, SEDPPOD = Number of seeds per pod, HSEDW = Hundred seed weight

Means with the same letter are not significantly different at 5% level of probability by the Least Significant Difference (LSD)

The primary leaf of IT86KD-G400NL (7.51 cm) was significantly longer than IT86KD-237-57 (5.35 cm). High value of heritability (0.97) recorded in the primary leaf length showed that the trait is highly heritable in cowpea.

The primary leaf breadth of IT86KD-237-57 (3.36 cm) was significantly longer than IT86KD-G400NL (1.43 cm). The highest heritability estimate observed for this trait showed that the narrow primary leaf mutant trait is highly heritable. Above 96% of the V_P observed between two lines was contributed by V_g (23.68) in this trait. This showed that the trait is highly heritable. IT86KD-237-57 (15.39 cm^2) was significantly larger in area than IT86KD-G400NL (8.5 cm^2).

The plant height observed in IT86KD-237-57 (86.79 cm) was significantly taller than IT86KD-G400NL (43.84 cm). High value of heritability (0.74) was observed for this trait.

IT86KD-G400NL (72.13 cm) was significantly larger in area than IT86KD-237-57 (37.34 cm). The V_g accounted for 62% of the variation observed between the mutant and the parental line.

The V_p recorded in this trait was partitioned to approximately 50% each of the V_g and V_e. The number of branches observed in IT86KD-237-57 (4.88) was significantly higher than IT86KD-G400NL (3.9).

The mean length of branches in IT86KD-237-57 (90.43cm) was significantly higher than IT86KD-G400NL (51.06cm).

The genotypic variance (V_g) was responsible for only 2% of the V_p recorded in this trait. No significant difference was observed in the length of peduncle between IT86KD-237-57 and IT86KD-G400NL.

The V_g (27.52) was responsible for 94% of V_p observed in this trait. The number of peduncle recorded in IT86KD-237-57 (29.88) was not significantly different from IT86KD-G400NL (27.7).

The V_e (0.43) recorded in the number of pod per peduncle account for 100% of the V_p. No significant difference was observed between IT86KD-237-57 and IT86KD-G400NL.

The observed V_e component (1.41) of V_p in the length of pod was greater than the V_g (1.02). The mean pod length in IT86KD-237-57 (13.17cm) was significantly greater than IT86KD-G400NL (11.71cm).

The V_e component (3.05) observed in the number of seeds per pod account for 100% of the V_p between the cowpea lines. This showed that the trait is non-heritable in the lines evaluated. No significant difference was observed between IT86KD-237-57 and IT86KD-G400NL.

The mean 100-seed weight observed in IT86KD-237-57 (17.51g) was significantly higher than IT86KD-G400NL (15.1g). Very high heritability (0.83) was observed for this trait.

4.4.4 Variation in growth habit traits and yield components of IT90K-284-2 cowpea and its mutant derivatives

The mean values of the growth habits and yield component traits observed among IT86KD-237-57 cowpea line and its mutant derivative are presented in Tables 4.18 and 4.19 respectively. Among all the growth habit traits studied, observed V_e was greater than V_g except in the length of branches per plant where observed V_g (9.98) was higher than V_e (6.13). However, the V_g observed among the yield components was greater than V_e for number of pods per plant, number of pod per peduncle and seed width where 82.1, 0.5 and 0.22 respectively were observed as V_e.

IT90K-284-2 was observed as the tallest line with the mean height of 43.16cm but not significantly different from IT90K-BS-1 and IT90K-SP with the mean height of 40.85cm and 42.59 cm respectively. There was no significant difference between IT90K-BS-3 and IT90K-BS-4 but they were significantly different from other lines. IT-284-FPL was significantly shorter than other lines with the mean height of 27.67 cm. Low heritability value (0.43) was observed for this trait.

The largest mean terminal leaflet area (50.49 cm^2) was observed in IT90K-SP which was not significantly larger than IT90K-284-2 (48.31 cm^2) but significantly

Table 4.18. Mean values and variance components of five growth habits of IT90K-284-2 cowpea line and its mutant derivatives

Genotype	PLHSW	TLA	NB	LBPPL	PEDL
IT90K-284-2	43.16a	48.31a	3.68a	30.56a	27.57b
IT-284-FPL	27.69c	42.20b	3.68a	21.45e	20.69c
IT90K-BS-1	40.85a	37.91c	3.73a	26.99c	30.13a
IT90K-BS-3	31.66b	39.31bc	3.75a	26.61cd	28.63ab
IT90K-BS-4	33.92b	41.68b	3.65a	25.75d	30.44a
IT90K-SP	42.59a	50.49a	3.75a	29.38b	27.69b
Mean	36.65	43.31	3.70	26.79	27.53
SD	9.33	9.06	0.48	3.87	6.29
CV	19.88	18.31	12.95	9.24	27.53
V_p	93.34	86.48	0.23	16.11	41.85
V_g	40.25	23.57	0.00	9.98	11.90
V_e	53.09	62.91	0.23	6.13	29.96
H	0.43	0.27	-0.02	0.62	0.28

PLHSW = Plant height at six weeks after planting, TLA = Terminal leaflet area, NB = Number of branches, LBPPL = Length of branches per plant, PEDL = Length of peduncle

Means with the same letter are not significantly different at 5% level of probability by the Least Significant Difference (LSD)

Table 4.19. Mean values and variance components of seven yield related traits of IT90K-284-2 cowpea line and its mutant derivatives

Genotype	PEDPPL	PODPPED	PODL	SEDPPOD	SEDL	SEDW	HSEDW
IT90K-284-2	16.23a	2.95ab	19.47a	13.45a	0.78d	0.67b	19.90c
IT-284-FPL	10.70c	3.03a	16.37c	9.40b	0.79d	0.64b	13.40e
IT90K-BS-1	12.70b	2.75ab	19.50a	4.73d	1.24a	0.73ab	28.54b
IT90K-BS-3	11.75bc	2.65b	19.82a	4.80d	1.20b	0.72b	28.10b
IT90K-BS-4	12.65b	2.78ab	17.57b	5.73c	1.26a	0.93a	32.85a
IT90K-SP	12.48b	3.00a	8.71d	2.55e	1.04c	0.62b	18.13d
Mean	12.75	2.86	16.91	6.78	1.05	0.72	23.49
SD	3.31	0.71	4.32	4.06	0.21	0.48	6.89
CV	22.48	24.81	11.48	27.57	6.07	65.07	4.36
V_p	11.48	0.51	21.59	19.10	0.052	0.23	56.58
V_g	3.27	0.01	17.83	15.61	0.048	0.01	55.53
V_e	8.21	0.50	3.76	3.49	0.004	0.22	1.05
H	0.28	0.02	0.83	0.82	0.92	0.03	0.98

PEDPPL = Number of peduncle per plant, PODPPED = Number of pods per peduncle, PODL = Pod length, SEDPPOD = Number of seeds per pod, SEDL = Seed length, SEDW = Seed width, HSEDW = Hundred seed weight
Means with the same letter are not significantly different at 5% level of probability by the Least Significant Difference (LSD)

different from other lines. The mean terminal leaflet area of IT-284-FPL, IT90K-BS-3 and IT90K-BS-4 were not significantly different form one another. IT90K-BS-1 was significantly different from other line but not different from IT90K-BS-3. The V_e (62.91) accounted for 73% of the variation observed in this trait.

There was no significant difference in the mean number of branches among all the lines. The V_e (0.23) recorded in the number of branches account for 100% of the V_p. This showed that the trait is non-heritable among the cowpea lines evaluated.

The mean longest branch was observed in IT90K-284-2 (30.24cm) which was significantly different from other lines. However, IT-284-FPL produced the shortest branch (21.45cm) which was significantly different from other lines. IT90K-BS-1 and IT90K-BS-3 were not significantly different from each other but different from other lines. IT90K-BS-4 was not significantly different from IT90K-BS-3.

The longest mean length of peduncle observed in IT90K-BS-4 (30.44cm) which was significantly not different from IT90K-BS-1 (30.13cm) and IT90K-BS-3 (28.63cm) but different from other lines. IT-284-FPL produced the shortest mean peduncle length (20.69cm) which was significantly different from other lines. IT90K-284-2, IT90K-BS-3 and IT90K-SP were not significantly different.

The largest mean number of peduncle per plant (16.23) was observed in IT90K-284-2 which was significantly different from other lines. IT90K-BS-1, IT90K-BS-3, IT90K-BS-4 and IT90K-SP were not significantly different. IT-284-FPL was significantly different from other lines but not different from IT90K-BS-3.

The V_e component (0.5) observed in the mean number of pod per peduncle account for 98% of the V_p among the cowpea lines. IT-284-FPL was significantly different from IT90K-BS-3 but not different from other lines. IT90K-BS-3 was significantly different from IT90K-SP and IT-284-FPL but not different from other lines.

The mean pod lengths (19.82 cm, 19.50 cm and 19.47 cm) observed in IT90K-BS3, IT90K-BS-1 and IT90K-284-2 respectively were not significantly different from one another but different from other lines. IT90K-BS-4 and IT-284-FPL were significantly different from other lines. IT90K-SP produced the shortest peduncle (8.71

cm) and was significantly different from other lines. High heritability estimate (0.83) was observed for this trait among the lines evaluated.

The highest number of seed per pod (13.45) was observed in IT90K-284-2 which was significantly different from other lines. However, IT90K-SP was significantly different from other lines and recorded the least (2.55) number of seed per pod. IT-284-FPL was significantly different from other lines. IT90K-BS-1 and IT90K-BS-3 were not significantly different. IT90K-BS-4 was significantly different from other lines. Genotypic variance (15.61) accounted for 82% of the variation observed for this trait among the line.

The longest seed (1.26 cm) was observed in IT90K-BS-4 which was not significantly different from IT90K-BS-1 but different from other lines. IT90K-SP was significantly different from other lines. IT-284-FPL was not significantly different from IT90K-284-2 but different from other lines. This trait was observed for high heritability (0.92) among the lines evaluated.

A similar trend to the seed length was also observed in the mean seed width. IT90K-BS-4 produced the widest seed (0.93cm) which was not significantly different from IT90K-BS-1. However, IT90K-284-2, IT-284-FPL, IT90K-BS-3 and IT90K-SP were not significantly different. Environmental variance (V_e = 0.22) accounted for 97% of the variation observed among the lines evaluated.

The mean 100 – seed weight (32.3 g) observed in IT90K-BS-4 was significantly higher than other lines. IT90K-BS-1 recorded the second highest and was not significantly different from IT90K-BS-3. The least value of 100 – seed weight (13.4) was observed in IT-284-FPL which was significantly different from other lines. The trait is highly heritable (H = 0.98) among the six cowpea lines evaluated.

4.5 Molecular characterization of cowpea mutants

4.5.1 Genetic diversity assessment

The result of genetic diversity study of the mutants using microsatellite markers is presented in Table 4.20. Sixteen labeled SSR primers were screened for the molecular study. Out of the sixteen, four primers (Vm34, Vm37, Vm54 and Vm57) revealed polymorphic loci (Plate 4.25) while monomorphism were observed with the other twelve primers. The polymorphic markers were used to evaluate the genetic diversity of 32 cowpea lines. A total of 15 alleles were produced on these four loci with an average of 3.75 alleles per SSR locus. The primer, Vm57 revealed the highest diversity index among all the lines and the polymorphic information content (PIC) varied from 0.33 to 0.63 with a mean of 0.51.

4.5.2 Phylogenetic analysis: The dendogram generated by UPGMA method showed the genetic relationship among cowpea lines (Figure 4.7). Seven distinct branches were revealed in the phylogenetic analysis of all the mutant lines including their parents. Comparing the clustering of the mutants with their parents, IB-ER and IB-ER-2 were observed in the same cluster (Group I) together with their parent IB, while other mutant derivatives of IB clustered in group VI and VII. IT90K-284-2 clustered with its mutant lines IT90K-BS-4, IT90K-500EM, IT90K-284TRV, IT90K-BS-3 and IT90K-284FPL-2 in group I, while its other mutant lines were in group II and IV.

IT86D-719 was observed in cluster IV, while its mutant derivatives clustered with group I, III, V and VI. IT89KD-374-57 was observed in cluster IV, while other mutant derived from it clustered with group I and II. These results show that the mutants were diverse in their genetic makeup from their parental lines.

Table 4.20. Summary statistics of 4 microsatellite markers used in cowpea mutant characterisation

Marker	Major allele frequency	No of observation	No of allele	Genetic diversity	PIC
Vm34	0.375	32	4	0.6797	0.6179
Vm37	0.75	32	3	0.3887	0.3336
Vm54	0.6875	32	4	0.4902	0.4545
Vm57	0.4063	32	4	0.6855	0.6257
Mean	0.5547	32	3.75	0.561	0.5079

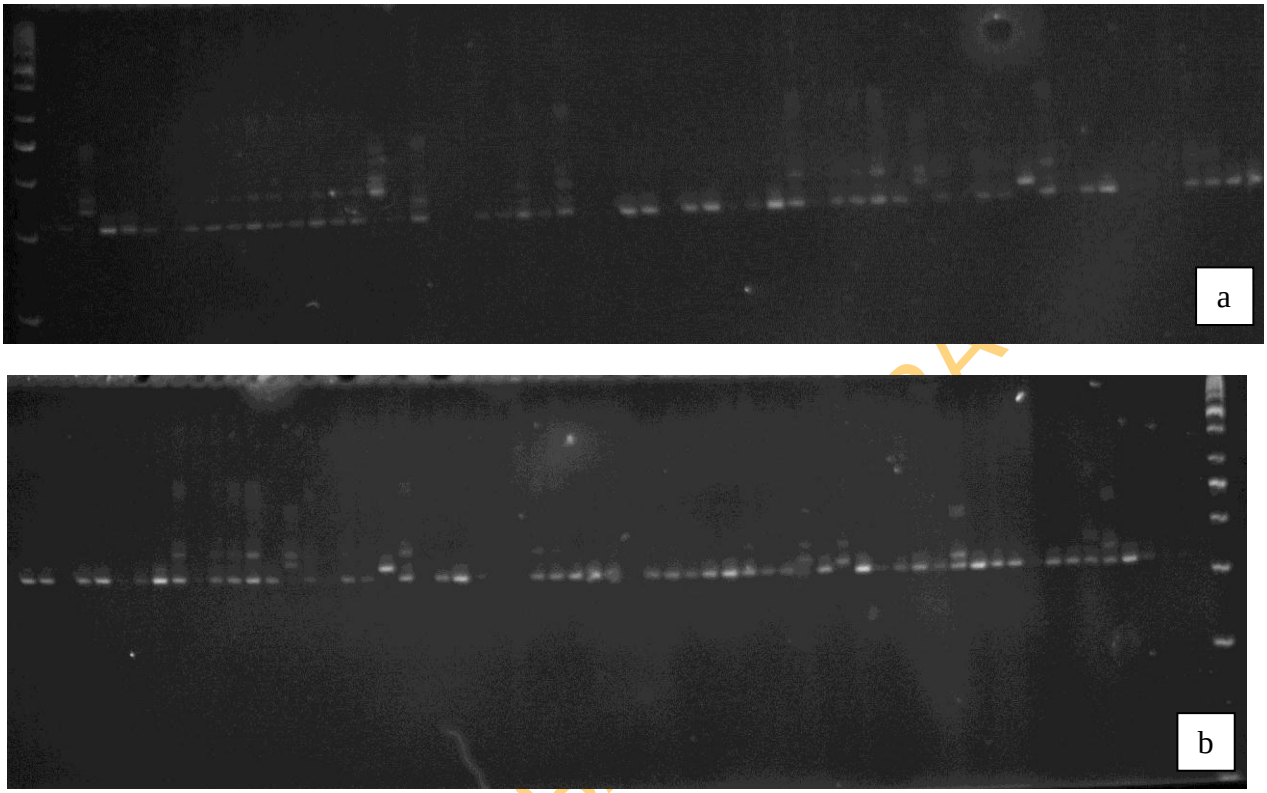

Plate 4.25. The picture from 6% acryl amide gel showing polymorphic bands of amplified primers; (a) polymorphic bands from primers Vm37 and Vm34, (b) polymorphic bands from primers Vm57 and Vm54

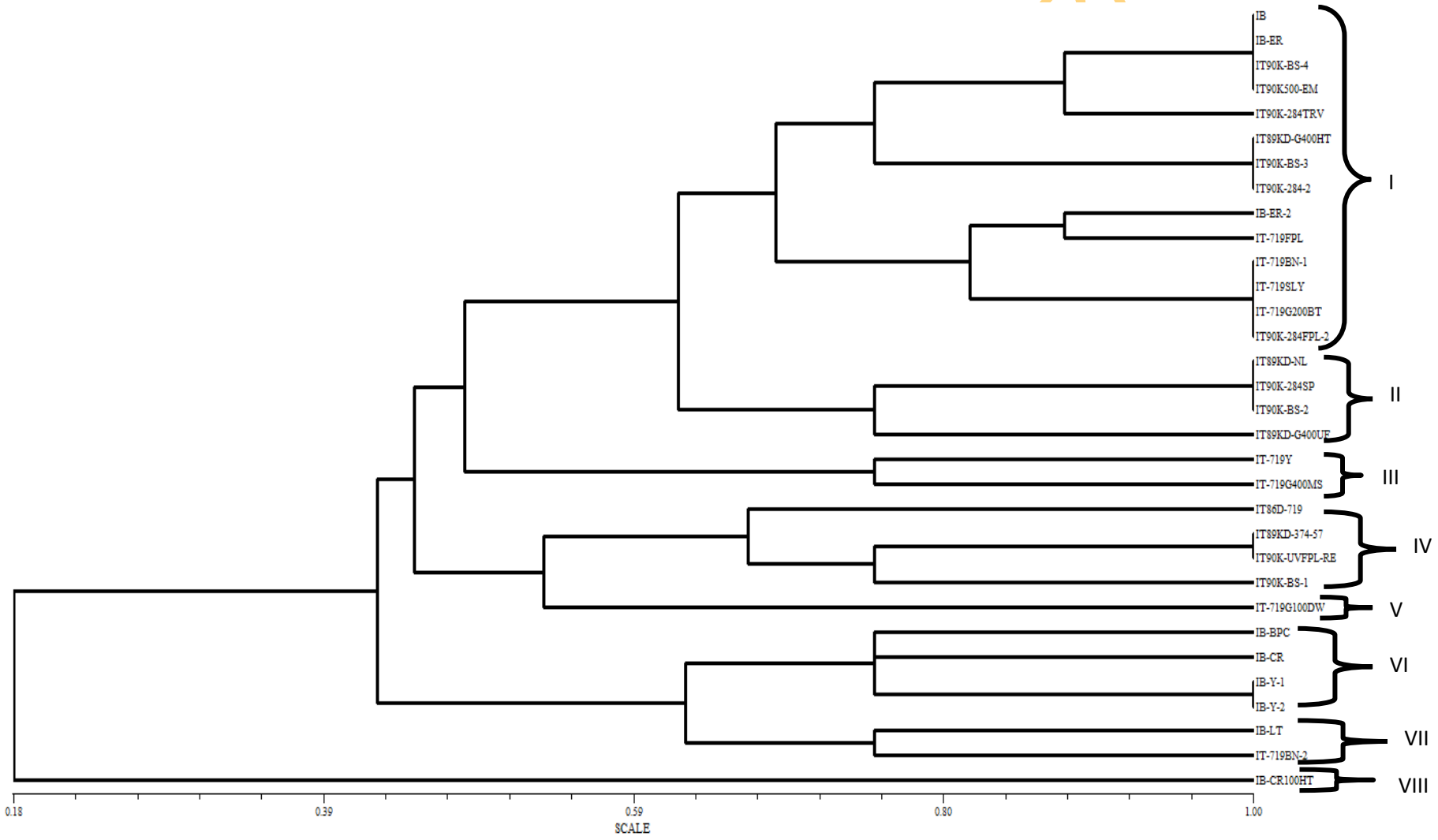

Figure 4.7. Dendogram showing genetic diversity between the parents and mutant cowpea lines

4.5.3 Sequence analysis

The one band amplicon obtained from RBCL primer on 1.5% agarose gel is shown in Plate 4.26, while the mutant lines and their parental sequence alignments with the RBCL reference sequence are presented in Figure 4.8.

The sequence data presented in Figure 4.8 includes new mutants selected from gamma mutagenesis in this study and pre-existing mutants of IB produced by spontaneous mutations. As a result of mutagenic treatment of cowpea lines with gamma rays, insertion of T and G was observed in the RBCL regions 2 and 3, respectively of IB-ER when compared with its parent IB, while G was substituted for A at region 4 of the sequence. A was deleted in IB-ER at position 129 of its sequence when compared with IB sequence. In IB-LT, insertion of AT was observed at positions 1-2, while base substitutions of T for G at region 3 and G for A at position 4 of its parent IB-CR sequence. In addition to these, deletion of 5 bases (AATTC) in IB-LT was observed at position 128 to 132 when compared with IB-CR. The sequence data also revealed the type of changes that occurred in the RBCL region of IB line as a result of spontaneous mutations that produced IB-BPC, IB-CR, IB-Y-1 and IB-Y-2. There was deletion of G and A at regions 4 and 129, respectively in IB-BPC. In IB-CR insertion of T and TTC was observed at positions 3 and130-133, respectively. Deletion of A was observed at position 129 in IB-Y-1, while in IB-Y-2 there was insertion of G at position 3 and deletion of A at position 129.

From the RBCL sequence data there were variations in the observed effects of mutagenic treatment of IT86D-719 with gamma rays on the resultant mutants in this study. In mutant line IT-719BN-1, G and A nucleotides were deleted at positions 3 and 128, respectively. G nucleotide was deleted at position 3, while ACC bases were substituted for CAA at region 4-6 in IT-719BN-2. In IT-719FPL and IT-719G100DW, C was substituted for A at region 6, while there was insertion of A at region 129 of the sequence in IT-719G100DW. At regions 3-4 and 128 of IT-719G200BT, GA and A were deleted, respectively. In IT-719G400MS, GA was deleted at region 3-4, substitutions of

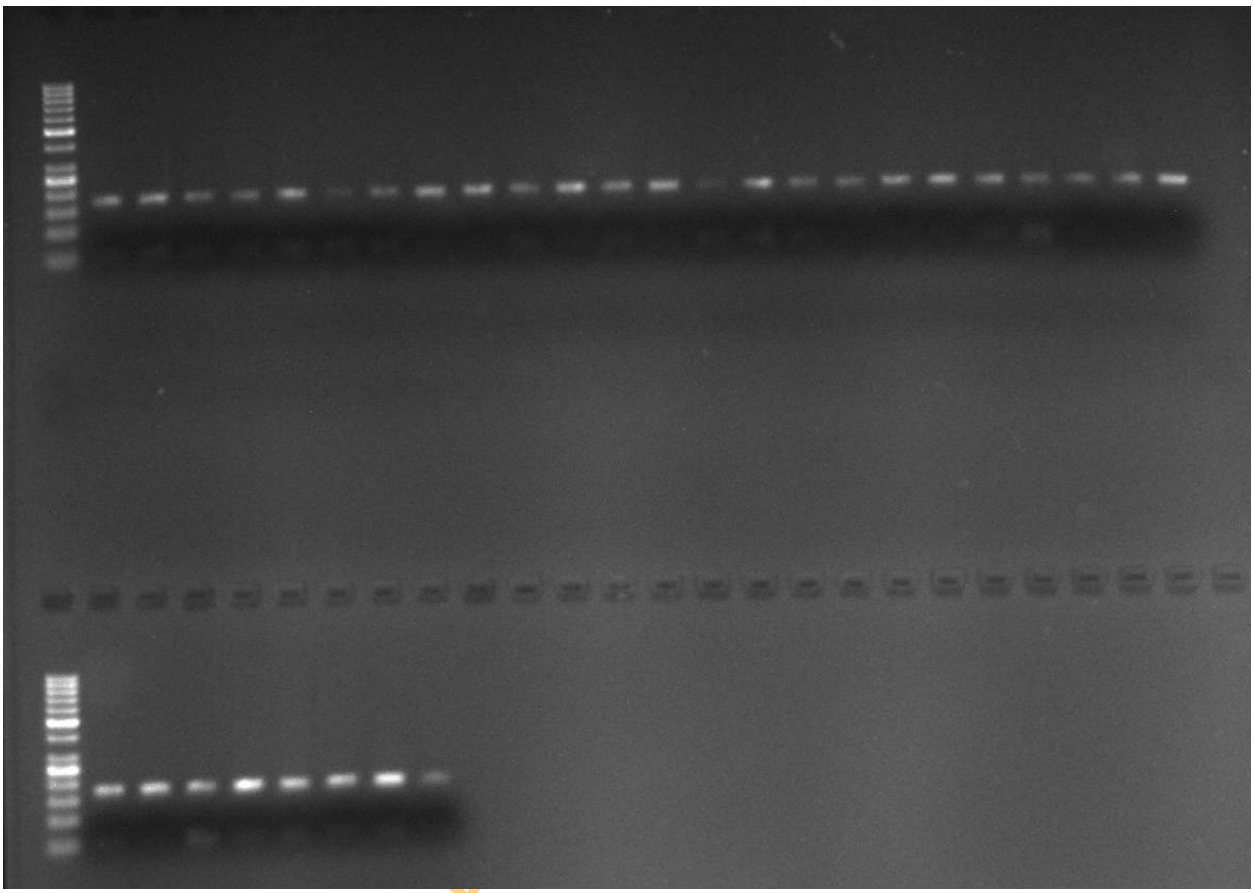

Plate 4.26. The picture from 1.5% agarose gel (RBCL primer) showing monomorphic bands of the samples indicating the presence of homozygous alleles before sequencing

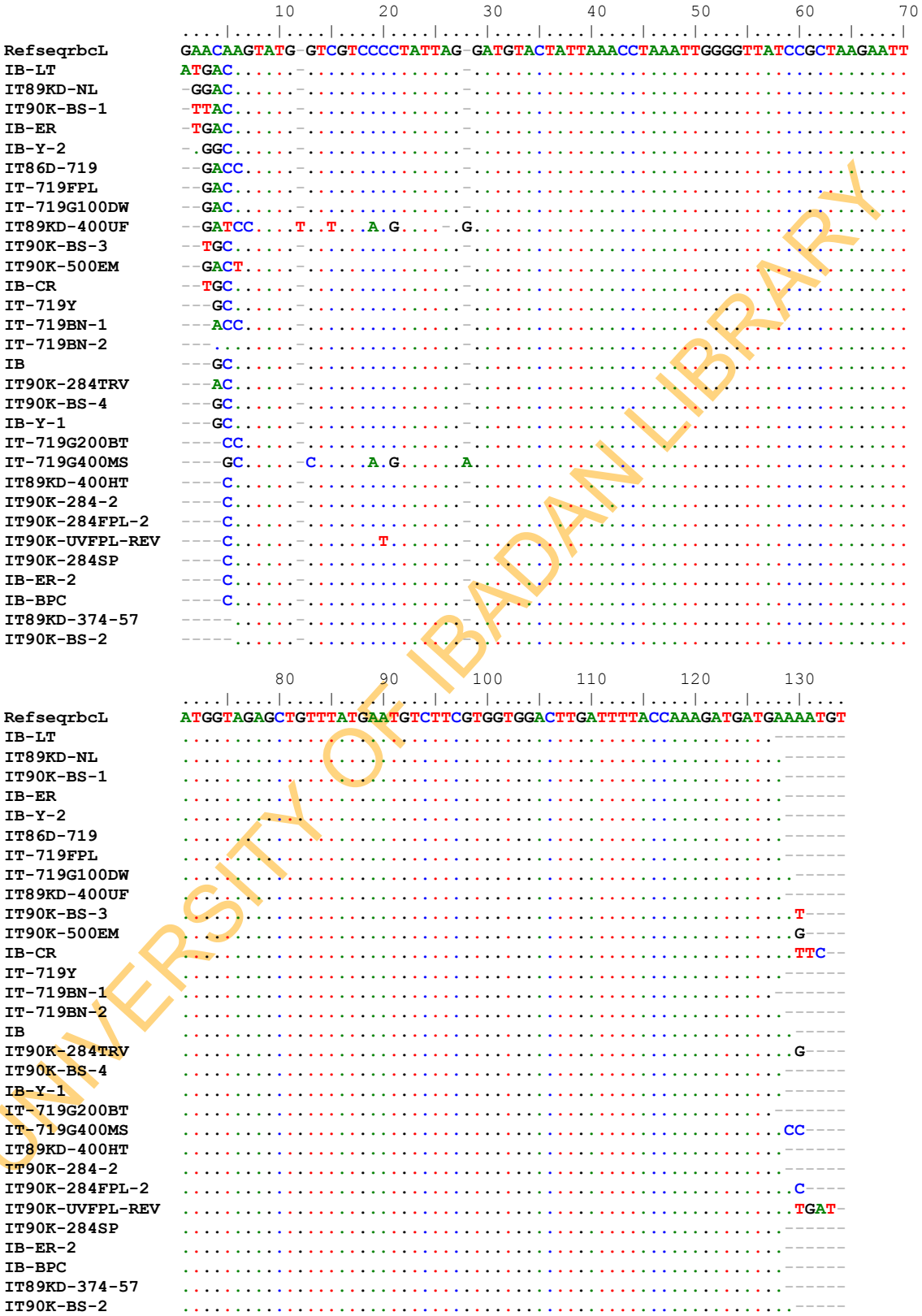

Figure 4.8. RBCL sequence alignment of cowpea mutants with their parents

C for G, G for C, C for A and C for G were respectively, observed at regions 5, 13, 19 and 21 and insertions of A and CC were revealed at regions 28 and 129-130, respectively. However, in IT-719Y, G was deleted at region 3, while A and C were respectively, substituted for G and A at regions 4 and 6.

The mutants derived from IT89KD-374-57 were different from their parent with respect to rbcl sequence. There was an insertion of C at position 5 of IT89KD-400HT. In IT89KD-400UF, the sequence data revealed insertions of GAT, T and G at positions 3-5, 12 and 28 respectively, while A, G, C, C and C were respectively, substituted for C, C, T, A and G at positions 6, 7, 15, 19 and 21. Insertion of four bases (GGAC) was observed at position 2-5 of IT86KD-NL.

The RBCL sequence data shows various changes that occurred in the mutants produced from gamma irradiation of IT90K-284-2 line. In IT90K-284FPL-2, there was insertion of AC at position 129-130 of the sequence, while in IT90K-284TRV, A and AG were inserted at positions 4 and 129-130, respectively. In IT90K-500EM, there were insertions of GA, T and G at positions 3-4, 6 and 130 respectively. In IT90K-BS-1, insertion of TTA bases was observed at position 2-4, while there was deletion of C and insertion of A in IT90K-BS-2 at positions 5 and 129 respectively. TG and T were respectively, inserted to regions 3-4 and 130 in IT90K-BS-3, while there was only an insertion of G to region 4 in IT90K-BS-4. IT90K-UVFPL-REV was a four-primary leaf mutant derived from the UV treatment of pollen prior to pollination in IT90K-284-2. In this mutant, a base substitution of C for T at region 20 and insertion of a fragment TGAT at region 130-133 was observed.

Analysis of the rbcl sequence of the mutants shows the presence of insertions and deletions (indels) and point mutations (base substitutions) as the two main classes of mutations induced in the plastid DNA of the mutants studied. The sequence data revealed that 45.45% of the mutations were insertions, 23.64% was recorded as deletions, while the rest were base substitutions of which 7.27% of the total was transition and 23.64% was observed as transversion. Only one stable UV induced mutant plant was included in the sequence analysis, hence the type of mutation induced from the UV source could not be quantified by the sequence results.

4.5.4 Molecular Phylogenetic analysis by Maximum Likelihood method

The analysis involved 30 nucleotide sequences out of the 32 samples. There were a total of 120 positions in the final dataset. Four main cluster groups of cowpea lines were revealed from the phylogenetic tree (Figure 4.9). The number of genotypes grouped within cluster I, II, III and IV was 1, 23, 4 and 2, respectively (excluding 2 samples with bad sequence data). The grouping which was irrespective of the mutant origin indicated that similarity within the mutant populations was independent of the RBCL sequence data. The RBCL reference sequence was found within cluster II, while the GU140278_COIgene sequence (check) was separated as an out-group which confirmed the validity of these results. The result indicates that IB is similar to its mutant derivatives except IB-CR. IT86D-719 is different from all its mutant lines except IT-719G200BT, IT-719BN-1 and IT-719G400MS. IT89KD-374-57 is similar to its mutant lines except IT89KD-400UF, while IT90K-284-2 is different from IT90K-500EM only.

4.6 Inheritance pattern of some cowpea mutant traits

4.6.1 Inheritance of erect-tall cowpea mutant

In Table 4.17, the data on the inheritance of erect-tall expression studied in crosses IB-ER x IB and IB-ER x IT89KD-374-57 are presented. In both crosses F_1 plants and the progeny of the backcrosses to creeping/short parents were creeping. The results of Chi-square tests of the data for backcrosses to erect-tall parent gave a goodness-of-fit to the 1 creeping: 1 erect-tall ratio, while F_2 data gave a goodness-of-fit to the 3 creeping: 1 tall-erect segregating ratio, indicating monogenic recessive inheritance of the erect-tall gene in cowpea. Thus, the gene *et* with the homozygous recessive line having genotype *et et* and the dominant lines having the genotype *Et* _.

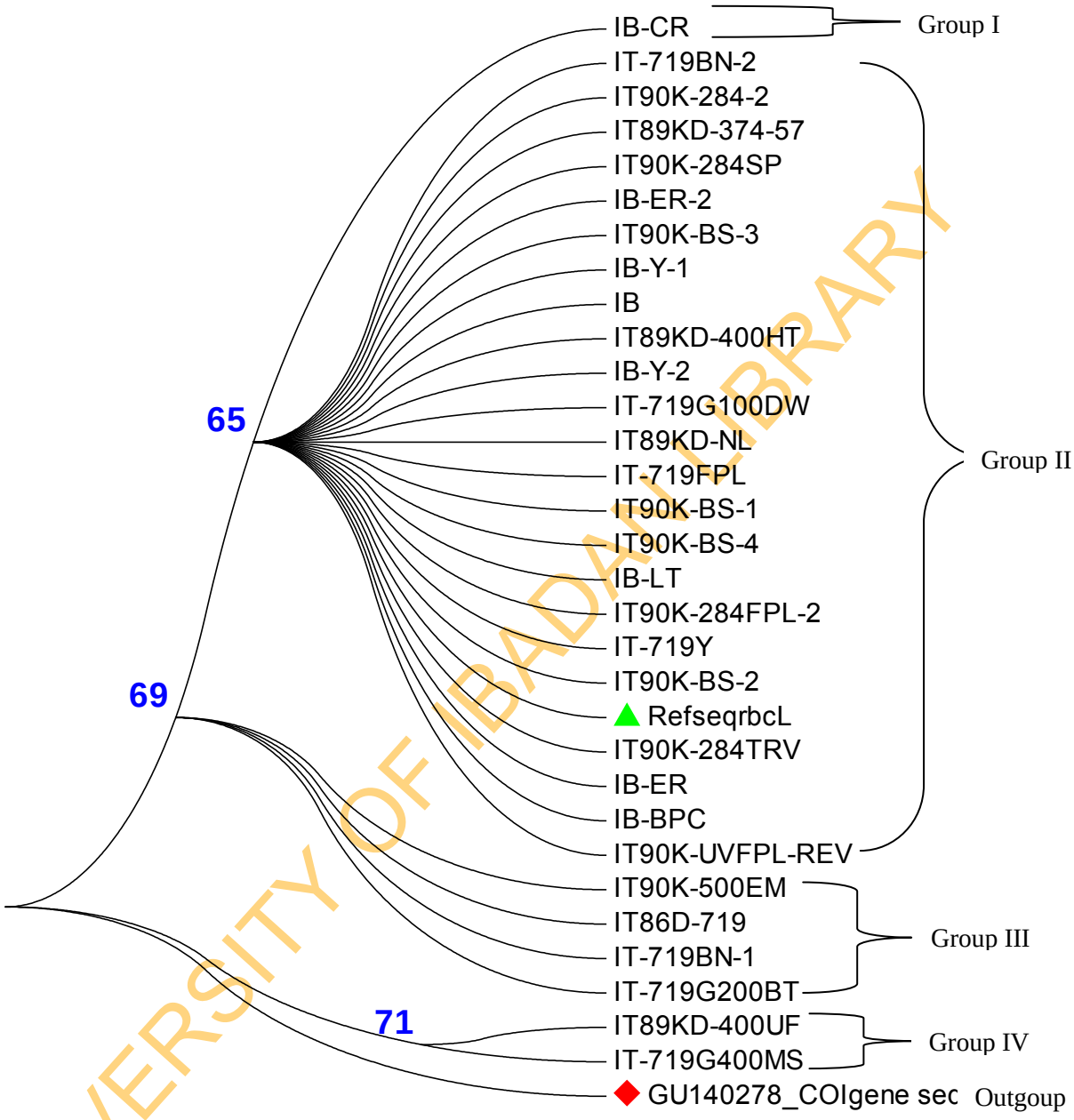

Figure 4.9. Molecular Phylogenetic tree of cowpea mutants inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model and the percentage of trees in which the associated taxa clustered together is shown above the branches.

Table 4.21. Inheritance of erect-tall plant in crosses of erect-tall x creeping-short lines of cowpea *Vigna unguiculata* (L) walp

Crosses and Generations	Number of plants			Expected Ratio	χ^2	P
	Creeping	Erect	Total			
IB-ER x IB						
IB-ER		20	20			
IB	12		12			
F_1	45		45			
IB-ER x F_1	435	441	876	1:1	0.0411	0.8394
IB x F_1	972		972			
F_2	614	196	810	3:1	0.2782	0.5979
IB-ER x IT89KD-374-57						
IB-ER		17	17			
IT89KD-374-57	11		11			
F_1	64		64			
IB-ER x F_1	523	538	1061	1:1	0.2121	0.6452
IT89KD-374-57 x F1	866		866			
F_2	1028	382	1410	3:1	3.2917	0.0696

4.6.2 Inheritance pattern of lettuce-leaf cowpea mutant (IB-LT)

Data on the inheritance of lettuce leaf trait in the cross of the mutant line IB-LT and IB-CR (crinkled parent) are shown in Table 4.22. The F_1 plants and backcross to the crinkled line (BC_2) had crinkled leaf. The data thus suggested that the lettuce leaf in cowpea is controlled by recessive gene(s). The backcross to the lettuce leaf (BC_1) mutant produced 1crinkled leaf: 1 lettuce leaf segregation ratio, while the F_2 progeny data gave a goodness-of-fit to the 3 crinkled leaf: 1 lettuce leaf ratio, indicating a recessive inheritance for lettuce leaf trait in cowpea (Appendix 25).

Data on the inheritance studies of lettuce (crinkled twisted-pale) leaf in the crosses involving the mutant (IB-LT) and 2 normal leaf cowpea lines (IB and IT86D-719) are presented in Table 4.23.

In the two crosses, F_1 plants and all backcrosses to the normal leaf parental lines had normal leaf. This indicated that the gene controlling normal leaf in cowpea is dominant to that of lettuce (crinkled twisted-pale) leaf. However, the backcrosses to the lettuce leaf parent gave a 1 normal leaf: 1 crinkled leaf: 1 smooth twisted-pale leaf: 1 lettuce leaf, while the F_2 plants gave a goodness-of-fit to the 9 normal leaf: 3 crinkled leaf: 3 smooth twisted-pale leaf: 1 lettuce leaf segregation ratio. These results indicated that there was genetic interaction among the traits observed in the crosses. The gene controlling crinkled leaf interacted with the gene controlling twisted-pale leaf both in homozygous recessive form to produce lettuce leaf phenotype. The 1:1:1:1 segregation ratio observed from the backcrosses to lettuce leaf parent and the 9:3:3:1 ratio obtained from the F_2 plants indicated a dihybrid inheritance pattern of the genes controlling crinkled leaf and twisted-pale leaf traits in cowpea. The backcross to the lettuce leaf mutant produced 1 normal leaf: 1 twisted-pale leaf joint segregation ratio, while the F_2 progeny data gave a goodness-of-fit to the 3normal leaf: 1twisted-pale leaf joint segregation ratio. This result indicated that twisted-pale leaf trait is controlled by a single recessive gene with *tp tp* representing twisted pale leaf (homozygous recessive) and *Tp*

Table 4.22. Inheritance of lettuce leaf and crinkled leaf in cowpea *Vigna unguiculata* (L) Walp

Cross and Generation	Number of plants			Expected Ratio	χ^2	P
	Crinkled leaf	Lettuce leaf	Total			
IB-LT x IB-CR						
IB-LT		12	12			
IB-CR	14		14			
F1	38		38			
IB-LT x F1 (BC_1)	471	458	929	1:1	0.182	0.67
IB-CR X F1 (BC_2)	883		883			
F2	749	255	1004	3:1	0.085	0.77

Table 4.23. Inheritance of crinkled leaf and twisted-pale leaf in crosses of lettuce leaf and normal leaf lines of cowpea, *Vigna unguiculata* (L) walp

Cross and Generation	Number of plants				Total	Expected ratio	χ^2	P
	Normal leaf	Crinkled leaf	Smooth twisted-pale leaf	Lettuce leaf				
IB-LT x IB								
IB-LT				16	16			
IB	13				13			
F1	98				98			
IB-LT x F_1	213	206	208	210	837	1:1:1:1	0.1278	0.9883
IB-LT x F_1	429		408		837	1:1*	0.5269	0.4679
IB-LT x F_1	421	416			837	1:1**	0.2990	0.8628
IB x F_1	671				671			
F_2	549	189	182	62	982	9:3:3:1	0.1806	0.981
F_2	738		244		982	3:1*	0.0122	0.912
F_2	738	244			982	3:1**	0.0122	0.912
IB-LT x IT86D-719								
IB-LT				14	14			
IT86D-719	10				10			
F1	125				125			
IB-LT x F_1	213	206	198	210	827	1:1:1:1	0.6131	0.8934
IB-LT x F_1	419		408		827	1:1*	0.1463	0.7021
IB-LT x F_1	411	416			827	1:1**	0.1463	0.7021
IT86D-719 x F_1	859				859			
F_2	439	144	146	48	777	9:3:3:1	0.0353	0.9983
F_2	583		194		777	3:1*	0.0004	0.9835
F_2	583	194			777	3:1**	0.0004	0.9835

* Normal and crinkled plants combined into untwisted phenotype while lettuce leaf and twisted-pale plants combined into twisted-pale phenotypic trait

** Normal and smooth twisted-pale plants combined into smooth phenotype while lettuce leaf and crinkled plants combined into crinkled phenotypic trait

Tp being the non-twisted-leaf (normal leaf) dominant genotype. The backcross to the lettuce leaf mutant produced 1normal leaf: 1 crinkled leaf joint segregation ratio, while the F_2 progeny data gave a goodness-of-fit to the 3 normal leaf: 1 crinkled leaf joint segregation ratio, indicating a monogenic recessive inheritance for crinkled leaf trait in these crosses. The symbol *crl* had been assigned to the crinkled leaf allele (Kehinde,

1994; Fawole, 1997) and the crinkled leaf plant is *crl crl* (homozygous recessive), while the dominant genotypes (normal leaf) was *Crl* _. Thus the normal parents used in the crosses had homozygous dominant alleles for crinkled leaf and twisted-pale leaf traits, while the lettuce leaf mutant parent had homozygous recessive alleles (*crl crl tp tp*) for crinkled leaf and twisted-pale leaf traits. Therefore, the new mutation produced from the IB-CR by gamma irradiation was the twisted-pale mutant (Appendix 27).

4.6.3 Inheritance of yellow leaf (IT-719Y) mutant

Data on the inheritance of yellow leaf colour in crosses IT-719Y x IT86D-719 and IT-719Y x IB are presented in Table 4.24. In all the two crosses F_1 progenies and backcrosses to the green leaf parents were green leaf plants. This suggests that the green leaf trait is dominant to the yellow leaf condition. The backcross to yellow leaf parent gave a 1 green leaf: 1 yellow leaf segregation ratio, while the F_2 progeny gave a goodness-of-fit to the 3 green leaf: 1 yellow leaf ratio, indicating a monogenic recessive inheritance for this yellow leaf in the cowpea crosses.

Results of the crosses involving the yellow leaf mutant, Ife brown yellow1and Ife Brown yellow2 **(**IT-719Y x IB-Y-1 and IT-719Y x IB-Y-2) are presented in Tables 4.25 and 4.26. In the cross of IT-719Y x IB-Y-1 all the F_1 plants were green leaf indicating that the genes controlling yellow leaf traits in the mutant, IT-719Y is non-allelic to that of Ife Brown yellow1 (IB-Y-1). The backcross to yellow leaf mutant parent (IT-719Y) gave a 1 green leaf: 1 yellow flush segregation ratio, while the backcross to IB-Y-1 gave a 1 green leaf: 1 bright yellow ratio indicating that the yellow leaf traits in IT-719Y and IB-Y-1 are controlled by recessive genes. The yellow foliage colour of IB-Y-1 is determined by a

Table 4.24. Inheritance of yellow leaf in crosses of yellow leaf and green leaf lines of cowpea, *Vigna unguiculata* (L) Walp

Crosses and Generations	Number of plants		Total	Expected Ratio	χ^2	P
	Green leaf	Yellow leaf				
IT-719Y x IT86D-719						

IT-719Y		16	16			
IT86D-719	10		10			
F_1	82		82			
IT-719Y x F_1	502	489	991	1:1	0.1705	0.6796
IT86D-719 x F_1	712		712			
F_2	717	246	963	3:1	0.1526	0.686
IT-719Y x IB						
IT-719Y		20	20			
IB	14		14			
F_1	118		118			
IT-719Y x F_1	319	327	646	1:1	0.0991	0.7529
IB x F_1	664		664			
F_2	922	314	1236	3:1	0.1079	0.7426

Table 4.25. Allelic test between yellow leaf (IT-719Y) mutant and Ife Brown Yellow-1 (IB-Y-1)

Cross and Generation	Number of plants				Total	Expected Ratio	χ^2	P
	Green leaf	Bright yellow	Yellow flush	Whitish yellow				
IT86D-719Y x IB-Y-1								

IT86D-719Y			12		12			
IB-Y-1		14			14			
F_1	58				58			
IT86D-719Y x F_1	372		365		737	1:1	0.0665	0.7965
IB-Y-1 x F_1	402	391			793	1:1	0.1526	0.6961
F_2	508	160	171	53	892	9:3:3:1	0.6119	0.8937

Table 4.26. Allelic test between yellow leaf (IT-719Y) mutant and Ife Brown Yellow-2 (IB-Y-2)

Cross and Generation	Number of plants			Total	Expected Ratio	χ^2	P
	Green leaf	Yellow spec	Yellow flush				
IT86D-719Y x IB-Y-2							
IT86D-719Y			12	12			

IB-Y-2		9		9			
F_1	46			46			
IT86D-719Y x F_1	317		311	628	1:1	0.0573	0.8108
IB-Y-2 x F_1	445	433		878	1:1	0.164	0.6855
F_2	637	208	279	1124	9:3:4	0.0822	0.9597

recessive gene *yfc* and the dominant allele for its contrasting green foliage colour is *Yfc* (Fawole, 2003). The symbol proposed for the yellow foliage gene of the IT-719Y mutant is *yfl*, while the dominant allele of normal green foliage will be *Yfl*. The Chi-square test of F_2 progeny of the cross confirmed a goodness-of-fit to the 9 green leaf: 3 bright yellow leaf: 3 yellow flush: 1 whitish yellow ratio. These results fit a two gene recessive epistasis interaction model with new a phenotype (whitish yellow) resulting from interaction between both homozygous recessive. The homozygous recessive genotype (whitish yellow) will be *yfc yfc yfl yfl* while the dominant genotype will be *Yfc _ Yfl _*.

In the cross of IT-719Y x IB-Y-2 all the F_1 plants were green leaf indicating that the genes controlling yellow flush trait in the yellow leaf mutant (IT-719Y) and yellow spec in Ife Brown yellow2 (IB-Y-2) are non-allelic. The yellow spec trait of the IB-Y-2 is controlled by a recessive gene *yfc-3* (Porbeni, 2009) and the dominant allele for its contrasting green foliage colour is *Yfc-3*. The backcross to yellow leaf mutant parent (IT-719Y) gave a 1 green leaf: 1 yellow flush segregation ratio, while the backcross to IB-Y-2 gave a 1 green leaf: 1 yellow spec ratio indicating a monogenic recessive inheritance each for the yellow leaf traits observed in IT-719Y and IB-Y-1. The F_2 progeny confirmed a goodness-of-fit to the 9 green leaf: 3 yellow spec: 4 yellow flush segregation ratio. These results indicated that a complete dominant interaction exists at both gene pairs, but one gene when homozygous recessive (*yfl yfl*) is epistatic to the other (*yfc-3 yfc-3*) i.e. in genotype *yfl yfl yfc-3 yfc-3*, *yfl yfl* masked the effect of *yfc-3 yfc-3* (as if we have *yfl yfl Yfc-3* _).

4.6.4 Inheritance of four-primary leaf mutants

Two of the four-primary leaf mutants selected (IT-719FPL-2Fas and IT-284-FPL-2) were studied for inheritance pattern of the trait. Observations from the inheritance studies of four-primary leaf in crosses of the mutant (IT-719FPL-2Fas) and normal leaf cowpea lines as well as the crosses of the second mutant, IT-284-FPL-2 and normal leaf lines are presented in this section.

Data on the inheritance of four-primary leaf trait in the cross of the mutant line IT-719FPL-2Fas and normal two-primary leaf cowpea lines (IT86D-719 and IB) are shown in Table 4.27. In the two crosses the F_1 plants and backcross to the normal parents gave two primary-leaf seedlings. Backcrosses to the four-primary leaf parents gave 1 two-primary leaf (normal) seedlings: 1 four-primary leaf seedlings, while the F_2 progenies gave 3 normal seedlings: 1 mutant seedling ratios. These suggested that the four-primary leaf seedling type in cowpea is recessive to the two–primary leaf type and is controlled by a single recessive gene. The symbol *fpl* is therefore assigned to the gene

controlling four-primary leaf in IT-719FPL-2Fas, while the normal two-primary leaf genotype will have *Fpl* _.

Table 4.28 shows the results of inheritance studies of four-primary leaf in two crosses IT90K-284-FPL-2 x IT90K-284-2 and IT90K-284-FPL-2 x IB-CR. F_1 progenies in each cross and backcrosses to the normal leaf parents gave two-primary leaf (normal) plant suggesting that the second four-primary leaf is controlled by a recessive gene. However in the F_2 generation and backcrosses to the four-primary leaf parents, 3 seedling phenotypes observed were two-primary leaf plants, three-primary leaf and four-primary leaf mutant plants. The backcrosses to the mutant parent produced 50 normal seedlings: 3 mutant (three-primary leaf + four-primary leaf) seedlings ratios against the expected 1:1 segregation ratio. Data on the F_2 progenies revealed 3 normal seedlings: 1 mutant (three-primary leaf + four-primary leaf) ratios, indicating a monogenic recessive inheritance of four-primary leaf trait observed in IT-284-FPL-2. The mutant gene was not stable but some reverted from four-primary leaf to three-primary leaf in the crosses suggesting that this mutation might have occurred from the action of transposable elements.

Table 4.27. Inheritance of four primary leaf in crosses of the mutant and two primary leaf (normal) lines of cowpea, *Vigna unguiculata* (L) Walp.

Crosses and Generations	Number of plants		Total	Expected Ratio	χ^2	P
	Tpl	Fpl				
IT-719FPL-2Fas x IT86D-719						
IT-719FPL-2Fas		21	21			
IT86D-719	13		13			
F_1	76					
IT-719FPL-2Fas x F_1	383	375	758	1:1	0.0844	0.7714

IT86D-719 x F_1	648		648			
F_2	738	251	989	3:1	0.0758	0.783
IT-719FPL-2Fas x IB						
IT-719FPL-2Fas		17	17			
IB	10		10			
F_1	88		88			
IT-719FPL-2Fas x F_1	337	325	662	1:1	0.2175	0.6409
IB x F_1	648		648			
F_2	406	138	544	3:1	0.0392	0.843

Tpl = Two-primary leaf (normal) seedling, Fpl = Four-primary leaf (mutant) seedling

Table 4.28. Observed numbers of phenotypic classes in crosses between four primary leaf mutant and two primary leaf (normal) cowpea lines

Cross and Generation	Number of plants			Total	Observed Ratio (Normal: Mutant)	Expected Ratio (Normal: Mutant)	χ^2	P
	Normal (Two primary leaf)	Mutants						
		Three primary leaf	Four primary leaf					
IT90K-284-FPL-2 x IT90K-284-2								
IT90K-284-FPL-2			14	14				
IT90K-284-2	17			17				
F_1	83			83				
IT90K-284-FPL-2 x F_1	480	10	18	508	50:3	1:1	402.1732	<0.0001
IT90K-284-2 x F_1	664			664				
F_2	579	76	104	759	3:1	3:1	0.668	0.4138
IT90K-284-FPL-2 x IB-CR								
IT90K-284-FPL-2			21	21				
IB-CR	13			13				
F_1	115			115				
IT90K-284-FPL-2 x F_1	708	14	30	752	50:3	1:1	586.2979	<0.0001
IB-CR x F_1	751			751				
F_2	513	80	72	665	3:1	3:1	1.6286	0.2019

All the 92 F_1 plants produced from the cross between the 2 four-primary leaf mutants (IT-719FPL-2Fas and IT-284-FPL-2) were two-primary leaf seedlings. This showed that the genes controlling four-primary leaf in the two mutants were non-allelic.

4.6.5 Inheritance of double standard petals flower mutant trait

Data on the inheritance of double standard petal flower in crosses of IT-719FPL-2Fas x IT86D-719 and IT-719FPL-2Fas x IB are presented in Table 4.29. In all the crosses F_1 plants and backcrosses to the one standard petal flower (normal) parents were one standard petal flower plants. The backcrosses to double standard petal flower parents gave a 1 one standard petal flower: 1 two standard petal flower mutant segregation ratios, while the F_2 progeny gave a goodness-of-fit to the 3 normal flower: 1 two standard petal flower ratios, indicating a monogenic recessive inheritance for two standard petal flower in the cowpea crosses. However, further studied would be required to confirm if the joint segregation observed between the four-primary leaf trait and the double standard petal trait was as a result of linkage or pleiotropic effects.

4.6.6 Inheritance of fasciated stem mutant trait

Results of the inheritance study of fasciated stem obtained from the crosses of IT-719FPL-2Fas x IT86D-719 and IT-719FPL-2Fas x IB are presented in Table 4.30. The F_1 offspring and backcrosses to non-fasciated parents gave non-fasciated stem plants. The progenies of backcrosses to fasciated stem parents produced 1 non-fasciated stem: 1 fasciated stem ratios, while data on the F_2 plants gave a goodness-of-fit to 3 non-fasciated stem: 1 fasciated stem ratio, implying a monogenic recessive inheritance for fasciated stem mutant observed in this study. However, further studied would be needed to confirm if the joint segregation observed between the four-primary leaf trait and the fasciated stem trait was to linkage or pleiotropic effects. This mutant is also characterized by the production of extra floral parts in various numbers suggesting that the mutation might have occurred due to the action of transposable elements. The variation observed in the

Table 4.29. Inheritance of double standard petal trait in crosses of two standard petal mutant one standard petal lines of cowpea, *Vigna unguiculata* (L) Walp.

Crosses and Generations	Number of plants		Total	Expected Ratio	χ^2	P
	OSP (normal)	TSP				
IT-719FPL-2Fas x IT86D-719						
IT-719FPL-2Fas		21	21			
IT86D-719	13		13			
F_1	76					
IT-719FPL-2Fas x F_1	383	375	758	1:1	0.0844	0.7714
IT86D-719 x F_1	648		648			
F_2	738	251	989	3:1	0.0758	0.783
IT-719FPL-2Fas x IB						
IT-719FPL-2Fas		17	17			
IB	10		10			
F_1	88		88			
IT-719FPL-2Fas x F_1	337	325	662	1:1	0.2175	0.6409
IB x F_1	648		648			
F_2	406	138	544	3:1	0.0392	0.843

OSPF = One standard petal flower (normal) plant, TSPF = Two-standard petal flower (mutant) plant

Table 4.30. Inheritance of fasciated stem trait in crosses of fasciated stem mutant and non-fasciated stem lines of cowpea, *Vigna unguiculata* (L) Walp.

Crosses and Generations	Number of plants		Total	Expected Ratio	χ^2	P
	Non-Fasciated stem	Fasciated stem				
IT-719FPL-2Fas x IT86D-719						
IT-719FPL-2Fas		21	21			
IT86D-719	13		13			
F_1	76					
IT-719FPL-2Fas x F_1	383	375	758	1:1	0.0844	0.7714
IT86D-719 x F_1	648		648			
F_2	738	251	989	3:1	0.0758	0.783
IT-719FPL-2Fas x IB						
IT-719FPL-2Fas		17	17			
IB	10		10			
F_1	88		88			
IT-719FPL-2Fas x F_1	337	325	662	1:1	0.2175	0.6409
IB x F_1	648		648			
F_2	406	138	544	3:1	0.0392	0.843

number of petals, stamens and carpel was such that an increase in the number of one floral part complemented a reduction of the other floral part.

Joint segregation data on inheritance of four primary leaf, two standard petal and fasciated stem traits in dihybrid crosses of IT-719FPL-2Fas x IT86D-719 and IT-719FPL-2Fas x IB are presented in Tables 4.31 and 4.32. In the two crosses, all the F_1 and backcrosses to mutant the produced two primary leaf. However, the F2 and backcrosses to the normal cowpea (IB) resulted in joint segregation ratio 3:1. This showed that there is linkage between the genes controlling four primary leaf, two standard petal and fasciated stem traits in the mutant.

4.6.7 Inheritance of burnt leaf cowpea mutant

Data on the inheritance pattern of burnt leaf obtained from the crosses IT86D-719 x IT-719BN-1, IT90K-284-2 x IT-719BN-1, IT-719BN-2 x IT86D-719 and IT-719BN-2 x IT90K-284-2 are presented in Table 4.33. For each cross, all F_1 plants and backcrosses to the normal leaf parents all gave progenies with normal leaf. Chi-square tests of the data for the backcrosses to the burnt leaf parents and the F_2 gave a goodness-of-fit to the 1 normal: 1 burnt leaf and 3 normal: 1 burnt leaf ratios respectively, indicating monogenic recessive inheritance of the burnt leaf trait in IT-719BN-1 and IT-719BN-2 cowpea mutants.

Table 4.31. Inheritance of four primary leaf, two standard petal and fasciated stem traits in crosses of four primary leaf two standard petal fasciated stem mutant and one primary leaf one standard petal and non-fasciated stem lines of cowpea

Cross and Generation	Number of plants						Total	Expected Ratio	χ^2	P
	Two primary leaf	Four primary leaf	One standard petal	Double standard petals	Non-fasciated stem	Fasciated stem				
IT-719FPL-2Fas x IT86D-719										
IT-719FPL-2Fas		21					21			
IT86D-719	13						13			
F_1	76						76			
IT-719FPL-2Fas x F_1	383	375					758	1:1*	0.0844	0.7714
IT-719FPL-2Fas x F_1			383	375			758	1:1**	0.0844	0.7714
IT-719FPL-2Fas x F_1					383	375	758	1:1***	0.0844	0.7714
IT86D-719 x F_1	648						648			
F_2	738	251					989	3:1*	239.8069	< 0.0001
F_2			738	251			989	3:1**	239.8069	< 0.0001
F_2					738	251	989	3:1***	239.8069	< 0.0001

* One standard petal and non-fasciated stem plants combined into two primary leaf phenotype while double standard petal and fasciated stem plants combined into four primary leaf phenotypic trait
** Two primary leaf and non-fasciated stem plants combined into one standard petal phenotype while four primary leaf and fasciated stem plants combined into double standard petal phenotypic trait
*** Two primary leaf and one standard petal plants combined into non-fasciated stem phenotype while four primary leaf and double standard petal plants combined into fasciated stem phenotypic trait

Table 4.32. Inheritance of four primary leaf, two standard petal and fasciated stem traits in crosses of four primary leaf two standard petal fasciated stem mutant and one primary leaf one standard petal and non-fasciated stem lines of cowpea

Crosses and Generations	Number of plants						Total	Expected Ratio	χ^2	P
	Two primary leaf	Four primary leaf	One standard petal	Double standard petals	Non-fasciated stem	Fasciated stem				
IT-719FPL-2Fas x IB										
IT-719FPL-2Fas		17					17			
IB	10						10			
F_1	88						88			
IT-719FPL-2Fas x F_1	337	325					662	1:1*	0.2175	0.6409
IT-719FPL-2Fas x F_1			337	325			662	1:1*	0.2175	0.6409
IT-719FPL-2Fas x F_1					337	325	662	1:1*	0.2175	0.6409
IB x F_1	717						717			
F_2	406	138					544	3:1*	132.0294	0.0001
F_2			406	138			544	3:1*	132.0294	0.0001
F_2					406	138	544	3:1*	132.0294	0.0001

* One standard petal and non-fasciated stem plants combined into two primary leaf phenotype while double standard petal and fasciated stem plants combined into four primary leaf phenotypic trait
** Two primary leaf and non-fasciated stem plants combined into one standard petal phenotype while four primary leaf and fasciated stem plants combined into double standard petal phenotypic trait

Table 4.33. Inheritance of burnt leaf in crosses of burnt leaf mutant and normal leaf lines of cowpea, *Vigna unguiculata* (L) Walp.

Cross and Generation	Number of plants		Total	Expected Ratio	χ^2	P
	Normal leaf	Burnt leaf				
IT86D-719 x IT-719BN-1						
IT86D-719	15		15			
IT-719BN-1		17	17			
F_1	78		78			
IT86D-719 x F_1	629		629			
IT-719BN-1 x F_1	401	387	788	1:1	0.2487	0.618
F_2	453	146	599	3:1	0.1252	0.7235
IT90K-284-2 x IT-719BN-1						
IT90K-284-2	16		16			
IT-719BN-1		22	22			
F_1	105		105			
IT90K-284-2 x F_1	802		802			
IT-719BN-1 x F_1	377	365	742	1:1	0.1941	0.6596
F_2	593	195	788	3:1	0.0271	0.8693
IT-719BN-2 x IT86D-719						
IT-719BN-2		23	23			
IT86D-719	13		13			
F_1	86		86			
IT-719BN-2 x F_1	483	477	960	1:1	0.0375	0.8465
IT86D-719 x F_1	582		582			
F_2	876	288	1164	3:1	0.0412	0.8391
IT-719BN-2 x IT90K-284-2						
IT-719BN-2		23	23			
IT90K-284-2	20		20			
F_1	95		95			
IT-719BN-2 x F_1	375	367	742	1:1	0.0863	0.769
IT90K-284-2 x F_1	709		709			
F_2	894	302	1196	3:1	0.0401	0.8412

Results of the cross between the two burnt leaf mutants IT-719BN-1 and IT-719BN-2 showed that all the 74 F_1 progeny were all burnt leaf, confirming that the recessive genes controlling burnt leaf in the two mutant lines were allelic. Therefore, the gene controlling burnt leaf trait was assigned symbol *bnl* with homozygous recessive lines having the genotype *bnl bnl* and the dominant (normal leaf) lines the genotype *Bnl* _. This mutation appeared to be deleterious to plant growth since the mutant plants were less vigorous when compared with the parent. However, the mutation did not show pleiotropic effects on the floral parts and the pod.

CHAPTER 5

DISCUSSION

The process of exposing living organisms to physical or chemical agents for the purpose of inducing mutation is referred to as mutagenesis. This process may produce immediate effects such as physiological disturbance on the organism and induced mutations which can be transmitted to subsequent generations (Sparrow, 1961). The results of gamma radiation mutagenesis for all the cowpea accessions used in this study showed definite trends which corroborate earlier findings (Mba *et al*., 2012; Horn and Shimelis, 2013). Generally, percentage seedling emergence and seedling survival were inversely related to radiation dosage. The higher the absorbed dosage of ionizing radiation, the lower the percentage seedling emergence and seedling survival in cowpea and vice versa. The low percentage seedling emergence and very low or no seedling survival observed at higher doses were due to severe damage of some vital embryonic cells or tissues. Lagoda (2012) and Mudibu, *et al*. (2012) among many authors reported that gamma (ionizing) radiation can damage and affect the morphology, anatomy, physiological and biochemical processes in plants depending on the radiation level. These effects can appear at various stages of plant development and may cause abnormal cell division, cell death, mutation, tissue and organ failure and reduction of plant growth. This finding therefore implies that high dosage of gamma-irradiation would limit the number of M_2 generation plants available for mutant screening in certain genotypes of cowpea.

The results of the radio-sensitivity test revealed a wide variation in the rate of seedling emergence and seedling survival among all the cowpea accessions studied. However, the lower rates of seedling emergence and seedling survival observed among IB and its derivatives when compared to the advanced cultivars with higher rates appeared to be due to inherent genetic properties of these cowpea accessions. The wide variation observed in the estimated LD_{50} among the eight cowpea accessions studied agrees with the reports of Horn and Shimelis (2013) who reported the LD_{50} of radio-

susceptible cowpea varieties Nakara and Shindimba was less than 200Gy whereas the radio-resistance cultivar Bira exhibited LD_{50} above 600Gy. The observed variation in the LD_{50} among the cowpea genotypes was as a result of the differences in their genetic constitutions which translate into variations in their seed characteristics and consequent differential responses to absorbed gamma irradiation. The absorbed radiation dosage is directly related to the surface texture of the seed coat. Cowpea seeds with rough testa surface appeared to be radio-susceptible in comparison to cowpea with smooth testa surface which were relatively radio-resistant to gamma irradiation. This implies that lower ionizing radiation dosage is required to reach LD_{50} for seedling emergence and seedling survival in cowpeas with rough testa and vice versa. There appear to be a direct relationship between testa thickness of cowpea and the susceptibility to gamma irradiation.

This study revealed that low level of gamma radiation treatment (100Gy) increased the primary leaf area, terminal leaflet area, seedling height and plant height at six weeks in cowpea. There appeared to be some stimulating effect of gamma radiation up to 100Gy on cowpea plant vigor. Similar observations were reported by Jones (1965) that low radiation doses up to 1.4×10^{12} neutrons/cm^2 and 5,000 r X-ray significantly increased seedling height of southern-peas. Horn and Shimelis (2013) also reported that low radiation doses are accompanied by early emergence, increased percent germination and field survival with healthy and vigorous seedlings. This suggests that low gamma radiation treatment (up to 100Gy) can be used to stimulate cowpea vegetative growth and plant vigor at M_1 generation. However, the reduction in primary leaf area and seedling height as radiation treatments increased in all the cowpea accessions indicate that seedling vigor appeared to be inversely related to gamma dosage intensity above 100Gy. This is because the primary leaf area and seedling height determine the plant seedling vigor and survival. Doses of 200Gy upward resulted in a marked reduction in the vigor of all cowpea accessions used in this study. One of the indices of plant vigor is plant height at six weeks. The observed plant height at six weeks across radiation treatments and cowpea genotypes shows that the vigor of M_1 cowpea plants reduces with increasing radiation dosage. These results are consistent with earlier research reported by Horn and

Shimelis (2013) in cowpea, Mudibu, *et al*. (2012) in soybeans, Harding *et al*. (2012) in rice, Kon *et al*. (2007) in long beans and Norfadzrin *et al*. (2007) in tomato and okra. However, radiation treatments did not appear to affect terminal leaflet area of cowpea plants at M_1 generation. The cowpea accessions IT86D-1010 and IT90K-284-2 were able to withstand the effects of gamma radiation up to 300Gy before noticeable reduction in the terminal leaflet area were observed at 400Gy and 500Gy probably due to the smooth surface and thick testa of their seeds.

Brown (2013) listed some negative consequences of radiation overdoses such as deletions of DNA nucleotide sequences that may cause reading-frame shifts, inactive protein products, or faulty transcripts. It therefore implies that seed treatment with gamma rays at higher doses has inhibitory effects on the vegetative growth of M_1 plants. This is because ionizing radiation at higher levels is injurious to some enzymes and growth hormones (Lagoda, 2012) which may have contributed to the reduction in cell multiplication and growth in plants. Consequently, this contributed to the low amount of seeds harvested at higher radiation doses. This reduction in the amount of seed harvested at high radiation treatments in some accessions was unexpected since there were very low plant survivals at those radiation treatments. This finding therefore implies that the lethal effect of high radiation doses would limit the number of M_2 generation plants available for screening and beneficial mutant selection in certain genotypes of cowpea.

Variation in the genetic constitution of plant material used may explain the differential responses of the 8 cowpea cultivars to gamma irradiation. In mutation induction, radio-sensitivity is performed with the purpose of selecting the optimal treatment for a specific genotype. The seed physical characteristic may help in the selection of dose or range of doses for radio-sensitivity test and mutation breeding in cowpea (*Vigna unguiculata*).

Treatment of fresh pollen grains with 30,000µWs/cm^2 UV rays for a short duration (60 minutes) before pollination appeared to enhance seed setting in cowpea, while exposure of pollen to the radiation for longer period (>60 minutes) has inhibitory effect on seed setting. The result of radiation dosage effect curves shows that seed setting in cowpea following pollination with UV irradiated pollen appeared to be dose

dependent. Nucleic acids are damaged when exposed to high level of UV radiation which may cause the pollen grains to lose viability. Britt (1995), Ravanat *et al*. (2001) and Lagoda (2012) reported that UV radiation has deleterious effects on cellular DNA which may be either mutagenic or toxic and the induced damage can lead to cell death due to photochemical damage. Variation in the genetic background of the cowpea accessions may explain the variability in LD_{50} observed in this study.

The results obtained from percentage seedling emergence and seedling survival suggests that there appeared to be some reversion of UV induced mutagenic changes in the plant genetic materials. The null effect of UV radiation on seedling emergence and seedling survival in the M_2 generation could also be attributed to the repairs of induced damages to the plants by certain biochemical mechanisms. This is consistent with observed reversion of the three-primary leaf and four-primary leaf seedling mutants when advanced from M_3 to M_4 generation and from M_3 to M_5 generation respectively. Since plants are unique in the obligatory nature of their exposure to UV, Britt (1995) hypothesized that they may have evolved particularly efficient mechanisms for the elimination of UV-induced DNA damages. Low frequencies of mutations recorded in this study revealed that cowpea plant is considered less amenable to the application of UV irradiated pollination as a practical breeding method. Similar observation was reported by Chin and Gordon (1989b) in rice pollinated with gamma irradiated pollen grains. The weak mutagenic effect of UV even at higher doses has been suggested to be the result of the occurrence of a dark repair system in plant cells (Britt, 1995; Gavazzi and Sanguineti, 1983).

The yellow and white seedling (albino) mutants observed at high frequencies in most treatments were chlorophyll deficient. The absence of chlorophyll has been attributed to a localized block of one of the intermediate steps in the pathway of chlorophyll synthesis (Ojomo and Chheda, 1975). Lack of chlorophyll in the primary leaves and stem produced lethal effect on these albino seedlings shortly after germination. High frequencies of mutations such as those observed for the albino trait have been attributed to the action of transposable elements (Fawole, 1988). Loss of these albino mutants at the seedling stage did not allow further study to be conducted on them.

However, several authors have reported on the inheritance of mutations resulting in chlorophyll deficiency in cowpea. Saunders (1960) and Kirchhoff *et al*. (1989) concluded that chlorophyll deficiency in cowpea mutants was controlled by single recessive gene.

One of the benefits of induced mutation is that it is used to create genetic variations and provides the raw materials for genetic studies and for the breeders to develop new varieties of plants and animals. Some cowpea mutants with novel phenotypes were selected from five out of the eight accessions used in this study. The tall-erect non-branching cowpea mutant (IB-ER) could be a useful mutant to breed for tall and erect cowpea varieties that may be used for mechanized farming. Fasciated stem mutant (IT-719FLP-2Fas) because of numerous pods produced on fasciated peduncle and the big seed mutants (IT90K-BS-1, IT90K-BS-3 and IT90K-BS-4) selected possess morpho-agronomic characters that could be used for the improvement of cowpea production. The fasciated stem mutants (IT-719FLP-2Fas) because of its vigor and numerous leaf productions may be a useful line for breeders to develop fodder cowpea varieties. Excess of floral parts produced by this mutant was as result of changes in its genetic material caused by gamma mutagenesis. Two and three carpel found in its flower implies that a cowpea variety with twin and triplet pods could be developed using this mutant line. Porbeni (2009) earlier reported a twin pod that arose from fasciated stem cowpea mutant having two or more styles. The four-primary leaf mutants (IT-719FLP-1 and IT-719FLP-2Fas) and narrow leaf seedling mutant (IT89KD-NL) could be used as genetic markers at seedling stage of plant growth. Burnt leaf mutants (IT-719BN-1 and IT-719BN-2), double standard petal flower mutant (IT-719FLP-2Fas) and lettuce leaf mutant (IB-LT) could also be useful as genetic marker. Yellow leaf mutants (IT-719Y and IT-719SLY) could be used to develop plant with aesthetic value. Porbeni (2009) selected some cowpea lines for their ornamental potentials. Diverse novel cowpea mutant lines selected and bred true in this study shows that new genetic variability could be created by induced mutation to widen the genetic diversity and increase germplasm collection of crop plants.

The yellow or white seedlings were lethal mutants because they were devoid of chlorophyll needed for photosynthesis. Among many authors, Olasupo (2004) reported

similar observations from cowpea seeds treated with ethyl methane sulphonate. Ojomo (1972) reported that the condition is controlled by two pairs of major genes in the homozygous condition. The three-primary leaf mutants selected could not breed true at M_3 generation probably due to reversion of the mutation. It seems as if there is genetic instability in the gene controlling three-primary leaf trait in cowpea.

The higher mutation rate observed in IT86D-719, IT86D-1010, IT86KD-374-57 and IT90K-284-2 than in Ife Brown (IB) and its derivatives corroborates earlier mutation study in cowpea by Ojomo and Chheda (1975). Several authors had suggested that this differences is related the genetic background or constitution of test varieties (Brook, 1965; Brook, 1967; van Harten, 1998; Mba *et al*., 2010). Ojomo (1972) proposed among other factors, that mutability of a gene may also be influenced by remaining genetic system in an organism. IT86D-719, IT86D-1010, IT86KD-374-57 and IT90K-284-2 were advanced cultivar which makes their genes more prone to mutation than IB and its derivatives.

The five mutant lines and their parent cultivar, IB showed wide genetic variability for all the traits studied. Variation observed among growth habit traits and yield components and their relatively high coefficients of variation implies that the lines are diverse in characters of interest. The genotypic variance which were higher than environmental variance indicate that observed variations in the traits studied were as a result of the difference in their genetic make-up. The longest plant height and peduncle length recorded in IB-ER implies that the mutant could be used to develop a cowpea variety for mechanized farming. In addition to this, longer peduncle could be an added advantage in cowpea because raised pods on it may be prevented from soil borne infections. The higher genotypic variance obtained in all the yield components evaluated except for the pod length implies that observed variations in the yield parameters were as a result of variation in the genetic constitutions of the cowpea mutants and parental lines. IB-ER competed averagely in all the yield components evaluated with the parental cultivar, IB and IB-BPC variety which demonstrates the agronomic potential of the mutant.

The fasciated stem mutant (IT-719FPL-2Fas) was more vigorous produced higher vegetative growth than the parent, IT86D-719 and other lines. This implies that this mutant has the potential to be used as fodder crop or integrated into dual purpose cowpea breeding progamme. The high values of environmental variance recorded for pod length, number of pod per peduncle and 100-seed weight suggest that there was little genetic contribution to the variation observed for these parameters in this experiment. Generally, high coefficient of variation was observed in the yield components studied. This demonstrates that induced mutation could create additional variability to serve as raw material for plant breeding or supplement existing germplasm of crop plants.

The narrow leaf mutant (IT86KD-G400NL) was significantly different from the parent in all morpho-agronomic traits studied. This revealed the extent of genetic changes caused by the mutagen in the plant genome. The long and narrow traits of this mutant could be used as genetic marker at seedling stage of growth. It could also be useful for physiological studies and linkage analysis. Fawole (1997) stressed the possible use of mutant for physiological studies and the development of genetic linkage map for cowpea. The large variability observed among the mutant lines derived from IT90K-284-2 in the quantitative growth traits (except in number of branches) and yield components demonstrates the creation of additional genetic diversity by induce mutation to supplement existing variability for cowpea improvement. These results agree with the report of Forster and Shu (2012) that improvement in plant breeding can only be made when sufficient variation for a given trait is available to the breeder. The big seed mutants could be of benefit in breeding for big seed cowpea varieties. The knowledge of the nature and amount of genetic diversity available in a germplasm is important in the sense that it forms the basis of genetic research and breeding programme. Detailed and accurate assessment of genetic variability among new mutants is vital for their preservation and utilization for crop varietal development.

Molecular characterisation has greatly complemented phenotypic evaluation among plants and animals species since the evolution of gene theory. PCR-based DNA markers are very valuable tools to plant geneticists and breeders because they are useful for precise estimates of genetic diversity and genome mapping. The results of genetic

diversity assessment in this study demonstrate that SSR markers could be used for molecular characterization of cowpea mutant lines. It has been previously reported that the use of SSR markers were effective for genetic diversity and phylogenetic relationships among cowpea accessions (Lee *et al*., 2009; Li *et al*., 2001; Ogunkanmi *et a*l., 2008; Asare *et al*., 2010; Xu *et al*., 2010; Badiane *et al*., 2012). Tan *et al* (2012) asserted that SSR is the most frequently used marker in the genetic diversity analysis of cowpea.

The relatively low percentage transitions (7.27%) as compared to percentage transversion (23.64%) mutations among the mutants studied is in contrast to the report of Lee *et al*. (2012) that transitions occur more frequently than transversions in mutational events. When compared to other regions of the sequences, high mutation rate was recorded between region 2-6 and 128-130 of all samples studied with regions 3-4 and 129 being the highest mutable sites. This result explains the variability in the observed mutant phenotypes in this study. Therefore, the effects of any gene mutation on an organism will vary, depending upon the type and where the mutation occurs.

The four groups obtained from the phylogenetic tree which is irrespective of the mutant origin indicated that similarity within the mutant populations is independent of the RBCL sequence data. The sequence data could not separate the parental lines from their mutant derivatives; this seems justified that each of the parents and their mutants are components within the single *Vigna unguiculata* entity. This observation indicates that the new cowpea mutant lines have inherent genetic potentials that could not be differentiated by the RBCL marker.

Results of inheritance studies for some of the mutant characters shows that induce mutation can create new alleles which could be useful to broaden our knowledge of genetic concepts. The pattern of inheritance of some of the mutations were in agreement with earlier reports of Mendelian segregation patterns in many plants, while deviations were recorded in the inheritance pattern of others. No published work was available to serve as background to the inheritance pattern of most of the traits studied because they were newly induced mutations. The erect tall mutation affects the plant architecture such that the mutant has erect, tall, long and raised peduncle with non-branching growth habit

and lacks the spreading attribute of the parent and other cowpea varieties. This mutation is heritable and under the control of a single recessive gene, *et*. The erect stem trait of the mutant is recessive to the normal crawling and branching stem of the parent. Further studies would be needed to determine the possibility of linkage in the genes controlling erect stem, branching and long peduncle in cowpea.

The results of inheritance pattern of lettuce leaf trait in the cross of the mutant and the crinkled leaf parent (IB-LT x IB-CR) showed a recessive inheritance for the mutant trait in cowpea. Fawole (1997) earlier reported that crinkled leaf trait is under the control of a single recessive gene, *crl*. Further studies on the lettuce leaf trait in crosses of the mutant and two normal cowpea lines having smooth and normal green leaf colour revealed that twisted-pale leaf trait was the new mutation actually induced by gamma rays in Ife Brown Crinkle (IB-CR). The twisted-pale leaf mutation affects the leaf structure and colour of the leaf which is twisted and pale in contrast to the coarse and dark-green leaf of the crinkled parent and the non-twisted and normal green leaf colour of Ife Brown and other cowpea lines. Analysis of the segregation pattern of the twisted-pale trait in crosses of IB-LT x IB and IB-LT x IT86D-719 showed that this mutation is under the control of a single recessive gene, *tp*. The interaction of the two genes, *crl* and *tp* in homozygous recessive (*crl crl tp tp*) condition gives the new lettuce leaf phenotype.

Yellow flush mutation of the cowpea line IT-719Y produced yellow foliage colour that appeared to be more pronounced in younger leaves of the mutant than in older leaves. This mutation is heritable and under the control of a single recessive gene. Yellow leaf mutants arose from Ife Brown cowpea had been reported and the genes controlling these traits were assigned *yfc* (Fawole, 2003) and *yfc-3* (Porbeni, 2009). However, allelic tests revealed that the new yellow foliage mutant is non-allelic to the pre-existing yellow foliage mutants, Ife Brown Yellow (IB-Y-1) described by Fawole (2003) and IB-Y-2 described by Porbeni (2009). Therefore the symbol *yfl* is designated to the recessive gene controlling yellow foliage colour in the mutant IT-719Y and the normal green foliage phenotype is assigned *Yfl* _. The allelic result of the cross between IT-719Y and IB-Y-1 fits a two gene recessive epistasis interaction model with new a phenotype (whitish yellow) resulting from interaction between both homozygous recessive genes (*yfc yfc yfl*

yfl). Similarly, allelic test between IT-719Y and IB-Y-2 shows that the new yellow foliage mutant is non-allelic to IB-Y-2. The result further revealed that a complete dominant interaction exists at both gene pairs, but one gene when homozygous recessive is epistatic to the other such that *yfl yfl* masked the effect of *yfc-3 yfc-3* in the homozygous recessive (*yfl yfl yfc-3 yfc-3)* condition. As a result of this, the expected 9:3:3:1 Mendelian ratio from the dihybrid cross was modified to 9 green leaf: 3 yellow spec: 4 yellow flush segregation ratio.

This study revealed that the four-primary leaf mutation in IT-719FPL-2Fas occurred in the nuclear gene of cowpea and it is heritable under the control of a recessive gene *fpl*. No previous mutation on four-primary leaf trait has been reported in cowpea. However, the four-primary leaf mutation observed in IT90K-284-FPL-2 showed a monogenic recessive inheritance pattern but the F_2 progenies of the crosses IT90K-284-FPL-2 x IT90K-284-2 and IT90K-284-FPL-2 x IB-CR. F_1 revealed some reversion of the four-primary leaf mutation back to three-primary leaf trait indicating genetic instability of the mutation in IT90K-284-2. This may be as a result of the structural disorder and damages caused by gamma radiation on the chromosome of the plant. Allelic test between the 2 four-primary leaf mutants (IT-719FPL-2Fas and IT90K-284FPL-2) showed that the genes controlling four-primary leaf in the two mutants were non-allelic. This indicates that the mutations occurred at different loci in the two cultivars. This result is consistent with the different segregation pattern demonstrated by the two mutants as earlier explained.

The monogenic inheritance pattern of fasciated stem mutant in crosses of IT-719FPL-2Fas x IT86D-719 and IT-719FPL-2Fas x IB agree with the findings of Adu-Dapaah *et al*. (1999) and Porbeni (2009). It appears that this mutant might have resulted from the action of transposable elements due to the fact that the mutation is associated with the variation in the number of petals, stamens and carpel. An increase in the number of one floral part complemented a reduction of the other floral part. This result corroborates the findings of Porbeni (2009).

The burnt leaf mutation affects the upper surface feature and colour of the leaves which is partially folded at the margin and pale green in contrast to the straight margin

and normal green colour of the parent. This mutant trait is heritable under the control of a single recessive gene *bnl*, while the normal genotype will be *Bnl_*. Allelic test between the two burnt leaf mutants, IT-719BN-1 and IT-719-BN-2 indicates that the mutations occurred at the same locus in the two cowpea lines. This mutation is deleterious to plant growth since the mutant plants were less vigorous when compared with the parent. However, the mutation did not show pleiotropic effects on the floral parts and the pod. The lines may be used in the physiological studies and in the development of genetic linkage map for cowpea.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Induced mutation is a valuable tool for creating new genetic variability to complement existing germplasm and broaden crop genetic base. Therefore, creating genetic diversity is needful for effective plant breeding programmes. The present study was initiated to assess the radio-sensitivity of cowpea accessions to seed and pollen irradiated with gamma and UV rays respectively, to select, characterize and determined the inheritance pattern of new mutants. Eight cowpea accessions were evaluated. Radio-sensitivity of the accessions to gamma and UV irradiations were respectively, determined at M_1 and M_2 generations. At M_3 and M_4 generations, the lines were phenotyped on field to select for new mutants with novel phenotypic and agronomic traits. SSR markers were used to determine the genetic diversity of the mutant and parental lines, while their sequence analysis and characterization were done using rbcl primers. The agronomic potential of these mutants and their inheritance nature were explored.

Gamma irradiation of cowpea seeds at 100 Gy did not affect the germination and seedling survival of most of the accessions at M_1 generation. However, seed germination and seedling survival reduced with increasing radiation dosage from 200 Gy and above. Generally, plant biomass at M_1 increased with gamma radiation up to 100 Gy, above which it reduced with increasing radiation intensity. This study has revealed that the susceptibility of cowpea to gamma irradiation is more associated with the texture and thickness of the seed testa than mean seed weight. The rate of mutation induced by gamma rays was higher in elite cultivars (IT86D-719, IT86D-1010, IT86KD-374-57 and IT90K-284-2) than in Ife Brown and its derivatives. No stable cowpea mutant was produced from pollen mutagenesis by UV irradiation in this study, indicating that the technique is not efficient for cowpea mutation breeding.

Several cowpea mutants were selected that show wide genetic diversity from the parental lines. In this study, induce mutation has been used to broaden the genetic base and increase germplasm collection of cowpea. Point mutations (base substitutions) and indels were the two main classes of mutations induced in the plastid DNA of selected mutants in this study. The tall-erect non-branching cowpea mutant (IB-ER) selected can be used to develop tall and erect cowpea varieties for mechanized cultivation. The big seed mutants (IT90K-BS-1, IT90K-BS-3 and IT90K-BS-4) selected can be used for the improvement of cowpea production. The fasciated stem mutants (IT-719FLP-2Fas) because of its vigor and increased biomass can be a useful line for developing improved fodder cowpea varieties. This study revealed that twisted-pale trait of IB-LT is under the control of a single recessive gene, *tp*. The interaction of the crinkled leaf gene (*crl*) and twisted-pale gene (*tp*) in homozygous recessive (*crl crl tp tp*) condition gives the new lettuce leaf phenotype. New yellow flush mutant (IT-719Y) selected in this study is non-allelic to Ife Brown Yellow-1 (IB-Y-1) and Ife Brown Yellow-2 (IB-Y-2) and it is under the control of a single recessive gene, *yfl*. The four-primary leaf mutation (IT-719FPL-2Fas) occurred in the nuclear gene of cowpea and it is heritable under the control of a recessive gene, *fpl*. This is the first four-primary leaf mutant trait reported in cowpea.

The following are the recommendations from this study:

1. Radio-sensitivity needs to be determined for each genotype prior to mutation induction.
2. Mutagen and the plant material to be used in the mutagenesis treatment are key factors that must be considered in mutation breeding for successful crop improvement.
3. There is the need to further investigate the mutagenic effect of pollination with irradiated pollen in cowpea using other physical mutagens.
4. There is the need for whole genome sequence of all the new cowpea mutants selected in order to add to our knowledge of the mutations and cowpea genetic structure.

5. Novel traits of morphological and agronomic values inherent in selected mutants in this study should be explored for the benefit of cowpea improvement.
6. Several mutant lines selected could be used for molecular studies of biochemical pathways, physiological studies and for the improvement of cowpea

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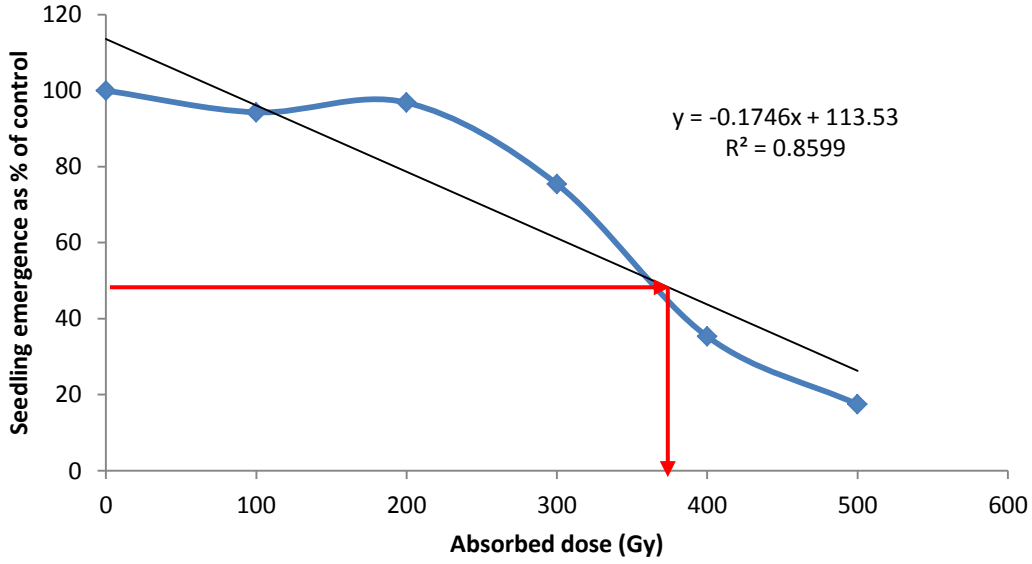
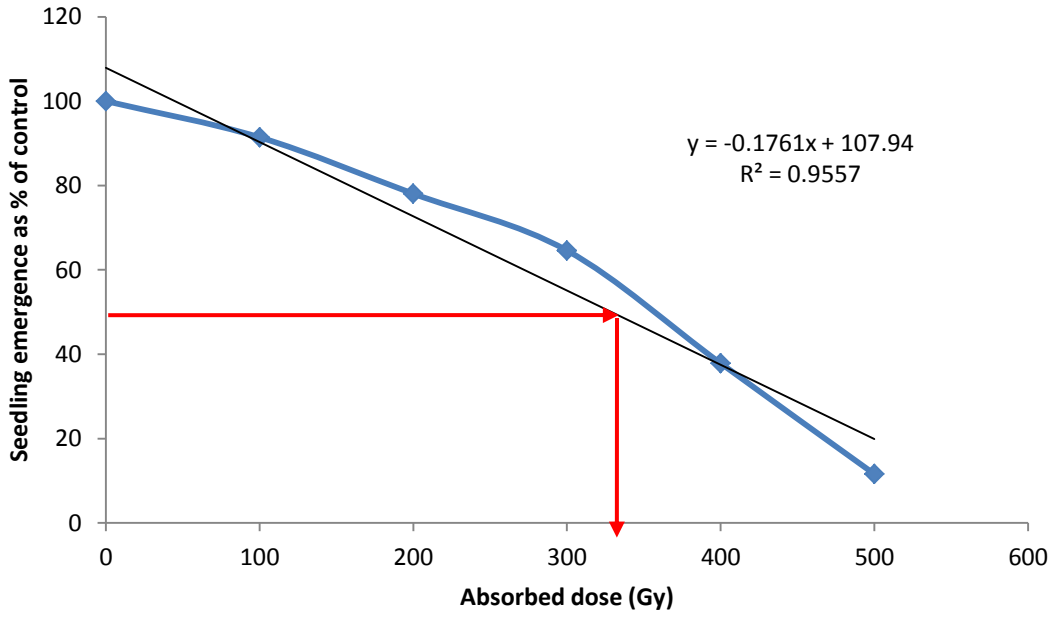
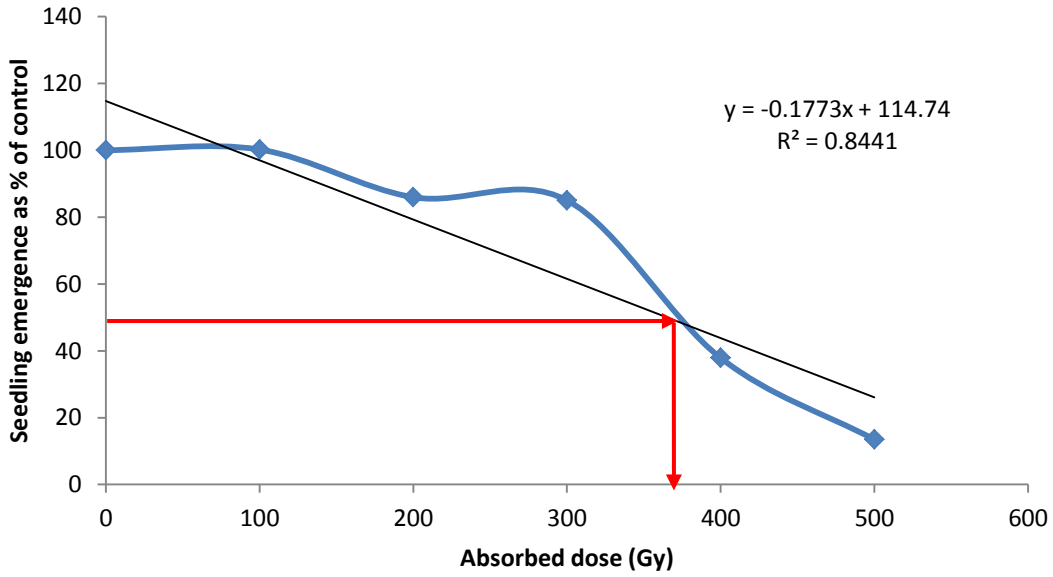
APPENDICES



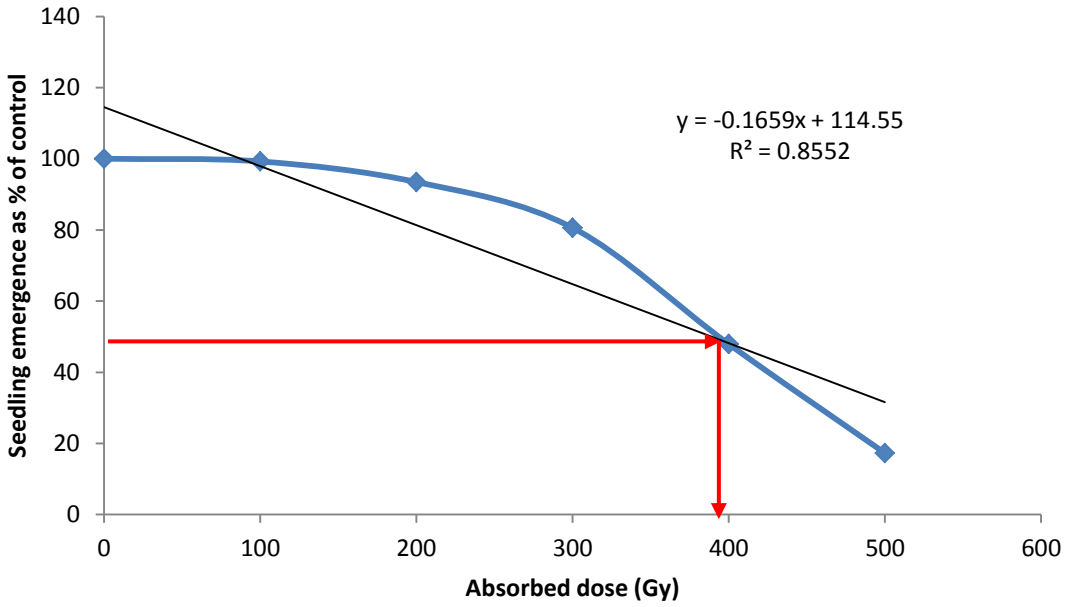
Appendix 1. Percentage of reduction in the seedling emergence of IB cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage



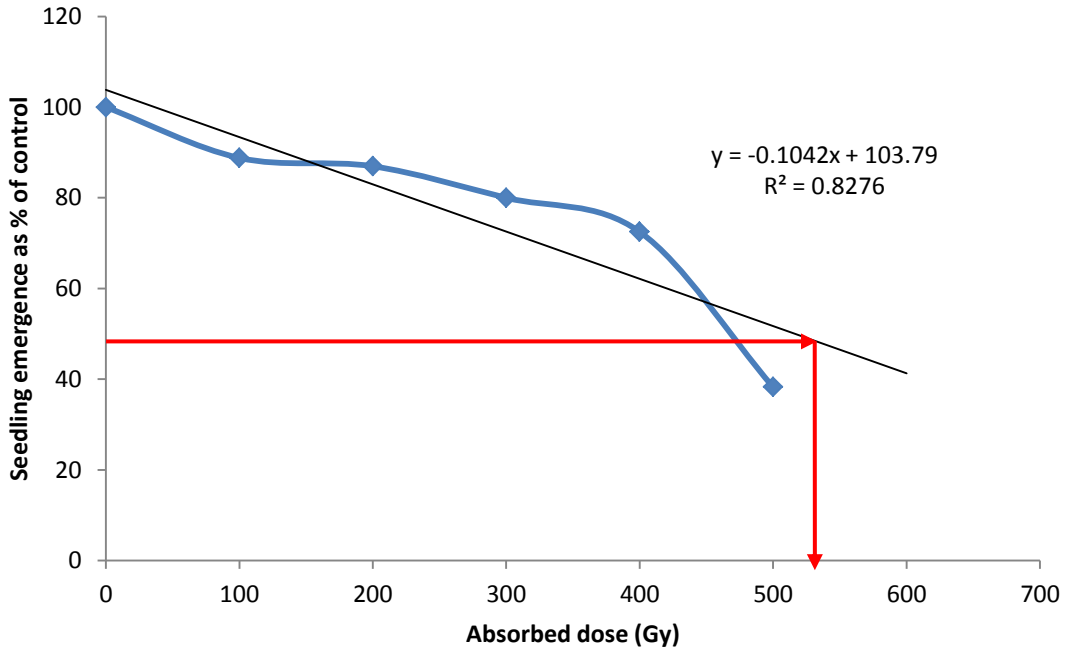
Appendix 2. Percentage of reduction in the seedling emergence of IB-Y-1 cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage.



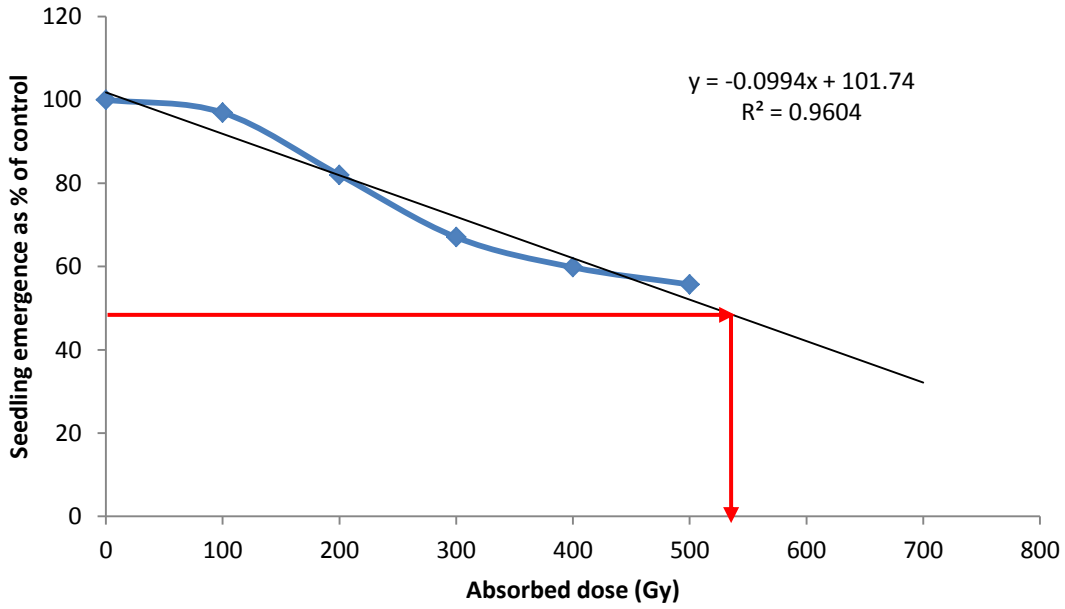
Appendix 3. Percentage of reduction in the seedling emergence of IB-CR cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage.



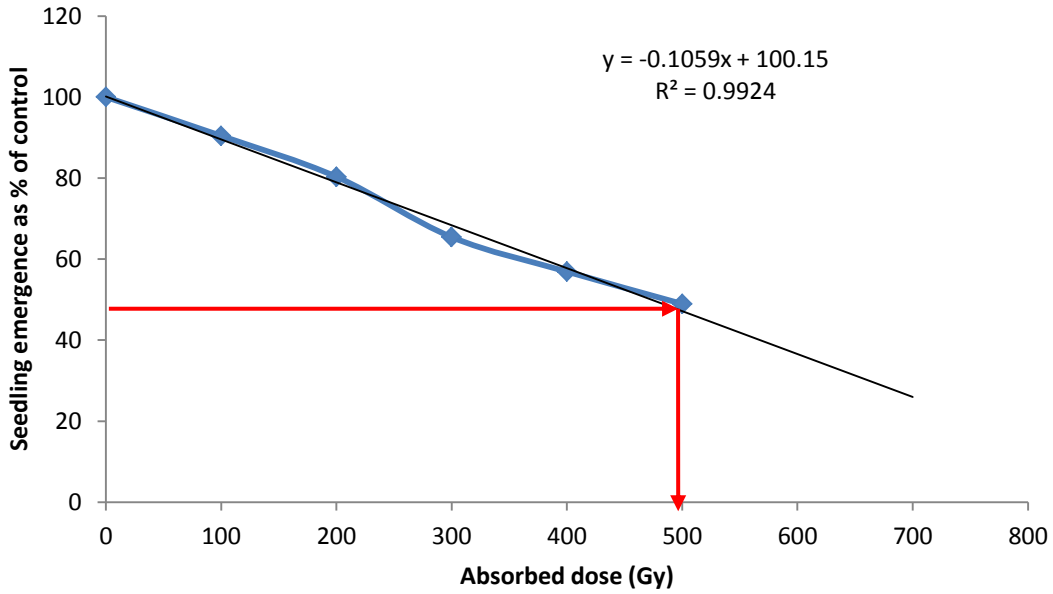
Appendix 4. Percentage of reduction in the seedling emergence of IB-BPC cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage.



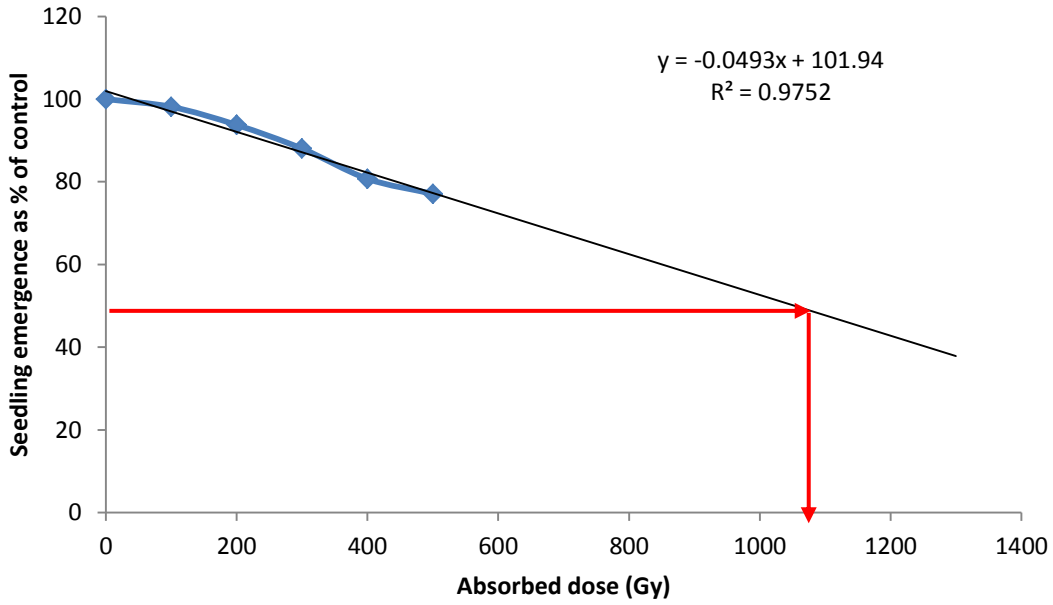
Appendix 5. Percentage of reduction in the seedling emergence of IT86D-719 cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage



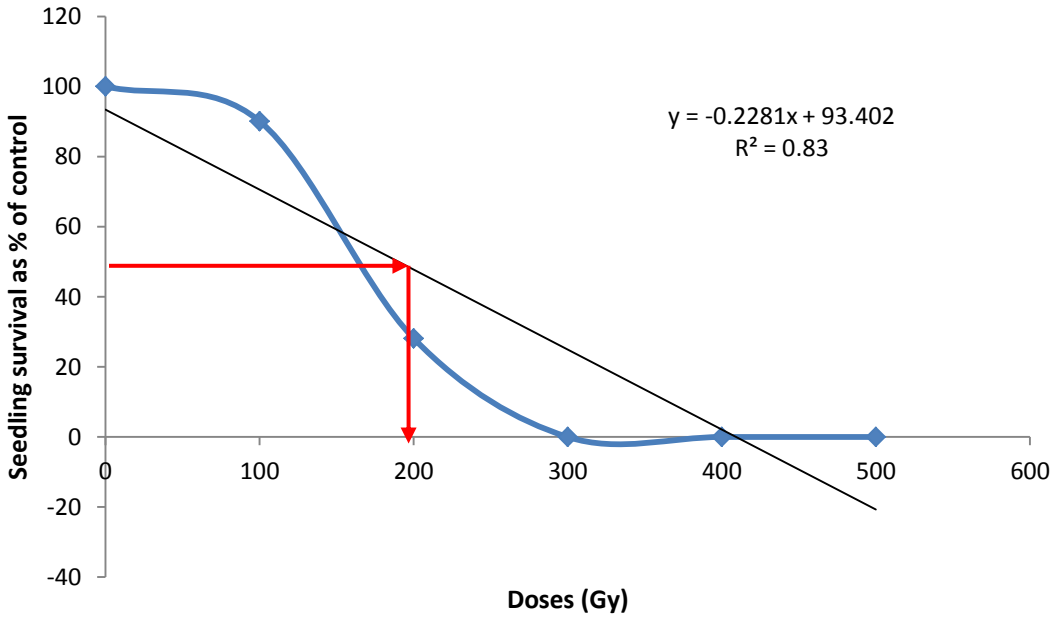
Appendix 6. Percentage of reduction in the seedling emergence of IT86D-1010 cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage



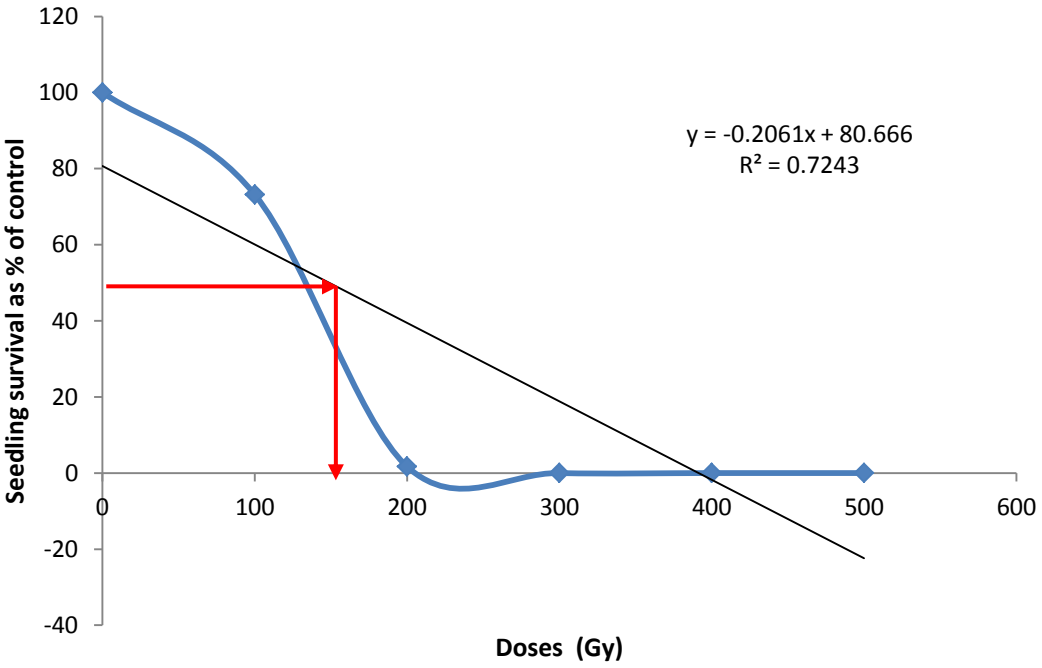
Appendix 7. Percentage of reduction in the seedling emergence of IT89KD-374-57 cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage



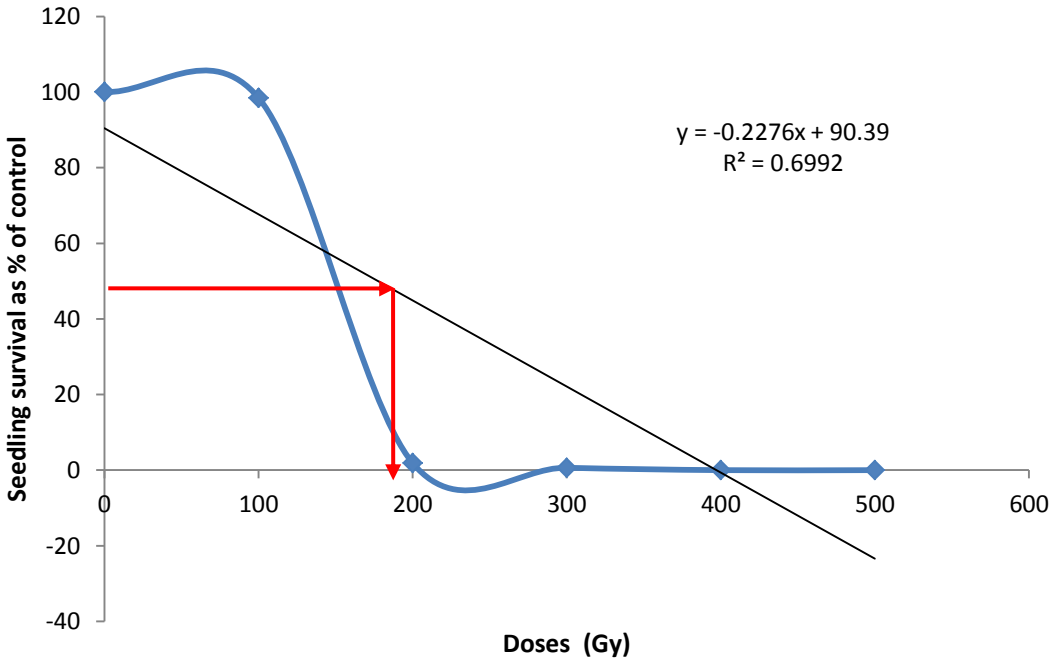
Appendix 8. Percentage of reduction in the seedling emergence of IT90K-284-2 cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage



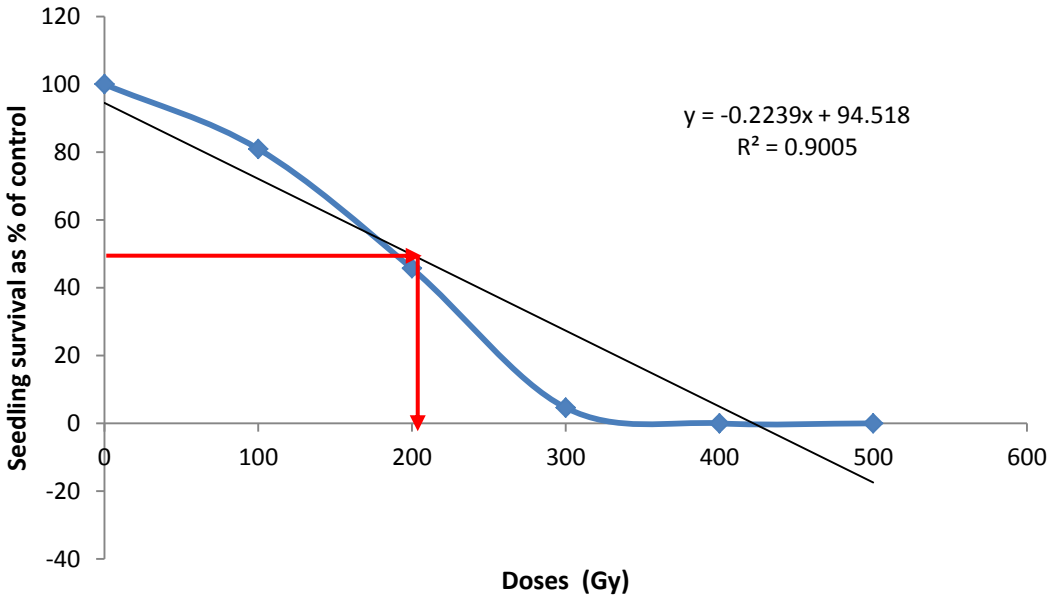
Appendix 9. Percentage of reduction in seedlings survival of IB cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage



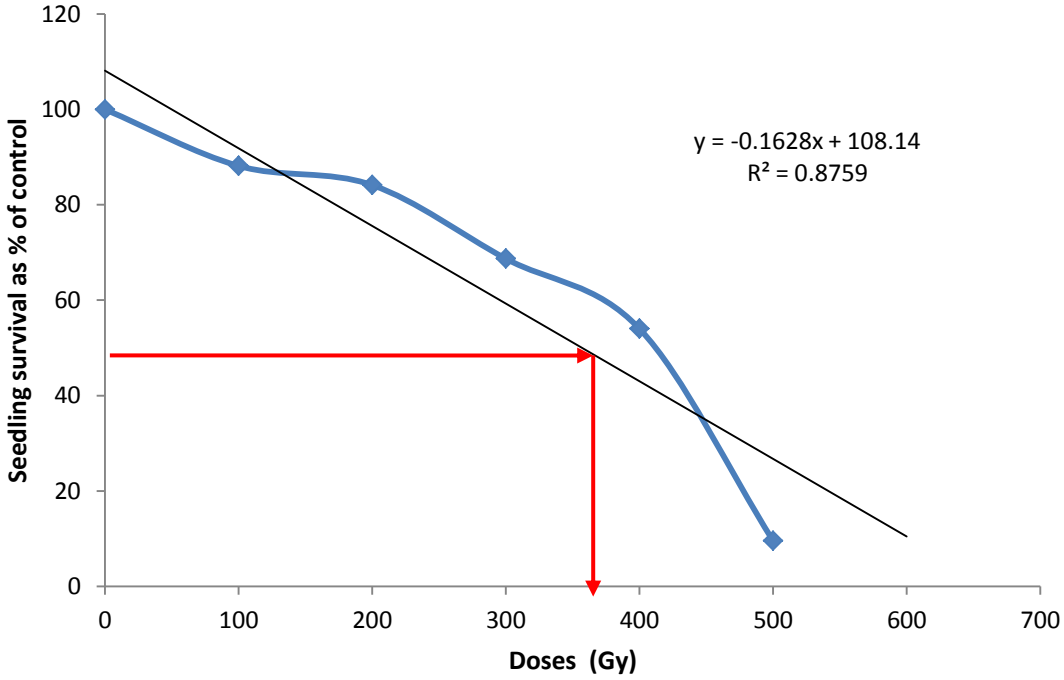
Appendix 10. Percentage of reduction in seedlings survival of IB-Y-1 cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage



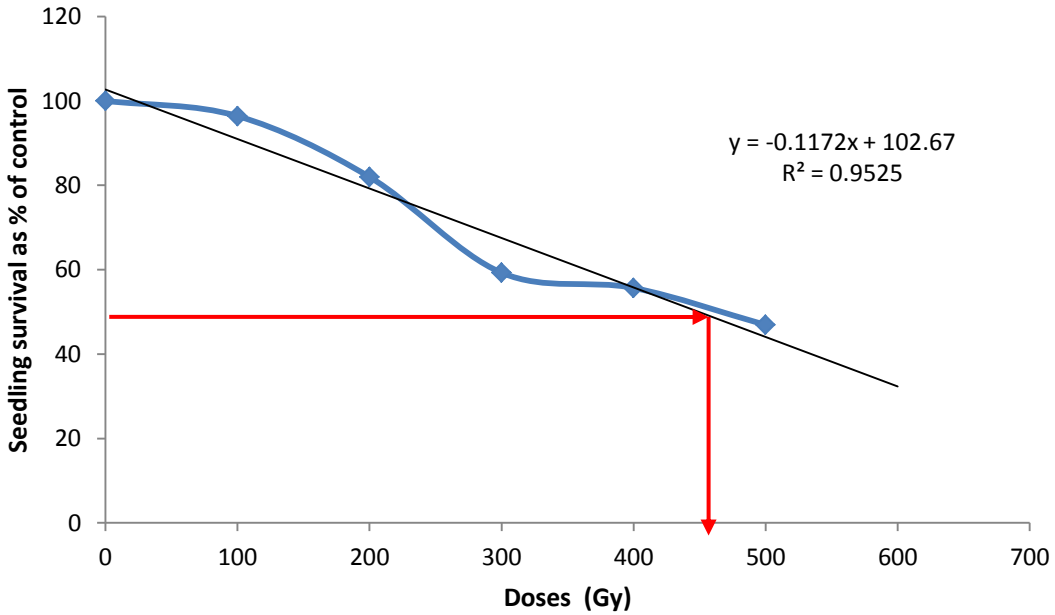
Appendix 11. Percentage of reduction in seedlings survival of IB-CR cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage



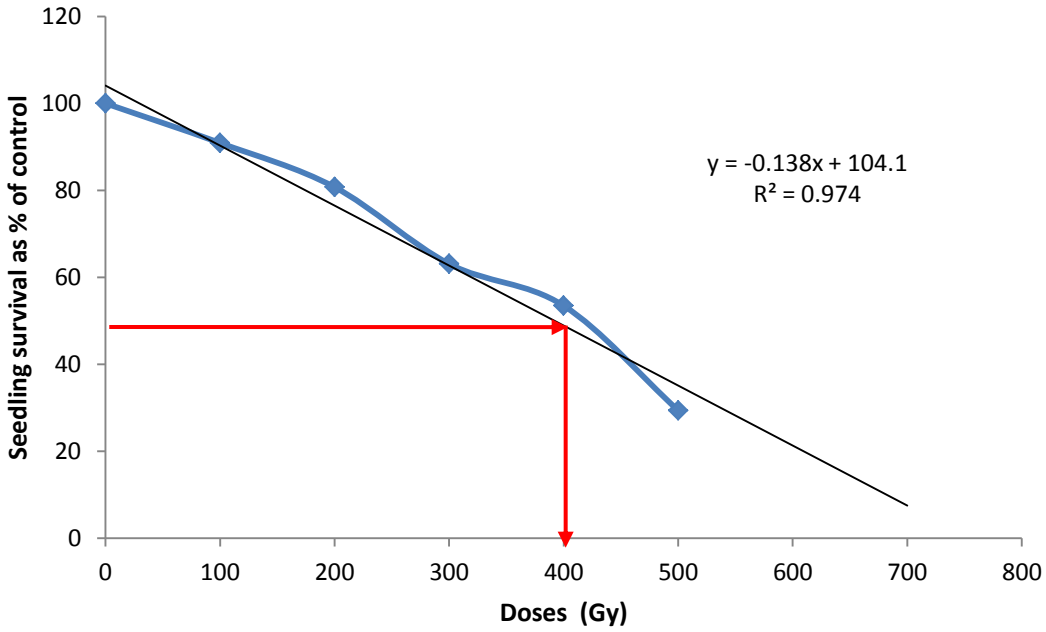
Appendix 12. Percentage of reduction in seedlings survival of IB-BPC cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage



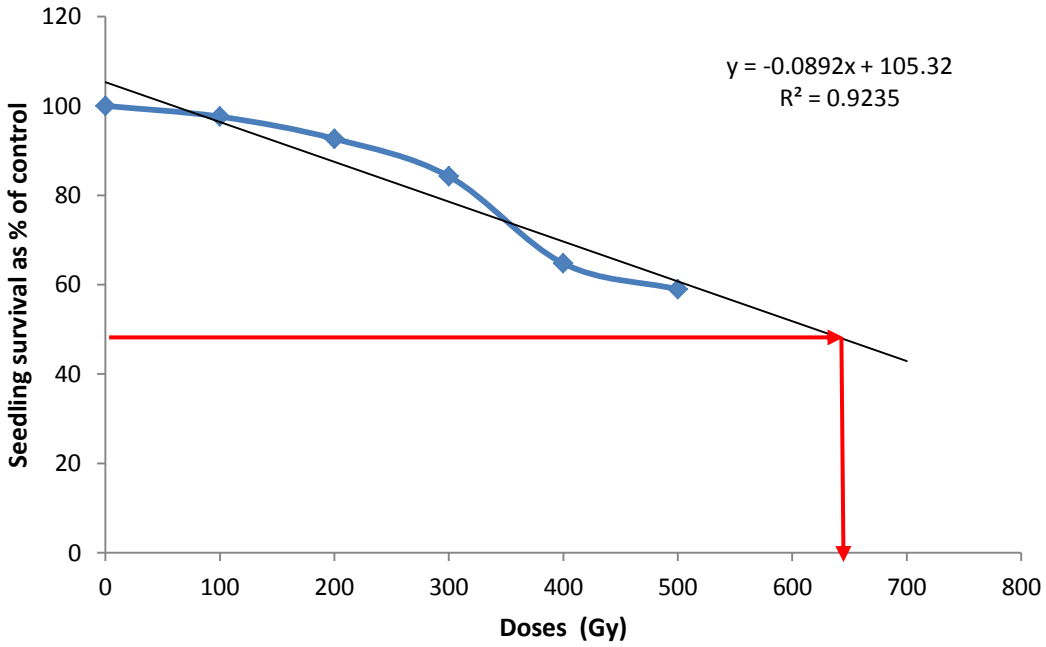
Appendix 13. Percentage of reduction in seedlings survival of IT86D-719 cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage.



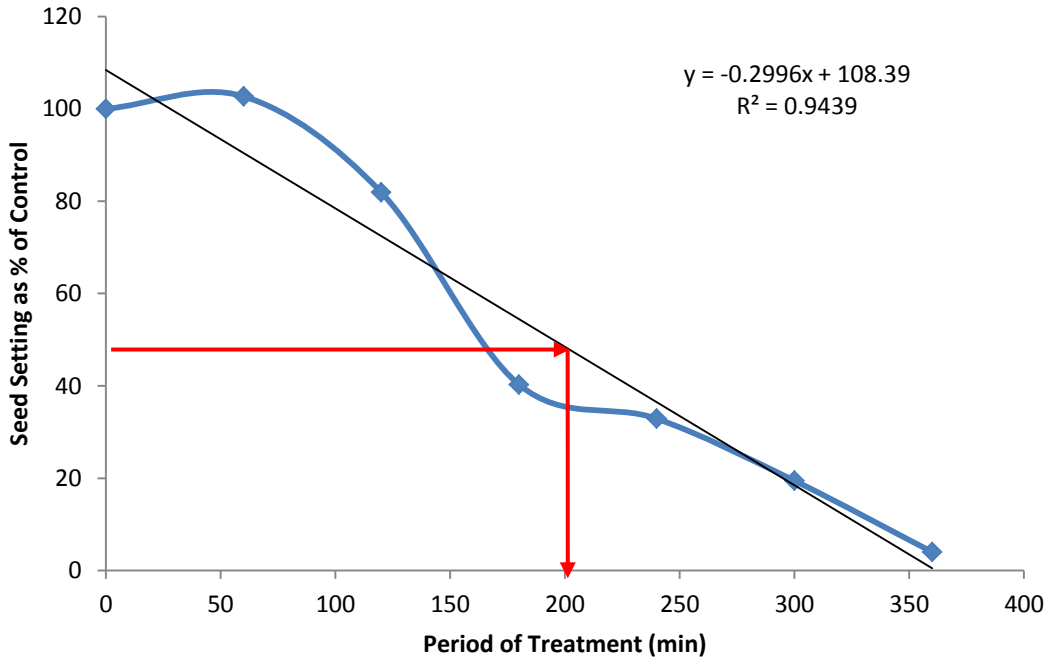
Appendix 14. Percentage of reduction in seedlings survival of IT86D-1010 cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage



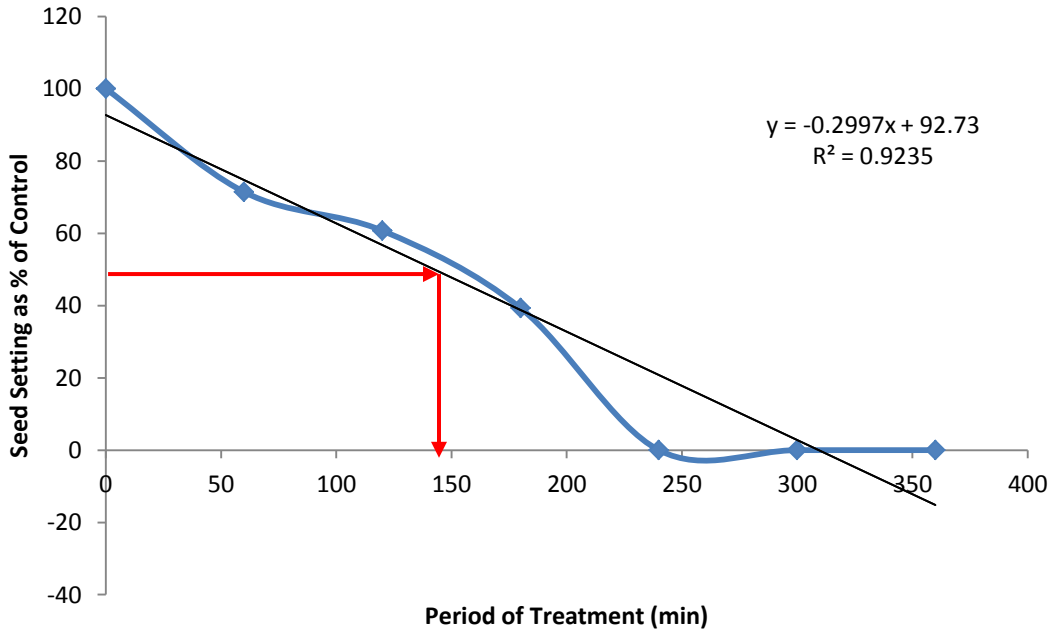
Appendix 15. Percentage of reduction in seedlings survival of IT89KD-374-57 cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage



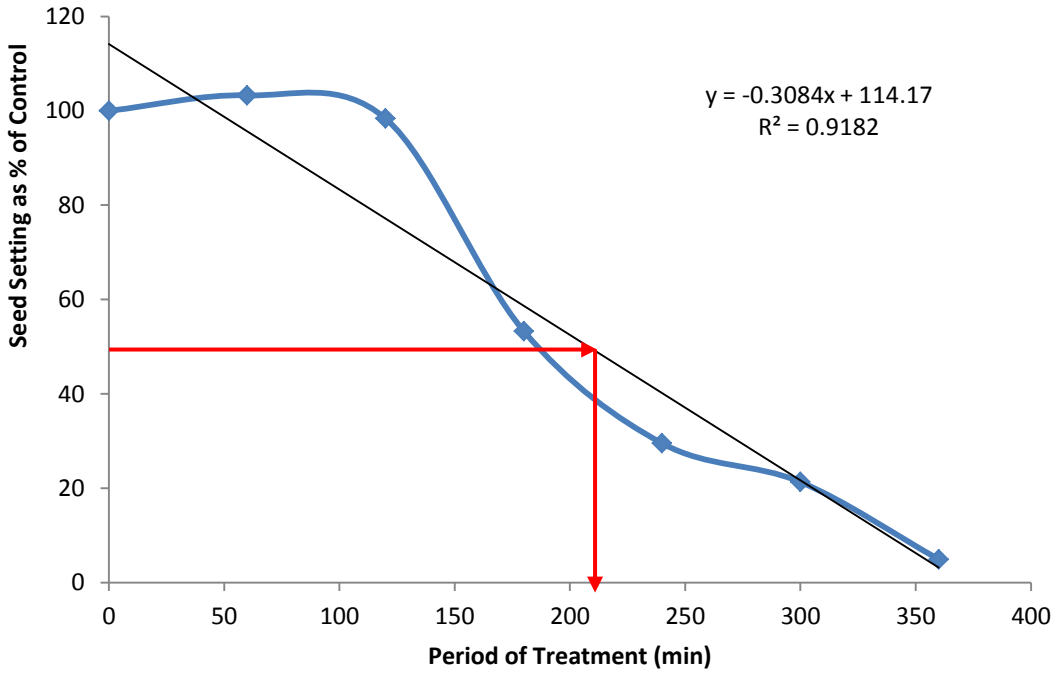
Appendix 16. Percentage of reduction in seedlings survival of IT90K-284-2 cowpea seeds exposed to gamma irradiation, plotted against gamma irradiation dosage



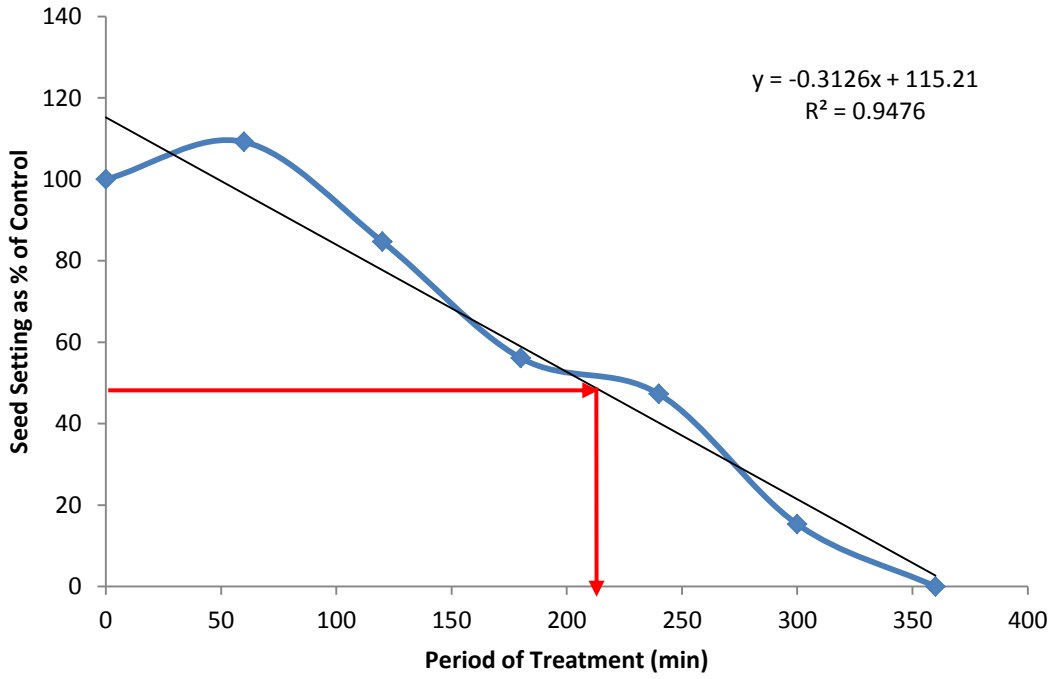
Appendix 17. Percentage of reduction in seed setting of IB cowpea pollen exposed to UV irradiation, plotted against UV irradiation period



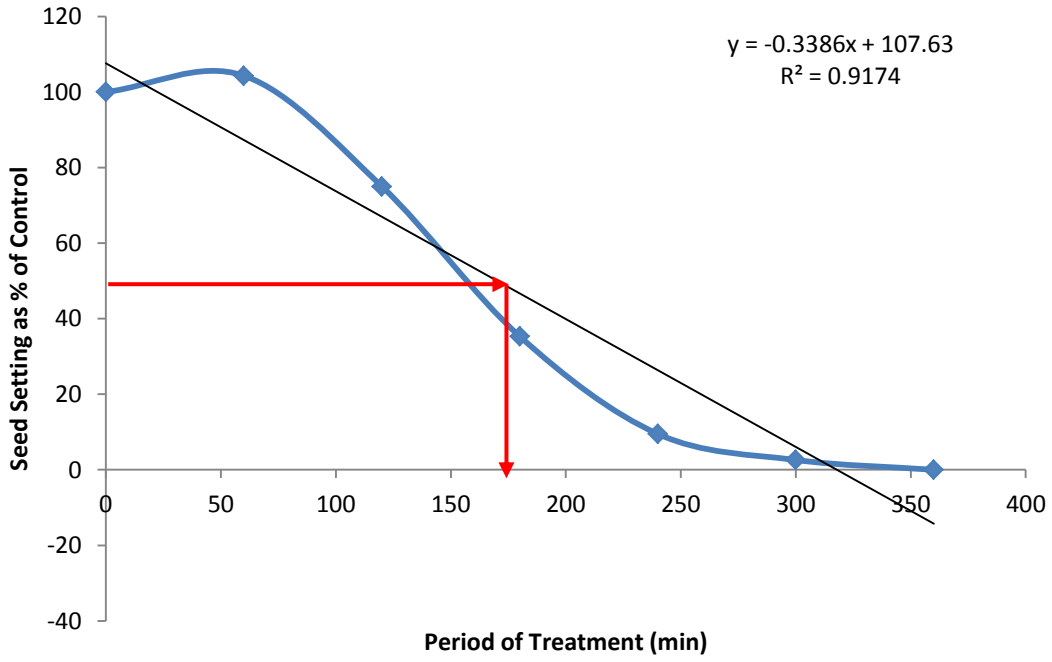
Appendix 18. Percentage of reduction in seed setting of IB-Y-1 cowpea pollen exposed to UV irradiation, plotted against UV irradiation period



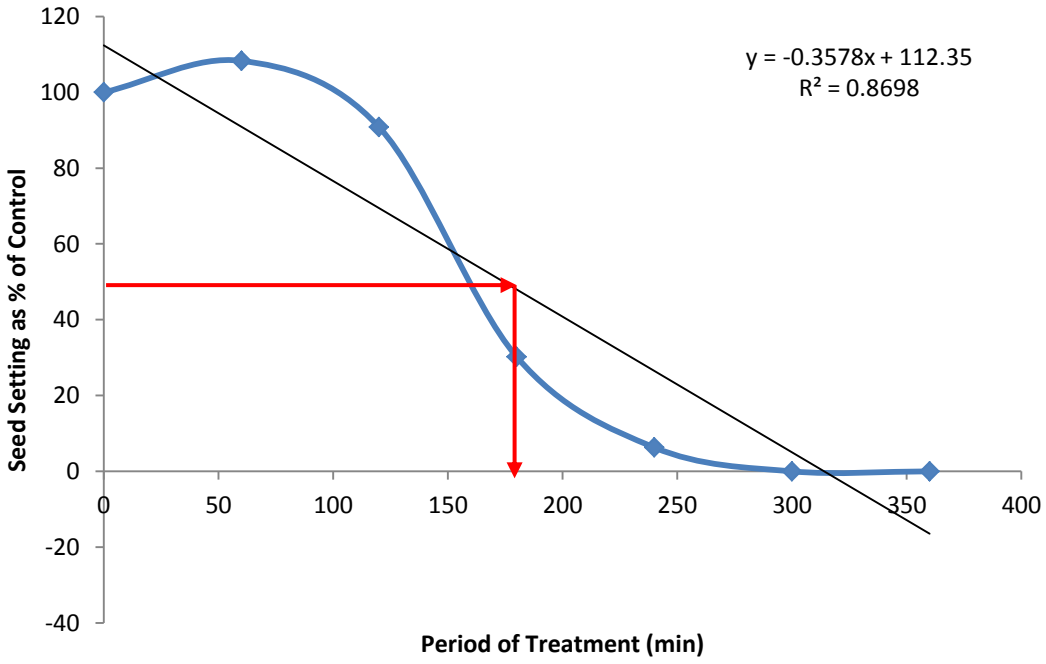
Appendix 19. Percentage of reduction in seed setting of IB-CR cowpea pollen exposed to UV irradiation, plotted against UV irradiation period



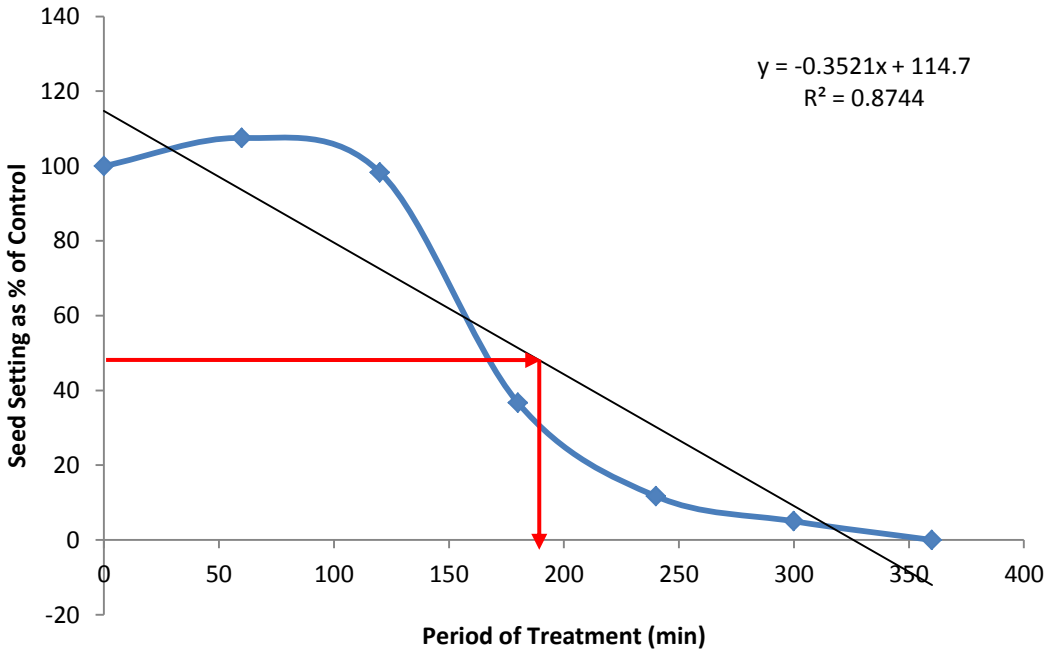
Appendix 20. Percentage of reduction in seed setting of IB-BPC cowpea pollen exposed to UV irradiation, plotted against UV irradiation period



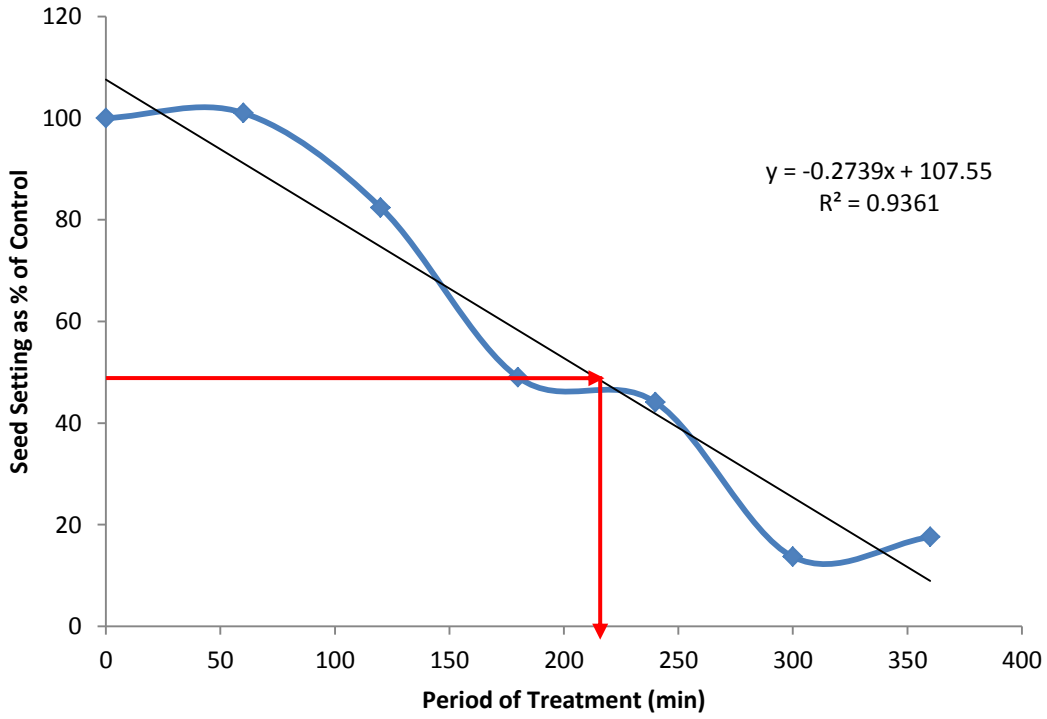
Appendix 21. Percentage of reduction in seed setting of IT86D-719 cowpea pollen exposed to UV irradiation, plotted against UV irradiation period



Appendix 22. Percentage of reduction in seed setting of IT86D-1010 cowpea pollen exposed to UV irradiation, plotted against UV irradiation Period

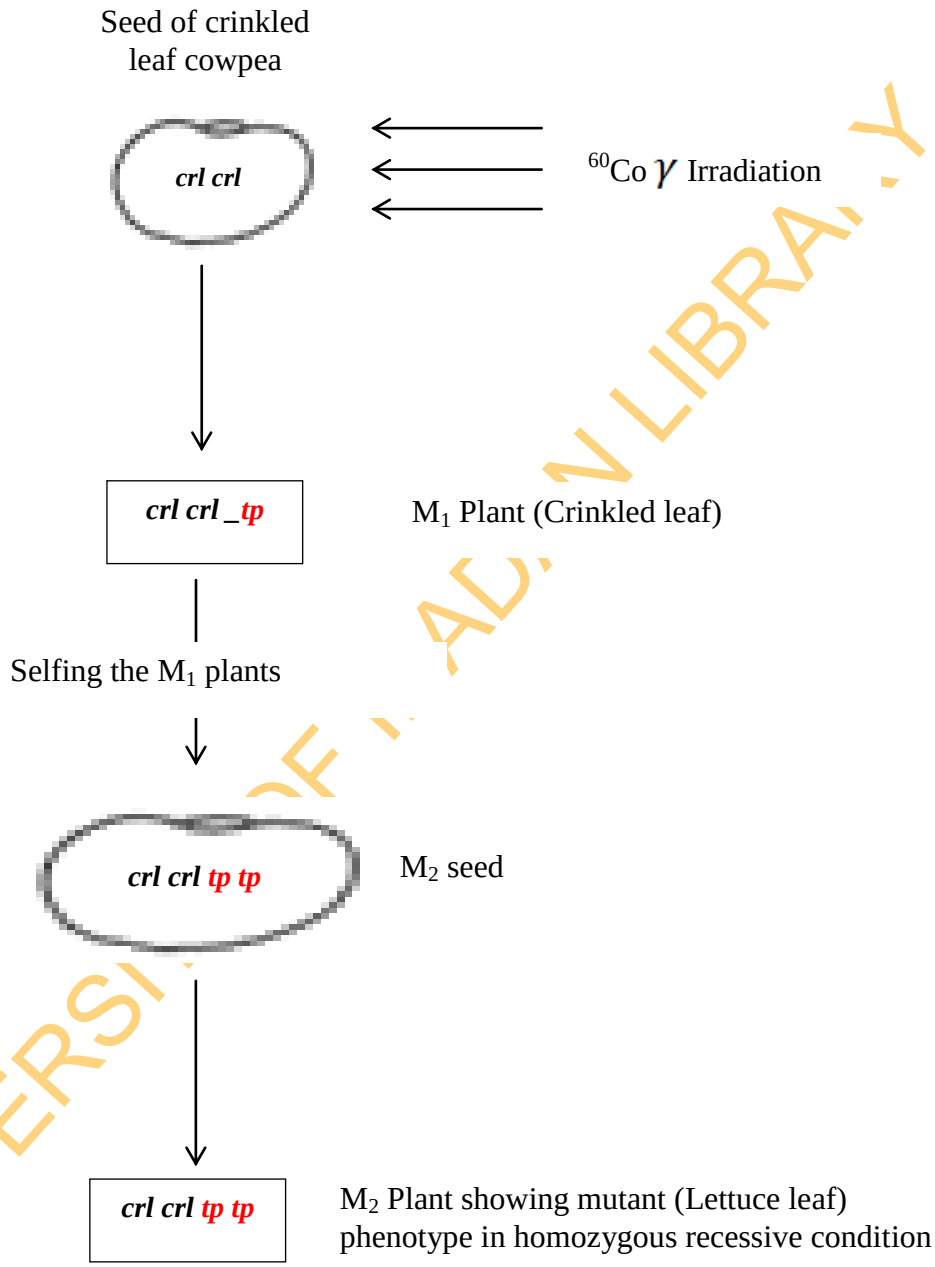


Appendix 23. Percentage of reduction in seed setting of IT89KD-374-57 cowpea pollen exposed to UV irradiation, plotted against UV irradiation period

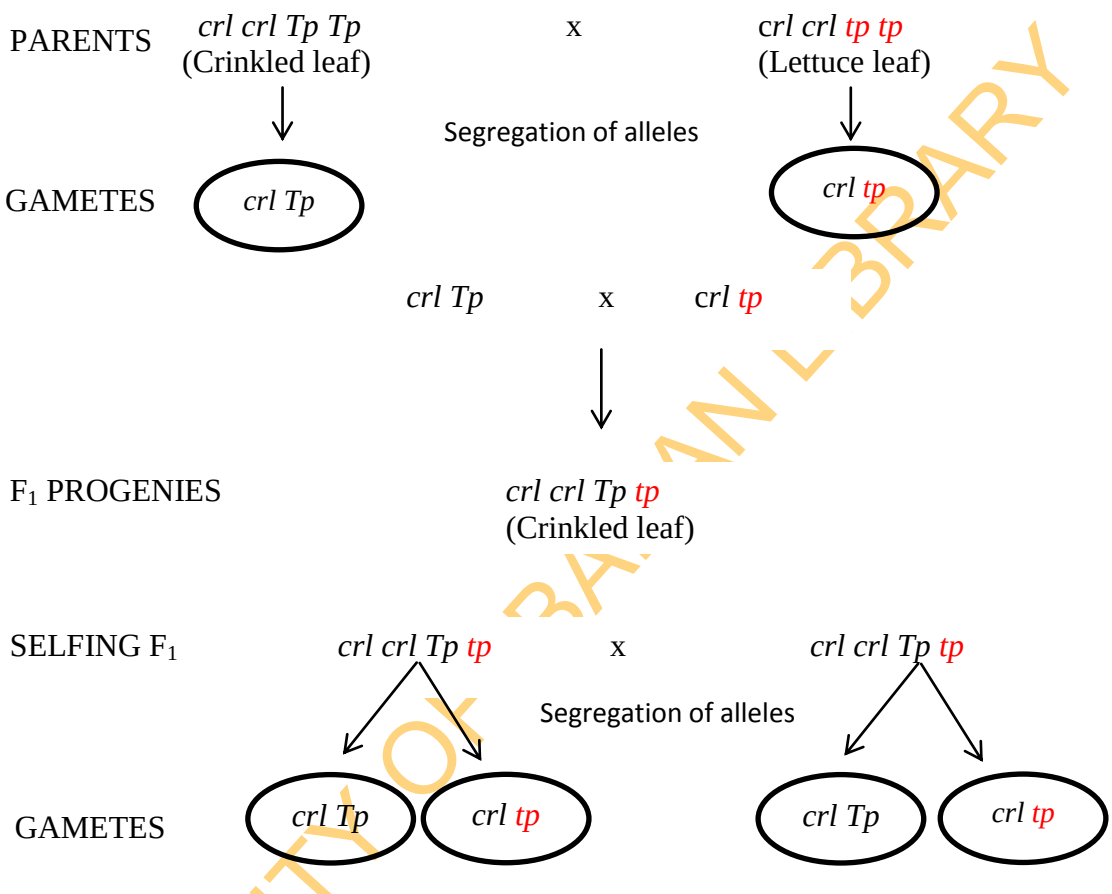


Appendix 24. Percentage of reduction in seed setting of IT90K-284-2 cowpea pollen exposed to UV irradiation, plotted against UV irradiation Period.

Appendix 25. Flow chart on the origin of lettuce leaf phenotypic trait in cowpea



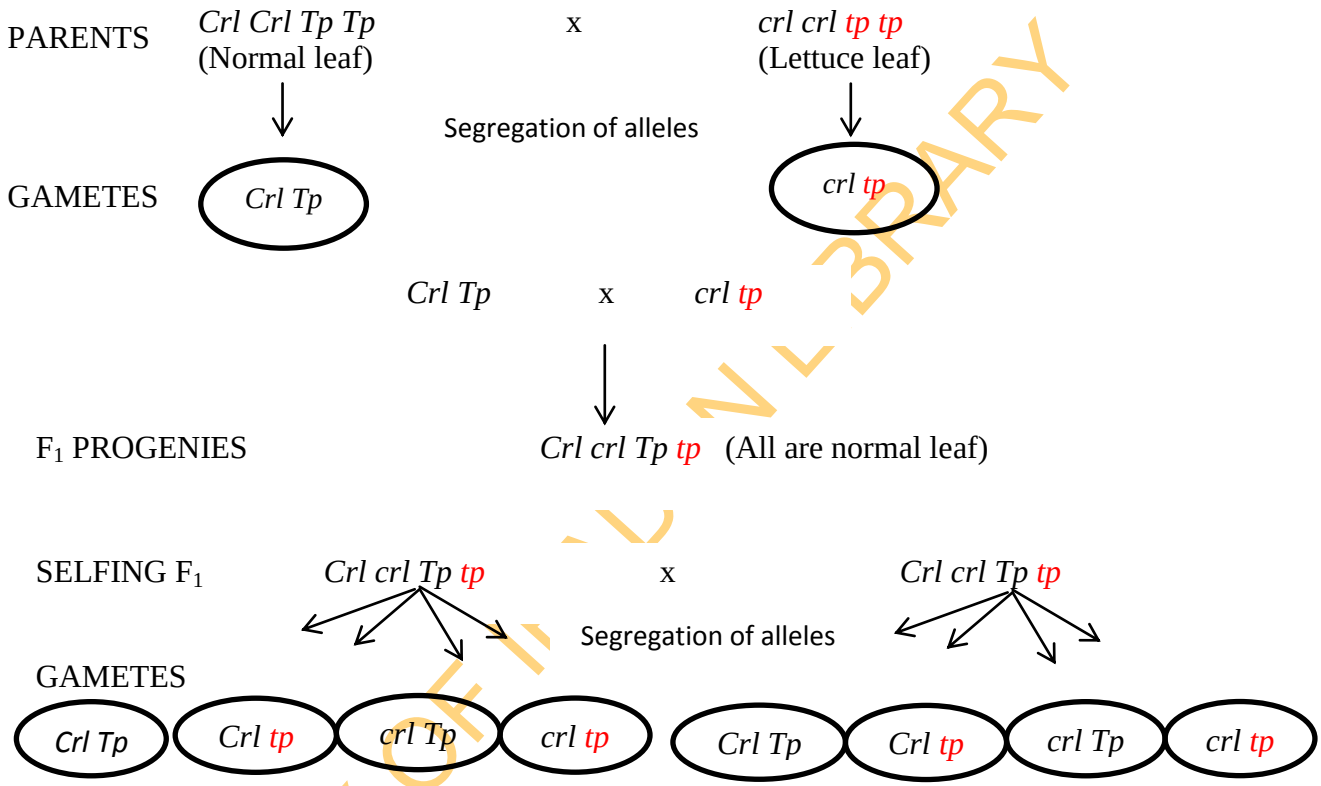
Appendix 26. Flow chart on the segregation pattern in a monohybrid cross between crinkled leaf (parent) and lettuce leaf (mutant)



	crl Tp	*crl tp*
crl Tp	*crl crl TpTp*	*crl crl Tp tp*
crl tp	*crl crl Tp tp*	*crl crl tp tp*

F_2 PHENOTYPIC RATIO = 3 Crinkled: 1 Lettuce Leaf

Appendix 27. Flow chart on the segregation pattern in a dihybrid cross between normal leaf cowpea and lettuce leaf mutant.



	Crl Tp	Crl tp	crl Tp	crl tp
Crl Tp	Crl Crl Tp Tp	Crl Crl Tp tp	Crl crl Tp Tp	Crl crl Tp tp
Crl tp	Crl Crl Tp tp	Crl Crl tp tp	Crl crl Tp tp	Crl crl tp tp
crl Tp	Crl crl Tp Tp	Crl crl Tp tp	crl crl Tp Tp	crl crl Tp tp
crl tp	Crl crl Tp tp	Crl crl tp tp	crl crl Tp tp	crl crl tp tp

F_2 PHENOTYPIC RATIO = 9 Normal leaf: 3 Crinkled leaf: 3 Twisted pale leaf: l Lettuce leaf