MORPHO-GENETIC DIVERSITY OF GAMMA IRRADIATED DOLICHOS BEAN (*Lablab purpureus* (L.) Sweet) GENOTYPES FOR CLIMATE CHANGE ADAPTATION.

BY

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DECLARATION

Declaration by the Candidate

This is my original work and has not been presented for any degree in any other University. No part of this thesis may be reproduced without prior permission of the author or University of Eldoret.

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DEDICATION

This thesis is dedicated to my wife Sharon and my children: Henry, Joy, Janice and Janelle, my parents Mr. and Mrs. Kimno and my siblings for their immense support and kind words of encouragement throughout this studies. May God bless you and to God be the glory and honour.

ABSTRACT

Dolichos bean (*Lablab purpureus (L.)* Sweet 2n=22 or 24 is a multipurpose legume mainly grown and used as a pulse, forage feed and in soil amendment for nitrogen fixation and green manure. Practically, it still yields below estimated potential of over 5000 $kgha^{-1}$. Induced crop mutagenesis is a safer conventional breeding method and has played a major role in increasing global food security. The main objective of the study was to contribute to climate change adaptability through gamma ray irradiation of dolichos bean genotypes and selection of climate smart allelic accessions. Specific objectives were to: evaluate the effect of gamma irradiation doses on morpho-agronomic traits of mutant dolichos bean accessions, assess genetic variability estimates, determine the genetic diversity, and evaluate nutritional and mineral composition and to evaluate the adaptability potential of mutant accessions in north rift Kenya. Four dolichos bean varieties (maridadi, cream, black I and black II) were irradiated with 300 gy and 400 gy gamma rays in 2018 in Austria. The M1 to M4 generations of the accessions of four dolichos bean genotypes were advanced by forward genetics protocol at University of Eldoret in 2019 through 2021.M2 accessions were evaluated for effect of mutation and genetic estimates, 95 M3s for genetic diversity based on 20 SSR markers, 24 M4s were screened for nutritional and mineral composition and yield and adaptability potential. The results showed that dose 300gy and 400gy significantly (p=0.05) increased leaf length, raceme length, dry seed yield per plant and plant height across the accessions. Qualitative phenotypic variations were present in all mutant accessions except black I. There was a higher genetic estimate variability for the yield associated traits measured for eldo maridadi than for eldo black I indicating difference in genotype and impact of mutation. Genetic diversity of 95 accessions based on microsatellite markers produced 20 polymorphic primers mapping an average of 5.25 alleles per locus, polymorphic information content of 0.58 with analysis of molecular variance (AMOVA) among population of 45% and among and within individuals 54% and 1%.The nutritional test showed that accession BF032 (28.86±0.18%), MT076 (74.88±0.59%), BF137 (9.69±0.34%), MT049 (12.55±0.57%) and BT188 (449.69±0.02 kcal) had significantly higher percent crude protein, carbohydrate, crude fat, crude fibre and energy. WT018, BT114 and BT039 had significantly higher phosphorous, potassium, calcium and zinc (0.58±0.21mg/l, 2.81±0.00 mg/l, 175.65±2.27 mg/l and 3.64±2.29 mg/l respectively). Accessions BT188 (3919 $kgha^{-1}$), MT049 (3315 $kgha^{-1}$), GT032 (3512 $kgha^{-1}$) and WT026 (4462 $kgha^{-1}$) were identified as adaptable and best yielding while Baringo as the best location for dolichos production. The use of gamma irradiation in generating genetic variability in Kenyan dolichos bean genotypes for climate change adaptation was effective. The best accessions on nutrition and yield adaptability are an important genetic resource for building resilience to climate change in Kenya.

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LIST OF ABBREVIATIONS

ATC: Agricultural Training Center

AMOVA: Analysis of Molecular Variance

ANOVA: Analysis of Variance

ANOVA: Analysis of Variance

AOAC: Association of Official Analytical Chemists

ASAL: Arid and Semi-Arid Lands

CF: Crude Fiber

CP: Crude Protein

DNA: Deoxyribonucleic Acid

DSC: Dry Seed Colour

EDTA: Ethylene Diamine Tetra Acetate

EV: Electron Volts

F2: Filial Stage 2

G X E: Genotype by Environment

GA: Genetic Advance

GAM: Genetic Advance of the Mean

GCV Genotypic Coefficient of Variation

GH: Growth Habit

GOK: Government of Kenya

Ha: Hectares

HCL: Hydrochloric Acid

HE: Expected Heterozygozity

HO: Observed Heterozygozity

IAEA: International Atomic Energy Agency

ILRI: International Livestock Research Institute

ISSR: Inter Simple Sequence Repeats

KALRO: Kenya Agricultural Livestock Research Organization

Kg: Kilogram

LD50: Lethal Dose 50%

LH: Lower Highland

LL: Leaf Length

LLL: Leaf Let Length

LSD: Least Significant Differences

LW: Leaf Width

MASL: Meters above Sea Level

MF: Mutation Frequency

MVD: Mutant Variety Development

NFBR: Number of Flower Buds Per Raceme

NRPP: Number of Racemes per Plant

PBGL: Plant Breeding and Genetics Laboratories

PCR: Polymerase Chain Reaction

PCV: Phenotypic Co effient of Variation

PH: Plant Height

Ppm: Parts Per Million

RCBD: Randomized Complete Block Design

RFLP: Restriction Fragment Length Polymorphism

RLC: Raceme Length in Centimeters

SD: Standard Deviation

SDS: Sodium Dodecyl Sulphate

SSR: Simple Sequence Repeats

TAE: Tris Acetate EDTA

UM: Upper Midland

UPGMA: Unweighted Pair Group Method with Arithmetic Mean

USA: United States of America

USDA: United States Development Agency

UV: Ultraviolet

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I wish you all God's blessings.

CHAPTER ONE

INTRODUCTION

1.1 Background

Dolichos bean (*Lablab purpureus L.* Sweet*)*, with 2n=22 or 24 chromosomes belongs to the family *Fabaceae* and is one of the most ancient crops among cultivated plants *(She et al*.,2015). Phylogenetically, dolichos bean is related closely with common beans (Liao *et al.,* 2019). It is a legume species that grows in the tropic and the sub tropic regions of the world from Asia, to America, Caribbean to Africa. Dolichos bean can grow favourably in arid, semi-arid and humid climates and in altitude ranges from sea level to 2500 meters above sea level (masl) (English *et al.,* 1999 &, Missanga *et al.,* 2021). Dolichos bean yields better on lower elevations, usually not above 2000 masl. Annual temperatures between 18 and 30°C also favours cultivation of dolichos bean. The least required temperature for growth of lablab is 3°C however, light frosts can damage leaves but will not exterminate the plant if not occurring for an extended time period (Tefera, 2006, Naeem *et al.,* 2020). High temperatures, on the other hand, have been shown not to affect the development of lablab (Grotelüschen, 2014a). Due to cosmopolitan distribution of dolichos bean, its farming can be achieved from areas of annual rainfall between 200 to 2500mm. Initial irrigation during the first two to three months after sowing is recommended if feasible for establishment a deep root system in drier regions (Smartt, 1990) .

Dolichos bean is believed to have originated in Asia and introduced to Africa from South East Asia in the eighth century (Deka *et al.*,1990). Dolichos bean is a drought tolerant

crop highly proteinous pulse when used as human food or as feed inform of forage legume. The protein content may vary distinctly among cultivars of a single species (Kamatchi *et al.,* 2010). It has been reported to vary between leaves and in grains between 14–33% with a good amino acid balance (Cook *et al.,* 2015). It is also rich in minerals (calcium, phosphorus) and vitamins A and D complex. Dolichos bean mends soil fertility through nitrogen fixation, as a green manure and as a cover crop.

Arid and semi-arid land mass constitutes 70-80% of the Kenya's land mass and hosts 70% of the livestock and a population of 14 million people (https://www.fao.org/kenya/resources/en/, 2021). It experiences repeated food and nutritional insecurity that necessitate promotion of production of early maturing, high yielding, nutritious and drought tolerant crops. Dolichos bean is classified as a minor or underutilized crop among the food crops (Pengell *et al.*,2001). Subsequently, the germplasms have not been for fully integrated into farming systems especially in potential arid and semi-arid lands (ASAL) agro-ecological environments such as North rift, Kenya.

A survey on dolichos bean production and utilization in Kenya showed that dolichos bean is produced in over 5000 hectares (ha) and is predominantly grown by small scale farmers in Eastern, Central and Coastal Counties. The grain yield of dolichos bean on farmers' fields indicate low production below one tone /hectare even where farmers grow the crop as pure stand or on terraces as a cover crop (Kinyua *et al.*, 2012). Its utilization was adversely affected by the bitter taste among some of the varieties and the long cooking time (Shivachi *et al*., 2012). However, the crop was reported to command a higher price in the Kenyan local markets than other legumes. Phenotypically the dolichos bean varies both in growth

characteristics, seed colour and seed size (Kinyua *et al*.,2012). Genetic studies by Shivachi *et al.,* (2013) and Kamotho *et al.,* (2016) on the cultivated Kenyan dolichos lablab varieties indicated very narrow genetic variability. This presents a serious limitation to dolichos improvement for resistance to biotic and abiotic constraints, nutritional and yield potential. Common pedigrees limits the ability of breeders to develop sustainable breeding materials among the cultivars for yield and adaptability traits. It is urgent that modern varieties and ecotypes of dolichos bean are developed to adapt to various and specific ecological conditions to meet food and nutritional security.

Nuclear techniques on crop improvement on yield and associated traits have been successfully applied by researchers (Rao *et al.,* 2018). Mutation breeding broadens the genetic base for selection of a traits of interest in a crop species, therefore is an effective approach in improvement of crops having narrow genetic base as is the case with Kenyan dolichos bean. Mutation induction have produced mutants with diverse and agronomically important traits that were screened by either phenotypic evaluation and selection of phenotype of interest (forward genetics) or by genotypic evaluation for detection of novel allele in gene of interest as well as study of gene function (reverse genetics) in cowpea, tepary bean, soybean and common bean (Mudibu *et al.,* 2012, *Rao et al.,* 2018). The effectiveness of gamma radiation in improving plant growth, drought and heat tolerance , seed quality and cooking time is also highly correlated to the levels of doses used (Hegazi *et al.,* 2010 ,Ulukapi *et al*., 2018). Diversity of putative mutant accessions can be monitored using morphological, agronomic biochemical and molecular characterizations studies. Globally mutation breeding technology has generated superior adaptable crops and increased food

security especially in marginal areas (Kharkwal *et al*., 2009, Nagarajan *et al.,* 2017). It has been used in Kenya as a method of breeding cereal crops : cowpea (*Vigna unguiculata*) (Pathak, 1991), wheat (*Triticum eastivum*) (Githinji *et al*., 2016) and barley (*Hordeum vulgare)* (Obare, 2014) .It has also been adopted in breeding and development of advanced Artemisia lines with high artemesinin content (Raymond *et al*., 2015) mutant potato (*Solanum tuberosum)* clones with resistance to bacterial wilt (*Ralstonia solanacearum)* (Chepkoech *et al.,* 2018). Therefore, based on this knowledge of use gamma mutation technology, this research study seeks to develop improved, farmers preferred, early maturing, and nutritiously rich, high yielding mutant dolichos varieties for deployment in Kenya to contribute to climate change resilience.

1.2 Statement of the problem

Dolichos bean is grossly underexploited compared to other species in the family of *Fabaceae* and is categorized among the orphan crops (Hendre *et al.,* 2019) . Major research programmes on it are majorly focused on its forage and in soil amendment qualities in agriculture vis-à-vis its yield and food security components (Kamotho *et al.,* 2016). Available germplasms and local landraces yield between 800-3000 $kgha^{-1}$ compared to potential yield of 5000$kgha^{-1}$ in research stations. They are also are characterized as having varying growth types, prolonged days to maturity up to 5 months, stay green effect, and unsynchronized maturity (*The Kenya Gazette*, 2015). There is insufficient information on genetic estimates and nutritive references of bean genotypes including dolichos bean improved through mutation programmes. The available genotypes also have narrow genetic base due to common breeding materials or "founder effect" therefore, the breeding value of these parental gene pools loosely responds to

improvement via hybridization, selection and or less genetic gain. Improvement through hybridization method of these germplasms also presents ambiguous genetic variation, and the linkage drag effects associated with transferring genes from elite × elite crosses or wild relatives. The major staple foods (Wheat, rice maize and potato) are a seriously limited in meeting food and nutritional security due to rising population, diminishing land sizes, increased biotic and abiotic constraints due to effects of global warming and climate change. The use of gamma ray induced mutation breeding technology could widen the genetic diversity of elite Eldo KT varieties for selection of traits for climate change adaptation in Kenya.

1.3 Justification

Climate change and its devastating effects on agriculture and more to productivity of staple food crops (wheat, maize ,rice and potato) have worsened in the 21st century (Kamenya *et al.*, 2021). It is therefore, important to exploit the other available genetic materials to enhance their resilience to climate change. Food security is one of the four pillars of the Kenyan government's Big Four Agenda (https://www.president.go.ke, 2019) and sustainable development goals number two of ending hunger. Leguminous food crops also known as "complete food" such are important in food security and wellbeing, poverty reduction and soil conservation. Dolichos bean has 21 to 38 % crude protein in the leaves and the seed contains 20 to 28% respectively (Kamotho.,2015). It also procures more revenue per unit quantity than common beans (*Phaseolus vulgaris* L.) (Rana *et al*., 2021). The current average per person intake of pulses is well below recommended levels of 21 g/person in the day. Hence, there is a need to promote diverse pulse production including dolichos bean for human and animal consumption (Röös *et*

al., 2018). Drought resilient crops that are adaptive to a wide range of climatic conditions like dolichos bean are needed as one of the mitigation strategies against climate change. Dolichos bean is a drought tolerant and a climatic stress crop that can withstand growth in dry lands with limited rainfall as low as 400ml.It is a food security crop in the arid and semi-arid lands where people are faced with food and nutritional crisis (Pauline, 2020).

Mutation techniques offers diverse breeding materials with non-linkage drag effects than the use of conventional hybridization techniques to solve the problem of limited breeding materials. It can also shorten long cropping time to maturity. Linkage drag is one of the major drawback while utilizing exotic and wild species in genetic improvement of legumes such as dolichos bean. The undesirable linkages mostly would hinder the transfer of desirable traits into cultivated backgrounds. A larger population, efficient selection pressure and additional generation of crossing among progenies prior to the selection or recurrent selection program over several generations would be needed for breaking linkage drag (Sharma., 2019) in conventional breeding. Spontaneous mutation rate is very slow to create urgent and meaningful genetic variability in plant breeding. Induced mutation is found to be potent and effective tool in the hands of a plant breeder to generate new genetic variability within short time for crops having narrow genetic base (Ahloowalia *et al.,* 2001). A mutation is a change in phenotype, which is sudden, heritable and is not produced due to segregation or recombination (Shu *et al.,* 2012). It has been utilized successfully to improve yield and yield components of various legume crops (Ahloowalia *et al.,* 2004, Mba, 2013; Al-Safadi *et al.,* 2000 ,Jankowicz-cieslak *et al.,* 2017). Gamma irradiation has been utilized by a number of countries globally and

national breeding programs to induce genetic variation and to develop mutant cultivars for resilience to climate change (Horn., 2016) as indicated in table 1.0 . It has proven to be environmentally acceptable, unregulated, and non-hazardous, because there is no difference between artificially induced mutants and naturally spontaneous mutants (Kato *et al.*, 2020). It has been used in Kenya as a method of breeding various crop such as cowpea, wheat and potatoes. Following rigorous selection of promising mutants, it is necessary to evaluate their phenotypic, biochemical and genetic composition and potential high yield production. Molecular characterization by simple sequence repeat markers are considered as the markers of choice for assessing genetic diversity studies, marker assisted selection and population studies. They able to detect variation in allele frequency at many unlinked loci, abundant in plants, have high level of polymorphism and are adaptable to automation (Keerthi *et al*., 2018). The estimate of heritability acts as a predictive instrument in expressing the reliability of phenotypic values. High heritability and genetic advance of the mean on yield and yield contributing characters is important in selection of potential accessions. Thus, keeping in view the above observations, the present study was planned with the aim to induce gamma irradiation on elite Eldo KT dolichos bean genotypes and evaluate isolated putative, genetically diverse, nutritious, high yielding accessions which can be released directly or deployed in future improvement programmes.

1.4 Objectives:

1.4.1 Main objective:

To contribute towards climate change adaptation through improvement of dolichos bean genotypes by gamma ray irradiation and selection of allelic accessions for earliness, nutritional composition, yield and adaptability potential.

1.4.2 Specific objectives:

1. To evaluate the effect of gamma irradiation doses on morpho-agronomic traits of dolichos bean accessions.
2. To assess genetic variability estimates of dolichos bean accessions based on yield and yield related traits.
3. To assess the genetic diversity of dolichos bean accessions using simple sequence repeat markers.
4. To evaluate nutritional and mineral composition of dolichos bean accessions.
5. To evaluate the adaptability potential of dolichos bean accessions in north rift Kenya.

1.4.3 Hypothesis

1. H_o: Gamma ray irradiations will not produce significant morpho-agronomic changes in dolichos bean accessions.
2. H_o: There will no significant difference in genetic variability estimates of dolichos bean accessions on yield related traits.
3. H_o: There will be low genetic diversity in dolichos bean accessions.
4. H_o: The nutritional composition of dolichos bean accessions will not be significantly different.
5. H_o: The dolichos bean accession will not differ significantly on adaptability in north rift Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Dolichos Lablab

2.1.1 Synonyms and common names

Dolichos bean has over 20 synonym including Lablab *purpureus (L.)* (Sweet). It belongs to the family *Fabaceae* among other legumes such as soybean (*Glycine max L*.Merr.) and common beans (*Phaseolus vulgaris (L.)*.(Reddy *et al.,* 2018). Almost every country or region identifies dolichos lablab by a unique common name. There are over 150 common names globally refereed to lablab crop that are scattered or unpublished according to Murphy *et al.,* (1999) and Maass *et al* ., (2010) . The collection of the names is indicative of its origin ,ancient existence, distribution and use .The most names include *Lablab, lablab bean, hyacinth bean, field bean, pig-ears, Rongai dolichos, dolichos bean, lab-lab bean, poor man's bean, Tongabean, bonavist bean, seim bean, Egyptian kidney bean.* In eastern Africa dolichos bean is known as '*Gerenge'* in Ethiopia, '*Lubia'(*Sudan), '*Fiwi* (Zambia) and '*njahi*'(Kenya) (Kamotho *et al*., 2016,Naeem *et al*.,2020). In India there are over 5 common names depending on states or region such as; *Shim (*Bengali, India), *Val (*Gujarathi) *Mochai*, (Tamil) *Sin bean* (Assam) *Wal (Marathi) and* Chikkudu (Telugu) Kankwatsa *et al*., (2018) and Singh *et al*., (2019).

2.1.2 Taxonomy

Taxonomically three subspecies (ssp.) are recognized, mainly based on differing characteristics of pods and seeds. The wild ancestral ssp. Unciantus includes the variety rhomboïdeus with pod size of approximately 40mm x 15mm mainly spread in East Africa. The second and third subspecies are the cultivated forms :Ssp. Purpureus having

larger pod sizes ranging from 100mm x 15mm and ssp. Bengalensis which has characteristically longer pods 140 mm x25 mm (Vaijayanthi *et al.*,2018).The dolichos bean species is known for being extremely diverse with over 200 genotypes being recognized and classified based on differences in size, shape and colors of pods, seeds, flowers and leaves, respectively (Naeem *et al*.,2020)

2.1.3 Diversity and distribution of dolichos bean.

The origin of dolichos bean is considered to be the Asian continent specifically the South or South-East Asia. This is because they have made great developments on improvement and utilization .Other evidence based on Robotham., (2015) indicates that this bean originated from the eastern Africa as early as 800 BCE and is now widespread throughout the tropics as indicated in figure 1 (Pengelly *et al*., 2001, National Research Council, 2006).

It is widely cultivated as an annual crop in regions of Africa , South and Central America, East and West Indies, China, South and South-East Asia and Australia where it has been used as a grain legume , forage and vegetable for over three centuries (Maass *et al.*, 2010).

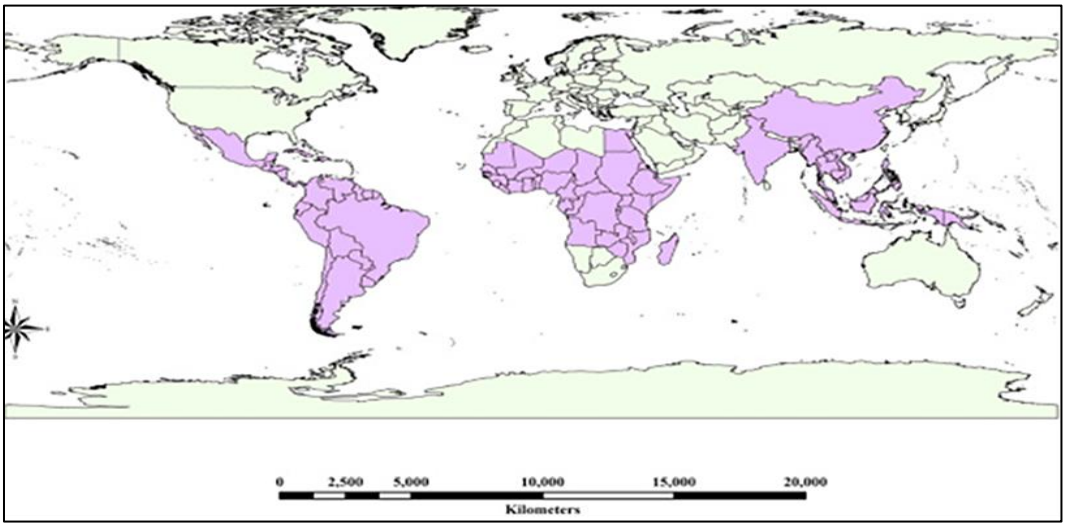

Figure 1: Intercontinental dispersal of dolichos bean. Light purple colour indicates the presence of the bean (Singh *et al.*, 2019)

Large agro-morphological diversity of dolichos bean is at South-Asia. However greater variation in wild dolichos bean have been recorded to occur naturally in Africa. In the wild, dolichos bean is found in grassland, bush land and in forest. (Maass *et al.*, 2010; USDA, 2012; Kankwatsa *et al*., 2018).

2.1.4 Distribution of dolichos bean in Kenya

Dolichos bean is grown and distributed in all ecological zones in Kenya. Dolichos bean accession have been collected from Lamu county at the coast region , Kitui , Machakos, Embu counties in the dry areas of Eastern Kenya, in Meru, Nyeri, Murang'a and Kiambu counties in the central region and Nakuru county in Rift valley region .Lamu county is the major dolichos bean growing region in Kenya (Kamotho *et al.*, 2016.)

2.2 Botanical description

Dolichos bean species is a summer-growing herbaceous plant that is frequently grown as annual or biennial crop.(Maass *et al.,* 2010).This legume shows greatest variation in its form and growth habit apart from heavily twining and trailing genotypes, also bushy, semi-erect and prostrate forms exist . Dolichos bean leaves are trifoliate; leaflets broad ovate-rhomboid formed and 7.5 to 15 cm long. They are thin, acute at apex, almost smooth above and short-haired underneath. The petioles are long and slender. Dissimilarity in size and color is high, whereas difference in shape of the leaflets is narrow. The inflorescences are axillary vertical, lax, fascicled, and of many-flowered racemes on rather elongated or short peduncles (Lim, 2012). Depending on the dolichos bean genotype, the flowers are 4 to 6 inch long and sweet scented , may be pink, red , white or purple, and usually arise from conspicuous tubercular thickenings on the peduncle. From there, up to four flowers are found at each node on an elongation raceme. Pods are very variable in shape, color and fragrance depending on the genotype; they may be curved flat or inflated with a curved beak and persistent style or even papery straight, hairy or smooth (Grotelüschen, 2014). The pod length can vary from 5 to 20cm, breadth from 1 to 5cm and usually contain three to six seeds each. The seeds colors range from cream, reddish, and purplish to tan, brown or black have a linear white with very obvious visible hilum and a long aril. Seeds are nearly as wide, flattened and oblong with rounded ends (USDA, 2012)

2.2.1 Rongai cultivar

This cultivar was derived from Rongai landrace in Nakuru county of Kenya and was first released in new wales, Australia in 1962. Rongai cultivar has green foliage with white flowers, light brown seeds plate 1. It is indeterminate and has a late maturity index it grows until it is cut or damaged by frost Cold tolerant varieties have been developed from this cultivar to endure winter time of year.

Plate 1: Rongai Lablab variety: (Graham *et al.*, 1986)

2.2.2 Highworth cultivar.

It originated from Coimbatore, South India and is morphologically similar to Rongai cultivar. Contrasts to Rongai cultivar occurs on the foliage, a purple band on the leaf axil and fresh pod, purple flowers and black seeds Plate 2. It is an early flowering cultivar with high seed yielding ability. It is suitable for pulse production and forage uses (Cameroon, 1988)

2.2.3 Koala cultivar

This cultivar is an early maturing grain type, released as a cultivar in Australia in 1995. Its wing petals of fresh flowers are violet to purple with violet-blue venation; seed cream coloured. Able to seed set before the onset of frost. It produces about 70% of the dry matter yield of 'Highworth' and 'Rongai' respectively (Mullen *et al.*, 2003). Koala dolichos lablab is cosmopolitan, adapted to a wide range of soils and it is suitable as a food and fodder.

2.2.4 Improved Cultivars

Plate 2: Highworth variety: (Graham *et al.*, 1986 ,)

2.2.4.1. Dash A: An annual forage cultivar moderately early-flowering selected from a single plant from within a population of ILRI 14437. Introduced to ILRI from Zimbabwe via the forage collection of the National Agricultural Research Centre.

2.2.4.2 Eldo-KT (Maridadi). A cultivar developed by the University of Eldoret, for the Central & North Rift as well as Western regions of Kenya, released in 2015, stay-green, late-maturing (> 5 months). It has spotted seeds, good flavour and short cooking time; the

cultivar has high forage production. Seed is maintained at University of Eldoret, Eldoret, Kenya.

2.2.4.3 Endurance A: This is a weakly perennial cultivar, developed from the strongly perennial (wild) line CPI 24973 and cv. Rongai, and released as a cultivar in 1998. It propagates well in the second and even into third year after grazing or cutting. It also has high dry matter production potential. Seed weight 5,500 seeds/kg. 'Endurance' seed is no longer commercially available.

2.2.4.4. KAT/DL-3 . It is an indeterminate, dual-purpose cultivar released in Kenya in 1995 by Kenya Agricultural & Livestock Research Organization (KALRO)-Katumani.

2.2.4.5. Rio Verde'(Reg. No. CV-280, PI 648441): Developed at the Texas A & M AgriLife Research and Extension Center at Overton, Texas and released by Texas A&M AgriLife Research in 2006; it is the first lablab cultivar released in the USA. A vining, herbaceous tropical legume with high nutritive value as a forage or browse for ruminant animals. It is drought tolerant, highly palatable, nutritive, excellent forage yield and adaptation to diverse environmental conditions; tolerance to defoliation.

2.2.5 Promising Accessions

2.2.5.1 CPI 67639: This forage type accession with black seeds, greater resistance to seed borers, possibly through its thicker seed testa.

2.2.5.2 CQ 3632, CQ3633, P5305, P5310, and Q6879: These are Australian-registered accessions with comparable agronomic attributes to Highworth and Rongai cultivars.

2.2.5.3 CPI 29399, CPI 30701, CPI 52506B, CPI 81364: All of these accessions produced over 5t/ha biomass in at 87 days after sowing on farmer's fields near Polokwane, Limpopo, South Africa.

2.2.5.4. Q 6880B: This is a consistent yielder dual purpose accession under dry land and or drought conditions of northern Tanzania and semi-arid Kenya (short growing seasons). It remains green for extended periods and intercrops relatively well with maize. The grain appears have rather somewhat bitter taste and so this accession may be unacceptable in the market.

2.2.5.5. CPI 24973 (Zimbabwe), **CPI 52437** (South Africa) and **CPI 60216** (Uganda) are vigorous perennial accessions. (https://uses.plantnetproject.org/e/index Lablab_purpureus_(PROSEA)&oldid=219238., n.d. Retrieved 09:50, April 11, 2022)

2.3 Agronomic Characteristics

Dolichos bean grows throughout the tropics and subtropics, ranging from 30° southern to 30° northern latitude, dolichos bean is suitably cultivated as a rainfed crop where annual rainfall is 600-900mm. Dolichos bean legume is well suited to most tropical environments supported by its great natural genetic diversity and distribution (Lim, 2012). It is adaptable to a wide range of environmental conditions ranging from near sea level to 2500 meters above sea level (masl) altitude levels (USDA, 2012 ,Kamotho, 2015) . It is more drought tolerant than other legumes such as *Phaseolus vulgaris* and *Vigna ungiculata* species. Dolichos bean thrives in warm climate regions where annual temperatures averages between 18 and 30°C, high temperatures have been shown not to affect the development of lablab however, light frosts can damage leaves but will not kill the plant if not occurring for a prolonged time period

(Abdallah *et al.*, 2015). Dolichos bean is predominantly self-pollinated, and cooler weather at flowering time can affect seed set. Dolichos bean cannot tolerate water-logging or standing in brackish water. It can survive in a wide variety of soil types and textures provided that they are well drained with in pH average range of 4.5-7.5. Dolichos bean exhibits tolerance to toxic aluminum soils and low-fertile soils. Yield performance is good on sandy loams with a pH of 6.5 and heavy clays with a pH of 5.0 (Grotelüschen, 2014)

2.4 Economic Importance

Dolichos bean is a multipurpose legume, primarily is used as a pioneer species to improve soil fertility and soil organic matter, a pulse crop for human consumption, as a fodder crop for livestock, as a rotational and cover crop, as an ornamental annual vine and as herbal medicine (Cook *et al*., 2015, Kahsay *et al.*, 2021).The leaves of dolichos make excellent hay and silage made from a mix of dolichos and sorghum raises the protein content by 11% with a ratio of 2:1 mixture of lablab :sorghum(Vaijayanthi *et al.*, 2018).When used as human food, green pods, mature seeds and leaves are traditionally eaten as vegetables in Africa, south and south-east Asia. Dolichos bean sprouts are also eatable and, thereby ,comparable to soybean or mung bean sprouts but need to be cooked before consumption. Immature pods contain 82% water, 4.5% protein, 2.7-4.2% crude lipid, 10% carbohydrates and 2% cellulose. Mature seeds contain 9.5% water, 20-25% crude proteins ,0.8% fat, 63-66% carbohydrates and 5-7% dietary fibre (Washaya *et al*., 2018, Purwanti *et al.*, 2019a). The mature seeds are sometimes soaked overnight before cooked thoroughly which is important in reducing anti-nutritional factors such as hydrogen cyanide and trypsin activity inhibitors. After

soaking, cooking duration usually range from two to three hours and normally include several water changes. In terms of soil amendments, dolichos bean dense green cover during the dry season can help to protect the soil from drying out and decreases erosion by wind and water when used as a cover crop (Mwangi *et al* ., 2015). According to Whitbread, (2004) , Maass *et al.*, (2010) and Okumu *et al.*, (2018) use of dolichos bean as green manure offers great potential for soil conservation strategies and stabilization of chemical and physical soil properties through increase of organic matter and mineral.

Dolichos bean roots and seeds have been exploited for its ethnobotany value in Asia and Africa. It is used to reduce fever, flatulence and stimulate digestion. It is also used as an antispasmodic and in treatment of heart conditions .As an ornamental crop dolichos bean is valued for its colourful flowers and purple peapods especially in United States (Sheahan, 2012).

2.5 Breeding of dolichos beans

Utilization of genetic variability present in any crop species including dolichos bean germplasm is the first principle on its improvement. Research programs on dolichos developed improved adaptable and high yielding dolichos cultivars through exploitation of genetic variability through hybridization (Ondabu, 2013). Induced mutations have been widely used in genetic improvement of various cereal crops including cow pea, wheat, soybean and dolichos bean through the use of artificial mutagenesis such as gamma rays, x-rays, and Ethyl Methane Sulphonate (Mba *et al.*, 2010). This has led to development and release of improved cultivars in Africa, Asia, and Latin America (Reddy, 2013). Additionally, most dolichos bean breeding initiatives aim at broadening

the genetic base of the crop to adapt various biotic and abiotic constraints, sensory and cooking qualities and enhanced nutritional quality (Shivachi *et al.,* 2012, Ondabu. 2013). The following breeding methods have been widely used in improvement of self-pollinated crops including dolichos bean:

2.6 Conventional Breeding

2.6.1 Pure-line selection

Danish botanist Johanssen in 1903 proposed pure-line selection concept on the basis of his studies on Princess beans (*Phaseolus vulgaris L.*). This method is suitable for highly self-fertilizing crop species such as beans. Promising individuals from a large number of segregating populations are selected following systematic crosses. Selected individuals are harvested individually and continuously selfed and selected to develop and release pure-line cultivars.

2.6.2 Pedigree breeding

Pedigree breeding retains complete record of the relationship between the selected plants and their progenies unlike pure-line breeding. In this method each progeny in every generation can be traced back to the F2 plant in case of hybridization and M2 if mutation technology is used from which it was selected from. It is commonly applied in selection of desirable plants from the segregating populations of self-pollinated crops. Pedigree method is valuable particularly when improving some definite attributes such as disease resistance, plant height or maturity deficient in an already established variety. Pedigree method key weakness is that yield is evaluated efficiently only at the end of the process, on inbred (hybridized or mutant) lines when seed is available for replicated trials. A large

number of lines have to be advanced, to widen possibility for improvement of yield potential. Consequently, the rate of progress for yield resulting from the pedigree method is normally modest, rarely exceeding 1% per year (Breseghello *et al.*, 2011)

2.6.3 Single seed descent

A single seed collected from each of the F2 plants is kept and bulked to grow the F3 generation. This process continues up to the F5 and F6 generations, whereby a desired level of homozygosity is achieved. Single plants in the F6 generation are selected in large numbers and their progeny grown separately. Selection of best performing lines for preliminary and national yield traits is carried out in the F7 and F8.

2.6.4 Mass selection

This method is also known as bulk population or population breeding. It was first used by Nilsson Ehle in 1908. It refers to a population grown in bulk plot from F1 to F5 with or without selection. A portion of the bulk seed is used to grow the next generation and individual plant selection is often started in the F6 or later generation. Bulk selection method is useful to increase the frequency of desirable types through positive mass selection. It is suitable for studies on the survival of genes and genotypes in populations and it offers greater chances of isolation of transgressive segregants than pedigree method.

2.6.5 Backcross breeding

It is mainly used to transfer few genes into a conventional cultivar. Backcrossing, leads to increased homozygosity allowing selection of desirable genotype in homozygous and desirable genetic backgrounds.

2.7. Mutation Breeding

Mutagenesis is the development of sudden heritable variations in the genetic information of an organism initiated by chemical, physical or biological agent. Gamma rays, x-rays, or EMS are vital sources of inducing genetic variation in plant breeding programs (Van Harten *et al,* 1989). Optimizing the dose of radiation is the first step in induced mutation breeding before large scale mutagenesis is undertaken on target genotypes. This is important because predictable value of mutagenesis dose guides the researcher in the choice of the ideal dose depending on the plant materials and desired outcome (Horn *et al.,* 2013). Induced mutations provides considerable genetic variation within a reasonably short period of time when natural genetic variation of the crop is limiting for breeding. Plant mutation breeding involves several processes of mutation induction, mutation detection, mutation fixation, mutant line development and release of new mutant cultivars. Induced mutations are highly effective in enhancing natural genetic variations in traits that appear to be recessive compared to the wild type plant. Mutagens bring about desirable changes including plant height, growth types, genetically, biochemical, physiological or morphological changes (Tshilenge-Lukanda *et al*., 2012). Ulukapi *et al*., (2018) reported that the mutation breeding process is a fast forward in developing diverse germplasm and it may take only up to 6 generations (M6). Data collected on registered varieties bred by various mutation approaches indicated a peak in the 1980-1990 figure 2. The International Atomic Energy Agency (IAEA) have registered 3,365 mutant varieties in its Mutant Variety Database (MVD) with over 1,000 new varieties are being utilized extensively. Seventy percent of the registered varieties were produced by classical gamma rays and X-rays irradiation.

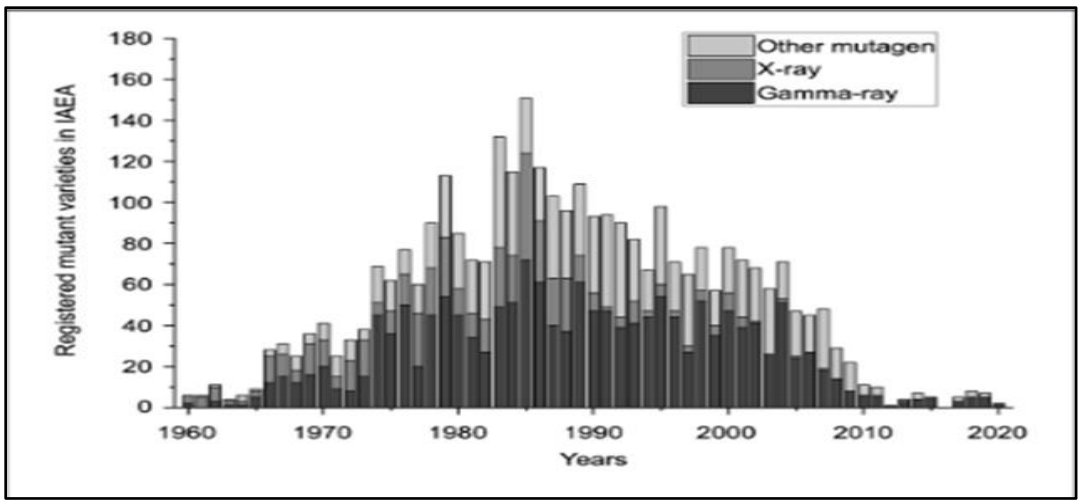

Figure 2: Numbers of mutant varieties registered in IAEA during 1960–2020 in IAEA Mutant Variety Database. Source(Ma *et al*., 2021)

2.7.1 Gamma irradiation

Gamma rays are electromagnetic radiation emitted by radioactive decay and having energies in a range from ten thousand (10^4) to ten million (10^7) electron volts (EV). They are ionizing radiations that result principally to lethal and mutagenic effects from incompletely or incorrectly repaired DNA lesions or multiple damaged sites (Ulukapi *et al.*, 2015). Among these lesions, strand breaks are considered to be the most important as they interrupt the continuity and integrity of the DNA double helix. Gamma irradiation (from a Co^{60} source) accounts for 80 % of mutant plant varieties released in China and 61 % of more than 200 direct-use mutant varieties released in Asia (Du *et al*., 2022)

2.7.2 Radio sensitivity test

The success of mutation breeding greatly depends on the rate of mutation, the number of screened plants, and the mutation efficiency. The mutation rate is affected by the total dose of the mutagen employed, and can be modified by physical and biological factors. Higher doses inevitably bring about mortality, high pollen and seed sterility and

deleterious mutations (Mba *et al*., 2010,Singh *et al.,* 2019). To avoid excessive loss of actual experimental materials, radio-sensitivity tests must be conducted to determine lethal dose-50 (LD50) (the safe dose at which half of the planting material survive) doses before massive irradiation of similar materials are accepted. The LD50 values vary with the plant parts and reference doses could be sourced from literature or obtained from the international Atomic Energy Agency (IAEA). This is helpful for researchers who lack the facility to conduct their own tests before the actual mass irradiation. Three mutants from the same mother have been recovered after irradiation of 1400 botanical seeds (Shu *et al.*, 2012). Three sister M2 genotypes were derived from the same M0 mother (progeny which was not irradiated serving as a source of many mutants) and that was a strong indication that within the same species, some cultivars are more radio-sensitive than the others. The radio sensitive test or LD50 dose of the similar test material must be known before mass irradiation of the test sample follows. The greater the number of test planting material to be irradiated, the higher the chances of finding a useful mutant (Du *et al.*, 2022)

In mutation-based development plant parts reproduced clonally results in creations of M1V1, M1V2 and M1V3 generations (Ahloowalia *et al.,* 2001). In these generations mutant individuals with the desired characteristics are easily detected via various stability tests. Salt-tolerant mutants of marfona potato node explants were treated with various dosages of gamma irradiation and the M1V2 and M1V3 clonal generations were developed in which 47 % difference was detected in mutant plants produced by 20 or 30 gray (Gy). In Kenya, mutation breeding has not been widely adopted as a method of

breeding, however, few crops has been developed through mutation such as cowpea (Pathak, 1996) and wheat (Kinyua, 2014)

2.7.3 A mutation breeding programme

The success of the mutation breeding programme is measured largely by the production of superior varieties, but also by the spectrum and quality of putative mutants induced, identified and recovered from a segregating mutant population (Nakagawa *et al.*, 2017). Even with a full consideration of the requirements for the mutation experiments there are other factors which could limit the success in recovering the targeted mutant trait (Setia *et al*., 2020).

2.7.3.1 Differences due to genotype

Much evidence exists that genetic differences, even when they are as small as single gene differences, can induce significant changes in radio-sensitivity, which in turn influences the total rate, the spectrum of recoverable mutations and the degree of background (Zaman *et al*., 2007). According to Bradshaw, (2016), it's difficult to predict the influence of a particular genotype on the mutation spectrum, therefore the choice of the parent material is a key factor of any programme in mutation breeding. The influence of the ploidy level on the mutation spectrum in diploid species shows that the great majority of mutations occur in single recessive genes. However, deviation from the normal 3:1 ratio due to deficiency of recessives has been very frequently observed.(Perez-Jimenez *et al.*, 2020) Dominant vital mutations are mostly lethal or semi-lethal in the homozygous condition, in contrast to diploid organisms, as the dose required to produce them is unlikely to result in viable plants. Many genes are re-duplicated in polyploids, which increases their ability to bear a high mutational load, including gross chromosome

aberrations, with no apparent negative effects. This results in the more frequent discovery of dominant and semi-dominant mutations amongst such species. According (Zengquan *et al.*, 2003, Rajarajan, *et al.*, 2014) the traits that exhibit greater variability in the background could be improved more easily and produce better expectation of mutant improvement.

2.7.3.2 Type of mutagen and dose

The difference in mutation spectrum among different sources of irradiation is obvious in the spectrum of induced flower colour changes following mutagen treatment(Singh *et al.*, 2019). For instance, densely ionizing radiations such as different sources of ion beam produce relatively more chlorophyll mutations of the albina, striata, and xantha type, whereas the frequency of the viridis type is highest following gamma-ray treatment. Thus, the chance of selecting desired mutants might be considerably increased by broadening the choice of mutagens. Another problem in the mutant quality is the number of mutation events that occur in the same meristematic cell at the time of treatment that are transmitted to later generations. The number of required mutation event is far less than the undesirable ones and consequently the number of mutant plants that carry only desirable changes will further decrease if more than one mutation per cell is induced (Jankowicz *et al.*, 2017). To avoid this undesirable result several measures can be taken. Firstly, one should not apply too high a dose of any mutagen. Super-mutagens, that give mutation rates of at least 50 percent on the basis of plant or spike progenies, may not be at all advantageous for mutation-breeding purposes and if high mutation rates have been induced, they should be allowed to segregate, and selection for useful types should be conducted in M3 or later generations (Forster *et al.*,2012)

2.7.3.3 Pleiotropy and linkage

Generally, it almost impossible to find a mutation in an organism that results in only one single divergent phenotype compared with its initial wild genotype (Gill *et al.,* 2015). Mutations resulting in pale green plants also result in decrease in general plant growth and delayed maturity and, in most cases a group of distinct variants can be observed and this group as a whole is transferred from one mutant generation to the next showing mostly a 3:1 segregation ratio. Three possible interpretations for this behaviour have been theorized as a single mutant gene is responsible for the whole complex of deviating characters; a tiny portion of a chromosome has been lost containing several genes; and several closely linked or neighbouring genes have mutated (Gottschalk *et al.*, 2012)

2.8 Mutation induction for yield improvement

The most important objective of most plant breeding programmes is production of a stable and high yield cultivar over a range of environmental conditions. Yield is a complex trait strongly influenced by other breeding objectives, such as, plant architecture, maturity, nitrogen utilization efficiency, resistance to biotic and abiotic stresses, etc. It is difficult to use mutation breeding to improve the yield potential of crops that are well established and which have been subject to intense and refined breeding over long periods of time (Sadiq., 2008). Low frequency, perchance at 1/1000 to 1/500 plants in an M2 population give positive yield mutations (Saeed *et al.,* 2009). Large populations are needed for yield improvement in order to increase the probability of finding high yielding mutants. Yield as an important traits is highly influenced by environment, it is difficult to recognize yield performance from observations on a single-

plant basis alone. Selection methods, therefore, deserve more attention than do the selection methods for isolating mutants for qualitative characters. The selection method for a character like yield is further complicated by the fact that spontaneous or induced mutants can react differently from the mother genotype to environmental changes (Badigannavar *et al*.,2017) .Changes in genotype × environment interactions may be utilized in practical evaluation of yield potential. Yield is practically governed by many loci, each of them having a relatively small effect. The progeny testing can only start in the M3 families at the earliest. In practical breeding work on self-pollinating crops, it may sometimes, be advisable to delay the progeny testing until later generations, M5 or M6. By that stage a fairly high degree of homozygosis (uniformity) is attained, and selected families need not be re-selected before they are handed over for testing on a large scale in field trials (Kusaksiz *et al.,* 2010). Globally across continents mutation induction has proven to be environmentally acceptable (Kato *et al.,* 2020). It has been used in Kenya as a method of breeding various crop such as cowpea, wheat and potatoes and across different countries as indicated in table 1 for yield improvements.

Table 1: List of mutants with improved yields developed through gamma irradiation in various countries

Crop	Mutants/Cultivars	Country
Rice (*Oryza sativa)*	Zhefu – 8 cultivars	China
Barley (*Hordeum vulgare)*	Golden Promise	United Kingdom
Banana (*Musa sp)*	Al Beely	Sudan
Groundnut (*Arachis hypogea)*	TAG24	India
Groundnut (*Arachis) hypogea*	TAFRA-1	Sudan
Blackgram (*Vigna mundo* L.)	TAU-1	India
Cotton (*Gossypium sp.)*	NIAB 78	Pakistan
Wheat (*Triticum aestivum*)	Eldo Baraka,Eldo mavuno	Kenya
Cassava (*Manihot spp*)	Tech bankye	Ghana

2.9 Heritability

Progress in crop improvement programs does not only depend on the amount of genetic variation present in the population but also the knowledge of heritability and extent to which the desirable characters can be transmitted from one generation to the other (Arulbalachandran *et al.,* 2010). Heritability estimates the relative contribution of genetic factors to the phenotypic variability observed in a population and is subject to prediction of gain from selection and determination of the relative importance of genetic effects. Heritability values can be used as a measuring scale to determine genetic relationships between parents and progeny (Wang *et al* ., 2011). Better heritability values points to the possibility of improvement in the parameters so that attention may be focused on important traits while synthesizing genotypes, it proportion the total phenotypic variance (variation in a trait after accounting for variance attributable to known fixed effects and it is composed of both genetic and environmental parts) attributable to genetic effects, both

of which are subject to change both within and between populations. Phenotypic variation observed among plants or varieties is due to differences in their genetic makeup, environmental influences on each genotype and interaction of the genotype and the environment (Memon *et al.*, 2007, Ahmed *et al.*, 2007)

$$\delta_P^2 = \delta_g^2 + \delta_e^2$$

Where δ denotes variance, and p, g and e refer to phenotype, genotype and environmental parts, respectively (Falconer *et al.*, 1996).

Genotypic variance encompasses the additive effects of genes, as well as dominance and epitasis, i.e. the effects of genes at the same locus, and interactions between genes at different loci. Environmental variance in this sense means all variation not due to genetic influences as well as measurement error and individual stochastic effects.

2.10 Genetic advance

Genetic advance indicates the magnitude of the expected genetic gain from selection cycle. It is the measure of the expected genetic progress that would result from selecting the best performing genotypes for a character being evaluated .Selection is a major important process in breeding for improvement of one or more plant attributes which involves the retention of the desired genotypes and elimination of the undesirable ones. The genetic advance achieved through selection depends on the factors of total variation in the population in which selection was conducted (Acquaah, 2007).

High value of heritability and predicted genetic advance clarifies that the selection among genotypes would be effective for yield and yield components. The studies conducted by various researchers have shown that high heritability alone is not enough for selection in

advance generations; it must be accompanied with substantial amount of genetic advance *(*Memon *et al*., 2007). However, if a character or trait is controlled by non-additive gene action, it gives high heritability but low genetic advance, while the character ruled by additive gene action, heritability and genetic advance both would be high (Anuj *et al*., 2017).

2.11 Marker techniques

The characterization of genetic diversity is also important for cultivar identification, cultivar protection as well as to ensure the trademark and intellectual property rights. Information on genetic diversity is used in co-ancestry/ pedigree studies to avoid closely related parents and hence inbreeding depression in a crop like beans (Fernández *et al.*, 2005). Genetic diversity is determined by genetic markers representing genetic differences between genotypes or species used. There are three major types of genetic markers: (1) morphological (‘classical’, ’phenotypic’ or ‘visible’) markers themselves are phenotypic traits or characters; (2) biochemical markers include allelic variants of enzymes called isozymes; and (3) DNA (or molecular) markers, which reveal sites of variation in DNA sequence (Coombs *et al.*, 2004)

2.11.1 Morphological markers

Phenotypic identification of plants has been used as a powerful tool in the classification of genotypes and to study taxonomic status, based on morphological traits recorded in the field. Most important agronomic characteristics are controlled by multiple genes and are subjected to varying degrees of environmental modifications and interactions. Morphological characterization has been used for various purposes including studies of

genetic variation patterns, identification of duplicates and correlation with characteristics of agronomic importance (Bekele., 2014)

2.11.2 Biochemical markers

Biochemical markers, like enzymes are routinely used to detect differences between individuals. These markers only sample actively expressed regions of the genome hence limits their use in certain aspects of plant biology and genetics as co-dominant neutral genetic markers due to lack of adequate polymorphism Tanksley *et al.,*1983, Kumar *et al*., 2018)

2.12 Molecular and mutational genomic analysis

Molecular markers are the most widely used mainly because they are much more numerous, they do not disturb the physiology of the organism. They reveal more sites of variation at the DNA sequence level which might be nothing more than a single nucleotide difference in a gene or a piece of repetitive DNA (Mutthanthirige *et al.*, 2018). Because polymorphisms are DNA sequence variations, these markers are applicable to any tissue and are independent of growing conditions. They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandem repeated DNA (Pandurangane *et al.,* 2022).

Mutational genomics is a valuable tool to differentiate and understand the mutants improved via mutation breeding programs. It is also an easy way to determine the genetic resemblances and characterize the variants between the mutants at the DNA level. The new mutational genomics technologies give opportunity to the plant breeders to make it quick and definite (Penna *et al.,*2012). Molecular markers are widely used to

differentiate the genetic differences between the mutant and the mother plants through characterizing the variations at DNA level (Ahloowalia *et al.,*2001). Through high-throughput genomic platforms such as random amplified DNA polymorphism (RAPD), cDNA amplified fragment length polymorphism (AFLP), single-strand conformational polymorphism (SSCP), allow rapid and in-depth analysis and classification of variations in mutants (Biswas *et al.,*2010). RAPD molecular markers is an inexpressive and a rapid method to use in many genetic diversity studies in mutation breeding programs Barakat *et al.,* (2010), Kaul *et al.,* (2011) used it to study genetic variability in mutants of *Chrysanthemum*. Soybean Atak.,(2004), Yaycılı.,(2012) characterized salt-tolerant potato (*Solanum tuberosum* L.) mutants irradiated with gamma irradiation. ISSR method is an easy to apply more informative than RAPD, reliable, and inexpensive (Sen., 2012). ISSR primers are designed by using microsatellite sequences to amplify the genomic regions flanked by microsatellite repeats. By using one primer, it is possible to amplify multiple fragments as a result of ISSR analysis (Baliyan *et al.,* 2014).

Sen *et al.,* (2012) used ISSR method to segregate the drought-tolerant sugar beet mutant improved via gamma ray irradiation of shoot tip. Nineteen (19) inter simple sequence repeat (ISSR) primers resulted 91 polymorphic bands they obtained of 106 PCR fragments. Wu., (2011) showed genetic similarities between the mutants of chlorophytum treated by three kinds of chemical mutagen using 60 ISSR primers that yielded 60 informative polymorphic bands.

The SSR or microsatellites (sometimes referred to as a variable number of tandem repeats or VNTRs) are short segments of DNA that have a repeated nucleotide sequences. They are tandem di to tetra-nucleotides sequence motifs flanked by sequences and are present

in most eukaryotes genomes (Robinson *et al* ., 2004). They are codominant molecular markers that distinguish homozygotic and heterozygotic individuals .They arise due to slippage like events occurring randomly in stretches of repetitive sequences. Microsatellites are mostly used in comparative and association studies, genetic diversity, marker assisted selection, population and evolutionary studies (Shi *et al*.,2011). Due to their high variability, they are especially good at distinguishing closely related individuals. A number of microsatellites are now available for a wide range of crops , such as groundnuts (*Arachis hypogaea*), (Cuc *et al.*, 2008), Pegion pea (*Cajanus cajan*), (Saxena, *et al*., 2010), Bambara groundnut (Basu *et al*., 2007) and Common bean (*Phaseolus vulgaris*) (Blair *et al.*, 2011).The SSR markers have been confirmed to be the most informative and appropriate because of their valuable attributes. These attributes of all SSR markers are high reproducibility which is the most important in genetic analysis. While reproducibility of SSR profile is as robust as RFLPs. The SSRs markers have polymorphic genic information contents. The hyper variable nature of SSRs produces very high allelic variations even among very closely related varieties. The SSRs are also codominant. Although homoplasious bands can be misleading in scoring SSR profiles ,the SSR bands produced from the same set of primers are intuitively orthologous (Asadi *et al.*, 2020). Homoplasy is a phenomenon wherein different copies of a locus are identical in state despite not identical by descent (Estoup., 2002). In SSR analysis, homoplasy can occur if two bands are similar in size but not identical in sequence. SSRs marker system are preferentially associated with non-repetitive DNA (Varshney *et al*., 2005). Genomic sites of SSRs markers, derived from genomic libraries fall into either transcribed region (genic SSRs) of the non-transcribed region (genomic SSRs). The

SSRs, derived from expressed sequence tagged sites (ESTs) or cDNAs, are mostly genic SSRs, which have the potential for application in areas as gene function characterization, association analysis for gene tagging and quantitative trait loci (QTL) analysis (Zeng., 2007).

2.13 Nutritional value of dolichos bean

The nutritional composition of the dolichos bean may vary depending on the varieties (Minde *et al*., 2020). Several authors have reported variations on different proximate values exhibited by different varieties globally. Major variations on percent crude protein (Alghamdi, 2009), percent crude fat, total carbohydrates, percent ash and percent moisture content (Kalpanadevi *et al.,* 2013).

Dolichos bean has a low-fat content compared to groundnuts and soybeans. Crude fats levels lablab range between 3.14–10.84g/100g in different lablab varieties Mortuza et al., (2009) and Grotelüschen., (2014)). The ash content is not intensely different from that of dolichos bean from India (Sarma., 2010) and Kenya (Kilonzi., 2017) ranged from 3.40 to 4.11% (wet mass) and from 4.11 to 4.90% (dry mass). Dolichos bean are also a good source of energy with carbohydrate content of about 60% (Adhikari *et al* ., 2017). The beans are believed to have appetite suppressive peptides that can induce satiety by stimulating cholecystokinin (CCK) secretion (Cornara *et al.,* 2016).

The investigated seeds of dolichos bean show they are good and rich sources of primary and secondary mineral composition. Large mineral composition variability of dolichos bean varieties have obtained from phosphorous, potassium and magnesium content in Indian varieties (Davari *et al.,* 2018).

2.14 Genotype by Environment Interaction (GEI)

Living organisms develop variations due to either genetic effects or environmental effects or both, genetic variation result from change in the genetic sequence due to genetic effects and the variation due to environmental effects is defined as the environmental variation (Zakir, 2011). The concept of genotype-environment interactions (GEI) leads to quantification of the agronomic stability of the genotype and under the biological concept stable genotype is one, whose phenotype shows little deviation from the expected character level when performance of genotype is tested over a number of environments (Dasgupt, 2017).

The idea that G X E is a pleiotropic effect of specific variants across environments indicates that any given trait when evaluated across more than one environment can be analyzed as genetically correlated traits (Malosetti *et al.,* 2013) .In this case, the magnitude of such a correlation indicates the degree of shared genetic control and the sign of the correlation indicates the direction of the allelic effect for the environments being considered. This perspective has provided an important framework for interpreting and handling G X E in plant breeding programs. As with most other areas of quantitative genetics, this has led to the development of statistical as opposed to biological parameters to quantify, understand, and interpret G XE in plant breeding (Malosetti *et al.,* 2013)

2.15 Adaptability under G XE Interaction

The interest of a plant breeder is in the stability of performance of economically important characters. The desirable accessions should have low G x E interactions for important characters, so as to get desirable performance of hybrids over wide range of environmental conditions. Under the biological concept stable genotype is one, whose

phenotype shows little deviation from the expected character level when performance of genotype is tested over a number of environments(Yan *et al* ., 2020). A univariate nonparametric stability methods are not affected by data distribution and these methods are based on rank order of genotypes, a genotype is considered stable if its ranking is relatively constant across environments (Tamene *et al.,,* 2015, Hahzad *et al.*, 2019) .Analysis of interaction of genotypes with locations and other agro-ecological conditions would help in getting information on adaptability and stability of performance of genotypes .An ideal genotype should have both high mean yield performance and high stability across environments(Elias *et al* .,2016)

2.16 Climate change adaptation

The resilience to climate change through reducing the vulnerabilities in biological and social aspect is termed as climate change adaptation. Adaptation approach is primarily developed to overcome the climate change effects (Lipper *et al* ., 2019). Adaptation to climate change plays a very critical role in reduction of effects of global warming and vulnerabilities globally. Adaptation to climate fluctuation is a very critical issue in the food security discourse(Wang *et al.,* 2018). Adaptability is principally relying on the availability of genetic variability within and between crop species. The widest genetic variation of crop species offers the greater chances for improvement to adapt to ecological conditions. Agriculture productivity and growth are mainly susceptible to different problems especially climate change and extreme weather conditions where extreme temperature at reproductive stages can cause grain sterility and yield reduction (Palombi., 2013). Adaptation strategies is plays key role through offering opportunities to confront climate change challenges and sustain food production and improvement

programs . The development of early maturing, drought and heat tolerant under climate change to sustain productivity is adaptation where new cultivars increase their production per unit area under moisture stress and extreme temperatures (Begna., 2021)

More complementary strategies are suggested so as to develop new superior technologies to make agriculture resilient to climate change (GOK, 2018) .Conventional plant breeding is dependent on phenotypic selection whereas genomics research is very important in developing the right adapted genotypes for the right environments without environmental impacts that complicates conventional breeding (Sultan., 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Development of mutant population

Four dolichos bean genotypes: Eldo-KT Maridadi, Eldo KT cream, Eldo-KT Black 1, Eldo KT Black II and denoted in the study onwards as (maridadi, cream, black I and black II) (table 2) were each packaged into one kilogram and sent for irradiation with a cobalt 60 (^{60}Co) source at Plant Breeding and Genetics Laboratories (PBGL) in Seibersdorf laboratories, Vienna, Austria in 2018. The irradiation treatments include 0gy, 300gy and 400gy of gamma rays. A sample comprising of 0.75kg or 3/4kg M1 irradiated seeds per genotype were grown as M1 generation then M2 and finally M3 generation at University of Eldoret biotechnology research field.

Table 2: Dolichos bean genotypes and their attributes as sent for irradiation at PBGL laboratory Austria in 2018

Release Name	Maturity Duration	Yield (t)/ha	Special Attributes
ELDO-KT Black I	4.5-5 months	3-5	Black seeded, good clearance, uniform maturity, short cooking time
ELDO-KT Cream	3-4.5 months	3.5 - 5.5	Good flavor, short cooking time, early maturity- cream seeded
ELDO-KT Maridadi	Over 5 months	2-4	Good flavor, short cooking time, late maturity, stay green, high forage, spotted seeds
ELDO-KT Black 2	4-4.5 Months	3.5 - 6.5	Black seeded, medium maturity

Source (*The Kenya Gazette*, 2015)

3.2 M1 generation

The 0.75kg M1 generation of dolichos bean seed per genotype per irradiation dose were grown in May to November, 2019 at University of Eldoret biotechnology field Uasin-Gishu County. The site is located 10 km north east of Eldoret town at an altitude of 2180 meters above sea level (masl), it consists primarily of an agro ecological zone LH3; latitude of 0°15' 31.64" S and longitude 35° 18' 17.96"E (Jaetzold., 2006). The average annual rainfall is 900 mm to 1000mm of a bimodal distribution. The mean air temperature ranges from 15 to 28 °C. The soil type is rhodic ferralsols non humic cambisols with low nutrient availability and moisture storage. The site was selected because it is among the major wheat growing regions with acidic soils. This site was

selected to promote dolichos bean production as an alternative crop that is drought tolerant and can improve the impoverished acidic soils of University of Eldoret.

3.2.1. Evaluation of effect of irradiation on M2 accessions of dolichos bean genotypes.

3.2.1.1Experimental site: University of Eldoret biotechnology field

Evaluation of effect of irradiation on M2 qualitative traits

3.2.1.2 Materials and methods

A thousand M2 seed for each 300gy and 400gy accession per genotype were evaluated at University of Eldoret biotechnology field. The M2 generations were grown during the rainy season of April to September 2020 in a spacing of 60cm by 40cm. The selection procedure was undertaken based on methods adapted from Oladosu *et al.*, (2016) where M2 seeds were planted in the field as M2 population in the form of progeny rows for individual plant selection. Screening for each mutant treatment was carried out by scoring the M2 plants from germination to maturity for any change in morphological trait observed then compared with the parent plants (checks). The mutants were grown with no fertilizer applications in all treatments to avoid a bias increase in the yield contributing traits . Normal recommended cultural practices and plant protection measures were followed. In total 7 qualitative characters were selected for evaluation of effect of mutation on dolichos bean accessions at M2.Descriptive statistics were used to analyze the data as follows : percent germination (%G), Abnormalities (ABN) observed, where 0= normal, 1= Albino, 2= leafy type, 3 = upright single stem, 4=seedless pods and 5= short dwarf pods as described by (Horn *et al.*, 2016) while flower bud colour (FBC), where 1=white,2=cream ,3=light yellow,4=pink ,5=purple, Growth habit (GH) :where

1=determinate, 2=semidetermnate,3=indeterminate,4=others, LS=leaf shape where1=round ,3=ovate,5=ovate lanceolate ,7=lanceolate ,9=linear lanceolate. Seed shape SS where1=round, 2=oval, 3=flat and 4 =others. Dry seed colour (DSC) where 1=white, 2=green,3=cream,4=purple,5=brown , 6=black and 7=others as described by Byregowda *et al* .,(2015) in table 3.

Table 3: Plant data based descriptors used for evaluation of dolichos bean accessions

Trait	Denotation	Method of evaluation
Leaf length	LL	Measured on the terminal leaflet of third trifoliate leaf (Middle portion of the leaf) from10 plants (cm)
Leaf let length	LLL	Measured on the terminal leaflet of third trifoliate leaf (Middle portion of the leaf) from 10 plants (cm)
Leaf width	LW	Measured on the terminal leaflet of third trifoliate leaf (Middle portion of the leaf) from 10 plants (cm)
Flower bud length	FBL	Average of 10 randomly chosen buds (mm)
Flower bud width	FBW	Average of 10 randomly chosen buds (mm)
Number of flower buds per raceme	NFB/R	Average of 10 randomly chosen racemes
Number of raceme per plant	NRPP	Average of 10 randomly chosen plants
Number of buds per node	NB/N	Average of 10 randomly chosen racemes
Number of nodes per raceme	NN/R	Average of 10 randomly chosen racemes
Raceme length in centimeters	RLC	Average of 10 randomly chosen plants (cm)
Days to 50% flowering	DTF	Number of days from sowing to the stage when 50% of the plants have begun to flower
Pod length in centimeters	PL	Average of 10 randomly chosen pods (cm)
Pod width in centimeters	PW	Average of 10 randomly chosen pods (cm)
Days to 80% maturity	DMT	Number of days from sowing to stage when 80% of the pods have matured and turned yellow
Plant height in centimeters	PH	Height from the base of the plant to tip of the longest raceme
100 seed weight in gram	SW	One hundred seeds randomly counted and weighed
Dry seed yield per plant in gram	DSYPP	Weight of all seeds harvested in a plant
Seed length in millimeters	SL	Average of 10 randomly chosen seeds and measured longitudinally (cm)
Seed width in millimeters	SW	Average of 10 randomly chosen seeds and measured medially (cm)
Dry seed colour	DSC	1=White, 2=Green, 3=Cream, 4=Purple, 5=Brown, 6=Black
Seed Shape	SS	1=Round, 2=Oval, 3=Flat, 4=Others (specify)
Growth habit	GH	1=Determinate ,2=Semi determinate ,3=Indeterminate ,4=others (specify)
Leaf shape	LS	1=Round, 3=Ovate, 5=Ovate lanceolate, 7=Lanceolate, 9=Linear lanceolate
Flower bud colour		1=White, 2=Cream, 3=Light Yellow, 4=Pink, 5=Purple

Source: (Byregowda *et al* ., 2015)

3.2.1.3 Evaluation of effect of irradiation on quantitative traits of M2 dolichos bean accessions

A total of 21 individual M2 families (10 families per mutant accession plus one family of control per genotype were planted. Eighteen (18) seeds per family were sown 60 cm by 40 cm. The experiment was laid out in randomized complete block design with two replications as per Amri *et al.,* (2018) . This resulted in 1512 M2 plants. The experiment was set at University of Eldoret biotechnology field. The M2 families were selected based on the number of seeds harvested in selected M1 plants. The M1 families were grown during the short rainy season of October 2020 to February and supplemented with drip irrigation.

Screening for each irradiation treatment was carried out by scoring three M2 plants per replicate from germination to maturity for the following morphological trait per dose per genotype. Data on quantitative traits was collected based on (Byregowda *et al.,* 2015) as described on table 3 above . Data were subjected to analysis of variance using SAS version 8.2. (SAS Institute Inc 2013). Main effects were separated by least significant differences (LSD) at P = 0.05 level.

Experimental model

$Y_{ij}= \mu + D_i + B_j + E_{ij}$: Where μ: Grand mean. $D_{i:}$ effect of i^{th} dose of irradiation (Treatment). $B_{j:}$ effect of j^{th} block and E_{ij} Error term

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3.3 Evaluation of genetic estimates of accessions of dolichos bean genotypes

3.3.1 Experimental materials: A random sample of thirty M2 mutant accessions per dose per genotype of black I and maridadi were used as test materials of the study. The materials were chosen based on their different and unique utilities: Maridadi is a dual purpose genotype brown with brown seeded, used as food and feed it also has a stay green effect after harvest while black 1 is black seeded, good clearance, uniform maturity and has short cooking time only used for household consumption as described by (The Kenya Gazette, 2015)

3.3.1.1 Experimental design: The trial was laid out in RCBD. Each of the accessions were planted in a plot size of 1.80 m^2 (3 rows, 1.5m row length, 50 cm between rows and 60 cm between plants within row and spacing of 1m, 1.5 m between plots and blocks, respectively)

3.3.1.2 Analysis of variance (ANOVA): The data collected for each quantitative trait was subjected to analysis of variance (ANOVA) for RCBD design. Analysis of variance was done using Proc ANOVA of SAS version 8.2 (SAS Institute Inc 2013). Treatment means were tested for significance (LSD) at p=0.05 probability levels.

3.3.1.3 Data collection: Data was collected either on plot basis or from randomly taken 3 plants on days to 50% flowering, leaf length, number of flower buds per raceme, raceme length (cm), plant height (cm), number of nodes per raceme, pod length (cm), 100 seed weight (g), dry seed yield per plant (g).

3.3.1.4 Estimation of variance components: The phenotypic and genotypic coefficients of variation were estimated according to the method suggested by Burton., (1952) as follows:

Environmental variance (σ2e) = Mse

$$\text{Phenotypic variance } (\sigma^2 p) = (\sigma^2 g + \sigma^2 e)$$

$$\text{Genotypic variance } (\sigma^2 g) = \frac{\text{Mse} - \text{Mst}}{r}$$

Where:

Mse	=	Mean square error
Mst	=	Mean square treatment
r	=	Replication

Phenotypic coefficients of variation (PCV)

$$\mathbf{PVC} = \frac{\sqrt{\boldsymbol{\delta}_p^2}}{\boldsymbol{\mu}} \times \mathbf{100}$$

Where μ is the grand mean value of the trait.

Broad sense heritability (H^2) in percentage was estimated in each character using variance components as described by DeLacy *et al.,* (1996).

$$h^2 B = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where:

$\sigma^2 p$	=	Phenotypic variance
$\sigma^2 g$	=	Genotypic variance
x	=	Grand mean of a character

3.3.1.5 Estimation of genetic advance: Genetic Advance (GA) and percentage of the mean (GAM) assuming selection of superior 5% of the genotypes was estimated in accordance with the methods illustrated by Johnson *et al*., (1955) as:

$$GA = \frac{K \times \sqrt{\sigma^2 p} \times \sigma^2 g}{\sigma^2 p}$$

Where:

GA	=	Expected genetic advance
K	=	Standardized selection differential at 5% selection intensity (K = 2.063)
$\sigma^2 p$	=	Phenotypic variance
$\sigma^2 g$	=	Genotypic variance

The genetic advance as percentage of mean (GAM) was computed as:

$$GAM(\%) = \frac{GA}{x} \times 100$$

Where:

GAM	=	Genetic advance as percentage of mean
GA	=	Expected genetic advance
x	=	Grand mean of a character

Heritability values was classified according to Singh (2001), greater than 80% as very high, values from 60-79% as moderately high, values from 40-59% as medium and values less than 40% are low.

Genetic advance as percentage of mean (GAM) was classified according to Johnson 1955; values from 0-10% as low, 10-20% as moderate and 20% and above as high

3.4 Evaluation of genetic diversity of dolichos bean genotypes accession

3.4.1 DNA extraction and Polymerase Chain Reaction (PCR)

Total genomic DNA was extracted from 95 accessions from dolichos bean genotypes (91 mutants and 4 as checks). Ninety one M3 accessions leaves picked from three week old plants for DNA extraction using modified Dellaporta protocol (Dellaporta *et al.*, 1983). A total of 0.3 gm per leave tissues were grinded in a mortar and pestle and placed in a 1.5 millimeter (ml) eppendorf® tube containing 600 µl of extraction buffer ([0.1 M of Tris-Hydrochloric acid (Tris-HCl) pH 8.0, 0.05 M of (w/v) Ethylene diamine tetra acetate

(EDTA), 0.5 M Sodium chloride (NaCl), 1 % of Polyvinylpirrodine (PVP), 0.07 % β mercaptoethanol and 20% (0.7µl) of (w/v) sodium dodecyl sulphate (SDS) added separately]). The mixture were incubated at 65 ℃ for 15 minutes with agitation every 5 minutes. Then the samples were then placed at room temperature for 5 minutes followed by addition of 350 µl of ice-cold 5 M potassium acetate then incubated at -20 ℃ for 20 minutes. After incubation the samples were centrifuged for 15 minutes at 13,000 revolutions per minute (rpm) at room temperature. The supernatant was then transferred to another tube and 480 µl of ice-cold isopropanol added and mixed gently. The mixture was kept at -20 ℃ for one hour or overnight and then centrifuged for 15 minutes at 13,000 rpm. The pellet was then left to dry at room temperature by inverting the tubes on paper towels until all isopropanol droplets disappeared from the walls of the tubes. The supernatant will be removed and the pellet washed with 700 µl of 70 % ethanol and the pellet dried at room temperature followed by a brief centrifugation of 5 minutes at 13,000 rpm. The above process from the addition of ice-cold isopropanol will be done twice and the incubation will be done for 20 minutes at -20 ℃. The pellet air dried and later resuspended in 50µl of Tri-EDTA (TE) 10:1 mM buffer and then incubated at -4 ℃.

DNA quantity and quality of each accession was determined by running samples on 1 % (w/v) agarose gels for 1 hour at 80 volts diluted in 100 mL 1X TAE buffer (0.89 M Tris base, 0.89 M boric acid, 20 mM EDTA pH 8.0) and 900 mL of distilled water. A standard undigested lambda DNA with a range variation of 10, 20, 50, 80 and 100 ng was further used as a comparison to determine the DNA concentration of the dolichos mutants' accessions by comparing band sizes and intensities. The gel was then stained in gelred (10mg/ml) for 30 minutes before viewing under ultraviolet trans- illuminator. The

concentration and quality was further assayed at optical density (OD) readings of 260 nm and 280 nm using Nanodrop spectrophotometer. The concentrations were used to guide the normalization of DNA of each ample to 20 ng/ul. The ratio of 260/280 nm optical densities per sample given by the nanodrop machine provided also an indication of the quality of the extracted DNA based on purity. A value of 1.8 indicated pure DNA while a deviation signifies contamination in the extracted DNA and could impede on PCR analysis.

3.4.2 Simple sequence repeat markers.

The simple sequence repeat markers with prefixes Lab T, PV, VA and BMd, were selected from various sources (Shivachi *et al.,* 2013, Abdallah., 2015 and Kamotho *et al.,* 2016). These SSR markers represent different linkage groups of dolichos bean and exhibit high polymorphism information content value. The 24 SSR primers (table 4) were selected from a pool of 46 SSR markers documented to have high polymorphic content and heterozygozity.

Table 4: List of SSR molecular marker used in the study

Primer name	Primer sequence	
	Forward 5'-3'	Reverse 3'-5'
Lab T1	ACCAGAATGGT	GGTGAACCTT
	TTCTCAAGTTCCT	CCTACACCATGACT
Lab T2	GTGCGCGTC	CAATATCT
	ACTTATTAGTTCTTA	TCACGTAACCACGGTA
Lab T3	CAGATCGAT	CCTCCTTA
	TGGTAGCTGGATTTC	CAGAAAG
		GGTAGCCTATGT
Lab T6	TCAATCGT	GTCTCCTT
	TGTTGGAAGAGGGTAT	CAACTGTGTCCACTGA
Lab T7	CAGCAGTGT	TGTACTTAG
	TGCCTCATACAGAAC	CCAAGATCAGGCACA
Lab T14	GGCATGGTG	AGAAGCAGA
	AAGATTGAAGAAGAC	GGACAGGTGAATTGT
Lab T24	GATCAGCT	TAACCCTCC
	CCAGACTGCTGACG	ATTCATTGTCCATTC
Lab T25	GGGTTGAAG	CCAATGA
	CTCACACAAATTCTT	TGGTTGTATGAGTTAGCAC
Lab T28	CTTTCTCC	CCTGTAAAT
	ATGCAGACCAAACTTC	AACTGTCCTGGGAAGC
BM143	GGGAAATG	ATGTTGGGA
	AACAGAGGAAA	ACTTTTAGTGTG
PV-atgc001	TGCCACCACA	TATGAGAG
	GCTTTCTCCTC	AAGCGGTTGGCACG
PV-cct001	GAGGGTGT	TTCATGGAT
	TTCACTATTGTCACTGC	GGTGGAGGAACAG
VA-ag001	GGGTAGTA	CCACCTTCT
	AAGGAAAGAGAAGAAAGAG	GTACTCGTTCCATG
PV-gaat002	AAACACACAA	TTCGTGAGGT
	AAAGTTGGACGCAC	AGGAGTTTGGTGG
PV-ag004	TTGATGACGT	AAAGGGCTAGG
	GGATGCATTGC	GAGAGTAAGTTGG
PV-at006	CCGTTGCCT	CGTGTGAAGTC
	GTATTTCCCCAT	ATCTGGAGTGGTC
BMd-20	GTTGCCACCG	GTGAGGCAA
	GTGATAATCT	GAAGCCTTCAA
BMd-22	GGTCACTT	CGGGAAAT
	CCGGAGCATTC	GGAAGTCACAGT
BMd-44	GGCAGCTT	TTCCTTCCC
	ACTAACCCGAAA	CTTTCTTCTCC
BMd-45	GGTTGGGA	ATCTTCGAC
	AGCCTCATACAG	CCACCTTGCT
BMd-53	TGCTGACCA	GGAGGAGGCT
	AGGAAATTCAG	TAAGCACAAA
BMd-1	CAAATCGCA	GTCGGAGCCA
	ACACCTCACAA	TCATCTGTTT
BMd-12	CATCAACA	GCAGCTGGCG
	AGGACAGCCTCA	GGTAAAACAG

3.4.3 Primer optimization

The 24 SSRs primers were optimized to avoid non-amplification. All primes that produced double bands (1 primers), with >50% genotypes missing amplification (2 primers) and non-amplified (2 primer) were discarded. 20 primers producing good bands amplified at annealing temperature of 55-59˚C were selected for final analysis with M3 selected early and high yielding mutant lines.

3.4.4. DNA amplification

The PCR reactions was performed in a Master cycler (Eppendorf®) using in a final volume of 20µl Bioneer AccuPower® containing 4µl pre-mix (1U Top DNA, 250µM each dNTP, 10 mM Tris-HCl pH 9.0, 30 mM Kcl, 1.5 mM $MgCl_2$, stabilizer and tracking dye), 0.5 ng/µl of each forward and reverse primer, 0.5 ng of DNA template, and 6 µl of double distilled water (ddH_2O). The PCR cycles consisted of initial denaturation at 95 °C for 5 minutes, followed by 35 cycles at 95 °C for 30 seconds, annealing at 54.6°C to 58.5 °C (depending on primer) for 1 minute, extension at 72 °C for 1 minute, and a final extension at 72 °C for 5 minutes.

3.4.5 Gel electrophoresis of PCR products

Agarose powder was dissolved in Tris Acetate EDTA (1X TAE) buffer by slowly boiling in the microwave oven. The agarose was allowed to cool and 0.5 ug/mg gel red staining dye was added to the gel. The warm agarose 60^0c solution was then poured into the gel tray in which combs had been inserted to form sample wells. The gel was then allowed to cool and solidify for 1hour. Following solidification of the gel, the gel container was

orientated to the negative terminal in the electrophoretic machine (Galileo™) before adding TAE buffer until the comb was fully immersed with the buffer and the comb removed. The samples were run alongside 2.0ul 100kb ladder on 2 % agarose gel run at 100 volts (V) for one hour and thirty minutes. The amplified PCR products were visualized, scored in ultraviolet (UV) Trans -illuminator at 312 nm.

3.4.6 Data collection

The bands generated from the SSR markers were scored for base pairs for all the M3 accessions.

3.4.7 Genetic diversity analysis

Molecular data were subjected to the GenALex (Peakall *et al.*,2006) and Power Marker software package (Liu *et al.*, 2005) to calculate the following summary statistics; percentage of polymorphic loci, mean number of alleles per polymorphic locus, observed heterozygozity (HO), expected heterozygozity (HE), polymorphic information content (PIC and genetic variance within and among populations (AMOVA) (Venkatesha *et al*., 2007)

3.4.8 Molecular phylogenetic and principal coordinate analyses

Phylogenetic trees were produced using genotyping data with 20 SSR markers using the hierarchical clustering method based on the dissimilarity matrix calculated with Manhattan index, as implemented in the DARwin software (version 6.0.9). The data matrices of the genetic distances were used to create the dendrogram using the unweighted pair group method with arithmetic mean allocated (UPGMA) based on the estimates of genetic similarity of the M3 populations. Principal coordinate analyses were also performed with DARwin 6.0.9 (Perrier *et al.*, 2006).

3.5 Selection at M3 generation

Ninety one M3 mutant accessions were selected from M2 mutant population based on earliness and dry seed yield. The M3 seed from selected M2 dolichos bean plants and parental accessions were planted in randomized complete block design of three replicates as M3 families at University of Eldoret biotechnology research field in 2020 for evaluation of earliness and yield potential. Twelve seeds were planted per accession per replicate in two rows of six seeds each in a spacing of 50cm by 60cm. The M3 plants were evaluated in the field using morphological and agronomical attributes. The accessions were monitored for germination percentage, seedling and plant vigor to time of maturity. Individual M3 accession that were early in flowering relative to the parental accession, with 90% germination, uniform flower colour, with more than 50 pods per plant, dry seed yield weight per plant over 180grams and were selected for advancement to M4 generation. A total of 20 M3 families were selected for advancement as M4 accession as indicated in figure 3.

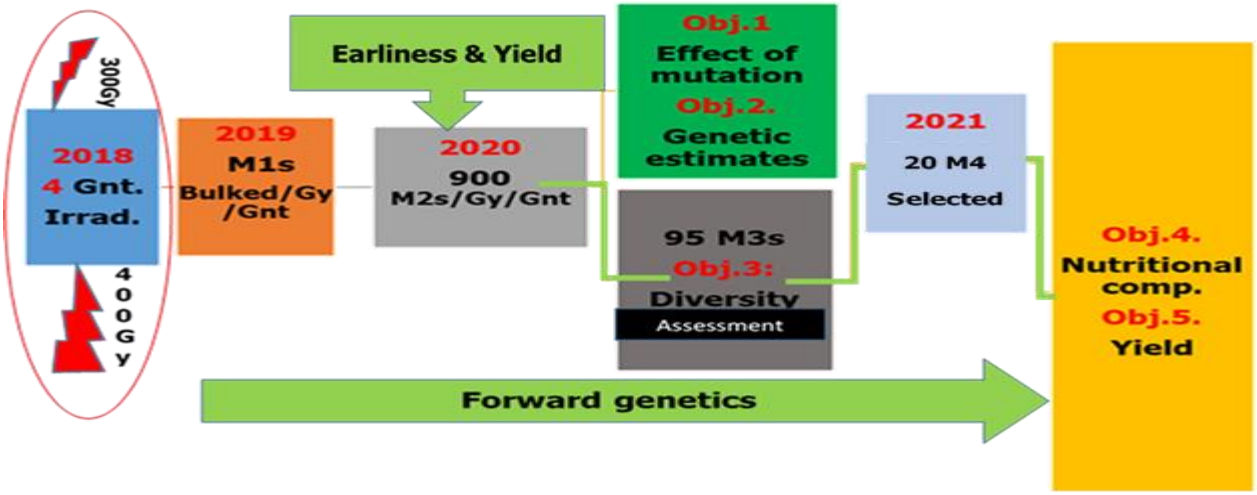

Figure 3: Framework on development and selection of mutant dolichos bean from M1 to M4 generation in four dolichos genotypes based on earliness and yield potential from 2018 to 2021.

3.6. M4 Accessions

A total of twenty one mutant M3 plant families (table 5.0) were selected based on the above criteria as indicated in section 3.5. The M3 accessions seeds were harvested bulked as M4 seed and used for evaluation for nutritional and mineral composition diversity and potential yield performance in four experimental sites. The naming of the accessions was developed based on the coding given the genotypes prior to irradiation .G represented black I, W: black II, B: maridadi; M: cream. Letters "T" and "F" coded the irradiation dose where: T (300gy) and F (400gy). The numeric values represented the plant selection number based on earliness at M2 generation. For example GT032 was an accession of black I, irradiation 300gy and plant number 032. While BF137 was a selection of maridadi irradiation of 400gy and plant number 137 and etc. as indicated in the table 5.

Table 5: Selected M4 dolichos bean accessions evaluated for nutritional and mineral composition and potential yields in different locations

Accession	Rad. dose	Seed size	Accession	Rad dose	Seed size
GT032	300gy	Small	**Black II**	Released var.	Large
BF137	400gy	Medium	**BF032**	400gy	Medium
MF048	400gy	Small	**Cream**	Released var.	Medium
GT076	300gy	Small	**BF105**	400gy	Large
WT018	300gy	large	**GT095**	300gy	Small
BT183	300gy	Large	**BT114**	300gy	Medium
BT039	300gy	Medium	**BT154**	300gy	Medium
MT110	300gy	Medium	**BT166**	300gy	Medium
Black I	Released var.	Small	**WT026**	300gy	Large
BT046	300gy	Medium	**MT049**	300gy	Small
BT188	300gy	Large	**MT076**	300gy	Medium
Maridadi	Released var.	Medium	**MF015**	400gy	Medium

3.7 Evaluation of nutritional and mineral composition

3.7.1 Materials and methods

The accessions used comprised twenty one M4 mutant lines (Table 5) that had been bred from gamma ray irradiation at 300gy and 400gy and selected on earliness and yield as indicated in section 3.6 and figure 3.0 and their four parental genotypes maridadi also

abbreviated as (B), cream (M), black I (G) and black II (W7) and their mutant accessions as (BT or BF) (MT or MF), (GT or GF.) and (WT.) respectively. The study was conducted in Egerton university animal science department and University of Eldoret biotechnology laboratories between the months of February and April, 2021.

3.7.2 Nutritional value component analysis

Dry dolichos bean grains with moisture content of below 13.0% were used in the study. The grains of the accessions were finely ground to fine flour and kept at 4–6°C in sealed and labeled polyethylene bags for nutritional analysis. The following nutritional components of the accessions were analyzed in duplicates among the samples: percent moisture, percent ash, and percent crude fat, percent crude protein, total starch and percent carbohydrates according to AOAC19 methods as described below (Association of Official Analytical Chemists., 2005).

3.7.3 Determination of moisture

Three grams of bean flour were accurately weighed in a pre-weighed petri-dish and dried in a hot air oven for 12hrs at 100±2ºC. The dish with the sample were then cooled in desiccators and weighed. This exercise was repeated until the difference in weight between two successive weighing became constant. From the weight loss during drying, amount of moisture was calculated using the following formula $Moisture\ (\%) = \frac{W1-W2}{W} 100$

W1 = Weight of sample with Petri dish before drying

W2= Weight of sample with Petridish after drying

W = Weight of sample

3.7.4 Determination of ash content

One gram of dried sample was accurately weighed into pre-weighed, clean crucible. The crucible was heated to the point of charring of the sample on a hot plate. The crucible with the carbon residue obtained as a result of ignition, was placed in muffle furnace at temperature of 650°C until the carbon residue disappeared. The sample was allowed to cool and then weighed. From the difference in weight obtained the ash content was calculated using the formula:

$$Total\ ash\ (\%) = \frac{Weight\ of\ crucible\ with\ Ash(g)}{Weight\ of\ crucible\ with\ sample} 100$$

3.7.5 Crude fat estimation

Ten grams of sample in a thimble were taken and plugged the top of the thimble with a wad of fat-free cotton. The thimble was dropped the into the fat extraction tube of a Soxhlet apparatus. The bottom of the extraction tube was attached to a Soxhlet flask. 75mL of hexane was poured through the sample in the tube into the flask. The top of fat extraction tube was attached to the condenser and the sample extracted for 6hrs on a heating mantle at 40°C. At the end of the extraction period the thimble from the apparatus was removed and the extract concentrated at rotavapor at 40°C. It was then dried at 100ºC for 1hr, cooled and weighed. The difference in weights gave the ether soluble material present in the sample.

$$Crude\ fat\ (\%) = \frac{Weight\ of\ hexane\ soluble\ material}{Weight\ of\ sample} x100$$

3.7.6 Determination of crude fiber (CF)

One gram of milled dolichos accession sample was taken into the beaker and 60ml of boiling sulphuric acid added. It was then connected to the digestion apparatus. The sample was boiled for 30 minutes, filtered through a filtering cloth, washed with hot water until it was free from acid. The residue was then transferred on the cloth into the flask with 200ml of boiling sodium hydroxide solution. Immediately it was connected to the flask with the digestion apparatus and boiled further for exactly 30 minutes. The flask was removed and immediately filtered through Gooch crucible. It was washed with hot water until it was free from alkali and then with 10ml of alcohol. It was then dried at 105-110°C in an air oven for about 2 hours. Cooling was done at room temperature in desiccator and weighed. The process was repeated 30 minute after drying, cooling and weighing until the difference between two successive weightings was less than 1mg. The lowest weight was noted as the weight of crucible and contents after drying.

The contents were then incinerated in the crucible in the electric muffle furnace at 620°C for 30minutes. It was then cooled to room temperature in desiccator and weighed. The difference between the two weightings was the weight of crude fiber.

$$Crude\ fibre\ ,(\%)\ by\ weight = \frac{(W1 - W2)}{W} x100$$

Where, W is weight (grams) of sample

W1 is weight (g) of crucible and contents after drying.

W2 is weight (g) of crucible and ash after incinerating.

3.7.7 Determination of crude protein (CP)

The test for protein measurement was based on the nitrogen content (Kjeldahl method). 0.5g of accession sample and digestion mixture (copper sulphate and potassium sulphate) was weighed into a Kjeldahl flask and 10ml of concentrated $H2S0_4$ was added. The Kjeldahl flask was then heated on a mantle (in slanting position) until colour of solution changed to pale blue green. The clear solution was made up to 25ml under cold conditions. The Kjeldahl apparatus was set up for protein estimation. 20ml of 4% boric acid and 1ml of mixed indicator (bromocresol green) was taken in conical flask and placed under condenser. 5ml of sample with 20ml of 40% NaOH and 10ml water were added to distillation tube through funnel. When water started boiling inside the round bottom flask the steam that was produced was then passed into distillation tube. The ammonium (NH3) gas that was evolved in distillation tube was then trapped in boric acid. Upon ammonia evolution, the colour of boric acid changed to blue. For maximum ammonia evolution, the process was continued for a further 20min. The solution was then titrated with standard HCl (0.01N) until blue colour of the solution disappeared. The amount of nitrogen in the samples were calculated by the following equation

$$\%\ of\ Nitrogen = \frac{14\ XNormality\ of\ HCl\ X\Delta VX100}{Weight\ of\ Sample\ 1000} x100$$

% Protein = % of Nitrogen × 6.24

3.7.8 Determination of carbohydrate

Total starch was found by difference method and expressed as

Percentage of total carbohydrates.

Total carbohydrate (%) = 100 − [Moisture + Ash + Fat + Protein]

3.7.9 Minerals composition determination

Determination of phosphorous, potassium, Zinc, Calcium and Magnesium

The mineral content of the bean samples was determined by dry ash method (AOAC, 2005). Five grams of accession sample was ashed at 550ºC for 8 hours then drops of 6N hydrochloric acid (HCL) were added and evaporated. The samples were incinerated further for 1 hour and diluted using 1N nitric acid. The samples were placed in 100 ml volumetric flasks and made to 100 ml using 1N nitric acid. Standards were prepared using the 1N nitric acid and the absorbance read in the atomic absorption spectrophotometer (Shimadzu, AA-6200, and Tokyo, Japan).

The dilution factor for all minerals except Magnesium (mg) was 100. For determination of Mg, a further dilution of the original solution was done by using 0.5ml original solution and enough distilled water was added to it to make the volume up to 100ml. Also for the determination of Ca, 1.0ml lithium oxide solution was added to the original solution to unmask Ca from Mg. The concentrations of minerals were recorded in terms of "ppm" are converted to milligrams (mg) of the minerals by multiplying the ppm with dilution factor and dividing by 1000.

3.7.10 Data analysis

The data obtained were subjected to Analysis of Variance (ANOVA) using SAS (Version 9.1). The mean values were displayed with standard deviation (SD). The statistical comparison between means for the treatments were made using LSD and significant difference were reported at P=0.05.

3.8 Evaluation of adaptability of selected M4 accessions

3.8.1 Materials and methods

3.8.1.1 Experimental procedure

The experimental land was prepared to fine tilth before planting in four agro ecological locations; Farmers field in Chepararia (West Pokot county) and in Maili saba -Kitale (Trans-Nzoia county), University of Eldoret research field (Uasin Gishu county) and Agricultural Training college farm – Koibatek (Baringo county). Twenty four dolichos bean accessions as described in table 5.0 were planted in each of the four experimental sites in a randomized complete block design (2.0 m x 1.2 m) with three replications. Each accession was grown in four rows at spacing of 60 cm x 50 cm between and within rows, respectively. The field was kept clean by hand weeding, first and second weeding was done on the 4^{th} week and the 8^{th} week from the date of planting.

3.8.1.2 Experimental sites

The University of Eldoret ($0^{o}34'N, 35^{o}18'E$), experimental location has an elevation of 2153 meters above sea level (MASL) predominantly a LH3 zone and experiences one rainfall season between march and September. The annual average rainfall is 1085 mm and mean temperatures range between 11-24^{o}C. The soils in Eldoret are *rhodic ferralsols*.

The farmers field in Maili Saba Kitale ($0^{0}95'N, 35^{0}28'E$), experimental location is located within the predominantly a LH3 –LH4 agro ecological zone. The highest rainfall falling between April/May and July/August the dry spells set in December to February. The annual average rainfalls is 900-1200 mm and mean temperatures range between 11-27^{o}C.

Koibatek ATC (latitude 1^0 35' S and longitude 36^0 66' E) lies at an altitude of 1890 MASL in agro-ecological zone UM4 with low agricultural potential. Average annual rainfall is 500-800mm and mean temperature ranges between 18.2-24.3^{0}C. Mean minimum and maximum temperatures are 10.9°C and 28.8°C respectively. Soils are vitric endosols with moderate to high fertility, well drained, deep sandy to loam soils(Jaetzold. R. *et al.,* 2006)

The farmers field in Chepararia –west Pokot county Pokot south sub county (latitude $1^0$21' N and longitude 35^0 12' E) lies at an altitude of 1701masl. Average annual rainfall is 700-1250 mm and mean temperature of 28.8 ^{0}C. Agro-ecological zone UM4 with low agricultural potential. The soils are sandy clay loam.

3.8.1.3 Data collection

Data was collected on selected set of traits associated with yield as per Nanthakumar *et al.*, (2021). A random sample of three plants was used to derive the mean performance with respect to plant height, number of nodes per raceme, raceme length, number of pods per plant and 100 seed weight and yield kgha^{-1}in. Days to maturity was taken from date of sowing to physiological maturity in more than 85% per cent of the plants of accession. Days to 50% flowering was recorded as days taken from date of sowing to the day when 50 per cent flowers had opened for each accession in the plot.

3.8.1.4 Data analysis

Analysis of variance was performed for quantitative trait using SAS software 9.1 statistical package using the following generalized liner model:

$$Y_{ijkl} = \mu + G_i + E_j + R_k + GE_{ij} + \sum_{ijkl}$$

Where;

$Y_{ijk:}$ Mean of different traits for the i^{th} genotype, in l^{th} replicate of j^{th} location

μ: Grand mean.

$G_{i:}$ Mean deviation for i^{th} genotypes.

$E_{j:}$ Mean deviation for the j^{th} location.

$R_{K:}$ Mean deviation for the i^{th} replicate of the j^{th} location.

GE_{ij}: interaction term for the combination of the i^{th} genotype with the j^{th} location.

$\sum_{ijk:}$ Residual error

CHAPTER FOUR

RESULTS

4.1.1 Effect of Irradiation on Qualitative Traits of M2 dolichos Bean Accessions

The effect of mutation in qualitative traits of dolichos bean accessions are presented in table 6. The germination percentage of M2 mutant accessions ranged between 75 to 89%. Cream, black II and maridadi at irradiation dose of 400 gy recorded germination percentage below 80. While black I at irradiation dose of 300 gy had the least germination percentage of 78. Phenotypic abnormalities due to gamma irradiation included: albinism and variation in leaf colour in cream (300 gy and 400 gy), maridadi (300 gy and 400 gy) plate 3, leaf deformity on all accessions and dose 300 gy and 400 gy, single stem in maridadi (400 gy), seedless pods in 400 gy of cream and maridadi and short pod sizes in cream 400 gy. Variation in flower bud color was noted in 300 gy and 400 gy of cream from white to color cream and in maridadi from purple 0 gy to white and pink in 300 gy and 400 gy.

Table 6: Qualitative characteristics observed M2 generation of dolichos bean due to effect of gamma ray mutagenesis

%G= percent germination, ABN= Abnormalities observed, where 0= normal, 1= Albino, 2= leafy type, 3 = upright single stem, 4=seedless pods and 5= short dwarf pods, FBC =flower bud colour, where 1=white,2=cream ,3=light yellow,4=pink ,5=purple, GH =Growth habit where 1=determinate, 2=semidetermnate,3=indeterminate,4=others, LS=leaf shape where1=round ,3=ovate,5=ovate lanceolate ,7=lanceolate ,9=linear lanceolate. SS= Seed shape where1=round, 2=oval,3=flat,4=others. DSC= dry seed colour where 1=white, 2=green,3=cream,4=purple,5=brown , 6=black 7=others

GNT	Dose (Gy)	G%	ABN	FBC	GH	LS	SS	DSC
	0	97	0	1	2	5	3	3
Cream	300	89	0,1,2	1,2	1,2,3	3,5	2,3	1,2,3,7
	400	78	0,1,2,4,5	1,2	1,2,3	3,5	3	1,2,3,5
	0	94	0	5	1	5	2	6
Black II	300	84	0,2,3	5	1,2,3	3,5	1,2,3	3,6
	400	78	0,2,3,4	5	1,2,3	3,5,7	1,2,3	3,5,6
	0	96	0	5	1	5	2	5
Maridadi	300	84	0,1,2	1,4,5	1,2	3,5	1,2	5,6
	400	75	0,1,2,3,4,	1,4,5	1,2,3	3,5,7	1,2,3	5,6
	0	95	0	5	1,2	5	1	6
Black I	300	78	0,2	5	1,2	3,5,7	1,2	6
	400	84	0,2	5	1,2	3,5	1,2,3	6

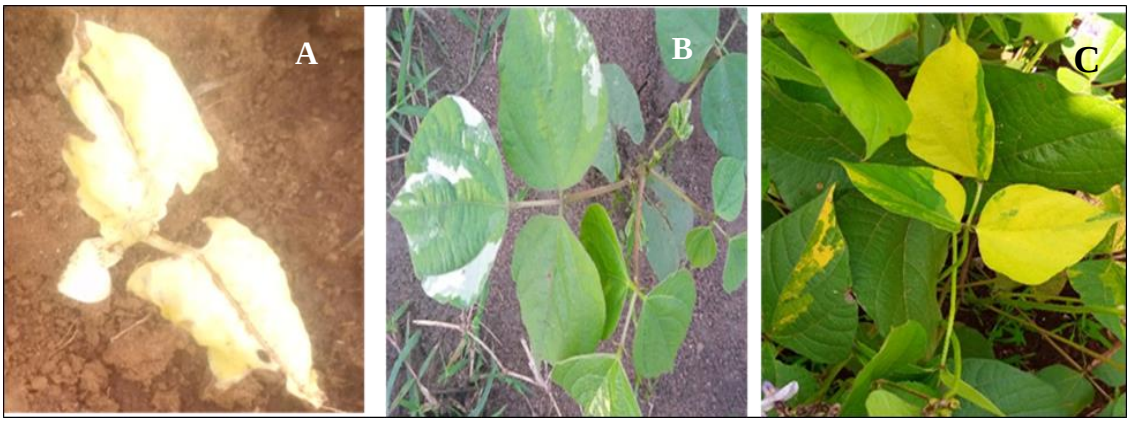

Plate 3: Phenotypic abnormalities (Albinism (A) and white (B) and yellow (C) leaf variegation on dolichos bean accessions due to gamma irradiation.

Growth habit variations ranged from determinate, semi determinate and indeterminate these were mainly observed among cream 300 gy and 400 gy. In black II 300 gy and maridadi 300 gy produced indeterminate mutant accessions compared 400 gy. Semi determinate accessions were identified in black I 300 gy and 400 gy accession. Leaf shape changed from ovate lanceolate to ovate and lanceolate with the two variations being largely pronounced by 400 gy black II, maridadi and 300 gy in black I.

Plate 4: Variation due to leaf shape A: normal non mutated leaf, B mutant leaf sample

Variations in seed shape ranged from flat to oval in cream 300 gy while no change in 400gy, in black II it changed from oval to round and flat in both doses, in maridadi it varied from oval to round in 300 gy and round and flat in 400 gy while in black I it changed from round to oval and flat

Dry seed colour varied among cream and maridadi mutants depending on the dose plate 5. The variation in cream ranged between pale, white to deep brown with white tinge, while maridadi produced marked variation in seed colour of brown with black spot or

white with black spot. Brown seeds were isolated among black II mutants while black recorded no variations on seed colour

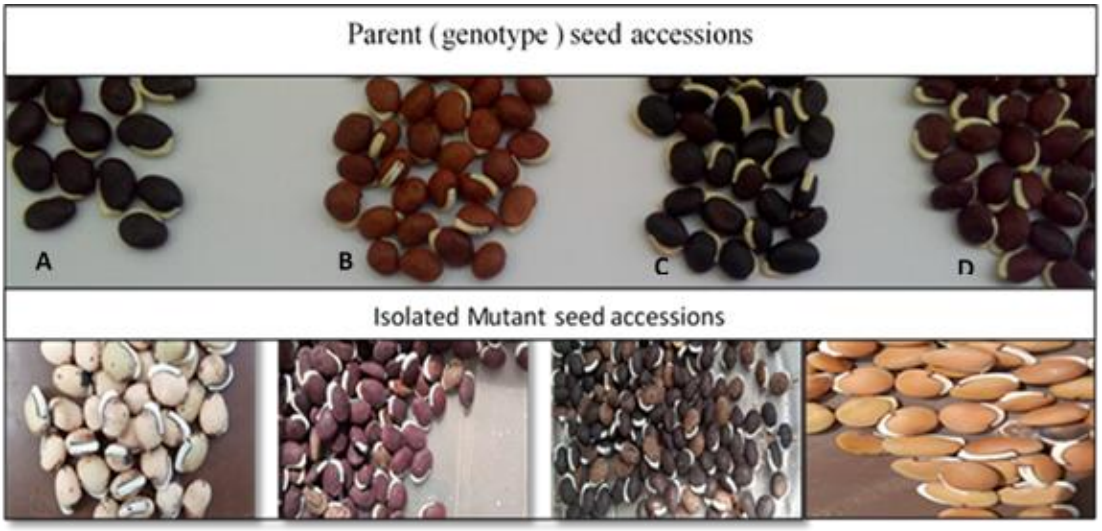

Plate 5: Comparison of (top) seed of non-mutated parent genotypes (A) black I, (B) maridadi, (C) black II and (D) cream and (bottom) isolated seed of mutant accessions showing colour variations due to mutation induction

4.1.2: Effect of Irradiation on Quantitative Traits of Dolichos Bean Genotypes

The effects of gamma rays irradiation (0gy, 300gy and 400gy) on leaf length, leaf let length, leaf width, number of flower buds per raceme, number of raceme per plant, raceme length (cm), number of nodes per raceme and number of buds per node of dolichos bean accessions M2 families are summarized in table 7. Leaf length ranged from 6.20cm-9.90cm (cream), 6.00-10.00cm (black II), 10.01-12.80 (maridadi) and 8.10-12.30 (black I), Significant differences $P \leq 0.05$ in leaf length between accessions and irradiation doses were recorded. Irradiation of dolichos accessions at dose 300gy significantly increased leaf length in all the accessions. The leaf length mean ranged from 7.96cm (cream) and 11.36cm (maridadi) as illustrated in figure 4. The leaf width length ranged from 7.06cm (maridadi) to 7.56 cm (black II). Significant difference in leaf width (LW)

only occurred in cream accession with the highest leaf width increase at 300gy (8.20cm). There was no significant impact among the accessions and on irradiation doses on leaf let length (LLL) which ranged from 20.13cm (cream) and 23.86 (black II). There was no significant difference in the number of flower buds per raceme (NFBR) that ranged from 15.93(maridadi) and 18.16(cream) and in the number of racemes per plant (NRPP).The number of racemes per plant was significantly different in cream accessions which ranged from 13.90 (400 gy) and 19.80 (300 gy) with mean of 16.73 $p \leq 0.01$. The mean variation in raceme length in centimeters among the cream, black II, maridadi and black I accessions was a significant P=0.001.There were significant differences among the mean of irradiation doses P=0.05 within the accessions. Irradiation dose of 400 gy produced the longest significant raceme lengths in all the accessions: cream (74.90 cm) black II (93.20 cm), maridadi (74.30 cm) black I (74.50). Number of nodes per raceme and number of buds per nodes were significantly different in maridadi with 0gy producing less 6.00 nodes per raceme while 400 gy yielding 7.80 nodes per raceme. The number of flower buds per node significantly decreased with increase of dose in maridadi 0 gy significantly produced more (3.50) number of buds per node while the least significant was produced at 400 gy (2.70).

Table 7: Mean of effects of gamma irradiation on dolichos bean vegetative traits

GN	Dose (Gy)	TRAITS							
		LL	**LW**	**LLL**	**NFBR**	**NRPP**	**RLC**	**NNR**	**NBN**
CREAM	**0**	6.20B	6.00B	20.20A	19.40A	16.50BA	36.10C	8.80A	2.80A
	300	9.90A	8.20A	21.50A	16.80A	19.80A	48.70B	7.70A	2.60A
	400	7.80B	7.30BA	18.70A	18.30A	13.90B	74.90A	9.30A	3.30A
	Mean	7.96***	7.16**	20.13	18.16	16.73*	53.23***	8.6	2.90
	Pr >F	***	**	NS	NS	*	***	NS	NS
	CV%	22.36	19.42	19.38	26.54	28.90	19.24	22.36	25.78
BLACK II	**0**	6.00C	7.60A	26.00A	15.60A	18.00A	39.20C	7.90A	2.50A
	300	10.00A	7.20A	21.10A	17.40A	16.10A	63.00B	8.70A	2.40A
	400	8.30B	7.90A	24.50A	16.10A	18.90A	93.20A	8.90A	2.50A
	Mean	8.10***	7.56NS	23.86NS	16.36NS	17.66NS	65.13***	8.50NS	2.46NS
	Pr >F	*******	NS	NS	NS	NS	*******	NS	NS
	CV%	20.86	15.11	22.28	27.42	30.22	18.91	22.25	21.92
MARIDADI	**0**	10.01B	6.80A	19.00A	16.40A	18.50A	36.00C	6.00B	3.50A
	300	12.80A	7.10A	21.40A	17.00A	19.50A	58.30B	6.60B	3.00BA
	400	11.20BA	7.30A	21.80A	14.40A	20.40A	74.30A	7.80A	2.70B
	Mean	11.36	7.06	20.73	15.93	19.46	56.20	6.80	3.06
	Pr >F	*	NS	NS	NS	NS	***	**	*
	CV%	18.36	9.85	20.73	21.17	23047	7.89	18.29	22.71
BLACK I	**0**	8.10C	7.1A	21.60A	14.40A	20.40A	37.90C	7.70A	2.90A
	300	12.30A	7.30A	24.30A	18.70A	17.90A	46.20B	8.90A	2.60BA
	400	10.60B	7.0A	22.80A	16.00A	17.20A	74.50A	7.70A	2.20B
	Mean	10.33	7.13	22.90	16.36	18.50	52.8	8.1	2.56
	Pr >F	***	NS	NS	NS	NS	***	NS	*
	CV%	15.59	16.69	19.47	29.21	30.54	11.59	21.59	28.3

Mean of * Significant at P<0.05, ** and P<0.01 and *** P<0.001: LL: Leaf length, LLL: Leaflet length, LW: Leaf width, NFBPR: Number of flower buds per raceme, NRPP: Number of raceme per plant, RL: Raceme length in cm, NNR: Number of nodes per raceme, NBN: Number of nodes per plant

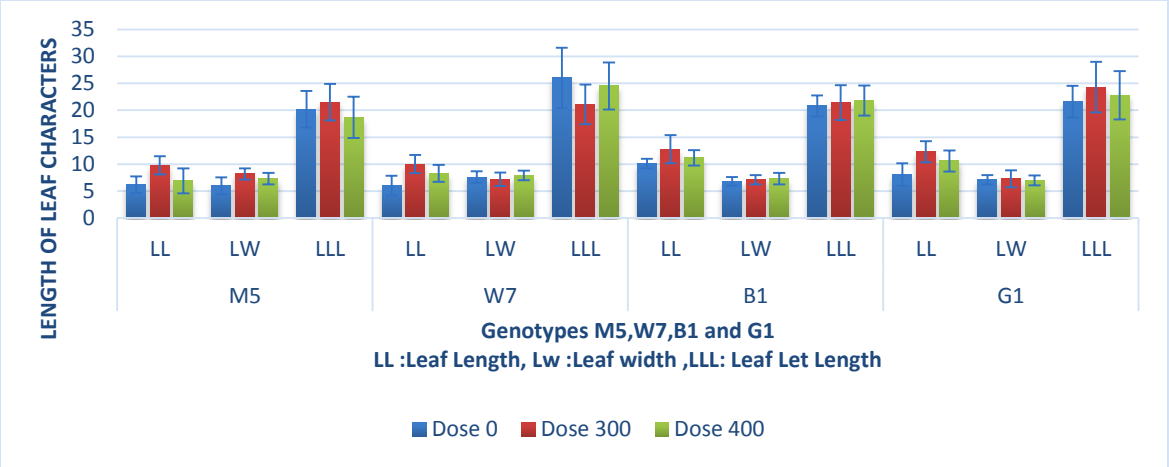

Figure 4: Effect of gamma irradiation on Leaf length (LL),Leaf width (LW) and Leaflet Length (LLL) in M2 generation of M5 (cream),W7(black II), B1 (maridadi) and G1(black I)

The effects of mutagenesis on pod traits (pod length and pod width), plant height, 100 seed weight, dry seed yield per plant seed length and seed thickness are summarized in 8. There was significant difference on pod length among irradiation doses and the accessions of cream and maridadi. Irradiation dose of 300gy significantly increased pod length across the accessions with cream (4.70cm), black II (5.29cm), maridadi (5.04cm) and black I (5.08cm). Maridadi accessions recorded significant decrease of pod width with increase of irradiation dose from 1.63 (0 gy) to 1.37 (400 gy) and the mean was 1.52 and significant P=0.001. Effect of radiation dose 300gy and 400 gy significantly reduced plant height of E. cream to 47.00 and 48.50cm compared to 60.30 cm of 0 Gy (check) while black II recorded significant reduction from 66.00 cm (0 gy) to 58.50 cm (300 gy) and in black I significant reduction was from 81.50 cm (0 gy) to 60.50 (400 gy).100 seed weight significantly reduced in cream from 24.90gm (0gy) to 22.90gm (400gy) and it significantly increased in black II on irradiation at 300 gy (32.10 gm) compared to 27.50 gm (400 gy) and 25.30 gm (0 gy) which were not significantly different there was no

significant variation in maridadi and black I in 100 seed weight in grams on irradiation at 0 Gy, 300 gy and 400 gy. All the genotypes recorded significant difference p=0.001 in dry seed yield per plant with average mean of 37.40 gm (cream), 73.80 gm (black II), 74.97gm (maridadi) and 58.65gm (black I). Mutagenesis with 300 gy on cream, black II and black I resulted in significant increase in dry seed yield per plant of 51.54 gm, 85.46 gm and 76.41gm while in maridadi significant increase of 88.82 gm in 400 gy was recorded. Accessions of cream (5.46 mm) and black II (8.86 mm) at irradiation of 300 gy and maridadi (9.54 mm) on irradiation at 400 gy produced significant increase in seed length. The average mean recorded were 4.62 mm (cream), 7.60 mm (black I), 6.83 (maridadi) and 9.02 (black II) and significantly different P=0.001 except for black II. Seed thickness was only significant in black II with 300 gy accessions having the highest seed thickness of 5.70 mm and 0 gy the least thickness of 5.03 mm. Seed thickness mean ranged from 1.68 mm (cream) to 5.63 mm (black II).

Table 8: Effects of gamma irradiation on pod traits (pod length and pod width), plant height, and 100seed weight, dry seed yield per plant seed length and seed thickness on dolichos bean genotypes

Genotype	Dose	DF	PL	PW	DTM	PH	SW	DSYPP	SL	ST
	0GY	61.00A	3.04C	1.64A	118.00A	60.30A	24.90A	36.15B	3.92B	1.45A
	300Gy	60.30A	4.70A	1.64A	118.00A	47.00B	23.60BA	51.54A	4.47B	1.77A
	400Gy	60.90A	3.64B	1.57A	116.70A	48.50B	22.90B	24.53C	5.46A	1.83A
Cream	**Mean**	60.73	3.79	1.61	117.86	51.93	23.80	37.40	4.62	1.68
	Pr >F	NS	***	NS	NS	*	*	***	***	NS
	CV%	2.11	11.15	7.88	2.63	22.53	7.80	22.51	20.29	35.25
	0gy	62.40A	4.80B	1.73A	139.30A	66.00A	27.30B	53.11B	7.55B	5.03B
	300Gy	63.00A	5.29A	1.85A	138.90A	58.50B	32.10A	85.46A	8.86A	5.70A
	400Gy	63.30A	4.90BA	2.05A	143.40A	63.50BA	25.30B	82.84A	6.39C	5.63BA
Black II	**Mean**	62.90	5.01	1.87	140.53	62.66	28.26	73.80	7.60	5.45
	Pr >F	NS	*	NS	NS	*	***	***	***	*
	CV%	3.74	9.09	23.75	6.23	12.70	10.92	18.40	12.94	11.89
	0Gy	63.40A	4.31B	1.63A	120.40A	65.50A	25.00A	57.07C	4.37C	4.04A
	300Gy	62.70A	5.04A	1.56A	114.60B	62.50A	25.68A	79.02B	6.57B	4.14A
Maridadi	**400Gy**	62.60A	3.72C	1.37B	116.70BA	60.50A	26.20A	88.82A	9.54A	4.21A
	Mean	62.90	4.35	1.52	117.23	62.83	25.62	74.97	6.83	4.13
	Pr >F	NS	***	***	*	NS	NS	***	***	NS
	CV%	2.87	9.68	9.01	4.20	15.79	6.07	11.84	28.42	22.54
	0Gy	64.30A	4.43B	1.79A	120.40B	81.50A	25.40A	36.692B	9.32A	5.77A
	300Gy	62.80A	5.08A	1.83A	123.30BA	66.50B	25.30A	76.41A	9.22A	5.21A
Black 1	**400Gy**	62.50A	4.68BA	1.65A	126.50A	60.50B	25.20A	62.86A	9.02A	5.36A
	Mean	63.20	4.7	1.75	123.40	69.50	25.30	58.65	9.19	5.45
	Pr >F	NS	*	NS	*	***	NS	***	NS	NS
	CV%	3.73	9.97	12.2	4.54	11.04	8.95	25.66	10.98	27.95

Mean of * Significant at P<0.05, ** and P<0.01 and *** P<0.001: GNT: Genotype, DF Days to flowering, PL: Pod length, PW: Pod width, PH: Plant height, DTM: Days to maturity; SW: Seed weight, DSYPP: Dry seed yield per plant, SL: Seed length, ST: Seed thickness.

4.2 Results of measurements of genetic estimates in dolichos bean accessions

4.2.1 Genetic estimates of yield associated traits of maridadi accessions

The genetic estimates of maridadi accessions are given in the table 9. below. The phenotypic variances were higher than genotypic variance among the traits measured in the accessions of maridadi. Higher phenotypic variation (PCV) was observed for number of nodes per raceme (27.05%) at 0 gy, number of racemes per plant (25.16%), Number of nodes per raceme (20.91%) at 300 gy and number of raceme per plant (35.38%), raceme length (21.09%) and plant height (32.68%) at 400 gy whereas leaf length (10.75%), number of raceme per plant (12.01%), raceme length (11.78%), pod length (15.04%), dry seed yield per plant (14.22%) at 0 gy, raceme length (18.29%), pod length (11.92%), plant height (14.27%), and dry seed yield per plant (11.33%) in 300 gy, leaf length (15.23%), number of nodes per raceme (16.27%) and pod length (14.60%) in 400 gy showed medium phenotypic coefficient of variation.100 seed weight, plant height and days to flowering in 0 gy, leaf length, days to flowering and 100 seed weight in 300 gy and days to 50% flowering 100 seed weight and dry seed yield per plant in 400 gy showed low phenotypic coefficient of variation.

Table 9: Genetic estimates of yield contributing traits of maridadi dolichos bean accessions

	TRAIT	σ^2P	σ^2G	%PCV	%GCV	%H	GA	%GAM
0gy	LL	1.19	0.35	10.78	5.82	29.11	0.65	6.46
	NRPP	4.94	8.45	12.01	15.71	171.05	2.48	13.42
	RLC	17.98	5.44	11.78	6.48	30.26	3.18	8.85
	NNR	2.64	1.11	27.08	17.56	42.05	2.18	36.26
	DTF	1.47	0.44	1.91	1.05	29.93	0.79	1.25
	PL	0.42	0.14	15.04	8.68	33.33	0.17	3.96
	PH	31.05	77.65	8.51	13.45	250.08	2.38	3.63
	SW	5.61	1.32	9.47	4.59	23.46	1.32	5.28
	DSYPP	133.00	41.47	14.23	7.94	31.18	4.75	5.85
300gy	LL	0.95	0.23	9.44	4.64	24.21	0.49	4.71
	NRPP	24.07	7.47	25.16	14.02	31.03	0.74	3.80
	RLC	40.26	12.75	18.29	10.29	31.67	1.49	4.29
	NNR	1.91	0.62	20.91	11.88	32.28	0.66	10.04
	DTF	12.58	4.11	5.66	3.23	32.64	0.77	1.22
	PL	0.27	0.07	11.92	5.90	24.53	0.10	2.24
	PH	74.56	18.05	14.27	7.02	24.21	0.20	0.33
	SW	4.27	0.32	8.04	2.19	7.39	0.03	0.12
	DSYPP	86.34	24.85	11.33	6.08	28.78	0.08	0.10
400gy	LL	2.71	0.55	15.53	7.00	20.30	0.69	6.49
	NRPP	52.09	11.68	35.38	16.75	22.42	0.79	3.85
	RLC	70.80	8.58	21.09	7.34	12.12	0.75	1.89
	NNR	1.61	0.45	16.27	8.60	27.95	0.53	6.76
	DTF	10.91	3.62	5.28	3.04	33.15	0.73	1.16
	PL	0.30	0.09	14.60	7.84	28.81	0.12	3.23
	PH	417.18	135.59	32.68	18.63	32.50	0.64	1.03
	SW	1.03	0.34	3.87	2.23	33.01	0.07	0.26
	DSYPP	37.51	13.58	7.31	4.40	36.20	0.07	0.08

LL: Leaf length (cm), NRPP: Number of Raceme per plant, RLC: Raceme length (cm), NNR: Number of nodes per raceme, DTF: Days to flowering, PL: Pod length (cm), PH: Plant height (cm), SW: Seed weight (grams) and DSYPP: Dry seed yield per plant

Medium genotypic coefficient of variation was recorded in number of racemes per plant 15.71%, 14.025 and 16.75% at 0 gy, 300 gy and 400 gy respectively, number of nodes per raceme at 0 gy (17.56%) and 300 gy (11.38%), plant height 0 gy (13.45%), 400 gy (18.63%) and raceme length (10.29%) in 300 gy the remaining traits showed low genotypic coefficient of variation (<10%). Large difference between PCV and GCV was produced in 0 gy on leaf length (10.78% and 5.82%), pod length (15.04% and 8.68%), seed weight (9.04% and 4.59%) and dry seed yield per plant (14.23% and 7.94%). The same trend was noted in 300 gy on pod length (11.92% and 5.90%), plant height (14.27% and 7.02%) seed weight (8.04% and 2.19%). On 400 gy leaf length (15.53% and 7.00%), number of raceme per plant (35.38% and 16.75%), raceme length (21.09% and 7.34%) number of nodes per raceme (16.27% and 8.60%) pod length (14.60% and 7.84%) and plant height (32.68% and 18.63%).

High heritability was only observed for 0 gy in number of racemes per plant (171.05%) and plant height (250.08%). Medium heritability was recorded in raceme length (30.26%), number of nodes per racemes (42.05%), pod length (33.33%) and dry seed yield per plant (31.18%) in 0 gy, number of racemes per plant (31.03%), raceme length (31.67%), number of nodes per raceme (32.28%) and days to flowering (32.64%) in 300 gy and days to flowering (33.15%), plant height (32.50%), 100 seed weight (33.01%) and dry seed yield per plant (36.20%) in 400 gy. The rest of the traits recorded lower heritability values <30%. In the study moderate genetic advance as percent of mean was estimated for grain yield $kgha^{-1}$ (11.98%) and other traits showed low genetic advances (<10%). High genetic advance of the mean (%GAM) was only recorded in at 0 gy in the number of nodes per raceme (36.26%). Moderate (10-20%) GAM was recorded in number of

racemes per plant (13.42%) in 0gy and number of nodes per raceme (10.04%) in 300 gy. 400 gy produced low %GAM among the traits studied.

4.2.2 Genetic estimates of yield associated traits of black I accessions

The coefficients of variation and heritability estimates of the traits of black I are summarized in table 10. The phenotypic coefficient of variation (PCV) and the genotypic coefficients of variation (GCV) were positive for all the traits. High PCV and GCV were observed in dry seed yield per plant (24.77% and 33.81%) in 0 gy, number of nodes per raceme (25.56% and 22.25%), pod length 106.60% and 150.76%) in 300 gy and leaf length (74.18% and 39.22%), number of nodes per raceme (54.16 % and 35.71%), days to flowering (141.37% and 51.22%) pod length (213.98% and 111.75%) and 100 seed weight (117.56% and 64.28%) in 400 gy per plant (144.8% and 141.6%), weight of seeds per plant (82.80% and 74.30%). Low phenotypic (PCV) and genotypic (GCV) coefficient of variations were observed in leaf length (9.00% and 6.15%), number of days to 50 % flowering (2.06% and 1.52%) pod length (5.76% and 2.26%) plant height (6.93% and 9.60%) and 100 seed weight (6.19% and 2.92%) in 0 gy. Plant height (6.14 % and 8.57%) and dry seed yield per plant (4.52% and 6.27%) in 300 gy. While number of raceme per plant (9.29% and 8.57%) plant height (6.27% and 6.67%) and dry seed yield per plant (4.41% and 2.26%) in 400 gy.

Traits with high heritability estimates were number of raceme per plant (196.27%), raceme length (97.20%), number of nodes per raceme (61.11%),plant height (192.16%), dry seed yield per plant (186.34%) in 0 gy, number of racemes per plant (75.78%), pod length (200%), plant height (185.96 %),100 seed weight (159.09%) and dry seed yield per plant (192.45%) in 300 gy. Number of raceme per plant (85.01%) and plant height

(113.25%). Moderate heritability 30-60% were recorded in leaf length (46.58%) and days to flowering (54.29%) in 0gy. Number of raceme per plant (37.59%) and number of nodes per raceme (43.48%) in 300 gy and 400 gy respectively.

Table 10: Genetic estimates of nine yield contributing traits of black I dolichos bean accessions

	TRAIT	σ^2P	σ^2G	%PCV	%GCV	%H	GA	%GAM
0 gy	LL	0.91	0.43	9.01	6.15	46.58	0.41	3.83
	NRPP	10.73	21.05	16.05	22.49	196.27	68.94	337.93
	RLC	21.89	21.28	11.73	11.56	97.20	99.53	249.46
	NNR	1.80	1.10	17.42	13.62	61.11	1.48	19.17
	DTF	1.75	0.95	2.06	1.52	54.29	1.26	1.95
	PL	0.07	0.01	5.76	2.26	15.38	0.00	0.06
	PH	31.88	61.25	6.93	9.60	192.16	345.80	424.30
	SW	2.48	0.55	6.19	2.92	22.22	0.87	3.41
	DSYPP	133.74	249.21	24.77	33.81	186.34	2882.01	6172.66
300 gy	LL	1.64	0.38	35.06	16.78	22.90	0.48	13.15
	NRPP	4.99	1.88	18.85	11.56	37.59	4.19	35.34
	RLC	18.64	4.88	10.24	5.24	26.16	21.05	49.93
	NNR	2.01	1.53	25.56	22.25	75.78	2.16	38.98
	DTF	3.45	0.50	25.10	9.56	14.49	0.93	12.55
	PL	0.06	0.11	106.60	150.76	200.00	0.03	11.73
	PH	17.81	33.13	6.14	8.37	185.96	139.80	203.35
	SW	2.20	3.50	18.78	23.68	159.09	5.19	65.71
	DSYPP	31.78	61.15	4.52	6.27	192.45	344.70	276.42
400 gy	LL	5.81	1.63	74.18	39.22	27.96	3.92	120.55
	NRPP	14.26	12.13	9.29	8.57	85.01	45.79	112.65
	RLC	8.60	0.30	20.22	3.78	3.49	0.88	6.07
	NNR	0.58	0.25	54.16	35.71	43.48	0.19	13.54
	DTF	6.48	0.85	141.37	51.22	13.13	2.16	120.16
	PL	0.11	0.03	213.98	111.75	27.27	0.01	6.42
	PH	25.94	29.38	6.27	6.67	113.25	149.60	184.13
	SW	3.76	1.13	117.56	64.28	29.90	2.18	132.25
	DSYPP	100.42	26.29	4.41	2.26	26.18	263.44	116.00

LL: Leaf length (cm), NRPP: Number of Raceme per plant, RLC: Raceme length (cm), NNR: Number of nodes per raceme, DTF: Days to flowering, PL: Pod length (cm), PH: Plant height (cm), SW: Seed weight (grams) and DSYPP: Dry seed yield per plant

High genetic advance of the mean was observed for the number of racemes per plant 337.93%, 35.34% and 112.65%, plant height (424.30%,203.35% and 120.16%), dry seed yield per plant 6172.66%, 276.42% and 116.00% in 0 gy, 300 gy and 400 gy respectively. Other traits that recorded high %GAM included raceme length 249.46% and 49.93% in 0 gy and 300 gy. Seed weight 65.71% and 132.25% in 300 gy and 400 gy, number of nodes per raceme 38.98% in 300 gy, leaf length 120.55% and days to flowering 120.16% in 400 gy. High heritability with low genetic advance was observed in pod length.

4.3 Results of Genetic Diversity of Dolichos Bean Accessions

4.3.1 Analysis of Molecular Variance (AMOVA) among populations of dolichos bean accessions

The variance components of mutant population of the dolichos bean under the study showed differences among populations, among individuals and within individuals using significance tests based on 1,000 permutations. The Analysis of Molecular Variation (AMOVA) revealed that in the diversity of the M3 population was 45%, 54%, 1% respectively as indicated in table 11 and figure 5 respectively.

Table 11: Analysis of Molecular variance (AMOVA) of dolichos bean accessions

Source	DF	SS	MS	Est. Var.	%
Among Population	7	536.441	76.634	3.002	45%
Among Individuals	87	642.759	7.388	3.665	54%
Within Individuals	94	5.500	0.058	0.058	1%
Total	188	1184.700		6.725	100%

DF: Degrees of freedom, SS: Sum of squares MS: Mean square and EST. Var.: Estimated variance

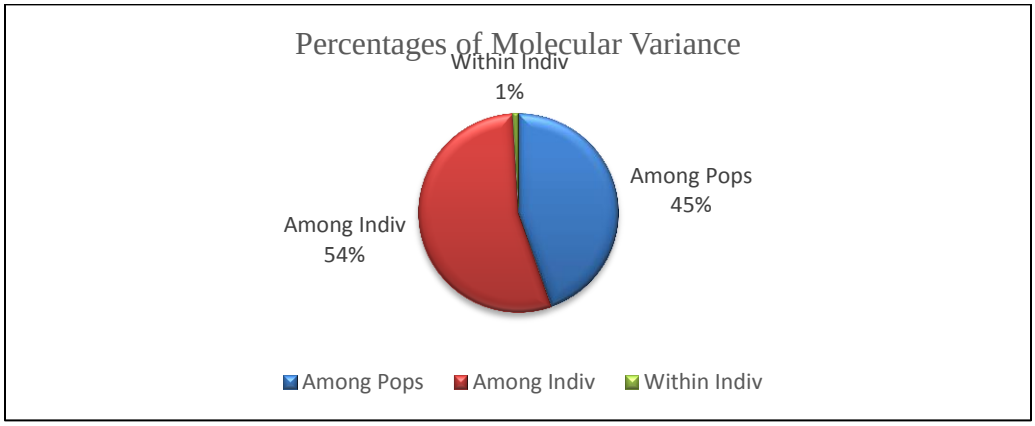

Figure 5: Pie chart showing the partition of genetic diversity in a population of dolichos bean accessions

4.3.2 Principal co-ordinate analysis (PCOA)

The genetic relationships among 95 M3 population were further investigated by 20 SSR markers using principal co-ordinate (PCoA) analysis (Figure 4.3). The first three major axis of differentiation (PC1, Pc2) explained cumulative percentage of 18.54%, 34.58 % and 44.47% of the total variation. The PCoA classified the 95 M3 accessions with some distinct major groups on as shown in figure 6 .The pattern of clustering was almost distinct for few genotypes of cream 400 gy ,maridadi 400 gy. Significant dispersion was observed on black I 300 gy and maridadi 300 gy.

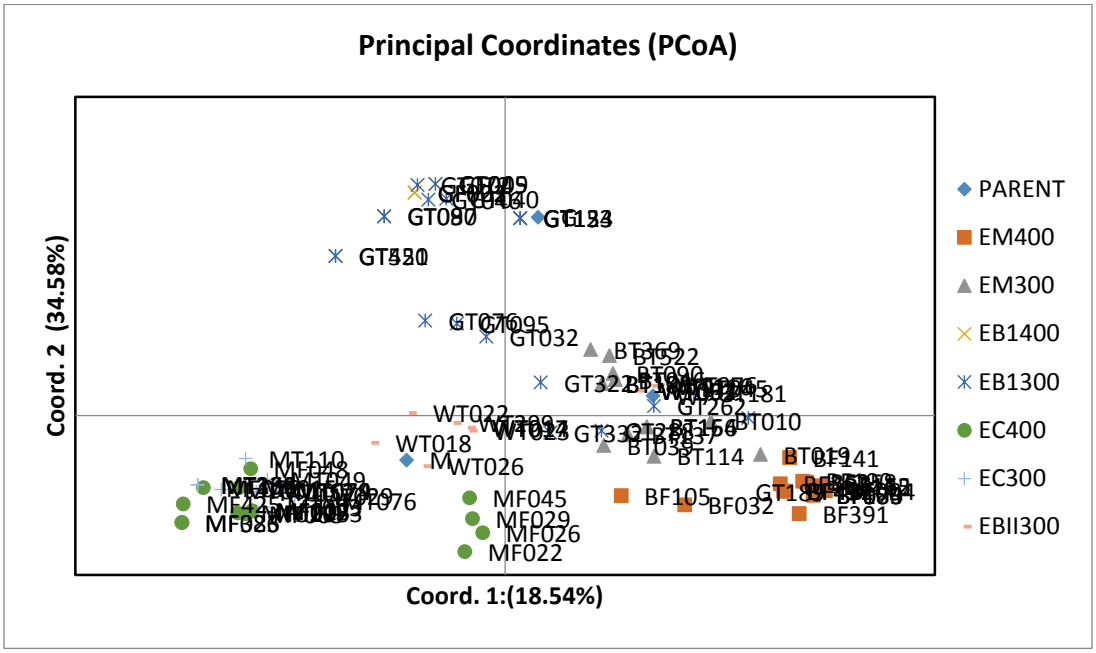

Figure 6: Scatter plot showing the clustering of dolichos bean accessions. EM400: maridadi 400gy, EM300: maridadi 300gy, EB1300: black I 300gy, EB1400: black I .cream 400: cream 400gy, EC300: cream 300gy and EBII300: black II 300 gy.

4.3.3 Genetic distances in population of dolichos bean accessions

The relationship between 8 populations of dolichos bean accessions was derived from commonly shared loci as per Nei 1983 using Genetic Analysis in Excel (GenAlEX) 6.2 software (Peakall, 2006) The relatedness through pairwise population genetic distance matrix range from a low of 0.14 between black 1 400 gy and black I 300 gy to a high of 1.50 maridadi 400 gy and black I 400 gy as shown in table 12. The other populations that recorded high genetic distance were between black I and cream 400 gy, maridadi 400 gy and cream 300 gy, black I 400 gy and black II 300 gy and black I 400 gy and cream 300 gy and genetic distance matrix .

Table 12: Pairwise Population Matrix of Nei Unbiased Genetic Distance

Checks	M400	M300	BI400	BI300	C400	C300	BII300	
0.000								Checks
0.341	0.00							M400
0.283	0.44	0.00						M300
0.282	1.47	0.63	0.00					BI400
0.231	1.21	0.76	0.06	0.00				BI300
0.327	0.91	0.84	1.35	0.95	0.00			C400
0.220	1.10	0.86	1.02	0.84	0.18	0.00		C300
0.325	1.08	0.86	1.20	1.02	0.87	0.72	0.00	BII300

Key: Checks: parents, M400: maridadi 400 gy, M300: maridadi 300 gy, BI400: black I 400 gy, BI 300: black I 300 gy, C400: cream 400 gy, C300: cream 300 gy and BII300: black II 300 gy.

A high level of unbiased pairwise genetic identity was identified to be between black I 400gy and black I 300 gy at 0.86 followed by cream 400 gy and cream 300 gy at 0.81. Maridadi 400 gy and black I 400 gy displayed the lowest genetic identity of 0.22 table 13.0.

Table 13: Pairwise Population Matrix of Nei Unbiased Genetic Identity

Checks	M400	M300	BI400	BI300	C400	C300	BII300	
1.000								Checks
0.711	1.00							M400
0.754	0.64	1.00						M300
0.755	0.23	0.53	1.00					BI400
0.794	0.29	0.46	0.93	1.00				BI300
0.721	0.40	0.43	0.25	0.39	1.00			C400
0.802	0.33	0.42	0.36	0.43	0.83	1.00		C300
0.723	0.34	0.42	0.30	0.36	0.42	0.47	1.00	BII300

Key: Check: Parent, M400: maridadi 400 gy, M300: maridadi 300 gy, BI400: black I 400 gy, B1300: black 1 300 gy, C400: cream 400 gy, C300: cream 300 gy and BII300: black II 300 gy.

4.3.4 Genetic differentiation based on F statistics of dolichos bean accessions

The estimates of genetic differentiation are tabulated in table 14. based on : Estimated correlation of genes within individuals within populations (F_{IS}), correlation of genes within individuals over all populations (F_{IT}); estimated correlation of genes of different individuals in same population (F_{ST}); and coefficient for gene flow between populations (NM) were calculated based on F Statistics model wrights 1951. Estimated correlation of genes within individuals within populations was high at 1.00 in 17 Loci and a total average of 0.98 and generally not heterozygous. The correlation of genes within individuals over all populations was high with an average mean of 0.99 with the LABT14, LABT33 and LABT28 recording the lowest correlation estimates. In this study F_{ST} ranged between 0.20 LABT25 to 0.69 LABT28 and an average mean of 0.47 implying a median differences in allele frequencies. The mean average gene flow coefficient was at a low of 0.31 between populations. It ranged from 0.110 LABT 28 to 1.002 LABT25

Table 14: F-Statistics and estimates of differentiation of dolichos bean accessions for each SSR marker locus

Locus	F_{IS}	F_{IT}	F_{ST}	NM
LABT3	1.00	1.00	0.41	0.36
LABT4	1.00	1.00	0.46	0.29
LABT1	1.00	1.00	0.45	0.30
LABT2	1.00	1.00	0.38	0.39
LABT6	1.00	1.00	0.47	0.28
LABT7	1.00	1.00	0.46	0.29
LABT14	0.97	0.98	0.47	0.28
LABT24	1.00	1.00	0.51	0.24
LABT25	1.00	1.00	0.20	1.00
LABT33	0.87	0.92	0.32	0.53
LABT28	0.86	0.95	0.69	0.11
BM143	1.00	1.00	0.62	0.15
PVATGC001	1.00	1.00	0.60	0.17
PVCC001	1.00	1.00	0.50	0.25
PVGAAT002	1.00	1.00	0.43	0.33
VAAG001	1.00	1.00	0.35	0.46
PVAG004	1.00	1.00	0.47	0.28
PVAT006	1.00	1.00	0.59	0.17
BMD20	1.00	1.00	0.62	0.15
BMD22	1.00	1.00	0.45	0.31
Mean	**0.98**	**0.99**	**0.47**	**0.32**
SE	0.01	0.01	0.03	0.04

FIS: Estimate correlation of genes within individuals at the population, FIT: Estimate correlation of genes within individuals over populations,FST: Estimates correlation of genes of different individuals in the same population and NM: coefficient of gene flow between populations.

4.3.5 Mean allelic patterns across populations of dolichos bean accessions

The analysis of mean allelic patterns across 8 M3 accessions as shown in table 15 shows that the number of different alleles (Na) to range from 1.05 in black I 400 gy population to 2.50 in black I 300 gy. The number of effective alleles (Ne) was high in the parent population at 2.28 and lowest black I 400 gy. The Shannon diversity index (I) was the

lowest in black I 400 gy and highest in the parent and black I 300 gy populations at 0.67. The number of private alleles was low among the populations ranging from 0.00 (Parent, maridadi 300 gy and black I 300 gy) to 0.60 in black II 300 gy population. Expected heterozygozity (He) was low and ranged from 0.02 in black I 400 gy to 0.40 black I 300 gy population's figure 6. The unbiased expected heterozygozity was low in black1 400 gy and the highest in the parent and cream 400gy population at 0.03 and 0.44 respectively.

Table 15: Mean allelic patterns across dolichos bean populations studied

Population	check	M400	M300	BI400	BI300	C400	C300	BII300
Na	2.35	1.90	2.05	1.05	2.50	2.30	2.15	2.40
Ne	2.28	1.42	1.70	1.04	1.86	1.97	1.63	1.69
I	0.67	0.38	0.52	0.03	0.67	0.66	0.56	0.57
No. Private Alleles	0.00	0.05	0.00	0.00	0.30	0.05	0.20	0.60
He	0.39	0.24	0.33	0.02	0.40	0.42	0.36	0.34
uHe	0.44	0.25	0.34	0.03	0.41	0.44	0.37	0.36

M400: maridadi 400gy, M300: maridadi 300gy, BI400: black I 400gy, B1300: black 1300gy, C400:cream 400gy, C300:cream 300gy and BII300:black II 300gy.

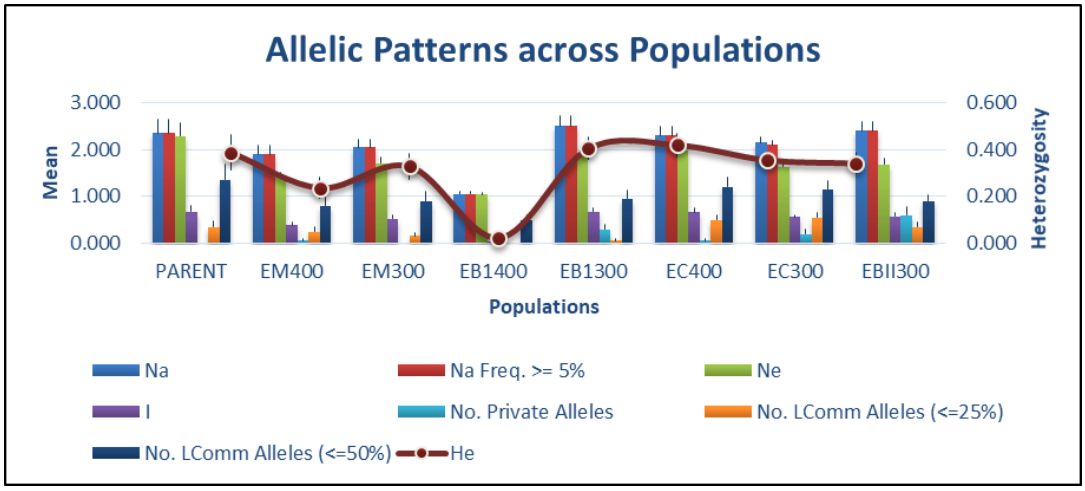

Figure 6: Bar graph indicating allelic patterns across *selected M3 mutant* populations

Na. number of different allele; Na (Freq >= 5%), Number of Different Alleles with a Frequency >= 5%; Ne, number of Effective Alleles; I, Shannon's Information Index; No. Private Alleles, which is the number of Alleles Unique to a Single Population; No. LComm Alleles (<=25%), No. of Locally Common Alleles (Freq. >= 5%) Found in 25% or Fewer Populations; No. LComm Alleles (<=50%), No. of Locally Common Alleles (Freq. >= 5%) Found in 50% or Fewer Populations.

4.3.6 Genetic distance among different populations of dolichos bean accessions

The sampled mutant populations exhibited unbiased genetic identity that ranged between 0.13 and 0.70 with the lowest being between cream 400 gy (C400) and cream 300gy (C300) and the highest genetic identity of 0.70 was between maridadi 400 gy (M400) and black I (BI400) as shown in table 16

Table 16: Genetic distance among different mutant populations and parent accessions

Checks	M400	M300	BI400	BI300	C400	C300	BII300	
0.00								Checks
0.21	0.00							M400
0.27	0.29	0.00						M300
0.47	0.70	0.45	0.00					BI400
0.17	0.35	0.22	0.19	0.00				BI300
0.30	0.38	0.33	0.54	0.29	0.00			C400
0.28	0.39	0.31	0.48	0.26	0.13	0.00		C300
0.21	0.34	0.29	0.50	0.26	0.32	0.31	0.00	BII300

Checks: Parent, M400:maridadi400gy, M300:maridadi 300gy,B1400:blackI 400gy,BI300:black I 300gy:C400:cream 400,C300:cream 300gy, BII300:black II 300gy.

The major allele frequency, genetic diversity, heterozygozity, number of alleles and polymorphic information content was computed using Power Marker version 3.25. The twenty SSR markers generated an average mean of 5.25 alleles, which were used to estimate the genetic diversity among the 95 dolichos bean accessions. The number of alleles revealed by each marker ranged from two (PVAT006) to ten (LABT1) as indicated in table 17, plate 6 and 7. The polymorphism information content (PIC) value for the SSR loci ranged from 0.16 (VAAG001) to 0.84 (VAAG001) with a mean of 0.58. The mean level of heterozygozity per SSR marker was 0.33 ranging from 0.00 to 1.00 for marker VAAG001, LABT7, PVATGC001

Table 17: Summary of allele frequency, genetic diversity, heterozygozity, number of alleles and polymorphic information

Marker	Allele. Frequency	Allele No	Gene Diversity	Heterozygozity	PIC
LABT3	0.37	7.00	0.78	0.00	0.76
LABT4	0.55	5.00	0.61	0.00	0.55
VAAG001	0.21	10.00	0.86	1.00	0.84
LABT2	0.39	7.00	0.72	0.00	0.68
LABT6	0.52	4.00	0.57	0.00	0.48
LABT7	0.31	6.00	0.77	1.00	0.73
LABT14	0.35	8.00	0.79	0.91	0.76
LABT24	0.71	4.00	0.46	0.00	0.43
LABT25	0.91	4.00	0.17	0.00	0.17
LABT33	0.74	6.00	0.43	0.05	0.41
LABT28	0.34	5.00	0.75	0.85	0.71
BM143	0.33	6.00	0.78	0.00	0.75
PVATGC001	0.36	6.00	0.74	1.00	0.70
PVCC001	0.31	5.00	0.78	0.00	0.75
PVGAAT002	0.37	5.00	0.68	0.90	0.61
VAAG001	0.91	3.00	0.17	0.00	0.16
PVAG004	0.36	4.00	0.69	0.00	0.63
PVAT006	0.51	2.00	0.50	0.00	0.37
BMD20	0.58	4.00	0.58	0.00	0.52
BMD22	0.48	4.00	0.64	0.90	0.58
Total	9.61	105	12.47	6.61	11.59
Mean	**0.48**	**5.25**	**0.62**	**0.33**	**0.58**

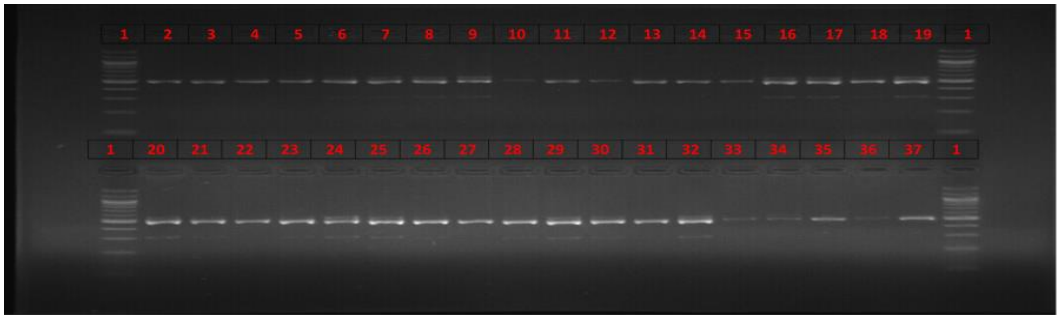

Plate 6: PCR amplification products of genomic DNA from subset of 37 dolichos accessions out of 95 based on LABT2 SSR marker (100kb ladder,2&20 checks), 3-19 and 21-37 mutant accessions.

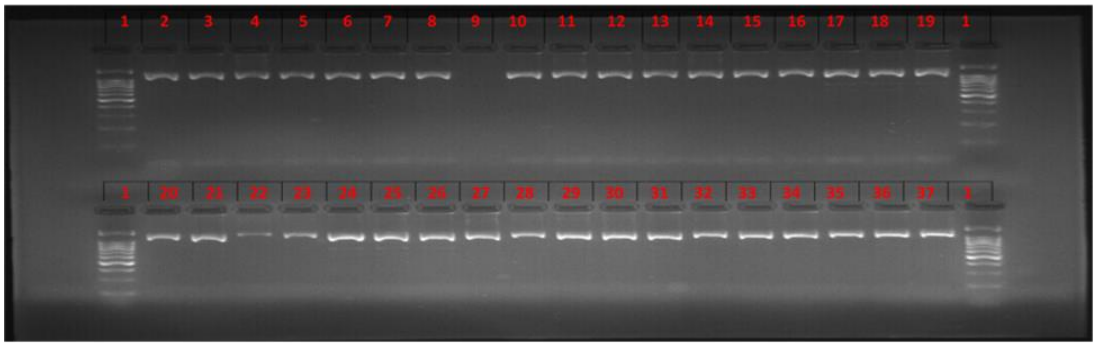

Plate 7: PCR amplification products of genomic DNA from subset of 37 dolichos accessions out of 95 accessions based on VAAG001 SSR marker (100kb ladder,2&20 checks), 3-19 and 21-37 mutant accessions

4.3.7 Cluster analysis of dolichos bean accessions

Based on unweighted neighbor-joining pair group UPGMA cluster analysis using the power marker .The genetic distances based on the UPGMA neighbor joining tree placed the 95 lablab accessions into two major clusters, 'I' and 'II'' Figure 4.5 and 1 and 2A, 2B, 2C and 2D as indicated in figure 4.6 with diversity in 2A and 2C, 2D with 2A and 2B of some accessions. Cluster I was the smallest cluster (2.10%) with comprising of black II

accessions WT018 and WT026 figure 7.0 and figure 8.0. Cluster 'II' was the largest cluster holding 93 accessions. It consisted of 5 major sub clusters that was further subdivided into mini clusters carrying mixed accessions and it also had unique isolated accessions of maridadi (BT046, BT188, BT137, BT 166, BT114 BF105) and cream (MF048, MT076, MF021) like cluster I .

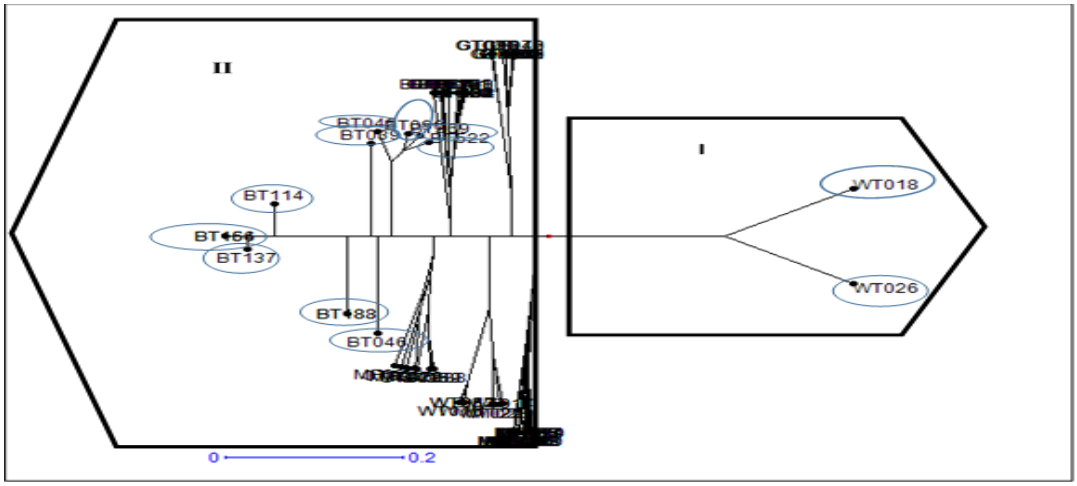

Figure 7: Radial tree diagram showing genetic relationship among 95 dolichos bean accessions based on 20 SSR markers

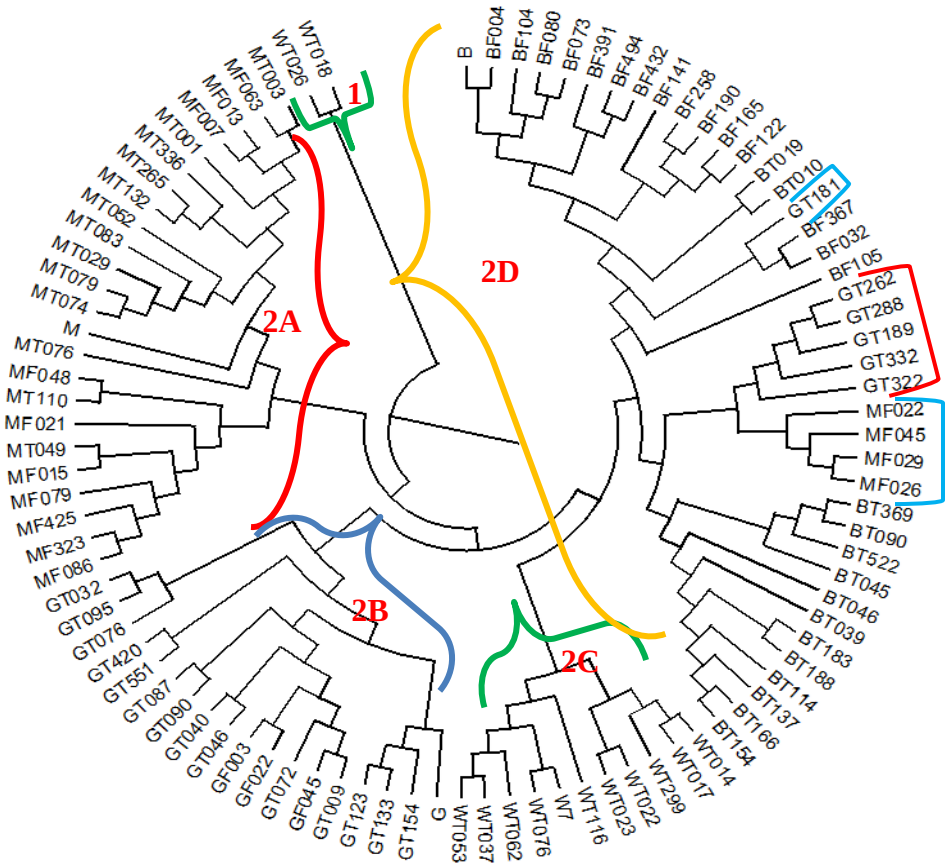

Figure 8: Circular dendrogram constructed based on 20 SSR markers using neighbor-joining methods, and the icons indicate groupings and the information of material source

4.4 Results of analysis of nutritional and mineral composition of dolichos bean accessions

The means of nutritional analysis for the different mutant accessions of dolichos bean are presented in table 18. There was significant difference ($P \leq .05$) among means of black I accessions in percent moisture content and crude protein levels. Accession GT032 recorded the highest significant difference in percent moisture content of 8.73±0.27 and

GT095 was the lowest at 7.35± 0.03. For percent crude fibre content it was vice versa at 9.16±0.02% and 10.40±0.25% respectively. The percent crude protein level among the mutant accessions of black I (GT032, GT076 and GT095) were significantly low compared to the parent.

The means of percent moisture content (8.03%), percent crude fat (4.18%), percent crude protein (24.85%) percent carbohydrate (68.55%) and energy (434.58 kcal) among the maridadi accessions were significant at P≤0.001. Moisture content ranged from 6.36 ±0.34% (BT114) to 10.61±0.62% (maridadi).Percent crude fat ranged from 2.53±0.23 (BT114) to 7.20 % (BT188) all being maridadi accessions dose 300gy. There was no significant difference in crude fibre content among the accessions. Crude protein content ranged from 28.86±0.18% (BF032) to 20.23±0.21% (BT154) while carbohydrate and energy ranged from 73.60±0.45% (BT154) to 63.50±0.38% (maridadi). BT188 had the highest significant energy values of 449.69±0.02 and the least was BF032 with 427.91±3.3.

The nutritional values of crude protein (23.08%) and total carbohydrates (71.55%) mean of cream accessions were significant at P=0.001. The percent average mean of moisture content was 7.58 where by MF048 had highest content at 8.77±0.40% and cream (parent-check) the least 6.39±0.78%. The crude fat mean was 2.97% with accession MT110 (4.11±0.16%) and MT049 (2.34±0.71%) having the highest and the lowest significant percentage. Crude fibre ranged from 12.55±0.57% (MT049) to 8.86±0.71% (MT110). MF015 (24.57±1.12%) and MF048 (26.45±0.17%) had the highest and the lowest significant percent crude protein among cream accessions while MT076 and MF048 having the highest and the lowest significant carbohydrate levels at 74.88±0.59% and

67.66±0.34% respectively. The total energy levels for cream were not significantly different.

There was no significant differences in among and within black II accessions in percent moisture content, percent crude fiber, percent crude protein, percent total carbohydrates and total energy. The crude fat mean 4.26% was significant at $P \leq 0.05$ with accession WT018 having the highest significant levels of 4.54±1.65% and the non-mutated parent-check having the least significant levels of 3.94±0.69%.

Table 18: Mean of nutritional composition of dolichos bean accessions

GN	ACC	MST	FAT	CF	CP	CHO	KCAL
BLACK I	GT032	8.73±0.27A	4.24±0.26A	9.16±0.02C	20.02±0.00B	72.88±0.50A	438.03±0.10A
	GT076	8.05±0.21BA	7.12±2.15A	9.34±0.49BC	21.86±2.12B	67.72±5.50A	446.02±0.27A
	G	7.84±0.19BC	9.05±0.70A	10.31±0.07BA	28.77±0.30A	59.80±0.29B	448.88±3.50A
	GT095	7.35±0.03C	7.08±2.09A	10.40±0.25A	21.66±0.11B	68.72±1.65A	448.48±10.33A
	MEAN	7.99*	6.7	9.8*	23.08*	67.33*	445.35
	CV	2.74	22.1	3.25	4.32	4.13	1.33
	LSD	0.69	4.83	1.01	3.17	8.86	18.92
MARIDADI	B	10.61±0.62A	5.37±0.02BC	8.98±0.53A	28.66±0.14A	63.50±0.38F	434.63±1.43CBD
	BF032	6.81±0.27CD	3.66±1.27ECD	10.81±0.07A	28.86±0.18A	65.00±1.72FE	427.91±3.36D
	BF105	7.39±0.30CBD	2.90±0.62ED	10.30±0.76A	21.48±0.21E	73.11±0.70BA	432.96±1.30CD
	BF137	9.69±0.34A	3.59±0.23ED	10.82±2.26A	27.48±0.42B	66.83±0.85DE	430.89±0.51CD
	BT039	8.35±1.24B	3.38±0.87ED	9.51±1.02A	22.53±0.25ED	71.52±1.70B	433.37±0.38CBD
	BT046	7.54±0.00CBD	5.48±0.17BA	10.16±0.75A	27.27±0.66B	64.84±0.89FE	436.81±3.19B
	BT114	6.36±0.34D	2.53±0.23E	10.96±2.26A	23.48±0.42D	71.55±0.85BA	429.63±0.51CD
	BT154	7.72±0.45CB	3.97±0.54BECD	9.68±0.46A	20.23±0.21F	73.60±0.45A	439.84±1.79B
	BT166	8.13±1.16CB	3.59±1.27ED	11.46±1.25A	25.20±1.07C	69.17±0.16C	432.55±6.21CD
	BT183	8.35±0.09B	4.46±0.14BCD	10.69±2.84A	25.67±0.01C	66.89±0.72DE	432.09±4.53CD
	BT188	7.41±0.17CBD	7.20±0.00A	10.23±0.00A	22.56±0.13ED	68.08±0.09DC	449.69±0.02A
	MEAN	8.03***	4.18***	10.32	24.85***	68.55***	434.58***
	CV	7.46	18.82	13.73	1.94	1.34	0.74
	LSD	1.33	1.75	3.16	1.07	2.06	7.25

CF: Crude fibre, CP: crude protein, CHO: Carbohydrate: Means with similar letters in the same column are not significantly different

Continued

GN: Genotype, ACC: Accession, MST: Moisture Content, CF: Crude fibre, CP: crude protein, CHO: Carbohydrate:

GN	ACC	%MST	%FAT	%CF	%CP	%CHO	KCAL
CREAM	M	6.39±0.78C	3.55±1.29BA	11.46±0.52BA	20.17±1.68B	74.14±0.41A	438.58±6.89A
	MF015	7.16±0.53BC	2.73±0.06BA	10.73±0.71BA	24.57±1.12A	70.25±0.76B	429.17±0.05A
	MF048	8.77±040A	2.72±1.02BA	10.00±2.04BA	19.45±0.17C	67.66±0.34C	428.44±6.38A
	MT049	7.61±0.18BA	2.34±0.71B	12.55±0.57A	22.14±0.19B	73.63±0.97A	428.79±0.89A
	MT076	7.70±0.06BA	2.39±0.66BA	12.12±0.34A	20.71±0.21B	74.88±0.59A	429.94±3.56A
	MT110	7.79±0.31BA	4.11±0.16A	8.86±0.71B	24.43±0.10A	68.77±0.10C	433.49±0.57A
	MEAN	7.58*	2.97*	10.95*	23.08***	71.55***	431.4
	CV	6.25	19.15	9.84	3.58	0.76	1.04
	LSD	1.21	1.46	2.77	2.12	1.4	11.54
BLACK II	W	6.93±0.75A	3.94±0.69C	11.04±0.26A	23.85±0.21A	7019±0.62A	434.89±2.19A
	WT018	7.72±1.63A	4.54±1.65A	11.77±0.08A	24.13±2.19A	71.31±4.02A	438.05±3.01A
	WT026	7.19±0.56A	4.31±0.25B	11.29±0.08A	25.22±4.56A	68.46±4.36A	435.40±6.24A
	MEAN	7.27	4.26*	11.36	24.4	69.99	436.11
	CV	15.15	1.56	1.56	14.22	6	0.97
	LSD	4.74	0.12	0.74	14.93	18.07	18.31

Means with similar letters in the same column are not significantly different

The ash and minerals (phosphorous and zinc) of black I accessions were not significant different at P≤0.05 table 19.0 .The average mean of potassium (2.35) and calcium (121.67) were significant at P≤0.001 .Accessions GT032 and GT095 had the highest significant potassium (2.79±0.04) and calcium (165.60±0.00) respectively. While GT076 and black I (2.04±00.03) and GT032 (65.45±0.49) had the lowest significant potassium and calcium levels.

Phosphorous (0.38), potassium (2.42) and calcium (142.20) were statistically different at P≤0.001 and zinc (2.59) at P≤0.05 for maridadi accessions. The average mean for ash level was 2.4% with accessions BT183 (2.98±0.88%) and BF137 (2.08±0.20%) having the highest and the lowest significant ash levels. The levels of accession BT046 (0.54±0.19) and BF032 (0.22±0.07), BT114 (2.81±0.00) and BT166 (1.91±0.00), BT114 (175.65±22.27) and BF137 (74.25±22.27) and BT039 (3.64±2.29) and BT166 (1.49±0.23) had significantly higher and lower phosphorous, potassium, calcium and zinc levels respectively.

The percent ash levels of cream accessions were not significantly different. The average mineral composition of the accessions were significant P=0.05 in phosphorus (0.38) and P=0.001 potassium (2.4), calcium(147.00) and zinc (2.56) at P=0.01. Accessions MF015, MF048, MT049 recorded the highest significant phosphorous levels while accession M, MT076, and MT110 had the least. The parent accession M (2.57±0.01) had the highest significant potassium levels while MT049 (2.25±0.00) was the least. The calcium levels significantly ranged from 114.65±6.86 (MF048) to 169.05±0.07 (MT049). Accession MT110 had the highest zinc level of 3.52±1.52 while MF015 (1.61±0.05) indicated the least significant levels.

Table 19: summary of mineral composition among dolichos bean accessions

GN	ACC	ASH	Mineral Elements in mg/l			
			P	K	Ca	Zn
BLACK 1	GT032	2.85±0.24A	0.43±0.00A	2.79±0.04A	65.45±0.49C	2.29±0.09A
	GT076	3.06±1.22A	0.41±0.13A	2.04±0.03C	123.55±0.07B	1.65±0.43A
	G	2.37±0.10A	0.27±0.03A	2.04±0.03C	132.10±6.50B	2.47±0.15A
	GT095	2.53±0.54A	0.38±0.21A	2.55±0.00B	165.60±0.00A	2.55±0.42A
	MEAN	2.7	0.37	2.35***	121.67***	2.24
	CV	28.55	8.2	1.51	2.73	15.92
	LSD	2.46	0.46	0.11	10.6	1.13
MARIDADI	B	2.46±0.26BA	0.26±0.01C	2.29±0.01DC	164.25±6.72BA	3.33±0.61BA
	BF032	2.46±0.28BA	0.22±0.07C	2.54±0.01BAC	142.70±7.07C	2.47±0.40BC
	BF105	2.50±0.28BA	0.23±0.15C	2.57±0.00BAC	154.00±13.58BC	2.30±0.62DC
	BF137	2.08±0.20B	0.47±0.21BA	2.61±0.00BA	74.25±22.27E	2.29±0.14DC
	BT039	2.56±0.58BA	0.46±0.00BA	2.06±0.01ED	141.35±0.07C	3.64±2.29A
	BT046	2.41±0.40BA	0.54±0.19A	2.45±0.28BC	145.60±0.00BC	2.51±0.18BC
	BT114	2.44±0.20BA	0.29±0.21C	2.81±0.00A	175.65±2.27A	2.46±0.14BC
	BT154	2.19±0.11BA	0.52±0.09A	2.57±0.34BAC	155.20±5.66BC	2.29±0.27DC
	BT166	2.16±0.04B	0.47±0.11BA	1.91±0.00E	160.45±0.64BAC	1.49±0.23D
	BT183	2.98±0.88A	0.43±0.14B	2.28±0.00DC	150.65±0.07BC	2.61±0.86BC
	BT188	2.16±0.04B	0.28±0.07C	2.55±0.00BAC	100.15±1.20D	3.18±1.16BC
	MEAN	2.4*	0.38***	2.42***	142.2***	2.59*
	CV	14.99	10.39	5.89	6.32	15.38
	LSD	0.8	0.08	0.31	20.04	0.89
CREAM	M	2.13±0.03A	0.28±0.07B	2.57±0.01A	141.80±3.96D	2.64±0.00B
	MF015	2.44±0.30A	0.56±0.00A	2.36±0.00C	151.35±0.21CB	1.61±0.05C
	MF048	2.61±0.51A	0.47±0.07A	2.36±0.04C	114.65±6.86E	2.40±0.42B
	MT049	2.37±0.45A	0.48±0.00A	2.25±0.00D	169.05±0.07A	2.55±0.11B
	MT076	2.51±0.28A	0.21±0.00B	2.39±0.00C	147.35±0.21CD	2.66±0.28B
	MT110	2.68±0.04A	0.31±0.07B	2.49±0.01B	157.80±0.42B	3.52±1.52A
	MEAN	2.45	0.38*	2.4***	147.00***	2.56**
	CV	14.45	13.52	0.62	1.97	8.67
	LSD	0.91	0.13	0.03	7.46	0.57
BLACK II	W	2.21±0.14A	0.51±0.06B	2.56±0.19A	168.30±29.27A	2.45±0.00A
	WT018	1.16±0.18B	0.58±0.21A	2.54±0.19A	130.30±0.28B	1.81±0.66A
	WT026	2.13±0.06A	0.49±0.00C	2.32±0.01B	166.25±0.07A	1.90±0.49A
	MEAN	1.83*	0.52***	2.47*	154.95***	2.05
	CV	9.2	0.77	1.57	0.34	16.66
	LSD	0.72	0.01	0.16	2.32	1.47

GN: Genotype, ACC: accession, P: Phosphorous, K: Potassium, Ca: Calcium and Zn: Zinc: Means with similar letters in the same column are not significantly different

The black II accessions produced significant ash of 1.83% at P≤0.05 with accession WT018 producing the least amount of 1.16±0.18% compared to the parental (check)

accession W (2.21±0.14%) and WT026 (2.13±0.06%). The average phosphorous mean was 0.52 and significant at P≤0.001 whereby the highest was WT018 (0.58±0.21) and WT026 (0.49±0.00) the least significant. The potassium and calcium levels among the black II accession ranged from 2.32±0.01 (WT026) to 2.54±0.19 (WT018) and 130.30±0.28 (WT018) and 168.30±29.27 (W) respectively. There was no significant difference among and within the zinc levels of black II accessions.

4.5 Results of evaluation of adaptability based on yield associated traits among accessions of dolichos bean genotypes

4.5.1 Days to flowering

Maridadi Accessions: The analysis of variance in days to flowering table 20.0 shows that there were significant differences with a mean of 63 days at P=0.001. The lowest and highest significant average of days to flowering were for Pokot (55 days) and Kitale (70days) at P=0.001. The accession B1 (check) significantly took longer duration of 66 days to attain 50% flowering in Baringo compared to all other accessions. In Pokot and Kitale BF137 recorded the lowest significant duration to attain 50% flowering. There was no significant difference in days to 50% flowering in Eldoret. Combined analysis of variance for maridadi accessions indicated that accession (B1) took significantly longer 69 days to flowering compared to the mutant accessions BT183, BT166, BT154, BT114, BT046 and BF137, BF105 and BF032. BT188 and BT183 were not significantly different.

Cream Accessions: Mutant accession MT049 and MF048 were significantly different in Baringo on days to 50% flowering. MT049 recorded shortest duration of 50 days and MF048 longest significant duration of 68 days. Accession MF015, MT049 and MT110

significantly had shortest duration to reach 50% flowering different in Pokot compared to B1 (65) days.

Black I Accessions: The mutant accessions: GT095 (55 days), GT076 (54 days) and GT032 (53 days) took significantly shorter duration to 50% flowering compared to parental (Check) G1 accession that took longer (65) significant days in Pokot. G1 took 70 significant days attain 50% flowering while GT032 (60 days) had the least significant days in Kitale. Generally the ANOVA of black I accessions specified significant difference between G1 with the highest days of 64 to 50% flowering while GT032 (60 days) and overall significant mean at P=0.001 of 62 days.

Black II Accession: The analysis of variation in the mean days to 50% flowering of parent (check)W7, WT018 and WT 026 shows that W7 was significantly different at P=0.05, with longer days (66) to 50% flowering compared to WT018 (55) and WT026 (56) with an average mean scores of 59 days in Baringo. Across the experimental sites the check (W7) attained significant longer days to flowering of 69 while WT018 and WT026 took 61 and 63 respectively. The general mean was 65 and significant at $P \leq 0.001$.

Table 20: Mean of days to flowering of various dolichos bean accessions planted in various locations

GN	ACN	LOCATIONS				Mean
		BRG	PKT	KTL	ELD	
MARIDADI	B1	66A	65A	75BA	80A	69A
	BT188	57B	53EFD	69BDC	75BA	65BA
	BT183	54B	57CEBD	67BDC	72BA	65BA
	BT166	54B	52EF	71BDC	76BA	64B
	BT154	57B	55CEFD	70BDC	79BA	63B
	BT114	51B	51F	70BDC	76BA	62B
	BT046	57B	53CEFD	67DC	71BA	62B
	BT037	54B	58CB	70BAC	72BA	62B
	BF137	51B	51F	50F	71BA	62B
	BF105	58B	60B	67DC	75BA	61B
	BF032	54B	57CBD	80A	70BA	61B
	MEAN	56	56***	68*	70***	63***
CREAM	M5	62BA	64A	69A	69A	66A
	MT110	62BA	54B	69A	68A	63A
	MT076	53BC	58BA	68A	70A	62A
	MT049	50C	54B	69A	71A	61A
	MF048	68A	58BA	69A	72A	65A
	MF015	58BC	52B	70A	69A	62A
	MEAN	59***	56*	69	70	63***
BCLACK I	G1	59A	65A	70A	64A	65A
	GT095	57A	55B	62BA	66A	62BA
	GT076	57A	54B	68BA	72A	60BA
	GT032	54A	53B	60B	71A	60B
	MEAN	57	57	65*	69	62***
BLACK II	W7	66A	67A	62A	70A	69A
	WT018	55B	53A	61A	70A	62B
	WT026	56B	54A	54A	71A	63B
	MEAN	59*	59	61	71	65***

GN:Genotypes,ACN:Accession,BRG:Baringo,PKT:Pokot,KTL:Kitale,Eld:Eldoret .Means with similar letters in the same column are not significantly different..

4.5.2 Days to maturity

The data on days to maturity of selected M4 dolichos accessions was analyzed and presented in table 21.

Maridadi Accessions: The accession with the shortest significant days to maturity were BT037 (98 days) and BT046 (101days) in Pokot and Kitale respectively compared to B1(check) accession that recorded significantly the longest period to attain 85% maturity of 109 days and 112 days. The accessions mean was 112 days and significant at $P \leq 0.001$. Accession BT037 was identified as the earliest maturing accession at 107 days relative to B1 116 days.

Cream Accessions. Mutant accession MT110 recorded 106 days which was the earliest maturing accession in Baringo while the check (M5) had 120 days and MF015 (119 days) the highest number of days to maturity. There was no significant differences among the accessions in other sites. Combined analysis results of the accessions across the experimental sites showed that accession MT110 (105 days) as the earliest maturing accession compared to the parental accession M5 and other mutant accession and the general mean of the cream accessions was 111 days.

Plate 8: Differences on days to maturity between a check accession (M5) and its mutant accession MT1110

Black I Accessions: The accession with the least numerical days to maturity in Baringo was mutant accession GT032 in 98 days. There was no significant differences among accessions in other sites.GT032 and GT076 recorded the earliest significant duration to maturity of 108 days against G1 at 115 days. The mean for days to maturity for Eldo black I accessions was 112 days.

BLACK II Accessions. Mutant accession WT026 recorded the earliest significant days to maturity in Baringo, Kitale and Eldoret with scores of 100,102 and 101 respectively. Combined evaluation of days to maturity shows that WT018 (109 days) and WT026 (109 days) was significantly shorter than W7 (check) (119 days).

Table 21: Mean of days to maturity of various dolichos bean accessions planted in various locations

		Locations				
GN	ACN	BRG	PKT	KTL	ELD	MEAN
MARIDADI	B1	118A	109A	112A	125A	116A
	BT188	115A	106BA	109BA	120A	113BA
	BT183	114A	105BA	105BC	121A	111BC
	BT166	111A	106BA	110BA	118A	111BAC
	BT154	114A	100BA	107BA	124A	111ABC
	BT114	116A	109A	109BA	122A	114BA
	BT046	111A	101BA	101C	124A	110BC
	BT037	110A	98B	104BC	115A	107C
	BF137	113A	107BA	110BA	118A	112BA
	BF105	117A	105BA	105BC	120A	112BA
	BF032	112A	106BA	108BA	127A	113BA
	MEAN	114	105*	107*	121	112***
CREAM	M5	120A	107A	109A	127A	116A
	MT110	106B	98A	99A	116A	105C
	MT076	109BA	103A	100A	126A	110BC
	MT049	115BA	105A	109A	123A	113BA
	MF048	115BA	104A	104A	116A	110B
	MF015	119A	100A	109A	126A	114BA
	MEAN	114*	103	105	122	111***
CLACK I	G1	119A	109A	107A	126A	115A
	GT095	112A	101BA	102A	119A	114BA
	GT076	117A	109A	109A	122A	109B
	GT032	113A	98B	101A	121A	108B
	MEAN	115	104.5*	105	122	112***
BLACK II	W7	113A	120A	119A	117A	119A
	WT018	108A	111A	107BA	96BA	109B
	WT026	100B	113A	102B	101B	109B
	MEAN	106*	114	109*	110.35***	112***

GN: Genotypes, ACN: Accession, BRG: Baringo, PKT: Pokot, KTL: Kitale, Eld: Eldoret. Means with similar letters in the same column are not significantly different.

4.5.3 Number of pods

Maridadi Accessions: The mean number of pods per plant was not significant in maridadi it ranged from 72 to 81 and an average mean of 77. Accessions with the highest significant number of pods were the mutants: BT188 (104) Baringo and Kitale (97),

BT037 and BT188 (93) Pokot, BT 137 (101) in Eldoret .Accession BT188 produced the highest significant

Number of pods (98) BT 154 (65) had the least number of pods across the experimental sites as presented in table 22.

Cream Accessions: The total number of pods ranged from 61 to 77 with a mean significant mean of 72 at P≤0.001. There was no significant difference in the accessions at Baringo, Pokot and Kitale. In Eldoret accession MT049 and MT015 produced the highest significant number of pods of 92 and 95. Significantly within cream accessions, MT015 produced the highest number of pods at 87.

Black I Accessions: The mean number of pods ranged from 60 to 81 with no significant difference in the sites and total mean of 74 at P=0.05 among accessions in Baringo, Pokot and Kitale and Eldoret. Accession GT032 (83) produced the highest number of pods per plant.

Black II Accessions: The mean of number of pods per plant was the least in Baringo at 83 and the highest was in Pokot at 91 and was not significant at P=0.05 with a mean of 85. Separation of means among the experimental sites showed that accessions W7, WT018 and WT026 produced significantly different number of pods per plant of 65, 87 and 102 respectively.

Table 22: Mean of number of pods in dolichos bean accessions planted in various locations

		LOCATIONS				
GN	ACN	BRG	PKT	KTL	ELD	MEAN
MARIDADI	B1	75BA	72BA	69BC	56G	68D
	BT188	104A	94A	97A	95BA	98A
	BT183	81BA	77BA	71BC	65GFE	74DC
	BT166	75BA	84BA	64BC	86BC	77DC
	BT154	60B	73BA	72BAC	55G	65D
	BT114	81BA	78BA	78BAC	80DC	79DC
	BT046	64BA	69B	56C	76DCE	66D
	BT037	90BA	93A	86BA	99A	92BA
	BF137	77BA	88BA	69BC	101A	84BAC
	BF105	83BA	79BA	69BC	63GF	74DC
	BF032	67BA	85BA	65BC	72DFE	72DC
	MEAN	78*	81***	72***	77***	77***
CREAM	M5	79A	73A	69A	27D	62C
	MT110	57A	61A	73A	48C	60C
	MT076	71A	71A	71A	56B	67C
	MT049	83A	86A	76A	92A	8BA
	MF048	76A	89A	73A	52CB	72BC
	MF015	87A	83A	83A	95A	87A
	MEAN	76	77	74	61***	72***
CLACK I	G1	63A	66A	91A	53A	68B
	GT095	76A	73A	76A	59A	75BA
	GT076	88A	76A	75A	59A	71B
	GT032	95A	89A	80A	68A	83A
	MEAN	81	76	81	60	74***
BLACK II	W7	67A	67A	73A	77A	65C
	WT018	115A	91A	70A	89A	87B
	WT026	91A	115A	105A	100A	101A
	MEAN	83	91	83	89	85***

GN:Genotypes,ACN:Accession,BRG:Baringo,PKT:Pokot,KTL:Kitale,Eld:Eldoret.

Means with similar letters in the same column are not significantly different.

4.5.4 Raceme length

The analysis of raceme lengths is summarized in table 23.

Maridadi Accessions: The mean raceme length of maridadi accessions at (P=0.01) was significantly different in Pokot with a mean of 29 cm. Accessions BT037 with a mean of 46 cm was significantly longer compared to PB1 and other accessions of maridadi. In Pokot and Kitale the accessions BT166 and BT 183 recorded the significantly the shortest and longest raceme length in centimeters of 31 cm and 48 cm BT183 scored as the best accession with the longest raceme length of 39 cm.

Cream Accessions. MF015 recorded significantly the longest raceme lengths of 48 cm and 49.00cm in Baringo and Eldoret. In Kitale the accession average mean was 31 cm and significant at (P=0.001). MF015 (39 cm) and MT076 (41 cm) were significantly the best accessions with the longest raceme lengths among the cream accessions in the study

Black I Accessions**:** The raceme lengths were between 21 cm to 40 cm in the 4 experimental sites. The general mean was 31 cm. Raceme lengths of accession G1 (44 cm) and GT032 (42 cm) and GT076 were significantly longest in Eldoret compared to other accessions of black I.

Black II Accessions: The raceme mean lengths were between 22 cm to 35 cm. The general mean was 30 cm. WT026 (44) produced significantly the longest raceme in Baringo whereas W7 had the shortest significant raceme length of 30 cm.

Table 23: Mean of racemes length of various dolichos accessions planted in various locations

		LOCATION				
GN	ACN	BRG	PKT	KTL	ELD	Mean
MARIDADI	B1	25E	19BDC	22F	41A	27D
	BT188	42BA	20BDC	34CB	46A	36BA
	BT183	42BAC	24BDAC	48A	42A	39A
	BT166	35EBDAC	31A	30CED	36A	33BC
	BT154	36EBDAC	26BAC	25FE	35A	31BCD
	BT114	32EBDC	29BA	25FE	37A	31BCD
	BT046	39BDAC	14D	36B	42A	33BC
	BT037	46A	21BDC	32CBD	42A	36BA
	BF137	30EDC	19BDC	35CB	39A	31BCD
	BF105	28ED	18DC	27FED	37A	28CD
	BF032	34EBDAC	21BDAC	32CBD	37A	31BCD
	MEAN	35***	22**	31***	40	32**
CREAM	M5	33B	24A	22C	34B	28B
	MT110	35BA	18A	23C	37BA	28B
	MT076	47BA	23A	50A	45BA	41A
	MT049	34BA	17A	33B	38BA	31B
	MF048	39BA	26A	20C	44BA	32B
	MF015	48A	22A	36B	50A	39A
	MEAN	40*	22	31***	41*	33*
CLACK I	G1	44A	44A	15B	36A	35A
	GT095	35A	31BA	28A	23B	30BA
	GT076	38A	42A	21BA	21C	29B
	GT032	42A	26B	20BA	35A	31BA
	MEAN	40	36***	21***	29***	31***
BLACK II	W7	30B	36A	25A	23A	29A
	WT018	31BA	40A	20A	18A	32A
	WT026	44A	30A	27A	25A	30A
	MEAN	35***	35	24	22	30

GN:Genotypes,ACN:Accession,BRG:Baringo,PKT:Pokot,KTL:Kitale,Eld:Eldoret.
Means with similar letters in the same column are not significantly different.

4.5.5 Plant height

Maridadi Accessions: The accessions of recorded plant height of between 46 cm to 52 cm with significant mean of 49 cm at P=0.001. The accessions in Eldoret had significantly taller height at P=0.001 of 51cm. Generally accession BT166 (54 cm) was significantly the tallest accession. While BT154 (43 cm) and BF137 (44 cm) were significantly the shortest accessions as summarized in table 24.

Cream Accessions: The accessions recorded plant height in centimeters that ranged from 43 cm to 53 cm with a significant mean of 48 cm at P=0.05.MT049 with a mean of 41 cm was considerably the shortest accession amongst the mutant in Eldoret while the tallest overall mutant accession was MF015 with 52 cm.

Black I Accessions: Plant height ranged from 46 cm in Pokot to 58 cm in Eldoret. The mean accessions height was 50 cm and significant at P=0.05. GT032 was significantly the tallest accession in Eldoret and during the study with a mean of 60 cm and 58 cm respectively.

Black II Accessions. The mean plant height among the accessions was 54 cm. Accessions in Baringo were the shortest at 51 cm while in Eldoret they were tallest 58 cm.WT018 52 cm was significantly the shortest accession while WT026 51 cm the tallest accession.

Table 24: Mean of plant height of various dolichos bean accessions planted in various locations

		LOCATION				
GN	ACN	BRG	PKT	KTL	ELD	MEAN
MARIDADI	B1	57A	48BA	44BA	55BC	51BA
	BT188	56A	53A	55A	59A	51BA
	BT183	54A	43BC	42BA	55BC	52BA
	BT166	57A	49BA	49BA	60A	54A
	BT154	40B	38C	42B	51C	43D
	BT114	53A	47BA	50A	37ED	51BA
	BT046	51A	45BC	51A	54BC	50BA
	BT037	51A	47BA	53A	42D	48BCD
	BF137	48A	43BC	42BA	42D	44D
	BF105	49A	44BC	44BA	58BAC	49ABC
	BF032	54A	48BA	48BA	49BAC	52BA
	MEAN	52	46***	47***	51***	49***
CREAM	M5	47A	43A	43A	42B	43C
	MT110	51A	36A	44A	57A	47BAC
	MT076	55A	46A	46A	53A	50BA
	MT049	52A	45A	45A	41B	46BC
	MF048	54A	41A	45A	53A	48BAC
	MF015	60A	46A	46A	57A	52A
	MEAN	53	43	45	50*	48*
CLACK I	PG1	45A	43A	52A	49B	47B
	GT095	50A	44A	44A	49B	46B
	GT076	51A	44A	46A	46B	47B
	GT032	60A	54A	59A	60A	58A
	MEAN	52	46	50	58*	50*
BLACK II	PW7	52A	53A	57A	64A	53BA
	WT018	53A	55A	47A	57A	54B
	WT026	47A	61A	53A	54A	57A
	MEAN	51	56	52	58	54***

Means with similar letters in the same column are not significantly different.GN:Genotypes,ACN:Accession,BRG:Baringo,PKT:Pokot,KTL:Kitale,Eld:Eldoret.

4.5.6 Number of racemes:

Maridadi Accessions**:** There was no significant difference as presented in table 25 in the number of racemes among the experimental sites on maridadi accessions. Kitale had the least number 9 whereas Baringo had the most at 13 the total mean was 11. BT114 produced the highest significant numerical number of racemes per plant of 14.

Cream Accessions**:** There was an average mean of 12 racemes per plant. But no significant difference in the number of racemes among the experimental sites. Accessions in Kitale produced the least number 10 of racemes whereas Baringo had the highest (13) number of raceme per plant. The check M5 recorded 13, and the mutant accessions also recorded the same number: MT076 (13) MF015 (13) produced the highest number of racemes, while MT049 produced the least significant number of racemes per plant of 9.

Black I Accessions**:** The number of racemes was not significantly different in Baringo, Pokot, Kitale and Eldoret. The average mean of number of racemes ranged from 10 in Kitale to 15 in Baringo with overall mean of 12. Within the accessions there was no significant difference apart from in Kitale where by GT076 and GT032 produced the highest in the number of racemes 13 compared to G1 accession with the least significant number of racemes of 10 per plant.

Black II Accessions: There was no significant mean difference in number of racemes in the experimental sites. Within the accessions WT026 in Kitale and Eldoret produced significantly higher number of racemes of 15 and 16 respectively.

Table 25: Mean of number of racemes per plant of various dolichos bean accessions planted in various locations:

		LOCATION				
GN	ACN	BRG	PKT	KTL	ELD	MEAN
MARIDADI	B1	13BAC	7ED	9BA	11CB	10ED
	BT188	13BAC	12BAC	11BA	14B	13BA
	BT183	11BC	9DC	7B	7D	9E
	BT166	11BC	11BAC	11BA	13B	12BAC
	BT154	10C	10BDC	10BA	13B	11BDC
	BT114	14BA	14A	14A	13B	14A
	BT046	16A	12BAC	9BA	12CB	12BAC
	BT037	14BAC	13BAC	6B	9CD	11BED
	BF137	14BAC	13BA	7B	17A	13BA
	BF105	13BAC	6E	9B	8D	9ED
	BF032	12BC	10BDC	7B	12B	10EDC
	MEAN	13***	11***	9***	12***	11***
CREAM	M5	15A	12BA	12A	13BA	13A
	MT110	14A	12BA	10A	11B	12BA
	MT076	12BA	13A	11A	13BA	13A
	MT049	9B	8B	9A	12BA	9B
	MF048	12BA	12BA	9A	11B	11BA
	MF015	12BA	15A	9A	12A	13A
	MEAN	13***	11***	10	12*	12*
CLACK I	G1	15A	12A	10A	10B	12A
	GT095	16A	11A	11A	12BA	13A
	GT076	14A	11A	12A	13A	13A
	GT032	13A	9A	7A	13A	11A
	MEAN	15	11	10	12	12
BLACK II	W7	11A	12A	12BA	12BA	12A
	WT018	11A	14A	9B	10BA	12A
	WT026	12A	12A	15A	16A	13A
	MEAN	11	13	12*	12.65*	12

GN:Genotypes,ACN:Accession,BRG:Baringo,PKT:Pokot,KTL:Kitale,Eld:Eldoret. Means with similar letters in the same column are not significantly different.

4.5.7 100 Seed weight:

Maridadi Accessions**:** 100 seed weight mean ranged from 23gm to 25 gm and an average of 24 gm across the experimental sites. There was no significant differences in Baringo

and Pokot within the accessions. In Kitale, BT046 (26 gm) produced the highest significant yield. In Eldoret BT166, BT114, BT037 and BF105 were the highest 100 seed weights. The total average mean of the accessions was 26 gm. The average mean of 100 seed weight was 24 gm and significant at P=0.01 of Eldoret. BT046 was the best accession with the heaviest significant 100 seed weight of 22 gm

Cream Accessions: The accessions of cream produced significant 100 seed weight mean of 24 gm at P=0.01. The accessions mean ranged from 23 gm in Kitale and 26 gm in Baringo at P=0.05. MF015 produced the highest significant seed weight of 28 gm in Baringo. There was no significant difference in Pokot and Kitale. MF048 produced the least 100 seed weight of 20 gm while MF015 26 gm as the accession with the highest significant 100 seed weight.

Black I Accessions**:** The mean of this accession for 100g seed weight was between 22 gm to 25 gm. There was no significant difference in the mean of the sites of experiment but the overall mean of all the sites was significant at P≤0.001 with mean of 23 gm. The accessions with the highest significant weights were GT076 (25gm) and GT032 (24 gm).

Black II Accessions: There was no significant difference in the 100 seed weight of black II accessions in Baringo, Pokot, Kitale and Eldoret. The average mean of the accessions across the sites was 25 gm. WT026 produced the highest significant 100 seed weight of 27 gm in Kitale. In Eldoret WT026 and WT018 were significantly better than W7. WT026 was identified as the best accession with the highest 100 seed weight of 27 grams as presented in table 25.

Table 26: Mean of 100 seed weight in grams of various dolichos bean accessions planted in various locations

GN	ACN	LOCATION				MEAN
		BRG	PKT	KTL	ELD	
MARIDADI	B1	23A	23A	24BA	23CB	23B
	BT188	27A	26A	23BA	22CB	25BA
	BT183	27A	24A	23BA	22D	24BA
	BT166	23A	24A	23BA	26A	24B
	BT154	24A	25A	23BA	22CD	23B
	BT114	25A	24A	24BA	26A	25BA
	BT046	27A	26A	26A	24B	26A
	BT037	24A	23A	21BA	26A	23B
	BF137	24A	24A	20B	24B	23B
	BF105	23A	23A	23BA	26A	24B
	BF032	26A	23A	25BA	24CB	25BA
	MEAN	25	24	23*	24**	24
CREAM	M5	25BA	24A	22A	24A	24BC
	MT110	25BA	24A	23A	26A	25BA
	MT076	27BA	25A	24A	25A	25BA
	MT049	25BA	23A	23A	25A	24BA
	MF048	23B	24A	22A	20B	22C
	MF015	28A	26A	23A	25A	26A
	MEAN	26	24	23	24**	24*
BLACK I	G1	21A	20A	20B	22D	21C
	GT095	25A	24A	24A	27A	22B
	GT076	23A	22A	21A	24C	25A
	GT032	24A	22A	25A	25B	24A
	MEAN	23	22	23	25***	23***
BLACK II	W7	25A	26A	25B	25B	25B
	WT018	28A	27A	25B	26A	24B
	WT026	25A	28A	27A	27A	27A
	MEAN	26	27	26*	27*	25

GN:Genotypes,ACN:Accession,BRG:Baringo,PKT:Pokot,KTL:Kitale,Eld:Eldoret.
Means with similar letters in the same column are not significantly different

4.5.8 Grain Yield in kgha^{-1}

Maridadi Accessions**:** A mean grain yield of 3114 kgha^{-1} was recorded across the four environments ranging from 2553 kgha^{-1} (Kitale) to 3792 kgha^{-1} (Baringo) presented in table 26. Accession BT188 yielded significantly (P=0.05) better than the other accessions in all the sites. A highest site mean of 4262 kgha^{-1}was recorded in Baringo. Accessions BF137 (2597 kgha^{-1}), BT154 (3283 kgha^{-1}), BF105 (2028 kgha^{-1}) and BT046 (2517 kgha^{-1}) yielded significantly (P≤0.05) lower than the other varieties in Baringo Pokot Kitale and Eldoret respectively.

Cream Accessions: There was no significant difference (P=0.05) in yield production on accessions of cream (M5, MT110, MT076, MT049, MF048 and MF015) in Baringo and Pokot .Accession MF015 (3272 kgha^{-1}) and MT049 (3157 kgha^{-1}) produced the highest significant yield in kitale.MF015 also was the highest significant yielder in Eldoret (3492 kgha^{-1}) and among all the accessions MF015 with mean yield 3362 kgha^{-1} and MT049 3315 kgha^{-1} were the best accessions of cream with high yields .

Black I Accessions**:** The mean of this accession for kgha^{-1} was between 2728 kgha^{-1} (GT076) and 3513 kgha^{-1} (GT032). Mutant accession GT032 was the overall best yielder in Baringo (4314 kgha^{-1}), Pokot (2603 kgha^{-1}), Kitale (2718 kgha^{-1}) and Eldoret (2988 kgha^{-1}) and among the accession of black I.

Black II Accessions: There was no significant difference in the yield kgha^{-1} in Kitale among the accessions of black II.WT026 accession yielded significantly the highest in Baringo (5020 kgha^{-1}), Pokot (4495 kgha^{-1}) and in Eldoret (3951 kgha^{-1}).It was the best significant yielder in kgha^{-1} with yield of 4462 kgha^{-1} among the black II accessions

Table 27: Mean of grain yield in kgha^{-1} of various dolichos bean accessions planted in various locations

		LOCATION				
GN	ACN	BRG	PKT	KTL	ELD	MEAN
MARIDADI	B1	2482 BC	3495ED	2651EBDAC	2928BDC	2889C
	BT188	4083A	4262A	3158A	4173A	3919A
	BT183	2629BC	3550EDC	2525EBDAC	3329BDAC	3008CB
	BT166	2475BC	4174BA	2073ED	3698BAC	3105CB
	BT154	2600BC	3283E	2286EDC	3036BDC	2801C
	BT114	2508BC	3506ED	2437EBDC	3027BDC	2870C
	BT046	2891BC	3792BDAC	3022BA	2517D	3055CB
	BT037	2689BC	4035BDAC	2817BAC	3861BA	3351B
	BF137	2597BC	3855BDAC	2743BDAC	3965BA	3290B
	BF105	3240BA	3688EDC	2028E	3522BDAC	3119CB
	BF032	2240C	4069BDAC	2346EBDC	2734D	2847C
	MEAN	2767	3792*	2553*	3345	3114
CREAM	M5	2352A	3512A	2163C	3028C	2764B
	MT110	2818A	3179A	2471CB	2742C	2803B
	MT076	2611A	3223A	2233CB	3407B	2868B
	MT049	2552A	3236A	3157A	3316B	3316A
	MF048	2182A	3558A	2614CB	3060C	2854B
	MF015	2672A	3590A	3272A	3915A	3362A
	MEAN	2539	3486	2652*	3301**	2994***
BLACK I	G1	2316B	3229B	2701B	2968A	284B
	GT095	2431B	3377B	2409C	2694A	2759B
	GT076	2319B	3053B	2790B	2873A	2728B
	GT032	3347A	4314A	2972A	3417A	3513A
	MEAN	2603	3493*	2718***	2988	2951***
BLACK II	W7	3330A	2488B	3061A	3161B	3023C
	WT018	2779B	2779B	2988A	2990B	3443B
	WT026	5020A	4495A	3931A	3951A	4462A
	MEAN	4191*	3254*	3326	3367*	3643***

GN:Genotypes,ACN:Accession,BRG:Baringo,PKT:Pokot,KTL:Kitale,Eld:Eldoret Means with similar letters in the same column are not significantly different.

4.5.9 Combined results of analysis of dolichos bean accessions yield and yield related components

Days to Flowering: The combined evaluation of variance for environments of the different accessions on flowering for the maridadi, cream, black I and black II genotypes revealed a highly significant mean square (P=0.05) for location table 28. Eldoret and Kitale had the highest significant days to flowering among the sites for all the accessions. In maridadi the days to flowering ranged from 70 Kitale and 56 Pokot with a mean of 63. It ranged from 69 (Eldoret) and 56 (Pokot) with a mean 63 for cream, 69 (Eldoret) and 57 (Baringo) with a mean of 62 for black I and 72 (Eldoret) and 59 (Pokot) with a mean of 65

Days to maturity. Highest site mean was recorded in Kitale ranged 120 to 122 days for maridadi, cream, black I and black II genotypes. The genotypes in Baringo recorded the lowest number of days of 104 to 105 to attain 85% maturity.

Raceme length: Eldoret and Kitale produced the longest raceme length for cream, black I and black II the accessions. Eldoret produced significantly the longest raceme length for maridadi of 40 cm and Pokot had the shortest significant raceme length for all the accessions in which it ranged from 21 cm to 22 cm.

Number of racemes: The average number of racemes range from 11 (maridadi) and 12 (black II).Eldoret produced the highest significant number of racemes maridadi (13) and black I (15). There was no significant difference among the environments in number of racemes of black II.

Plant Height: The accessions in Eldoret and Kitale experimental environments had significantly the tallest plants for maridadi, cream and black II ($P \leq 0.05$).There was no

significant difference on plant height for black II among the experimental sites. The mean plant height for the accessions was between 48 cm and 54 cm.

Number of pods per plant: Baringo produced the highest significant number of pods per plant ($P \leq 0.05$) for ranged between 76 and 81 among the genotypes. Eldoret produced the highest significant number of pods per plant at 91.

Seed weight: The average ranged of seed weigh produced among the genotypes and the location were between 23 and 26 grams

Yield in kgha^{-1}: Baringo yielded significantly the highest at 3992kgha^{-1}, 3486 kgha^{-1}, 3493 kgha^{-1} and 492 kgha^{-1} for maridadi ,cream ,black I and black II accessions respectively. Eldoret and Pokot yield significantly less for maridadi (2767 kgha^{-1} and 2553 kgha^{-1}), cream (2539 kgha^{-1} and 2652 kgha^{-1}) and black I (2604 kgha^{-1} and 2718 kgha^{-1}).The average yields in kgha^{-1} ranged from 2951 kgha^{-1} for black I and 3643kgha^{-1}for black II.

Table 28: Combined mean analysis of various traits of dolichos bean accessions associated with yield

		TRAITS							
GN	LOCATION	DTF	MT	RL	NR	PH	NPP	SW	$Kgha^{-1}$
MARIDADI	BRG	58B	105C	35B	11B	46B	81A	24BA	3791A
	ELD	69A	114B	40A	13A	53A	78BA	25A	2767C
	KTL	70A	121A	31C	12BA	51A	77BA	24BA	3345B
	PKT	56B	107C	22	9C	49B	72B	23B	2553C
	MEAN	63***	112***	32***	11***	49***	77*	24*	3114***
	LSD	3	5	3	1	5	9	1	240
	CV%	10	5	21	24	13	23	10	16
CREAM	BRG	59B	103C	40A	12A	43B	77A	25A	3486A
	ELD	70A	114B	41A	13A	53A	76A	23B	2539B
	KTL	69A	122A	31B	12A	50A	61B	24BA	3301A
	PKT	56B	105C	22C	10B	45B	74A	25A	2652B
	MEAN	63***	111***	33***	12	48***	72*	24***	2994***
	LSD	4	4	4	1	5	11	8	269
	CV%	10	6	19	23	16	22	15	13
BLACK I	BRG	57B	105C	36A	10B	46A	76A	22B	3493A
	ELD	69A	115B	40A	15A	52A	81A	23B	2604C
	KTL	65A	122A	29B	12B	51A	60B	25A	2988B
	PKT	57B	105C	21C	11B	50A	81A	23B	2718C
	MEAN	62*	112**	31***	12*	50	74*	23	2951***
	LSD	4	6	5	2	6	10	1	203
	CV%	8	6	20	25	14	17	7	8
BLACK II	BRG	59B	105C	35A	11A	51B	83BA	26A	4192A
	ELD	71A	114B	35A	12.A	56A	91A	27A	3254C
	KTL	71A	121A	28B	13A	56A	77B	23B	3798BA
	PKT	58B	108C	22C	12.A	52BA	87BA	24A	3326BC
	MEAN	65*	112***	30***	12	54*	85*	25*	3643***
	LSD	6	6	6	3	5	13	1	505
	CV%	9.	6	21	22	10	15	6	14

4.6 Analysis of variance and adaptability of dolichos bean accessions

The effect of genotype (G), replication (rep), location (L), and genotype x location interaction (GxL) are presented in table 29.0. Days to 50% flowering, days to 85% maturity, raceme length, number of racemes per plant, plant height, number of pods per plant,100 seed weight and yield in $kgha^{-1}$were highly significant at (P=0.001) in genotype, replication and location while genotype x location interaction (GxL) on raceme length was significant at ($P \leq 0.01$).

Table 29: Combined analysis of variance of yield and yield associated traits of 24 accession of dolichos bean

Source	df	DTF	MT	RL	NR	PH	NPP	SW	$kgha^{-1}$
G	23	2.00***	3.13***	4.12***	3.07***	3.64***	5.56***	4.63***	10.29***
Rep	2	12.83***	0.72ns	4.46*	1.51ns	8.38***	1.88ns	2.73ns	5.92***
L	3	95.61***	114.64***	109.53***	17.93***	17.01***	5.03**	7.17***	85.95***
G x L	69	1.29ns	0.49ns	2.24**	1.11ns	1.51ns	1.04ns	1.25ns	1.48ns
MEAN		63.22	111.64	32.02	11.29	49.66	76.43	24.22	3122.95
CV%		9.64	5.66	19.84	23.48	14.02	21.51	8.86	14.31
LSD		4.91	5.09	5.11	2.17	5.61	13.24	0.36	359.99

DF: Degrees of freedom, DTF50% Days to 50% flowering ,MT 85% Days to 85% flowering ,RL :Raceme length in CM,NR: Number of racemes ,PH :Plant height in cm, NPP: Number of pods per plant SW: 100 Seed weight in grams KG/Ha grain yield in kilograms per hectare:, G: Genotype, Rep: replicate, L:Location,GXL:Genotype x Location *:significant at 5% probability level; **: significant at 1% probability level; ***: significant at 0.1% probability level.

The dolichos bean accession with the longest significant days to flowering were parental accessions W7, B1 while mutant accessions BT046, GT032 and GT095 were significantly early flowering .Days to maturity was highly significant (P=0.001) with a mean of 112.Parental accession W7 was the latest significantly maturing accession whereas mutant accession MT110 earliest maturing mutant accession at 105 days as

indicated in table 30.0 .The mean of raceme length 32 cm was highly significant (P=0.001).Mutant accession MT076 and maridadi non mutated parental accession (B1) had significantly the longest 41 cm and the shortest 27 cm raceme length respectively (P=0.05) .The average mean number of raceme was significant (P≤0.001) on 12 with accession BT114 (14 cm) and BF105 (9) producing significantly the most and the least number of racemes per plant. Plant height mean 50 cm was significant (P≤0.001) .Mutant accession GT032 (52 cm) was significantly the tallest accession, while BF137 (44 cm) and non-mutated parental accession M5 (43 cm) were the shortest accessions. WT026 produced the highest significant number of pods per plant (102) whereas MT110 (60) produced the least number of pods per plant. The average mean of number of pods among the accessions was 76 and significant at(P=0.001).The 100 seed weight ranged from significantly the least 21 grams (G1) and the highest 27grams (WT026). The average mean was 24 grams. The average weight of grains in kilograms per hectare was 3123 $kgha^{-1}$, and significant at (P=0.001) .Accession WT026 (4462 $kgha^{-1}$) and significantly the highest yielder while GT095 (2728 $kgha^{-1}$) the least producer.

Table 30: Combined mean analysis of dolichos bean accessions on yield and yield associated traits

	TRAITS							
ACC	DF	MT	RL	NR	PH	NPP	SW	kgha^{-1}
BF032	64.75BAC	113.16^{FBDECG}	31.00^{EFDC}	10.33^{EDHFF}	52.08^{BDEC}	72.41^{HEFIG}	24.91^{BCD}	2847.10^{GF}
BF105	63.50BC	111.75^{FBDEHCG}	27.58^{EF}	8.75^{H}	48.66^{FGDECH}	73.58^{HEFG}	23.83^{FECD}	3119.40^{DFE}
BF137	64.92BAC	111.91^{FBDEHCG}	30.75^{EFDC}	12.66^{BDAC}	43.50^{H}	83.83^{EDC}	23.33^{FED}	3289.80^{DCE}
BT037	62.41BC	106.91^{IJ}	35.25^{BAC}	10.58^{EDHGF}	48.33^{FGDECH}	92.25^{BAC}	23.41^{FECD}	3350.70^{DCE}
BT046	60.50C	109.41^{FIEHG}	32.83^{EDC}	12.08^{EBDACF}	50.16^{FGDEC}	66.41^{HFIG}	25.83^{BA}	3055.30^{GFE}
BT114	61.08BC	113.91^{BDAC}	30.75^{EFDC}	13.66^{A}	51.41^{FDEC}	79.16^{EFDC}	24.50^{BCD}	2869.80^{GF}
BT154	63.08BC	111.41^{FBDIEHCG}	30.58^{EFDC}	10.75^{EDHGCF}	44.58^{GH}	65.25^{HIG}	23.41^{FECD}	2801.30^{GF}
BT166	62.16BC	111.33^{FBDIEHCG}	32.75^{EDC}	11.66^{EBDACF}	53.66^{BAC}	77.33^{EFDG}	23.96^{ECD}	3105.20^{DGFE}
BT183	62.16BC	111.16^{FDIEHCG}	38.83^{BA}	8.58^{H}	50.83^{FDEC}	73.50^{HEFG}	24.00^{BECD}	3008.00^{GFE}
BT188	61.500BC	112.66^{FBDECG}	35.58^{BAC}	12.58^{BDAC}	50.00^{FGDEC}	97.91^{BA}	24.75^{BCD}	3918.80^{B}
GT032	59.83C	108.33^{IJHG}	30.83^{EFDC}	10.58^{EDHGF}	58.25^{A}	83.16^{EDC}	24.00^{ECD}	3512.50^{C}
GT076	62.41BC	114.41^{BDAC}	30.41^{EFDC}	12.75^{BAC}	46.33^{FGEH}	75.00^{HEFG}	22.41^{FEG}	2758.90^{GF}
GT095	60.41C	108.33^{IJHG}	29.41^{EFDC}	12.67^{EBDAC}	47.08^{FGDEH}	71.25^{HEFIG}	25.00^{BC}	2727.80^{G}
MF015	62.00BC	113.58^{FBDEC}	38.83^{BA}	12.58^{BDAC}	52.25^{BDEC}	87.08^{BDC}	25.83^{BA}	3362.30^{DCE}
MF048	65.92BA	110.00^{FDIEHG}	32.08^{EFDC}	11.08^{EBDAGCF}	48.33^{FGDECH}	72.41^{HEFIG}	22.08^{FG}	2853.50^{GF}
MT049	61.16BC	113.08^{FBDECG}	30.66^{EFDC}	9.41^{HG}	45.66^{FGH}	84.25^{EDC}	24.16^{BECD}	3315.70^{DCE}
MT076	62.16BC	109.58^{FIEHG}	41.33^{A}	12.41^{EBDAC}	50.16^{FGDEC}	67.25^{HFIG}	25.16^{BC}	2868.20^{GF}
MT110	63.33BC	104.66^{J}	28.25^{EF}	11.58^{EBDACF}	46.91^{FGEH}	59.66^{I}	24.50^{BCD}	2802.50^{GF}
B1	69.083A	116.00^{BA}	26.83^{F}	10.08^{HGF}	50.91^{FDEC}	68.00^{HFIG}	23.33^{FED}	2889.20^{GF}
G1	64.50BAC	115.25^{BAC}	34.91^{BDC}	11.88^{EBDACF}	47.41^{FGDEH}	68.25^{HFIG}	20.83^{G}	2803.80^{GF}
M5	66.17BA	115.58^{BA}	28.25^{EF}	12.91^{BA}	43.41^{H}	62.08^{HI}	23.50^{FECD}	2763.70^{GF}
W7	69.41A	118.83^{A}	28.66^{EF}	11.66^{EBDACF}	52.91^{BDC}	65.41^{HIG}	24.91^{BCD}	3022.80^{GFE}
WT018	61.75BC	109.00^{FIJHG}	31.83^{EFDC}	12.08^{EBDACF}	51.58^{FBDEC}	87.41^{BDC}	24.00^{ECD}	3442.7^{DC}
WT026	63.16BC	108.83^{IJHG}	30.16^{EFDC}	13.08^{BA}	57.50^{BA}	101.58^{A}	27.33^{A}	4462.10^{A}
MEAN	**63.22*** **	**111.64*** **	**32.02*** **	**11.49*** **	**49.66*** **	**76.43*** **	**24.12*** **	**3122.95*** **
CV %	9.64	5.66	19.84	23.48	14.02	21.51	8.86	14.31
LSD	5.088	4.72	5.89	2.2	5.96	13.29	1.77	381.8

ACC: Accession, DF: Degrees of freedom, DTF50% Days to 50% flowering, MT 85% Days to 85% flowering, RL: Raceme length in CM, NR: Number of racemes, PH: Plant height in cm, NPP: Number of pods per plant SW: 100 Seed weight in grams kg/ha $^{-1}$grain yield in kilograms per hectare.

4.6.1 Effect of location on yield and yield related traits of dolichos bean accessions.

The yield associated traits of the dolichos bean accessions were significantly affected by location as indicated in table 31.There was significant difference between Baringo and Pokot with Eldoret and Kitale in days to flowering and plant height. The accessions took significantly the longest time to mature in Eldoret. Baringo produced significantly the highest number of pods per plant and $kgha^{-1}$. Eldoret produced the longest raceme length, number of raceme per plant and 100 seed weight. The accessions in Pokot were early flowering (56 days), maturity (106 days) shorter raceme lengths (22 cm), lower number of raceme per plant (10), shorter plant height (48 cm), and lower seed weight (24 gm) and yield per hectare 2702 $kgha^{-1}$ respectively. Baringo was significantly the best location for dolichos bean production with yield of 3715 $kgha^{-1}$

Table 31: Effect of location on yield and yield associated traits of dolichos bean accessions

	TRAITS							
LOCATION	DTF	MT	RL	NR	PH	NPP	SW	$kgha^{-1}$
BRG	58B	104C	36B	11C	46B	80A	24CB	3715A
ELD	69A	114B	40A	13A	53A	80A	25A	2744C
KTL	69A	122A	30C	12B	52A	70B	24B	3331B
PKT	56B	106C	22D	10D	48B	76A	23C	2702C
MEAN	63	112	32	11	50	76	24	3123
CV%	10	6	20	23	14	22	9	14
LSD	2	2	2	1	2	5	1	147

Key: Means with the same letters are not significantly different at $P \leq 0.05$; DTF: Days to flowering, MT: days to maturity, RL: Raceme length, NR: Number of racemes, PH: Plant height, NPP: Number of pods per plant, SW: Seed weight and $kgha^{-1}$: Kilograms per hectare.

CHAPTER FIVE

DISCUSSION

5.1. Effect of gamma irradiation on dolichos bean genotypes

The application gamma radiation on dolichos bean resulted in a range of variations among the phenotypic traits. Segregation due to genetic distortion was evident at M2 generation for quantitative and qualitative traits. These results were in conformity with the findings of Okamura *et al.,* (2003); Shikazono *et al.,* (2005) and Hase *et al*., (2020) that gamma ray exacts high mutation frequency (MF), extensive mutation spectrum coupled with abundant generation of large deletions and chromosomal rearrangements on seed. The significant results of increase or decrease in plant agro-morphological traits on the individual accessions per genotype in the present study were attributed to effects of mutagenesis based on the mutagen used, the genotype or the physical factors such as percent moisture content on the seeds during mutation. Various studies indicate that different genotypes of the same species also can respond differently on even the same dose of irradiation due to genotypic variations (Owoseni *et al.,*2006). Arulbalachandran *et al.*, (2010) and Horn, (2013) documented significant changes in various morpho-agronomic traits of black gram and cowpea that included variations in leaf length, pod length, raceme length, dry seed yield per plant, seed length, number of nodes per raceme, leaf let length and leaf width and plant height at M2 which are in conformity to this finding on dolichos bean. Gamma rays 300gy and 400gy produced by Co^{60} a source of mutation caused significant positive genetic variation with increase of leaf parameters which may contribute to increased biomass and seed yield due to increased photosynthetic area. Longer raceme length potentially contributes to increased number of

pods per plant hence increased dry seed yield per plant. High mutation doses causes significant reduction of plant height or dwarfing (Brunner, 1995, Bolbhat *et al.*, 2012). The induced shortness or decreased plant height among the accessions of dose 300 gy and 400 gy was considered a desirable effect of mutation in this study as this could enable increase in plant density per unit area to meet food security. Reduced plant height also fastens translocation of metabolites and such mutants materials can be utilized for studies of gene function and development of new varieties with anti-lodging resistance (Bhat., 2007). Days to maturity were significantly reduced in maridadi (300 gy) similar findings of dose 300 gy were noted in the mutation treatment of cowpea varieties by Horn., (2016) that documented positive reduction on days taken to 50% flowering on cowpea breeding lines while Maluszynski *et al.,* (2009) suggested that a high dose of a mutagen can return delayed maturity this suggestion matched the prolonged (143) days to 85% in black II at 400 gy. Variations in days to flowering and maturity are key factors to both plant breeders and farmers because they regulate planting time. The breeder will have a choice from a larger breeding pool for various breeding traits and purposes. Conversely, other scientists have also reported negative effects of increased mutagenic doses affecting various crops growth and developmental or survival for breeding (Chikelu., 2013).

The improved dry seed yield due to mutation at 300 gy in cream, black I and II and at 400 gy in maridadi is consistent with the findings of high yielding mutants that were isolated by (Veerabadhiran *et al.,* 2001) in lablab bean and in horse gram (Bolbhat *et al.,* 2012) when same dose was used. The leaf heterozygosis or chlorophyll mutations resulting as albino or variegated leaves (yellow or white) in irradiated accessions of

cream (300 gy) and maridadi (400 gy) can be used to monitor the effect of radiation on dolichos lablab. Similar result of occurrence of chlorophyll mutations in lentil were reported by Kumar *et al.,* (2019), in chickpea (Bara., 2007),vicia bean (Bhat., 2007) and in cowpea (Olasupo *et al.*, 2017). Maridadi and cream accessions exhibited plant with yellow or white variegated leafs, mutants with different flower bud colours, growth habit and leaf shape were among the mutant accessions derived in this study. Similar trends were observed in mung bean mutants obtained from gamma-rays irradiation at a rate of high doses of 300 gy-600 gy (Ahuja *et al.,* 2014). Additionally, this study exhibited the creation of new phenotypes in change of shape and colour of the dry seed of the mutant accessions. This diversity is very important since such accessions can be used to further improve dolichos germplasm for food and sensory values and the findings concur with those of cowpea research in Namibia by Horn., (2016). The current study also found that mutation treatment at 300 gy and 400 gy on did not significantly affect phenotypic traits of black I accessions a part from germination percent. It is important to initially carry out tests of radio sensitivity before actual mutation induction research (Ahloowalia *et al.,* 2001, Mba *et al.,* 2010).

5.2. Genetic estimates of maridadi and black I accessions.

The assessment of variability, expected genetic advances and heritability of agronomic components are significant in crops genetic improvement. Genetic status PCV and GCV in a breeding line is useful for comparing the relative amounts of phenotypic and genotypic variation among traits and to estimate the scope for improvement by selection. The evaluation of genetic estimates in maridadi and black I dolichos accessions at M2 showed relatively high phenotypic variance (σ^2p) to genotypic variance (σ2g) across the

mutation doses. This shows that these the characters were influenced by environment. Phenotype selection alone on the basis this trait cannot be effective for dolichos improvement. The % PCV values were slightly were higher than those of % GCV by almost half in leaf length, pod length, 100 seed weight dry seed yield per plant height, number of nodes per raceme and raceme length in 0 gy, 300 gy and 400 gy in both maridadi and black I. This effect could be due to the fact that the expression of this traits are highly influenced by environment. Similar findings have also been reported in other studies on soybean (Malek *et al.,* 2014) and (Jain *et al*., 2015) which is in agreement with this present study.

Heritability estimates can be utilized in selection of accessions based on phenotypic performance and can be used to predict the reliability of a phenotypic value (Akram *et al.,* 2016). Effective selection of a particular accession is based therefore, on high heritability of a specific trait of interest such as number of pods per plant. Heritability estimates are classified as low (<30 %), medium (30 - 60 %) and high (60 %) and above (Arulbalachandran *et al.*, 2010). There was high heritability in 0gy maridadi on racemes per plant and plant height while it was high in 0 gy and 300 gy than 400 gy accession of black I in raceme per plant, raceme length, number of nodes per raceme, plant height, dry seed yield per plant which indicated that the total variation in this traits are under genetic control and or additive gene action. Selection based on phenotypic levels of this traits in this accessions would be useful in the improvement of these accessions. Similar observations were made on different studies on hyacinth bean (Islam., 2011, Sen *et al.,* 2018), mutant soya bean (Nagarajan *et al.,* 2017). The low heritability in some traits at 300 gy and 400 gy (maridadi), 400 gy (black I) accessions might be due to some

influence of non-additive gene action effects due to mutation effects which reduces uniformity and stability of this traits. Selection for the traits under this dose can be achieved once the accessions have attained uniformity and stability like 0 gy.

Akram *et al.,* (2016) reported that estimated percent heritability is not very much useful because it includes the effect of both additive and non-additive gene effects. The genetic advance is therefore a useful indicator to achieve expected result on the trait of interest after selection. Genetic advance in percentage of mean is found to gives more precise result in comparison to only genetic advance (Basavaraj *et al.,* 2015). Genetic advance as percent mean is categorized from low (0-10%), moderate (10-20% and high (≥20%) in the accessions. In the present study based on various traits it indicated mixed variations low to high among the accessions. Maridadi (300 gy and 400 gy) accessions had low % GAM while black I recorded high % GAM. The existence of variability in the accessions can be attributed to varietal differences and mutagenic effects. Similar findings have been reported by Khan *et al.*,(2006) on yield contributing traits of green gram and mung bean. Bhat., (2007) also reported that following irradiation of seeds material with strong ionizing rays there was alteration of heritability values due to formation of further genetic variability.

The percent genetic advance of the mean was low in most some traits of maridadi which are indicative of non-additive genetic effects in this genotype. Similar results have been reported in other studies by Kharkwal., (2003) in chick pea mutant accessions in one of the three soya bean genotypes on pod clusters per plant ,plant height, number of seeds/pod, 100 seed weight and seed yield/plant (Malek.,2014, Mahbub *et al.*, 2015,Shivom *et al.,* 2020). The limited scope for the improvement of the characters is an

indication for creation of a broad germplasm base through alternative breeding methodology to utilize both additive and non-additive gene effects simultaneously.

5.3 Genetic diversity of dolichos bean accessions based on Simple Sequence Repeat (SSR) markers

Mutational genomics is becoming a valuable tool to differentiate the mutants improved via mutation breeding programs. Assessment of genetic variability in putative mutant population is a prerequisite to its improvement. In the present study, all the variation components confirmed that there was relatively substantial genetic diversity among individuals (54%) among individual and within population (45%) and least substantial in within individuals in the population (1%). Estimated variance were 6.725. These results showed that the two mutational doses 300 gy and 400 gy produced relatively average genetic differences in dolichos bean genotypes. The presence of relatively average percent of variation within individuals and among individual in a population can be attributed to limited irradiation doses (300 gy and 400 gy), selection pressure of the accessions where each accession was compared to the parent based on earliness and yield, previous breeding method and the fact that dolichos bean is a self-pollinated crop.

In the principal component analysis (PCoA) that helps in exploring and visualizing genetic differences among the accessions based on accrued genetic data, the first two principal components had nearly the same variation. PCoA plots confirmed that the population was highly associated and the computed unbiased genetic distance and identity showed low variability. This limited variability and close relationship based on the above finding points to similarity among the accessions and/or populations due to

mutation uptake, selection criteria or that these accessions were originally developed from elite cultivated forms that tend to have low levels of genetic diversity. Shivachi *et al.,* (2013) and David *et al.,* (2021) reported low genetic diversity of the cultivated dolichos bean in Kenya. A major component of diversity in this study is the wide dispersion and differentiation of accessions (WT018 and WT026) and BT 114, BT166, BT137, BT188 and BT046 as exhibited in the from the bulk of the mutant materials screened by use of the SSR markers . This is a direct impact of mutation of on the genome of genotypes and resulted in valuable accessions for use in further improvement of the dolichos bean crop.

The F_{ST} values as per Wright 1951 that formed putative populations was high and almost one implying that the mutant accessions selected based on earliness and yield potential was almost fixed. The F_{IS} values also exhibited that some SSR loci among the mutant accessions in the populations were heterozygous. Natural cross pollination of 6-10 % has been attributed to occur in lablab is could be linked to such heterozygozity similar findings have been reported by and Kamotho *et al*., (2016), Shivachi *et al.,* (2013) and Gnanash *et al* ., (2006). However, the observed coefficient of gene flow (0.34) in all accessions reflecting the high contribution of inbreeding index a characteristic of autogamous species such as dolichos bean, stringent selection pressure within the doses and accessions, limited irradiation doses 300 gy and 400 gy and common ancestry of the accessions.

The mean allelic diversity based on expected heterozygozity 66% was high in the population studied. High number of different alleles in the parents was exhibited than within the mutant accessions could be attributed to differences in diverse breeding

method of development as some were bred through hybridization maridadi, black I, and black II whereas cream has foundation of mutation (Ondabu., 2013, Kinyua., 2020) .

The SSR primer sets showed diversity when used for genetic diversity studies in the dolichos accessions and generated an average of 5.25 alleles per primer pair. Fourteen (70%) primers had PIC values more than 0.5 suggesting the discriminating nature of these markers. The study thus addressed effectiveness of SSR markers in analyzing genetic diversity of dolichos bean population. This was a relatively excellent amplification given that the primers had been pre-tested in other studies (Kamotho *et al.*, 2016, Keerthi *et al.*, 2018), and reflects the efficiency and reproducibility power of these microsatellites across laboratories. Rai *et al*., (2010) reported comparable findings in dolichos bean using SRRs with mean amplifications of 5.69 alleles per primer. Kamotho *et al*., (2016) and David *et al.*, (2021) recorded PIC values of between 0.05-0.88 and 0.19 to 0.75 in non-mutant dolichos bean varieties in Kenya . Markers with PIC more than 0.5 are efficient in discriminating genotypes and extremely useful in detecting the polymorphism rate at a particular locus (Amkul *et al.*, 2021). Lack of amplification of alleles in certain accessions was probably due to difference in the sequences flanking microsatellite or simply the production of undetectable amount of PCR product creating null alleles (Smulders *et al*., 1997, David *et al*., 2021).

5.4. Nutritional and mineral composition of dolichos bean accessions.

The nutritional composition in dolichos bean accessions of maridadi, cream, and black I, and black II genotypes was found to differ significantly. Mutation breeding is considered as a rational tool to create variability in a crop species in a very short period as compared to

hybridizations. Different workers have employed wide ranges of physical and chemical mutagens for the improvement of nutritional values traits in various food crops (Shu *et al*., 2012) .The moisture content in the accessions of dolichos bean in this research ranged from 7.27% to 8.03%. This results are not different from the findings on three (KAT/DL 1-3) varieties which was 8.1-9.8 % (Kilonzi *et al.,* 2017a) or 6.7%-8.5% of 25 genotypes of dolichos bean (Davari *et al.,* 2018). Information related to water content is deemed as pivotal insight due to its close connection with storage stability of seeds, and post-harvest loss due to aflatoxin contamination. Percent crude fat was categorized the lowest content (2.7%-6.7%) among nutritional components in the accessions studied but it compared to the findings of (Kalpanadevi *et al.,* 2013) that reported 5.6% . Lower percent crude fat 1.25% have been reported in F3 families by (Ondabu, 2013) , 1% (Hossain *et al*., 2016) and 2.6% (Kilonzi *et al.,* 2017a). Studies on % fibre in dolichos bean accession indicate to range between 9.8% and 11.36% and matched previous studies .Black I and cream accessions were significantly different this could be attributed to effects of mutation. Percent average mean protein was 24% and similar to the findings of Abhilash *et al.,* (2019). It was different from 27.60% by Kilonzi *et al.,* (2017) indicating that mutation creates genetic variability on levels of percent crude protein and the accessions are different from KAT/DL 1-3. Significant variation in the means of total carbohydrate and energy in calories in maridadi and cream indicates that mutation had significant impact on them in these two accessions. Generally the accessions had higher levels of carbohydrates and energy values in this study compared to those in common bean (*Phaseolus vulgaris L*.). The carbohydrates in legume grains have also been reported to have anti-diabetic properties (Liu *et al*., 2020) apart from being sources of energy and hence dolichos beans have the potential to be used in therapeutic management of diabetes (Kilonzi *et al.,* 2017, Nugraha *et al.,* 2020).

Mutation breeding for fortification in underutilized food crops can be a potential area of research. In the present study, high yielding mutant dolichos accessions were assessed for mineral micronutrients to level to determine variability. Accessions of black I showed significant variation in potassium and calcium while the other three accessions exhibited significant variation in three to four mineral elements. The existence 60%-80% variability on the mineral composition traits of the three accessions can be attributed to the effect of induced mutation. The low significant variability in black I accession could be associated with low uptake of gamma ray irradiations of 300 gy and 400 gy. Such insensitivity with respect to mutagenesis have been reported in various crops such as soya bean ,lentil (Laskar *et al*., 2018) and lablab (Purwanti *et al.*, 2019). Although there exist conflicting reports on linking high grain yield with high mineral or nutritional content through mutation breeding where some view to be difficult (Rehman *et al*., 2001) while Kozgar, (2014) reported the possibility .There was improvement in some nutritional components of the accessions compared to the check accession which indicated possibility of yield improvement and nutritional composition in dolichos bean.

5.5. Assessment of yield and adaptability of dolichos bean accessions in North rift Kenya.

Yield is one of the complex quantitative characters which is controlled by many genes interacting with the environment and is the product of many yield components (Carbonell *et al.,* 2016). Facts on yield and its associated traits is paramount for an efficient selection process for plant breeders to identify a novel high yielding cultivar. The employment of gamma rays 300 gy and 400 gy with the same variety gave different mutations among the wide range of yield contributing characters for which the study was about. The analysis

of variance revealed significant difference among the genotypes for days to flowering. The mutant accessions showed significant reduction on days to 50% flowering and days to maturity than the parental accessions for maridadi in the different experimental sites. Days to flowering in of GT095and BT046 of 60 and 61 is an improvement parental accessions of 64-69 and from the findings of Karanja., (2015) of 68-80 days and Kamotho.,(2015) of 92-102 days and Ayisi.,(2006) of 63-75 days. The findings of Ondabu., (2013) on crosses of accessions of cream (M1) recorded 50% flowering at 65 while in this study following mutation induction the accessions of cream significantly attained 50% flowering at 63days. Accessions BT037, MT110, GT032 and WT026 were the earliest maturing mutant accessions at 104-108 days. Dolichos bean is generally cultivated as rain-fed crop during the main rain season and time of sowing is heavily dependent on onset of long rains. Due to climate change unpredictable onset and delay in arrival of long rains, is now common. The number of days to maturity is a key factor that determines farmer's adoption where most farmers prefer early maturing cultivars. Early and extra early maturing cultivars hold great promise for some harvest and the opportunity for field to be cleared for the next crop before the start of the short rain season. Differences in the accessions for flowering and maturity in the four experimental sites are typical attributes of new accession of mutations or hybridization process. Earliness in flowering and maturity are some of the easiest mutations that can be easily induced and detected by simple observations. Several early maturing mutants have been released directly as new cultivars (Li *et al.*, 2019)

Raceme length, number of raceme per plant are important traits because the longer and higher the number of racemes the higher number of flower buds a bean can bear. Farmers

prefer varieties with high number of flower buds which is directly proportional to higher number of pods. Mutant accessions MT076 and BT114 expressed significant increase in raceme number and length per experimental site compared to the checks. The identification of mutant accession WT026 with significant podding capacity pointed toward its ability to be the best yielder. Increased podding tends to increase harvest index due to increased seed yield. Other studies have shown that bean lines with high podding had greater harvest indices and increased seed yield in favorable conditions (Wondimu *et al.*, 2018). Mutant accessions exhibited significant stability based plant height across the environments this included BT188 and GT032 and MF015. Plant height is an important trait in dolichos breeding .Farmers desire determinate accessions which could be highly branching and with synchronous maturity for ease of harvesting and also intercropping with other staple crops like maize and sorghum. Plant height range from 47-54 cm among the accession which is a significant contrast to the accessions of Karanja., (2015) of 121-163 cm. The 100 seed weight differences across the genotypes was small although there were significant differences in the overall mean .The mutants accession W7 had higher 100 seed weight than the parents. Seed weight is relatively a conservative character and it can vary depending on genetic and environmental conditions (Mudibu *et al.*, 2012). The high yielding accessions BT188, MF015, GT032 and WT026 with seed yields of 3918 $kgha^{-1}$, 3362 $kgha^{-1}$, 3513 $kgha^{-1}$ and 4462 $kgha^{-1}$ respectively. High variability among accessions was revealed in seed yield indicating the possibility to increase seed production through selection. The same high yielding accessions identified in this study could provide valuable sources of tolerance to climate-change related stresses and could be incorporated in future dolichos bean breeding programs.

Combined analysis of variance for mean squares revealed a highly significant variance among locations for the different traits per accessions. The results of all the accessions exhibited significant longer days to 50% flowering, 85% maturity, increased plant height and number of pods per plant in Eldoret and Kitale compared to Baringo and Pokot. The other traits including raceme length, number of nodes per raceme, plant height, 100 seed weight and yield $kgha^{-1}$ followed the same trend or varied slightly. The variation in grain yield and associated agronomic traits for the accessions in each environment are due to climatic conditions and the edaphic conditions. The high bean yields in Baringo may be attributed to the accessions earliness to flowering and maturity allowing good pod formation and grain filling, the high organic matter content, good drainage and soil moisture retention. These factors contribute to proper root anchorage and expansion that allow better nutrient assimilation and, therefore, good plant development. Additionally, the high yields can be attributed to the right amount and distribution of rainfall during the production cycle, especially during pod formation and filling. Similar reports on common bean have been documented by Gómez, (2017). However, the opposite i.e. low yields among the genotypes were observed in Eldoret where sufficient rainfall is usually recorded. The yields could have been limited by predominant concentration of aluminum and low concentration of exchangeable phosphorus and high pH in the soil (Obura *et al*., 2010) that could have affected the nutrient uptake for the dolichos accessions and subsequently low yields. The effect of genotypes and location was greater than that of the genotype and location interaction, which indicates that genotypes responded similarly to environmental changes. All the bean traits under this study were less influenced by genotype by environment interaction, thus a lesser need to plant multi-location trials

when selecting for these traits. This concurs with the study on evaluation of agronomic traits in new improved bean cultivars in tropical environments.(Carbonell *et al.*, 2016, Ligarreto *et al.*, 2021). With stringent selection following irradiation it is possible to identify stable accessions early at M4 shortening the breeding cycle in dolichos bean varieties.

CHAPTER SIX

CONCLUSION

Agricultural workers understand the need to create systems, technologies and products that not only feed an entire generation but is capable of feeding every generation to come irrespective of the climatic change. Plant breeding technologies have played a fundamental role in human history in revolutionizing agriculture to feed the ever-growing population. These technologies such classical plant breeding (hybridization) , genetic engineering ,plant tissue culture, use of plant molecular markers and mutation induction have contributed significant success in developing resilient varieties to effects of climate change . The current focus of plant breeding science is to develop genetically diverse germplasms with superior resilience to effects of climate change. This includes the improvement of potential underutilized crops such as dolichos bean. Through a structured breeding program which takes advantage of modern crop improvement tools and laboratories. A wide range of genetic variations both quantitatively and qualitatively was induced by gamma ray radiations in four dolichos bean genotypes (maridadi, cream and black I black II). The morphological characterization of M2 mutant accessions revealed that induced mutation of dolichos beans with dose 300 gy and 400 gy causes significant increase in leaf length, raceme length, pod length, dry seed yield seed yield and reduction in plant height. There was also phenotypic changes due to mutation frequencies in plant, leaf, flower and seed characters in maridadi, cream and black II accessions. However, black I accessions were not remarkably affected by gamma ray 300 gy and 400 gy irradiation doses.

Different doses of mutation affect differently the genetic estimates of dolichos bean depending on the genotype. Most of the yield contributing traits have high percent phenotypic coefficient of variability than genotypic coefficient of variability irrespective of application of mutation because of environmental dependence. High heritability were observed for characters such as such as plant height, number of racemes per plant, plant height and dry seed yield per plant depending on the genotype and radiation dose .This indicated that there was a lesser influence of environment in the expression of these characters which are amenable for selection. The characters of days to 50 per cent flowering, number of nodes per racemes and seed weight produced moderate level of variability. Hence, these characters are also amenable for selection by minimizing the environment influence.

The assessment of genetic variability among selected mutant accessions based on SSR molecular markers revealed average genetic diversity among individuals and within population selected based on earliness and yield potential. Highly clustered dispersion and few individual accession dispersion indicated by PCoA, unweighted pair group method with arithmetic mean revealed that the SSR markers were effective in differentiating unique mutant accession. WT018 and WT026 were identified to be distantly related to other selected mutants at M3.

It was observed that the genomes of induced mutants selected and analyzed in the study contain noble combinations of alleles for better nutritional and mineral composition. The findings also shows that there was a wide variability in nutritional and mineral composition traits among the mutant accessions per genotypes. As such mutants with

improved nutritive and mineral composition relative to the check were identified in the study such that for percent less moisture content accession :GT095 and BT114; percent crude fat (BT188, MT110, WT018), percent crude fibre (GT095, MT049, MT076), percent crude protein (GT032, BT154, MT076) percent total carbohydrate (GT032, BT154, M T076) and energy in kcal (BT188) energy were identified during the study .while accession with high mineral composition relative to the check genotype were BT046, MF015 and WT018 for phosphorous; GT032 and BT114 in potassium; GT095, BT114, MT049 in calcium and BT039 and MT110 in zinc.

The most important objective in breeding crops for climate change adaptation is production of a stable and high yielding genotype over a range of environmental conditions. Yield is a complex trait strongly influenced by polygenic effects and other breeding objectives, such as, plant architecture, maturity, resistance to biotic and abiotic stresses etc. The breeding of new resilient accessions quickly, economically and with greater precision is critical to ensure small-scale farmers can adapt to climate change. Therefore, the identification of positive yield improvement in mutant accessions BT188, MF015, GT032 and WT026 relative to the checks (B1, G1 M5 and W7) points toward the value of gamma induced mutation in creating new genetic variability in dolichos bean genotypes. It also indicates that induced mutation is still an important breeding method in the 21st century and can be used to improve orphan crops which have not been subject to intense and refined breeding over long periods of time.

CHAPTER SEVEN

RECOMMENDATIONS

1) The economically important genetic trait identified in each accession should be used for inheritance and genetic studies.
2) Further research for effective gamma ray irradiation doses for black I (black I) genotype for effective genetic variability.
3) Continued search for effective simple sequence repeat (SSRs) molecular markers that can stringently distinguish genetic diversity of within individual in a population.
4) The genotypes identified (high nutritional values &high yielding) be used as cultivars directly or as parents in dolichos bean breeding programmes.
5) The best three identified genotypes are recommended for advancement to preliminary yield trials
6) Increased use of synergistic novel plant breeding tools in crop improvement for effective mitigation of effects of climate change

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APPENDICES

Appendix I: Table 8: Combined Analysis of Variance

Table 1. Mean squares of eldo black I as affected by varietal trial in Eldoret, Kitale, Pokot and Baringo 2021 planting season

E.BLACK I SOURC E	DF	DTF 50%	MT 85%	RL	NR	PH	NPP	SW	KG/HA
VAR	3	2.01ns	3.08*	1.87ns	1.19ns	7.73***	3.24*	15.01***	28.56***
REP	2	2.27ns	0.38ns	0.08ns	2.49ns	2.84ns	0.57ns	1.85ns	0.49ns
ENV	3	15.59***	17.04***	21.7***	5.31***	1.35ns	7.7***	4.77ns	31.71***
VAR*ENV	9	1.9ns	0.22ns	4.31***	0.61	0.44ns	1.33ns	0.76ns	2.54ns
MEAN		61.79	111.64	31.39	11.83	49.77	74.41	23.06	2950.72
LSD		8.38	6.35	19.55	24.55	14.19	16.7	7.08	8.25

DF: Degrees of freedom, DTF50% Days to 50% flowering ,MT 85% Days to 85% flowering ,RL :Raceme length in CM,NR: Number of racemes ,PH :Plant height in CM,NPP:Number of pods per plant SW: 100 Seed weight in grams KG/Ha grain yield in kilograms per hectare:,*:significant at 5% probability level; **: significant at 1% probability level; ***: significant at 0.1% probability level

Table 1. Mean squares of eldo maridadi as affected by varietal trial in Eldoret, Kitale, Pokot and Baringo 2021 planting season

SOURCE	DF	DTF 50%	MT 85%	RL	NR	PH	NPP	SW	KG/HA
VAR	10	1.71ns	1.93*	3.39***	4.59***	2.93**	4.21***	1.43ns	5.12***
REP	2	6.67**	0.05ns	1.70ns	0.52ns	13.12***	7.29***	1.1ns	6.79***
ENV	3	48.7** *	54.71** *	42.99***	10.89***	9.1***	1.43ns	2.87*	43.22***
VAR*ENV	30	1.44ns	0.57ns	1.75*	1.56*	3.06***	0.62ns	1.15ns	1.54ns
MEAN		63.19	111.78	32.06	11.06	49.46	77.24	24.12	3114.05
LSD		3.1	4.74	5.36	2.13	5.04	14.34	1.89	398.58

DF: Degrees of freedom, DTF50% Days to 50% flowering ,MT 85% Days to 85% flowering ,RL :Raceme length in CM,NR: Number of racemes ,PH :Plant height in CM,NPP:Number of pods per plant SW: 100 Seed weight in grams KG/Ha grain yield in kilograms per hectare:,*:significant at 5% probability level; **: significant at 1% probability level; ***: significant at 0.1% probability level

Table 1. Mean squares of eldo cream as affected by varietal trial in Eldoret, Kitale, Pokot and Baringo 2021 planting season

Source	DF	DTF 50%	MT 85%	RL	NR	PH	NPP	SW	KG/HA
VAR	5	1.24ns	4.44**	9.37***	2.74*	1.94ns	6.34***	4.31**	5.44***
REP	2	1.8ns	0.96ns	1.45ns	0.53ns	1.37ns	0.66ns	3.28ns	2.44ns
ENV	3	18.92***	35.16***	37.48***	3.99ns	6.55***	3.85ns	4.09ns	24.68ns
VAR*ENV	15	1.01ns	0.71ns	2.61**	0.64ns	0.78ns	1.8ns	0.68ns	1.46***
MEAN		63.45	111.08	33.27	11.66	47.79	72.12	24.2	2994.4
LSD		5.41	5.46	5.12	2.22	6.47	12.89	1.8	329.4

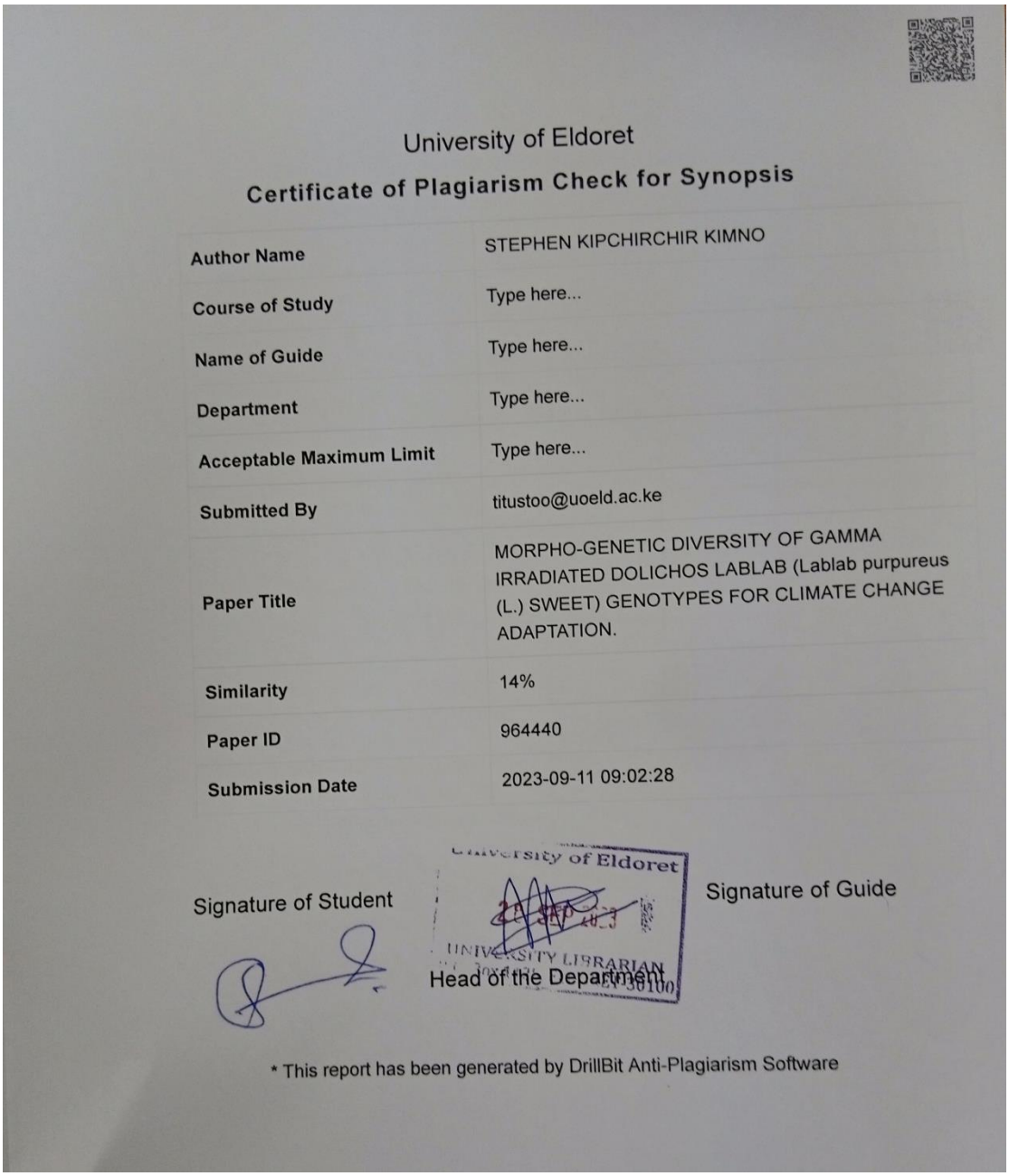
DF: Degrees of freedom, DTF50% Days to 50% flowering ,MT 85% Days to 85% flowering ,RL :Raceme length in CM,NR: Number of racemes ,PH :Plant height in centimeters , NPP: Number of pods per plant SW: 100 Seed weight in grams KG/Ha grain yield in kilograms per hectare:,*:significant at 5% probability level; **: significant at 1% probability level; ***: significant at 0.1% probability level.

Table 1. Mean squares of eldo black II as affected by varietal trial in Eldoret, Kitale, Pokot and Baringo 2021 planting season

Variable	DF	DTF 50%	MT 85%	RL	NR	PH	NPP	100 SW	KG/HA
VAR	2	5.71**	9.81***	0.73ns	0.86ns	3.78ns	24.19***	14.63***	24.61***
REP	2	2.4ns	3.06ns	2.19ns	0.28ns	3.41ns	2.72ns	3.8*	0.8ns
ENV	3	12.36***	10.74***	8.8***	0.59ns	2.29ns	1.91ns	9.67***	6.48**
VAR*ENV	6	1.01ns	0.25sn	2.77ns	1.12ns	1.92ns	0.46ns	1.44ns	1.23ns
MEAN		64.77	5.64	30.22	12.27	54	84.8	25.41	3642.52
LSD		5	5.36	5.43	2.29	4.68	10.86	1.32	437.57

DF: Degrees of freedom, DTF50% Days to 50% flowering ,MT 85% Days to 85% flowering ,RL :Raceme length in CM,NR: Number of racemes ,PH :Plant height in CM,NPP:Number of pods per plant SW: 100 Seed weight in grams KG/Ha grain yield in kilograms per hectare:,*:significant at 5% probability level; **: significant at 1% probability level; ***: significant at 0.1% probability level.

Appendix II: Similarity Report



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