INDUCING GENETIC VARIABILITY IN COWPEA (*VIGNA UNGUICULATA*, L. WALP VAR. *ASONTEM*) USING ETHYL METHANE SULFONATE

A THESIS PRESENTED

BY

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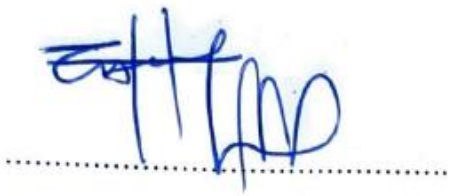
DECLARATION

I, the undersigned, Muhammed Opoku Gyamfi, the author of this work, hereby declare that this is my own work which was supervised by Dr. John Eleblu and Prof. Isaac Asante and this has not been submitted in part or in full by me or anyone to this institution or any other institution of higher education.



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ABSTRACT

Unique variants are desired in the development of genetically improved crops to meet farmer and market needs hence ethyl methane sulfonate (EMS) was used to induce genetic variability in cowpea (*Vigna unguiculata* cv. *Asontem*). The main objective of this research was to characterize induced variations in EMS chemically mutagenized population of cowpea (*Vigna unguiculata* L. Walp Var. Asontem) in the M_1 and M_2 generations. The optimum concentration (LD50) of EMS for generating the mutagenized population was determined by treating seeds with different concentrations of EMS (0.0%, 0.2%, 0.4%, 0.6% and 0.8% v/v) and observing the germination count after 5 days. Three thousand cowpea seeds were treated with the LD50 concentration to generate the M_1 population. Mutagenized seeds were sown with 500 untreated seeds serving as control (wild type). Data on qualitative and quantitative traits were collected during the evaluation of the M_1 generation using Cowpea Descriptor of the IBPGR (1983). M_2 seeds obtained after the M_1 generation were sown and evaluated for morphological mutations in quantitative and qualitative traits. Data analysis was done using statistical software R, STATA and excel. The optimum dose (LD50) determined was 0.4%. The percentage germinations in the mutagenized population and wild type were 17.8% and 61.6% respectively. Percentage survival was higher in wild type (98.38%) as compared with the M_1 population (78.46%). Frequency distribution analysis revealed variations in both the qualitative and quantitative traits observed in the M_1 and M_2 generations. Different phenotypic classes were observed in plant pigmentation, pod curvature, leaf shape, leaf colour, seed shape and seed coat colour. Individuals in the M_1 population were widely distributed for measurements of chlorophyll content, plant height, number of pods per plant, pod length, number of locules per pod and number of seeds per pods as compared with the wild type. Principal component analysis revealed that he quantitative traits in the M_1 population had first four principal components contributing to 77.22% of the total variability observed whiles the first four principal components in the wild type accounted for 75.26% variability. In the wild type, the

first three principal components of qualitative traits accounted for total variability whereas, the first four principal components of qualitative traits in the M_1 population accounted for 89.96% variability. During the M_2 generation, percentage germination in the M_2 population (74.03%) was lower than the wild type (80%). A wide spectrum of morphological abnormalities was observed in the M_2 population. There were 1.59% that had abnormal leaflet number, 0.4% had variegated leaves, 0.14% xantha and albino mutants, 0.55% with irregular leaves. M_2 individuals were widely distributed for days to flowering, number of pods per plant, number of seeds per pod, number of locules per pods, percentage seed set, pod length and number of seeds per plant. Chi-square test of associations of qualitative traits had only one insignificant association in the wild type while a total of 27 associations were observed in the M_2 with 13 significant associations. A total of 28 pairwise correlations were estimated among the 7 quantitative traits in the M_2 population and all correlations were significant. Principal component analysis showed that the quantitative traits in the M_2 population had first four principal components contributing to 95.58% of the total variability observed whiles the first four principal components in the wild type accounted for 92.20% variability. In the wild type, the first two principal components of qualitative traits accounted for total variability whereas, the first four principal components of qualitative traits in the M_2 population accounted for 76.77% variability. Assessment of diversity using both quantitative and qualitative traits grouped the M_2 generation into 7 major clusters. About 2.88% of the total M_2 population performed significantly higher than the wild type for number of seeds per plant, 2.59% performed significantly higher than the wild type for number of pods per plant. Top 20 individuals selected for number of seeds per plant had significantly higher performance in other yield attributing characters. In conclusion, the EMS mutagenesis was effective in inducing the unique variations that will be useful for breeding and development of new farmer preferred varieties.

DEDICATION

I dedicate this work to my parents and my brother A. Kwarteng Gyamfi for the reassurance and financial support.

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LIST OF ABBREVIATIONS

< - Less than

> -Greater than

% - Percentage

± - Plus or Minus

µl - microlitre

CC- Chlorophyll content

Cm – Centimetre

cv- cultivar

Dim -Dimension

DNA – Deoxyribonucleic Acid

EMS- Ethyl methane sulfonate

FAO - Food and Agricultural Organization

FAO/UN - Food and Agricultural Organization of the United Nation

FAOSTAT – Food and Agriculture Organization Corporate Statistical Database

ha – Hectare

IBPGR - International Board of Plant Genetic Resources

ICRISAT - International Crop Research Institute of Tropical for Semi-Arid Tropics

IITA - International Institute of Tropical Agriculture

kg – kilogram

l – Litre

LD50 – Letha dose 50

m – metre

Mb – mega base

mg - milligram

ml – millilitre

mm - millimetre

MOFA Ministry of Food and Agriculture

OECD Organization for Economic Co-operation and Development

PC -Principal Component

PCA – Principal Component Analysis

trt – Treatment

UC SAREP – Sustainable Agriculture Research and Education Program

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Induced mutagenesis has been recognised recently as an important supplement to conventional breeding in crop improvement programs. Since the prominent discoveries by Muller (1927) and Stadler (1928), large amount of genetic variability has been induced by various mutagens. The use of induced mutation over the years has contributed to the modern plant breeding programs. Many member states of the International Atomic Energy Agency (IAEA) have been supported by the agency to genetically improve various crops including cowpea using artificial mutagenesis (chemical and physical means) (Horn and Shimelis, 2013). About 3,500 varieties have been released around the globe through mutation breeding (IAEA 2021). The majority of mutants released all over the world are food crops (IAEA mvgs.iaea.org, 2021). Among the varieties released, very few are pulses. There are 10 mutant varieties of cowpea released in India. In Africa no variety of cowpea has been developed through mutagenesis although the application of induced mutation breeding techniques in cowpea has increased in most countries across the continent. The application of mutagenesis in breeding programs in Ghana has led to the development of two mutant varieties in two crops; *Manihot esculanta,* Crantz (high yielding cassava mutant with 40% dry matter content) and *Theobromo caocao* (a cocoa swollen shoot disease resistant mutant variety) (Danso *et al.*, 2008). In the current studies, cowpea was subjected to EMS treatment to create genetic variability in the cultivar. Induced mutation fits well in cowpea genetic improvement since most cowpea breeding programmes look at increasing the genetic bases of the crop to adapt to various cropping systems and agroecologies. Cowpea is a very important crop which is widely used around the world. The crop is in the Fabaceae family. Cowpea is a diploid (2n=2x=22 chromosomes) organism with a genome size of about 600 million base pairs. The seed is mainly used by people as food and the forage is

widely used as animal feed in the tropics and sub-tropics regions of the world. Cowpea is very nutritious since it is a high source of dietary proteins and carbohydrates (Pavadai *et al.*, 2009), minerals and antioxidants (flavonoids and phenols) (Asante *et al.*, 2016). In the tropical regions, cowpea is mostly cultivated by small scale farmers for food and income. It is mainly involved in mixed cropping farming systems with various cereals such as millet, sorghum, maize and some plantations (banana, plantain). Cowpea also has the capacity to fix atmospheric nitrogen through a compatible symbiosis with the developed root nodules and the soil bacteria (Rhizobium). According to Singh, (2003), cowpea grows well in soils with poor conditions (more than 85 % sand with less than 0.2 % organic matter and low levels of phosphorus). Therefore, the crop is regarded as multifunctional, providing food for man and animals, nourishing the soil and also generating earnings for producers and traders. However, the general production and yield around the world is low. It is estimated that the world cowpea total grain yield is about 3 million tons on 12.5 million hectares annually with west and central Africa regions producing about 74% of the estimated grains produced annually (FAO 2021). Ghana's production area is mainly in the northern regions and some part of the coastal savannah. Cowpea production in the northern Ghana is reaching a maximum of 46% of its potential (MOFA, 2016). According to studies by ACDI/VOCA 2020 (acdivoca.org), the low production is mainly as a result of low application of good agricultural practices and lack of high-quality seeds. Several methods have been adopted for improving yield in crops and induced mutation is one of them. Induced mutation increases genetic variations leading to the release of many developed cultivars with improved economic traits (Horn and Shimelis, 2013). Artificial induced mutagenesis increases the amount of genetic variability in crops. Genetic variability in an existing crop is very important for the improvement of the crop. Knowledge of genetic variability in the existing plant material and association among the various characters is a vital step for yield improvement (Vijayan 2005 as cited by Ajayi and Adesoye, (2013)). Induced mutation causes changes in the genome of an organism resulting in visible changes in

both qualitative and quantitative plant characters. According to Alan (2007), induced mutations play prominent role in changing the genetic make-up of an organism not only at a chromosomal but also at a molecular level creating variability in the existing genotype. To improve desirable traits of economically important plants, induced mutation in both seeds and vegetatively propagated crops is a technique used.

1.2 Problem Statement

While some plants species of cultivated crops have rich genetic diversity, others such as cowpea have very limited genetic variation (Shu, 2009). Cowpea is a self-pollinating crop and its fertilization reproduction process completes before the flower opens. This cleistogamic reproductive nature of the crop has restricted its inherent variability making breeders over exploit the existing variability. Many cultivars developed by breeders are based on exploiting the limited genetic variation. This has made the productivity of the crop lower as compared to other pulse crops (Nair and Mehta, 2014)

Cowpea's economic yield is low although most of the varieties have high biological yield. Ghana is among the lowest in the world in cowpea production (Ofosu-Badu *et al*., 2007). Ghana imports 10,000 tons of cowpea every year (Gomez, 2012). About thirty percent of the Ghanaian imports come from Burkina Faso and the rest from Nigeria and Niger.

1.3 Justification

Based on the limited genetic variations in cowpea, there is the need to conduct this study. Inducing genetic variability in the crop by chemical mutagen (EMS) is an important step to enhance level of variability in the crop. Ethyl methane sulfonate causes random mutation in an organism and it has the potential of hitting almost every gene in the genome of an organism. The variability assessed in the mutant generations would serve as platform for breeders to select wide range of desirable traits. Gregory (1955) postulated that artificial induction of genetic variability is one of the quickest methods for enhancing genetic variation in crop plants.

Genetic variability is what is needed for crop improvement (Novak and Burnner, 1992; Aliero, 2006; Bolbhat*et al*., 2012). This is because mutation and subsequent recombination created variability existing in all organisms including crop plants (Essel *et al*., 2015). The high level of variability induced by a chemical mutagen depends on the treatment conditions.

Lethal dose 50 (LD50) is the dosage concentration of a mutagen that causes 50% lethality out of the total number of seeds treated. Determination of LD50 of EMS in the current study is an important step in inducing genetic variability in cowpea. The frequency of viable mutations depends on the concentration of mutagen used. The LD50 when obtained in this research would enhance the level of variations. It can also be used in other mutation breeding programs of cowpea. According to Arisha *et al.,* (2014), determination of LD50 is required for producing high frequency of mutations. Characterization and evaluation of germplasm is an important aspect of breeding programs because it aids in selection of desirable traits. Characterising the M_1 and M_2 generations in this study would play a crucial role in detecting changes in the features of the '*Asontem*' cultivar to improve on it.

The study of associations among qualitative traits and pairwise correlation among quantitative traits is very useful in plant breeding programs. The associations among traits are mostly due to genetic linkage or pleiotropy, thus, mutation induced by EMS in this study in a certain gene may have effect on some or all associated traits simultaneously. This makes selection of one trait affecting the other associated ones. Selection of novel genotypes and high yielding mutants can also be incorporated into breeding programs for the development of cowpea.

1.4 Main Objective

The main objective of this research was to assess genetic variability in EMS chemically mutagenized population of cowpea (*Vigna unguiculata* L. Walp Var. Asontem) in the M_1 and M_2 generations.

1.4.1 Specific Objectives

The specific objectives of this study are to:

1. determine the lethal dose of EMS that cause 50% lethality in cowpea (LD50)
2. characterize M_1 and M_2 generations of EMS mutagenized populations of Cowpea
3. determine associations among morphological traits studied
4. identify and select mutants with higher performance in terms of yield and sub-yield characters

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History, Origin and Distribution of Cowpea

Cowpea (*Vigna unguiculata* L. Walp.) (2n=2x=22) belongs to the Phaseoleae tribe of the Fabaceae family. Many economically important pulses such as soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), and mungbean (*Vigna radiata*) belong to the Phaseoleae tribe (Timko *et al.*, (2007). According to Timko *et al.*, (2007), the name cowpea would have derived from the fact that the crop was used as feed for cows. Some important local names for cowpea around the world include "niebe," "wake," and "ewa" in much of West Africa and "caupi" in Brazil. These names reflect the traditional ways of how the people utilized the crop.

Cowpea is among the most ancient crops. The crop is believed to have originated from Africa somewhere near Ethiopia and afterwards was cultivated mainly in the farms of the African Savannah. (Duke, cited by UC SAREP). Due to lack of archaeological evidence, there has been contradictory views which supports Africa, Asia, and South America as origin (Coetzee, 1995; Tindall, 1983). Cowpea is widely adapted and cultivated all over the world (Aveling, T., 1999) with Africa being the continent with the highest production (about 74% produce coming from the region. FAOSTATS, 2013). The crop geographically ranges from Warm Temperature Thorn to Moist through Tropical Thorn to Wet Forest Life Zones. Hall *et al.*, (2002), stated that, cowpea is more adapted to high temperatures and drought compared to other crop species. There has been 1000 kg/ha of dry grain that has been produced in a Sahelian environment with only 181 mm of rainfall and high evaporative demand (Hall and Patel 1985). Cowpea dwells in hot environment with very little rainfall and low humidity. According to (Van Rij, N., 1999), long taproot and mechanisms such as turning the leaves upwards to prevent them from becoming too hot and closing the stomata, gives the cowpea an excellent drought tolerance. The cultivars of other crops available today cannot dwell in these conditions. Cowpea grows

well in low fertile soils because of its ability to fix atmospheric nitrogen through the effective symbiosis with mycorrhizae as compared to other crops. Obviously, the crop dwells in both favourable and harsh conditions.

2.2 Variability Study in Cowpea

Cowpea shows different qualitative and quantitative genetic features which are heritable and vary among cowpea in diverse manner. According to Amoatey (1987), variability studies in seed colour, pod length, number of seed per pod have been observed in some cowpea accessions in Ghana. Asante (1991) conducted a study on variability in flower bud tip and sepal, pigmentation of unripe pod tip, seed eye pattern and coat colour, flower colour and paleness in chlorophyll. Doku (1970) also studied the variability in local and exotic varieties of cowpea. He reported that there is an association between pod length, fruiting period and seed yield. Spillman (1913) observed that all varieties having coffee-colour or white or cream-colour seed had white flowers and devoid of anthocyanin in stem and leaves.

2.3 Cowpea Yield Attributing Characters and Character Association Studies of Cowpea

Studies conducted by Tamilselven and Das (1994) reported that in the improvement of high yielding cultivars of cowpea, quantity of clusters, number of pods per plant and 100 seed weight have to be utilized. Previous work by Aravindan and Das (1996) in cowpea had significant association between green seed yield and leaflet width and number of branches. Earlier studies conducted by Naidu *et al*. (1996) also revealed that number of pods per plant, number seeds per pods, hundred seed weight and seed yield per plant were higher in determinate genotypes, whereas in unclear varieties pod length was higher.

The degree of observed association between two traits can be referred as direct, aggregate phenotypic relationship. The measure of ecological impact on the co-variation between the two traits is also known as natural relationship. Previous study by Patil *et al*. (1989) revealed associations that existed between seed yield and pods per plant, 100 seed weight, pod length

and days to 50 % flowering during diallel cross which involved ten different indigenous lines and accessions of cowpea. Siddique and Gupta (1991b) also reported an association between days to 50 % flowering and seed yield per plant, number of pods per plant and days to maturity. Earlier work by Oseni *et al*. 1992 reported a positive correlation between seed yield and pods per plant, between number of days to 50% flowering and 100 seed weight and a negative association between days to 50% flowering and seed yield and between 100 seed weight, seed yield, days to maturity, 100 seed weight. Altinbas and Sepetoglu (1993) stated that both days to flowering and maturity had no associations with seed yield however, they observed positive associations between 100 seed weight and pod per plant and seeds per pod. Sawant (1994) also observed significant associations among seed yield and branches per plant, inflorescence per plant, pod per plant, pods length, seeds per pod, 100 seed weight and harvest index. During the study of three F_2 populations by Birader *et al*. (1996) there were significant correlations between pod weight per plant and seed yield, number of clusters and number of pods per plant, pod length and number of seeds per pod. Belhekar *et al*. (2003) also reported by evaluating F_2 generations, positive and significant correlations between seed yield per plant and plant height, number of flowers per plant, first pod growth and complete development, number of pods per plant and 100 seed weight both at the phenotypic and genotypic levels. Previous investigation by Venkatesan *et al*. (2003) revealed that number of branches per plant, number of clusters per plant, number of pods per plant, number of pods per cluster and pod yield were significantly associated with seed yield at the hereditary and phenotypic levels when association and path analysis were computed using observations from 20 genotypes of cowpea. Recent studies by Owusu *et al.*, (2021) showed positive and significant correlations between number of pods per plant and number of seeds per pod, pod weight and grain weight and between days to flowering and days to 90% pod maturity and 100 seed weight. Many characters of cowpea are associated and this might be due to pleiotropy or genetic linkage.

2.4 Cowpea Production and Economic Importance

Cowpea production around the globe is estimated to be on 12.5 million hectares with total grain yield of about 3 million tons annually (FAOSTAT, 2020). West and central Africa regions produce about 74% of the estimated grains produced annually which make them the largest production regions in the world. In West Africa, Nigeria, Burkina Faso, Niger and Ghana are the major producers and consumers, contributing approximately 69% of the total production in world (FAOSTAT, 2020).

In Ghana, cowpea is the second largest cultivated crop in terms of area under cultivation and quantity produced and consumed annually (Egbadzor *el al.*, 2013). Ghana's production area is mainly in the northern regions (Guinea savannah zone and Sudan savannah zones) and some part of the coastal savannah. Some areas in the transitional zones of Ashanti., Ahafo and Brong regions also serve as production areas in Ghana. Cowpea production in the northern Ghana is reaching a maximum of 46% of its potential (MoFA, 2017). Ghana has an average production rate of about 206,380MT per 146,600 ha making Ghana the fifth highest producer of cowpea in Africa (MoFA,2017).

Whiles cowpea remains the second most important food legume in Ghana (Egbadzor *el al.*, 2013), its consumption is higher than its production. Farmers do not have access to robust and high yielding varieties. Cowpea is an underexploited crop and little research has been done on the crop (Timko and Singh, 2008).

Insect pests are known to be major constraint to cowpea production in Africa (Adipala *et al.*, 2000; Asante *et al.*, 2001; Makoi *et al.*, 2010). Main pests in the growing fields of cowpea are the aphids (*Aphis craccivora*), thrips (*Megalurothrips sjostedti*) and Maruca pod borer (*Maruca vitrata*). Aphids attack the soft leaves and branches of cowpea plants resulting in poor growth and death of the plants. Maruca pod borer feed on developing pods and insert their bodies into the pods. According to Egbadzor *el al.* (2013), farmers around Ho confirmed pest and diseases attack as a major problem in the area.

Another major constraint to cowpea production in Ghana is drought. Although the Guinea savannah zone is the major production area, production can be done only within a short period in the year in these regions because of long period of drought. Coastal areas in Ghana are also known to receive very little rain in the year. Although cowpea is a short season crop with relatively high drought tolerance ability (Muchero *et al.*, 2009), farmers around Akatsi in the Volta region have to wait for rains before planting which delays production (Egbadzor *el al.*, 2013). Weed attack is also a limiting factor to cowpea production. *Cyperus spp.* is one of major limitations for farmers around the coastal savannah. Other limitations to cowpea production includes grain quality (easy to cook, storability, seed size and coat colour) and labour especially during harvesting.

Cowpea serves as quality protein for human and animals especially for resource poor families. According to Khalid and Elharadallou (2013), cowpea is regarded as nutrient rich food with low energy density. Its grain may contain 23-32% of protein17, 50-60% carbohydrate and about 1% fat (Kirse and Karklina, 2015) in dry basis. The total protein content of cowpea is greater than cereal and tuber crops; about 3 to 4 times higher. (Sebetha *et al.*, 2014 and Trehan *et al.*, 2015). Proteins derived in cowpea are also rich in lysine and tryptophan as compared to cereal grains making it a natural supplement food with carbohydrates such as tubers and cereals (Gonçalves *et al.*, 2016). But cowpea protein is low in amino acids like methionine and cysteine compared to animal proteins (Petchiammal and Hopper, 2014). Cowpea provides nutrients such as phenolic compounds, minerals, soluble and insoluble dietary fibre, and many other functional compounds which promote health. (Liyanage *et al.*, 2014 as cited by Jayathilake *et al.*, 2019). Cowpea contributes significantly towards alleviating lack of protein-energy malnutrition in children in areas where major food sources are carbohydrates.

Some cowpea cultivars are utilized in weed and soil erosion control due to their ability to grow extensively providing full ground cover. The root system of the crop has high ability of associating with the different species of *Rhizobia* bacteria in the soil to fix atmospheric nitrogen

and due to this, cowpeas are grown in rotation or mixed with many cereals and tuber food crops. Cowpea provides income for many rural farmers and traders in Ghana through cultivation of the crop, processing, and sales of cowpea products.

2.5 Mutagenesis and Crop Improvement

2.5.1 Mutation

Mutation is considered as the changes that occur in the genetic makeup of an organism. On the other hand, mutation breeding refers to the deliberate changes that occurs in an organism to create, harness desired and valuable traits, in the quest to develop elite breeding lines. Mutation breeding can also be referred to as variation breeding. Mutation can occur either under the influence of human activities (induced) or without human intervention (spontaneous) (Harten, 1998; Mba, 2013). A successful mutation breeding process can be achieved through appropriate means of inducing such changes in a plant and to detect the desired traits among various mutants produced. Mutation induction involves the means of causing genetic changes through the exposure of the seeds or propagules to either high radiation source (physical) or chemical mutagens (Ahloowalia and Maluszynski, 2001; Schaart and Visser, 2009). However, variation caused due to mutation can be classified into point mutation, chromosome mutation and genome mutation (Shu *et al.*, 2012). These changes occur as a result of the influence of mutation activities on deoxyribonucleic acid (DNA).

The production of sustainable food will always be a challenge in years to come. Today, more than 800 million people do not have access to sustainable food. According to the report by IAEA, (2014), the global population is expected to increase from seven billion people to 9.8 billion by 2050 and to feed everyone, farmers will by then have to produce 50% more food. Crop mutation breeding and the breeding of improved crop varieties with other technologies are important factors in curbing this future demand.

2.5.2 Induced Mutation

In the genetic improvement of crops, molecular plant breeders have the option of studying natural variations or induced variations. The induction of mutations involves the artificial processes of subjecting an organism (plant, animal, microbes, or any living system) to physical and chemical mutagens in other to create variations at the molecular level which can be observed as physical characters and may be stably inherited. Chemical mutagens such alkylating agents (ethyl methane sulfonate and methyl methane sulfonate), colchicine, sodium azide as well as physical mutagens, such as ionizing radiations have been used to increase the frequency of variations and mutations in organism (Predieri, 2001). Chemical mutagens are more efficient as compared to the physical mutagens. This is because chemical mutagens such alkylating agents cause point mutation which mostly results in a base pair change (GC →AT change) which are trackable and therefore useful in gene mapping and trait phenotype association studies. The change in base pair may affect expression of gene resulting to variations in characters of traits. Physical mutagens on the other hand cause deletions of part of DNA or chromosome. The random deletions caused by physical mutagen may result in deleting the gene of desirable traits thereby abolishing the function of the gene. According to Bhat et al. (2005), chemical mutagens enhance genetic variability in plants for successful breeding programs. Previous studies by Adamu and Aliyu (2007) reported that chemical mutagen induced a wide range of variation in morphological traits when compared with normal plants.

Induced mutation with chemical mutagen is a simple technique used for creating mutations in plants for the improvement of potential agronomic traits especially traits with low level of genetic variation. Chemical mutagenesis, therefore, has been proved as fundamental in the improvement of crop plants (Szarejko and Forster, 2007). In the study of genes, chemical mutagenesis is regarded as a vital tool employed. Thus, it helps in the production of raw materials for genetic improvement of crops.

2.5.3 Spontaneous Mutation

Basically, spontaneous mutation is the process whereby new genetic traits occurs in an organism by nature. Spontaneous mutation may result from the activity of transposons (mobile genetic elements) usually referred to as "jumping genes". Wessler, (2006) postulated that transposons can randomly insert themselves in different positions within the genome of a single cell and affect the functions of gene in which they are inserted.

Spontaneous mutations at the molecular level can be caused by these processes; tautomerism, depurination, deamination and slipped strand mispairing. Tautomersim occurs during replication in cell division. The change caused by this process is as result of the relocation of hydrogen atom leading to the changes in the hydrogen bonding patterns of the base. Depurination is the loss of a purine base (Adenine or Guanine) to form apurinic site (AP site). Deamination also occurs when the amine group of a base is replaced by a keto group as a result of hydrolysis. Examples include C → U and A → HX (hypoxanthine), which can be corrected by DNA repair mechanisms; and 5MeC (5-methylcytosine) → T, which is less likely to be detected as a mutation because thymine is a normal DNA base.

During replication, there is the denaturation of the new strand from the template followed by renaturation in a different spot leading to slipped strand mispairing. This process leads to insertions and deletions.

2.5.4 Plant Mutation Breeding

About ninety years ago, L.J. Stadler demonstrated the induction of mutations in barley and maize by using x-rays and radium (Stadler, 1928a and 1928b). This was the birth of mutagenesis in plants for improvement. According to IAEA (2014), plant mutation breeding is the process of treating planting material (seeds, cuttings, corms) and cell cultures with physical or chemical mutagens at a specific dose and then cultivating the mutagenized material. Mutation breeding relies on the implementation of either physical or chemical agents in order

to create variability in the population of interest. Mutations created by mutagens are incorporated in crop improvement programs. Crops that are obtained in mutation programs are readily accepted for public consumption (Jain 2007). About 3500 varieties of crop plants have been developed around the globe by mutation breeding (IAEA, 2021). Currently, the use of mutagens in crop improvement is being practiced in almost every part of the world; Asia, Northern America, Latin America, Africa and Europe.

During the process of mutagenesis, the genetic material (seeds) before mutagen treatment and after treatment are referred to as M_0 and M_1 respectively. Plants that are produced from the treated materials are also termed as M_1, which is followed by M_2, M_3, and M_4. In order to improve a crop, the mutated plants with improved agronomic traits that are obtained after selection from several generations, are then multiplied and examined for their agronomic performance. The selection of mutants with desirable traits usually starts in the M_2 generation. Mutants selected are usually called putative mutants (not truly mutants). Selected mutants are further evaluated in the subsequent generations for confirmation.

2.5.5 Importance of Mutation Breeding in Agriculture

One of the main challenges faced by decision making leaders around the world is the need to address the issue of famine and malnutrition in view of the rise in population (Cavagnaro *et al.*, 2011). Much attention is focused on how to provide highly nutritious and affordable foods to population since there has been a maximum utilization of land and other resources. Therefore, breeders identified simple ways of improving existing crop species, obtaining variabilities and producing food in abundance through mutation. Mutation breeding is seen as low cost and simplified technology in harnessing desired varieties among plants (Oladosu *et al*., 2016). Most researches have documented on improved traits such as high yield, change in plant architecture, resistance to diseases and insects being obtained from mutation induction (Ahloowalia *et al*., 2004; Haq *et al*., 2001).

The main objective of mutation breeding is to increase food production and provide sustainable nutrition (Goyal *et al*., 2009). Scientists can use mutagens to shorten the time it takes to develop new and improved plant varieties. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars in cereals, fruits and other crops (Lee *et al.*, 2002). IAEA (2014) stated that plant mutation breeding is not gene transformation process rather the plant's own genetic resources are used to mimic the natural process of spontaneous mutation which is the cause of evolution.

Previously, plant breeders used conventional breeding in producing crop species. With conventional breeding much focus was on the ability to select and cross plants with traits desired by farmers (Acquaah, 2016). In contrast, this approach takes a long period of time before traits of interest could be identified whilst mutational breeding is concise and takes a short period to obtain mutants carrying specific traits (Brock, 1971; Oladosu *et al*., 2016) Mostly, crops carrying specific traits including important agronomic traits are identified in the next two generations. Therefore, breeders identified mutational breeding to be a less time consuming, a precise way of improving and obtaining variability in crops compared to the various conventional breeding methods. Induced mutagenesis has led to increase in food supply and improved upon the livelihood of many small scaled farmers (Mba, 2013; Pathirana, 2011). Moreover, induced mutation has led to the creation of diversity in various crop species which serves a breakthrough from ancient breeding techniques (Novak and Brunner, 1992). Also, induced mutagenesis has provided a milestone from which important breeding work can continue (Martín *et al*., 2009).

Some of the lost alleles and useful variability in plants can be possibly recovered using mutation breeding approach. Mutation techniques employed in plant breeding programs have contributed significantly to crop improvement worldwide, and have made positive effect on productivity and economic value of some legume crops (Shanko, 2017). Mutation breeding technique has played a major role in generation of climate smart varieties. These crop varieties

have been shown to withstand wide range of environmental fluctuation. Globally millions of hectares of cultivated lands have been devoted for the cultivation of these mutant crop varieties and in turn billions of revenues have been generated (Jain, 2010).

2.5.6 Limitations of Mutation Breeding

Mutagens have the tendency of reacting with DNA as a result of causing changes in nucleotide sequences. But each mutagen has different mode of action. Also, a mutagen may effectively cause mutations, but its efficiency may be decreased by the undesirable effects like lethality or sterility associated with it. Therefore, in crop mutagenesis studies, much work must be done on the efficiency and efficacy of the mutagen on a particular crop in order to obtain high frequency of desirable mutations.

In crop mutagenesis, there is very low frequency of desirable mutations out of the total mutations. Therefore, for a successful identification of desirable mutant, large number (several thousands) of M_2 and other subsequent generations must be screened. This is laborious and time consuming. Efficient and quick selection techniques are needed to screen large populations and these are very expensive to employ.

Most often induced mutations results in pleiotropic effects. One way to reduce or remove pleiotropic effects is by transferring the gene into different genetic backgrounds by hybridizing the mutant with a range of other elite varieties. Mutations induced in quantitative traits are normally in the direction away from the selection history of the parent variety example yield and this makes the selection of hybrids of the desirable traits very difficult.

2.5.7 Effect of Ethyl Methane Sulfonate (EMS)

EMS is an alkylating agent responsible for causing point mutation in organisms by means of base pair substitution. A base-pair substitution occurs through the reaction of the mutagen with three of the four bases (Brookers, 1960). Alkylation of phosphate group by EMS might result in the breaking of the phosphate backbone from the DNA. Base substitution emerges through

a means known as transition or transversion (Freese, 1961; Krieg, 1963). Ethyl methane sulphonate induces variations in crop species by the alkylation of nitrogenous base, guanine at the position causing abnormal replacement of cytosine with thymine. The effect of this substitution results in a mispairing of guanine with thymine instead of adenine with thymine (Krieg, 1963). Substitution caused does not affect the reading frame of the double helix but an alteration in the codon.

Mutation induction with chemical mutagen like EMS produces functional mutations with a high probability of producing dominant traits in the M_1 generation which can be inherited in the next mutant generation (Arisha *et al.*, 2015; Shu *et al.*, 2012). Induced mutation has shown impressive results in crop improvement of diverse crops. Therefore, EMS as an alkylating agent causes viable mutations and chlorophyll mutations in cowpea different from their wild species (Gnanamurthy and Dhanavel, 2014). EMS causes point mutations hence can be used in forward and reverse genetics technique to detect site of variation and identifying gene function in the gene of interest (Greene *et al.*, 2003).

Auerbach *et al.*, (1947) suggested that, for a chemical mutagen to be considered as effective, it must be highly specific in its action and not destroy the chromosome carrying that particular gene. Therefore, chemical mutagen like EMS is chosen over physical mutagens due to simplicity and specificity in inducing mutation in various crop species (Drake, 1969). Ethyl methane sulphonate is usually used in mutagenesis due to numerous benefits it possesses.

2.5.8 Ethyl Methane Sulfonate Mutagenesis in Cowpea

EMS has been highly effective and efficient in producing diversity in agronomic traits of different varieties of legumes including cowpea. The efficiency of an EMS mutagen describes the rate at which the mutagen is able to induce desirable effects, which can be either negative or positive. Various mutagens including EMS are known to induce morphological mutation such as chlorophyll mutation and viable mutation (Gnanamurthy and Dhanavel, 2014). Induced mutation has an effect on the lethality and germination rates, chlorophyll content, viability and

other factors adding up to the improvement on the morpho-agronomic nature of cowpea plant (Dhanavel and Girija, 2009; Dhanavel *et al*., 2008). Bind and Dwivedi, (2013), in their study reported decrease in percentage germination, percentage plant survival and increase in pollen sterility in cowpea treated with doses of ethyl methane sulfonate (EMS).

Gnanamurthy and Dhannavel, (2014) reported a wide range of mutations in cowpea cultivar treated in different doses of ethyl methane sulfonate which included variations in plant height (very tall and dwarf plants observed), flower colour (changed from white to pink), leaf abnormalities and increasing number of pods. There was also wide spectrum of chromosomal mutations observed in the mutagenized population. Important traits including early maturing, high protein content, high yield and resistance to biotic stresses were observed in different cowpea cultivars when induced with ethyl methane sulphonate at different concentration (Odeigah *et al*., 1998). A study conducted by Nair and Mehta (2014) reported variability in cowpea mutagenized population as a result of the effect of EMS. They reported mutations affecting morphological changes in leaf morphology, plant, habit, flowers, pods, days to flowering and maturity, yield characters, seed coat colour, size and shape.

Various EMS concentrations produce wide range of mutation which is used to determine mutation frequency. Mutation frequency that occurs in a particular gene change from one plant to another. Mutation frequency can range from 1 mutation per Mb in barley (Caldwelletal., 2004) to 1 mutation per 170 kb in *Arabidopsis* (Greene *et al*., 2003). The frequency of viable mutations will depend on the optimum treatment condition used. The dose of EMS concentration and time taken have impact on the viable mutations produced by EMS. In order to produce a high frequency of desirable mutations in a crop, it is very important to determine the lethal dose 50 (LD50) (Hohmann *et al*., 2005; Arisha *et al*., 2014). LD50 is the EMS concentration that contributes to the 50% lethality of the total number of genetic materials that are subjected to the mutagenesis treatment.

The functional mutations in genes induced by EMS as a result of point mutation have a greater chance of being dominant or co-dominant mutations. During the M_1 generation, only dominant or co-dominant mutations are easily detected by visualization (Shu *et al.*, 2012). Some viable mutations in the M_1 generation include reduction in plant height, pollen sterility, late or early flowering, leaf chlorosis and curled leaves, occurrence of irregular leaf structure.

During the M_2 generation, there is segregation of mutated genes which leads to homozygotes for recessive and dominant alleles (Page and Grossniklaus, 2002). The segregation is due to the self-pollination of the M_1 individuals. At this time, visual screening is the method used to identify phenotypic mutations. Mutants with desirable traits are selected and further evaluated and advanced in the M_3 and M_4 generations.

CHAPTER THREE

3.0 MATERILAS AND METHODS

3.1 Experimental Site

The project was carried out at the University of Ghana farms, Legon. This area is coastal savannah zone of the country's agroecological zones. Amount of rainfall in a year is about 900 mm

3.2 Experimental Materials

Cowpea variety ('*Asontem*') was used for the study. IT82E-16 ('*Asontem'*) is a cultivar that was developed by International Institute for Tropical Agriculture (IITA), and released by Council for Scientific and Industrial Research-Crops Research Institute, Ghana. "*Asontem*" has intermediate growth pattern with days to maturity ranging from 70 to 76. The leaves are narrow (hastate) and the growth habit is intermediate. The *Asontem* seeds were obtained from the West Africa Centre for Crop Improvement, University of Ghana (WACCI) gene bank.

3.3 Sensitivity Test for Determination of Lethal Dose 50 (LD50)

Cowpea seeds were mutagenized with different EMS concentrations (0.0%, 0.2%, 0.4%, 0.6% and 0.8%). Each concentration had 100 '*Asontem*' seeds. Seeds were soaked in the solutions for 16 hours. After that, the chemical reaction and activity of the EMS was terminated using sodium thiosulfate solution to neutralize and stop the reaction by inactivating the EMS. The treated seeds were plated in petri dishes and number of seeds germinated were observed after 5 days.

3.4 Generation of M_1 Seeds

Three thousand cowpea (*Asontem*) seeds were treated with 0.4% EMS concentration. The variability of the seeds was generally low, however, obtaining new *Asontem* seeds was difficult

so the available seeds were used for this experiment. The 0.4% concentration was prepared by adding 2.2 ml of EMS to 547.8 ml of distilled water. M_1 seeds were obtained after soaking seeds in EMS concentration for 16 hours based on the protocol for EMS mutagenesis. Five hundred seeds were soaked in 0.0 % EMS concentration (distilled water) which served as control (Wild type).

3.5 Evaluation of M_1 Population

3.51 Experimental Layout

The wild type (non-treated seeds) and mutagenized seeds were sown on the field in a single row plot design. A total of nineteen rows were established for the study; sixteen rows comprised the mutagenized seeds and three rows were made of the control (which served as wild type).

3.5.2 Field Establishment

The experimental field was ploughed, harrowed and pegged for demarcations of blocks and plots. The field was divided into two blocks and each block had 10 rows. Glyphosate, a pre-emergence herbicide was applied on the field before planting to control weeds. Seeds were sown at 3 seeds per hill with four rows of mutant lines and 1 row of wild type in a sequential manner. The planting distance used was 0.75m inter-row and 0.3m intra- row. A total of sixteen rows and three rows were designated to the mutagenized population and wild type respectively. Wild type seedlings were thinned out to one plant per hill in the fourth week after planting. Agronomic practices such as weed control, irrigation and pest control were carried out in the field.

3.5.3 Planting of M_1 Plants

Seeds were sown on 18th October, 2019.

3.5.4 Morphological Characterization of M_1 Generation

The morphological characteristics studied were quantitative and qualitative traits. Quantitative and qualitative data on the following traits were collected based on the Cowpea Descriptor by International Board of Plant Genetic Resources (IBPGR) (1983).

Qualitative data collected were: plant pigmentation, growth habit, flower colour, terminal leaflet shape, pod shape/curvature, pod colour, seed shape and seed coat colour.

Quantitative data collected comprised: chlorophyll content, days to flowering, days to first pod maturing, number of pods per plant, pod length, number of locules per pod, number of seeds per pod and seed weight (100 seeds).

3.6 Evaluation of M_2 Population

3.6.1 Experimental Materials

M_2 seeds derived from the M_1 population were used. Three hundred and nineteen (319) mutant lines (M_1 parents) were used and twenty seeds were obtained from each mutant line. A total of 6,380 M_2 seeds and 100 wild type seeds were used for this experiment.

3.6.2 Experimental Layout

The wild type (non-treated seeds) and M_2 seeds were sown on the field in a single row plot design. A total of three hundred and twenty-four (324) rows were established for the study; three hundred and nineteen (319) rows comprised the M_2 lines and five (5) rows were made of the wild type.

3.6.3 Field Establishment

The experimental field was ploughed, harrowed and pegs were used in making demarcations. The field was divided into five (5) blocks and each block was made up of 65 rows. Each block had 64 rows of M_2 lines and one row of the wild type except the last block which comprised

63 rows of M_2 lines and one row of wild type. The rows were made up of twenty seeds from each mutant line (parent). Seeds were sown at one seed per hill. The planting distance used was 0.75m inter-row and 0.3m intra- row. The rows in each block were randomized to contain the mutant lines and the wild type. A total of three hundred and nineteen rows and five rows were designated to M_2 lines and control respectively. Agronomic practices such weed control, irrigation and pest control were carried out in the field.

3.6.4 Planting of M_2 Population

Seeds were sown on 25th September, 2020 at the University farms, Legon.

3.6.5 Morphological Characterization of M_2 Generation

The morphological characteristics studied were quantitative and qualitative traits. Quantitative and qualitative data on the following traits were collected based on the Cowpea Descriptor by International Board of Plant Genetic Resources (IBPGR) (1983).

Qualitative data collected and analyzed were: terminal leaflet shape, plant pigmentation, leaf marking, growth habit, leaf colour twinning tendency growth pattern, pod shape/curvature, pod colour, seed coat colour and seed shape.

Quantitative data collected and analyzed comprised: percentage germination, percentage survival, germination speed, days to flowering, number of pods per plant, pod length, number of locules per pod, number of seeds per pod, percentage seed set and number of seeds per plant.

Germination percentage was found by observing the emergence of the coleoptile at the surface of the soil. The total number of seeds germinated in each treatment was recorded after 21 days of planting.

Germination percentage was obtained as; % germination = $\frac{number\ of\ \ seeds\ germinated}{total\ number\ of\ seeds\ planted}\ x\ 100\%$

Germination speed was also calculated as described by Maguire (1962): **GS** = $\sum_{j}^{i} \frac{ni}{Di}$

ni is the number of seeds germinated on the i^{th} day and ***Di*** is the number of days after planting.

Percentage seed set was calculated as; % seed set = $\frac{number\ of\ \ seeds\ per\ pod}{number\ of\ locules\ per\ pod}\ x\ 100\%$

3.7 Selection of Putative Mutants

Regarding each character evaluated, the performance of the wild type was determined. Mutants or plants differing from the wild type plants with regard to each character studied was identified by simply comparing it with that of the control or wild type character. A test of statistical significance was then carried out to highlight the difference.

3.8 Statistical Analysis

Frequency distribution of qualitative variables in both the wild type and the mutagenized populations were computed using STATA statistical software version 14 (StataCorp., 2015). Distributions of quantitative traits in the populations were determined using R software version 4.0.1. R software was also used to compute principal component analysis and hierarchical cluster analysis using factoextra, factominer, cluster and dendextend packages. Pearson's correlation analysis among the quantitative variables was computed using R software and the results displayed in a heatmap (correlogram). Student T-test or Z-test was used to determine significant difference. Mutants with higher significant differences were determined using the general linear model prediction method for contrast analysis in STATA.

CHAPTER FOUR

4.0RESULTS

4.1 Sensitivity Test for Determination of LD50 (Lethal Dose 50)

The percentage germination ranged from 0.00 to 63.00% as described in **figure 4.1**. From the graph, percentage germination decreased as EMS concentration increased ($R^2 = 0.9543$). The highest percentage germination was recorded in the wild type and the lowest was recorded in EMS dose 0.8%. The EMS lethal dose 50 (LD50) was estimated to be approximately 0.4% concentration of EMS solution where 34% germinated. The 34% is approximately 50% of 63 (wild type individuals that germinated). The 63% germination in the wild type was assumed as 100% germination since there was no EMS treatment. Hence, 0.4% EMS was recorded as LD50 since the concentration caused 50% lethality comparatively with the wild type.

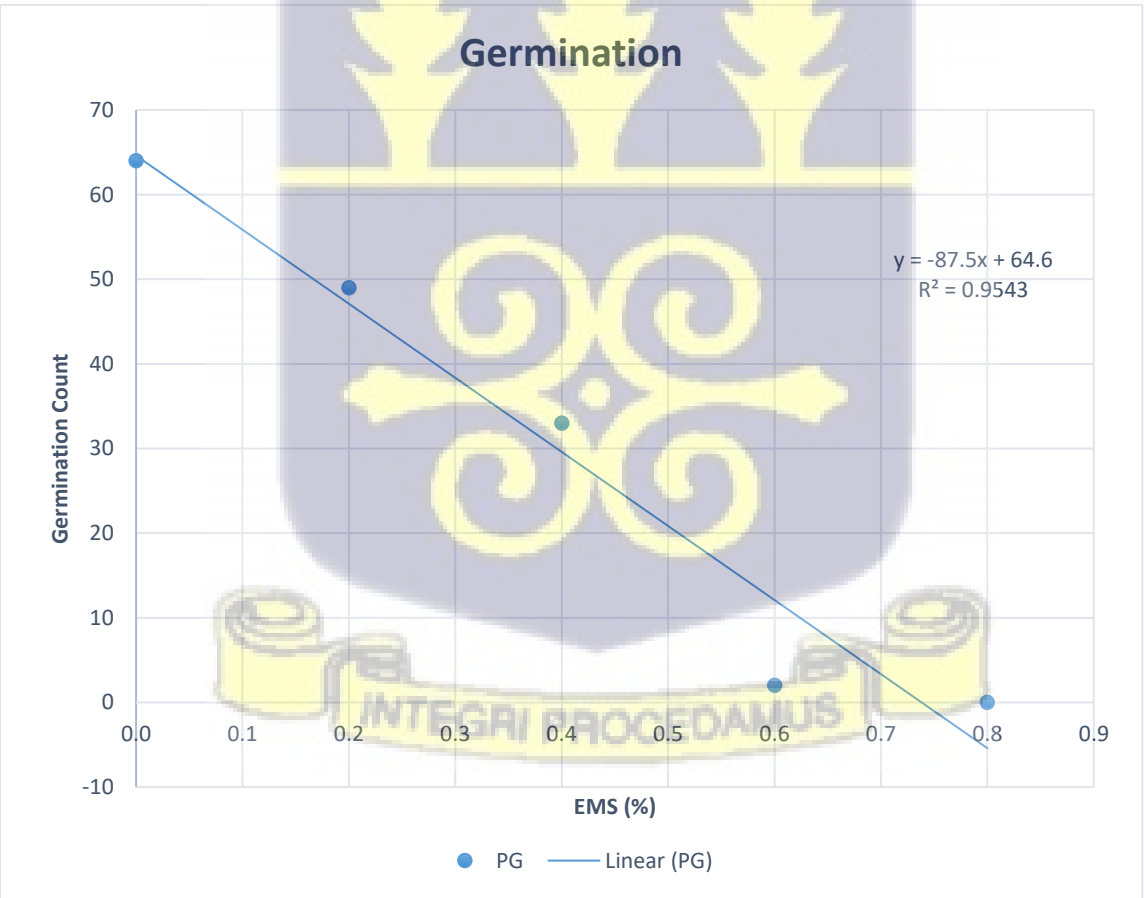

Figure 4.1: Germination percent (%) and line of best fit for estimation of the LD50 in *Asontem* cowpea genotype when subjected to five EMS doses for 16 hours. PG-Percentage Germination.

4.2 Percentage Germination and Lethality in the M_1 Generation

A total of 534 seeds germinated in the M_1 population out of the 3000 seeds sowed whiles 308 seeds germinated from the 500 wild type seeds (**Table 4.1**). The percentage survivals obtained in M_1 population and the wild type were 74.46% and 98.38% respectively.

Table 4.1: Percentage germination and percentage survival in the M_1 generation

EMS (%) Dose Treatment	Number of seeds sowed	No of germinated seeds	Percentage Germination	Percentage Survival
0.0	500	308	61.6	98.38
0.4	3000	534	17.8	78.46

4.3 Variability of M_1 Generation

4.3.1 Frequency Distribution of Qualitative traits in M_1 Generation

In the M_1 generation, most traits showed different descriptor states as the non-mutagenized population.

Stem pigmentation: There were five categories of stem pigmentation in the wild type and six categories in the M_1 population. The unique stem pigmentation described as solid was clearly absent in the natural control population but present in the induced mutant population. The frequency ranged from 4.61% (*very slight*) to 73.03% (*None*) in the wild type (**Table 4.2**). The highest frequency in the M_1 population was 78.91% (*None*) and the lowest was 2.61% (*Solid*) (**Table 4.2**).

Petiole pigmentation: Four different phenotypic classes were observed in the wild type whiles three classes were observed in the M_1 population. *Intermediate* class which was observed only in the wild type was the lowest (frequency =3.29%) and *None* class was the highest frequency (77.63%) in the wild type (**Table 4.2**). In the M_1 population, the frequency ranged from 4.50% (*Moderate*) to 84.60% (*None*). *Intermediate* class was absent in the mutant plants (**Table 4.2**).

Branch pigmentation: Branch pigmentation had six different phenotypic categories in both the wild type and M_1 population. Frequency distribution based on treatment ranged from 0.66% (*Solid*) to 39.47% (*Moderate*) for the wild type, while for M_1 population the range was between 1.66% (*None*) and 30.09% (*Moderate*) (**Table 4.2**).

Growth habit: There was only one category (*intermediate*) identified for this trait in the wild type, whiles seven categories were observed in the M_1 population. The six different categories that were present in the M_1 plants were absent in the wild type. The frequency ranged from 2.13% (*semi-erect*) to 31.75% (*Climbing*) in the M_1 population (**Table 4.2**).

Leaf shape: Only one phenotypic class (*Hastate*) was observed for this trait in both wild type and M_1 population (**Table 4.2**).

Flower colour: There was only one phenotypic category (*violet*) identified in both the wild type and M_1 population (**Table 4.2**).

Pod colour: Pod colour had only one phenotypic class (*Pale tan*) in both the wild type and M_1 population (**Table 4.2**).

Pod curvature: There was one category of pod curvature (*slightly curved*) in the wild type and three categories in the M_1 population. The highest frequency in the M_1 population was 37.68% (*slightly curved*) and the lowest was 25.12% (*curved*) (**Table 4.2**). The *curved* and *straight* categories of pod curvature were absent in the wild type.

Seed shape: Only one phenotypic class (*rhomboid*) was observed in the wild type whiles three classes were observed in the M_1 population. The different seed shapes that were observed only in the mutant population were *kidney* and *ovoid*. *Kidney* class had the lowest frequency (0.24%) and *ovoid* class had the highest frequency (77.59%) in the M_1 population (**Table 4.2**).

Seed coat colour: There was one phenotypic category (*walnut*) that occurred in the wild type whiles three different categories were obtained in the M_1 population. The different seed coat

colours described as *cream* and *chocolate* were observed in the M_1 plants. Frequency distribution ranged from 0.48% (*cream*) to 72.05% (*walnut*) for the M_1 population (**Table 4.2**).

Table 4.2: Frequency distribution of qualitative traits in M_1 generation

		frequency (%)	
Trait	**phenotypic class**	**Wild type**	**M_1**
Stem pigmentation	None	73.03	78.91
	Very slight	4.61	5.45
	Moderate	6.58	3.79
	Intermediate	7.24	5.69
	Extensive	8.55	3.55
	Solid	0.00	2.61
Branch pigmentation	None	15.13	28.91
	Very slight	5.26	4.98
	Moderate	39.47	30.09
	Intermediate	29.61	24.41
	Extensive	9.87	9.95
	Solid	0.66	1.66
Petiole pigmentation	None	77.63	84.60
	Very slight	12.50	10.90
	Moderate	6.58	4.50
	Intermediate	3.29	0.00
	Extensive	0.00	0.00
	Solid	0.00	0.00
Leaf shape	Hastate	100	100.00
Growth habit	Acute erect	0.00	7.82
	Erect	0.00	18.96
	Semi-erect	0.00	2.13
	Intermediate	100	12.09
	Semi-prostrate	0.00	12.80
	Prostrate	0.00	14.45
	Climbing	0.00	31.75
Flower colour	Violet	100.00	100
Pod curvature	Straight	0.00	37.20
	Slightly curved	100.00	37.68
	Curved	0.00	25.12
Mature pod colour	Pale tan	100	100
Seed shape	Rhombus	100.00	22.17
	Kidney	0.00	0.24
	Ovoid	0.00	77.59
Seed coat colour	Walnut	100	72.05
	chocolate	0	27.47
	Cream	0	0.48

4.3.2 Distribution of Quantitative Traits in M_1 Generation

4.3.2.1 Chlorophyll Content

From measurements of the chlorophyll content in the leaves of M_1 generation, the mutagenized population was widely distributed with some outliers as shown in **figure 4.2.** The wild type plants (*Asontem*) had a range of 20.9-51.00 mg/l for chlorophyll content and the mutagenized population had a range of 10.40-56.90 mg/l (**figure 4.2**). The median value obtained in the mutagenized population (36.45 mg/l) was slightly above the median value recorded in the wild type (35.70 mg/l).

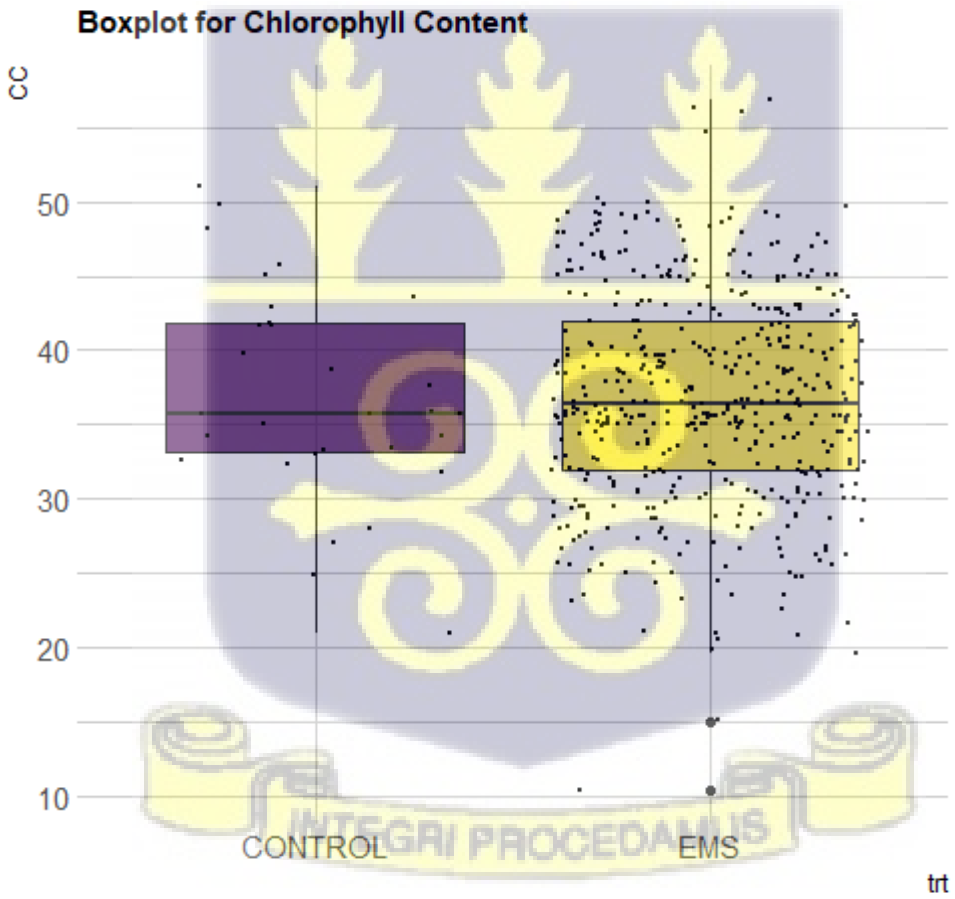

Figure 4.2: Distribution of chlorophyll content into 25th percentile, 50th percentile and 75th percentile in the wild type and mutant population. CC=Chlorophyll Content, trt=Treatment, EMS=Ethyl methane sulfonate. Black dots represent the dispersion and frequency distribution of the phenotypic data in each treatment.

4.3.2.2 Plant Height

There was high phenotypic variation for plant height (PH) in the mutagenized population of the M_1 generation whereas low variation was observed in the wild type (**figure 4.3**). The wild type had a range of 100 cm (from 100-200 cm) and the range of the mutagenized population was 180cm (from 20-200 cm) (**figure 4.3**). The individuals in the M_1 population were widely dispersed with a median value of 113 cm whereas 50% of the wild type were above 160.5 cm for plant height.

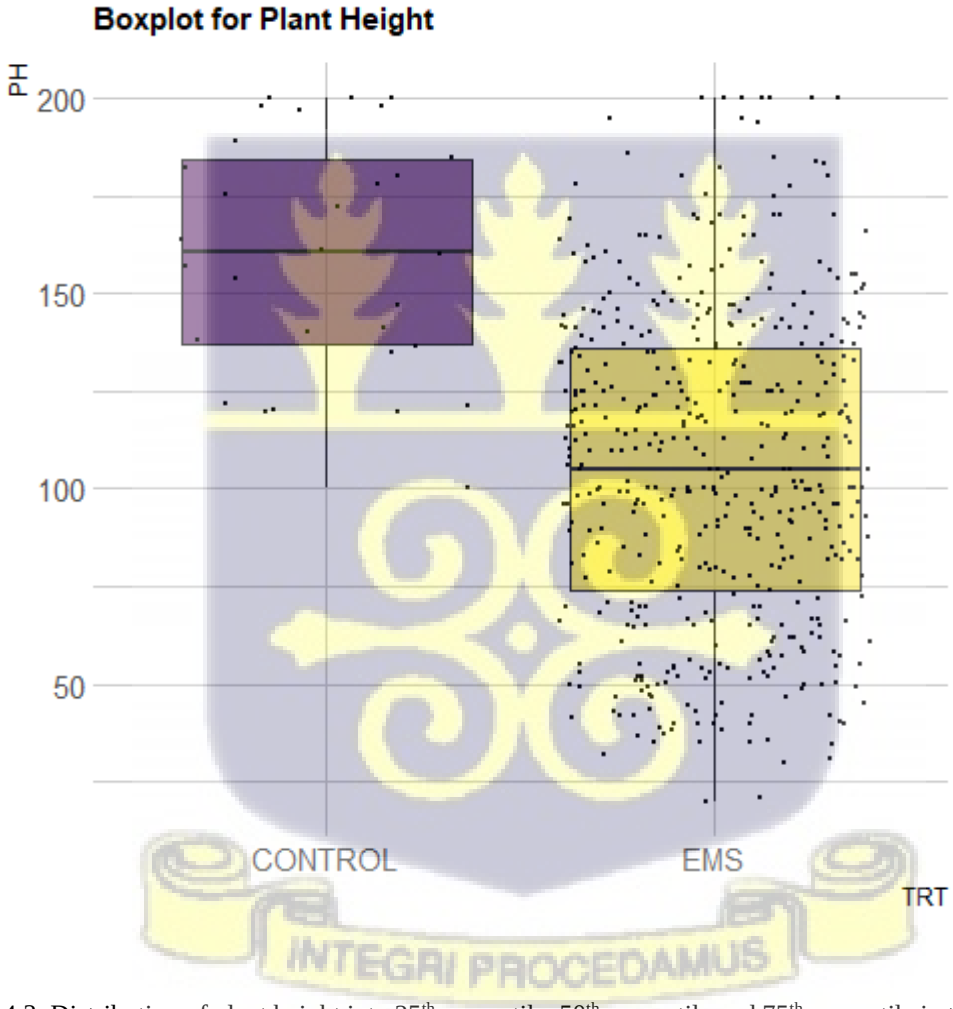

Figure 4.3: Distribution of plant height into 25th percentile, 50th percentile and 75th percentile in the wild type and mutant population. PH=Plant height, trt=Treatment, EMS=Ethyl methane sulfonate. Black dots represent the dispersion and frequency distribution of the phenotypic data in each treatment.

4.3.2.3 Days to Flowering

In the M_1 generation, there was low phenotypic variation observed in both the wild type and the mutagenized population for days to flowering. There were few outliers in both the treated and the wild type (**figure 4.4**). The mutagenized population ranged from 60-66 days and the wild type ranged 59-65 days (**figure 4.4**). A median value of 62 days was obtained in both the M_1 population and the wild type.

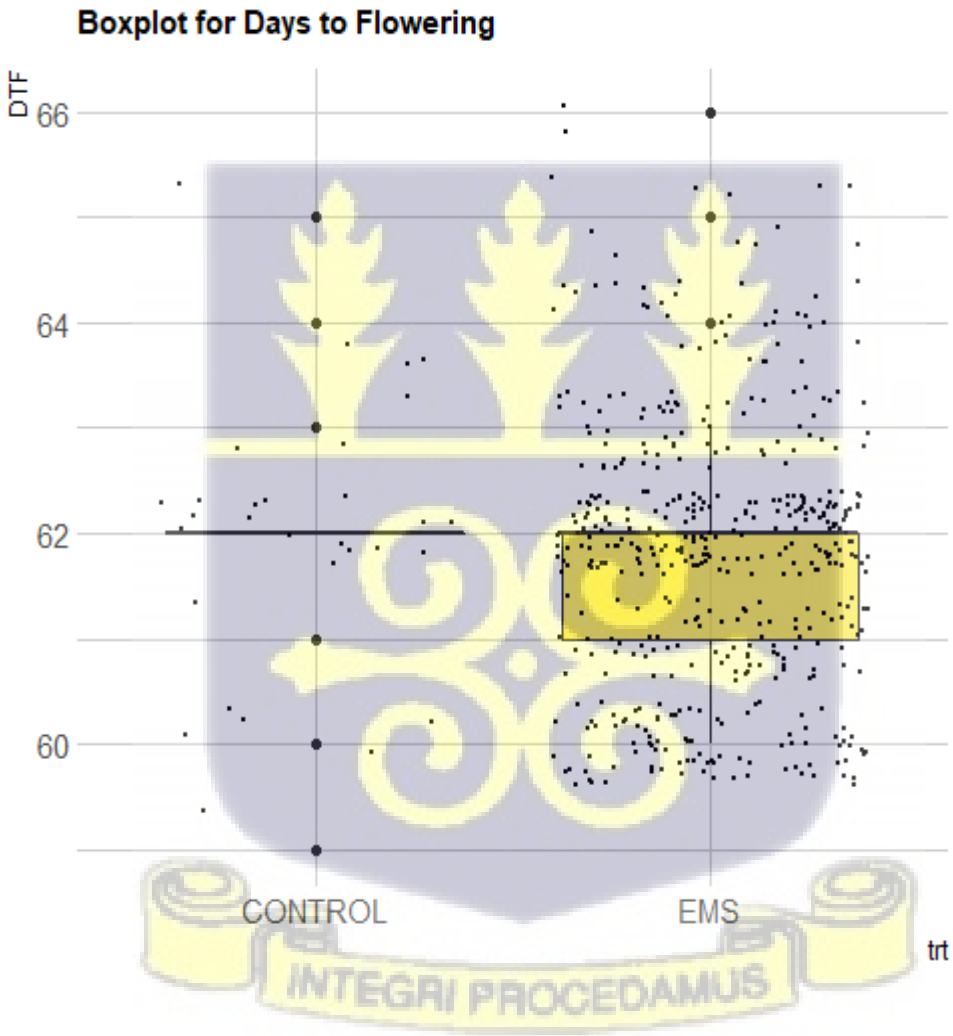

Figure 4.4: Distribution of days to flowering into 25th percentile, 50th percentile and 75th percentile in the wild type and mutant population. DTF=Days to Flowering, trt=Treatment, EMS=Ethyl methane sulfonate. Black dots represent the dispersion and frequency distribution of the phenotypic data in each treatment.

4.3.2.4 Number of Pods per Plant

In the M_1 generation, the mutagenized population was widely dispersed whiles the wild type was narrowly dispersed for number of pods per plant as shown in **figure 4.5**. The wild type ranged from 18-46 pods per plant and the mutagenized population ranged from 1-80 pods per plant (**figure 4.5**). Fifty percent of the mutagenized population had less than 12 pods and the median value in the wild type was 40 pods.

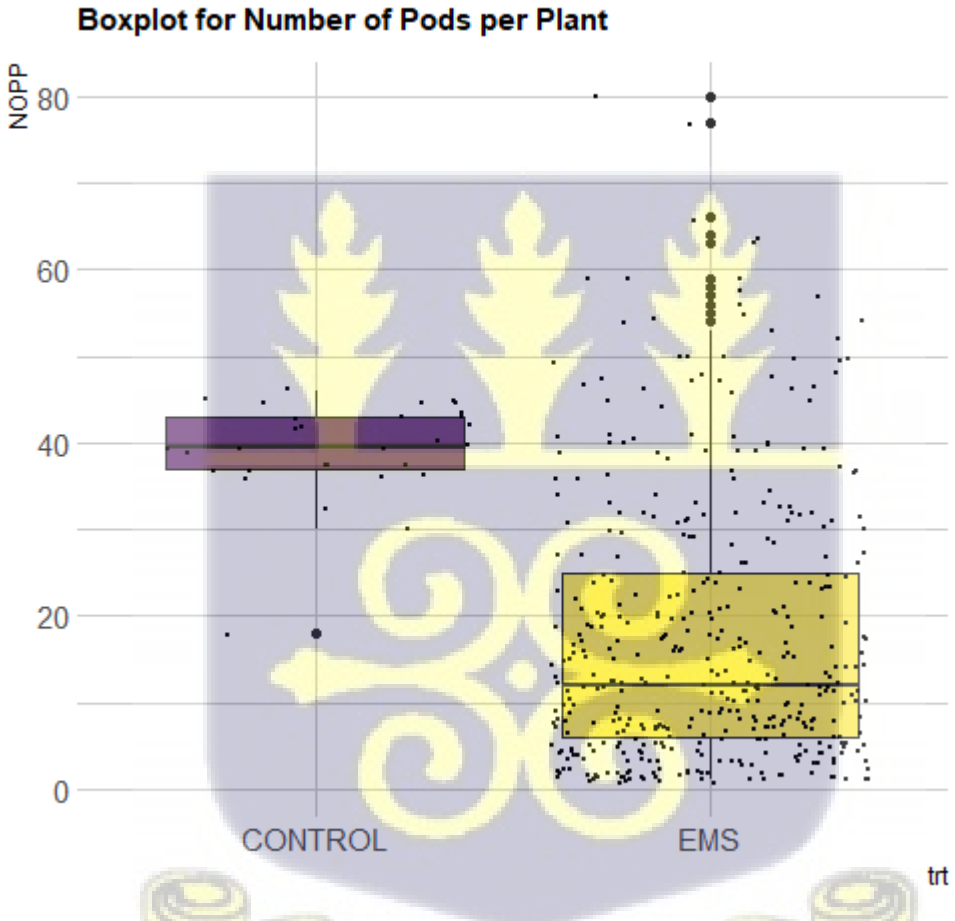

Figure 4.5: Distribution of number of pods per plant into 25th percentile, 50th percentile and 75th percentile in the wild type and mutant population. NOPP=Number of Pods per Plant, trt=Treatment, EMS=Ethyl methane sulfonate, No.=number. Black dots represent the dispersion and frequency distribution of the phenotypic data in each treatment.

4.3.2.5 Pod Length

High phenotypic variation was observed in the mutagenized population with many outliers for pod length (**figure 4.6**). The mutagenized population ranged from 3.0-18.20 cm and the wild type ranged from 12.9-18.1 cm in the M_1 generation (**figure 4.6**). The variation in the wild type was low. The median values recorded for this trait in the wild type and the mutagenized population were 15.4 cm and 14.3 cm respectively.

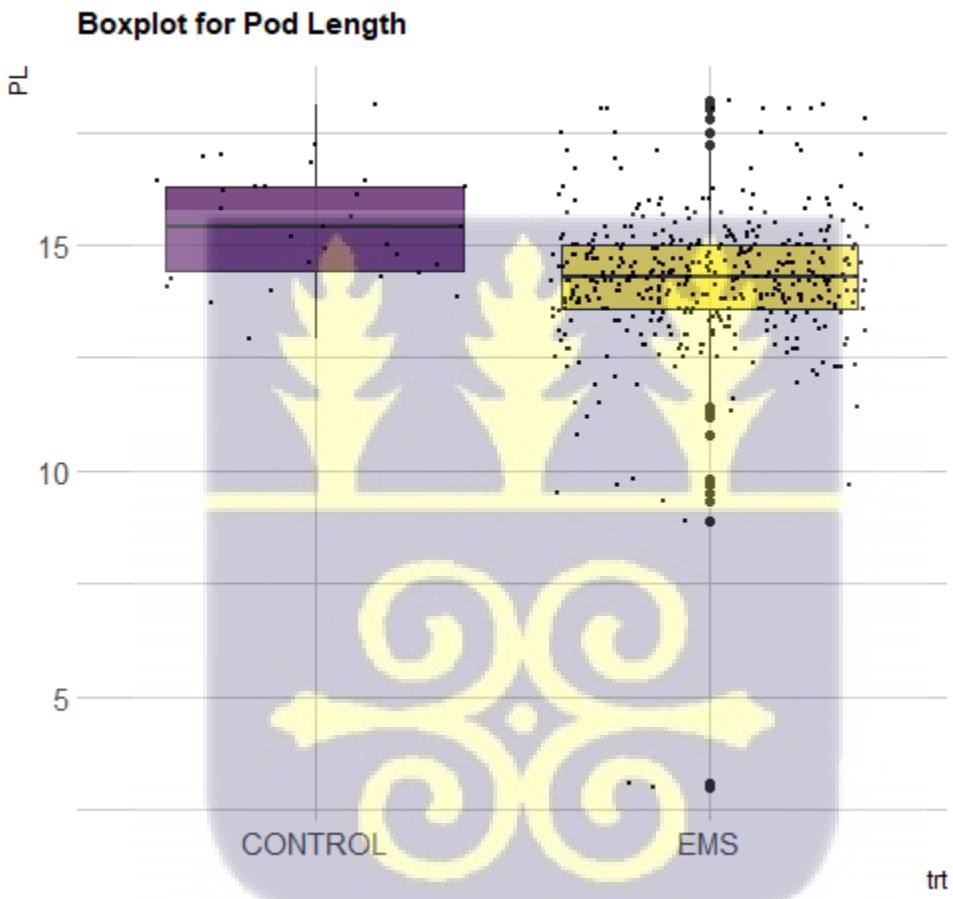

Figure 4.6: Distribution of pod length into 25th percentile, 50th percentile and 75th percentile in the wild type and mutant population. PL=Pods length, trt=Treatment, EMS=Ethyl methane sulfonate. Black dots represent the dispersion and frequency distribution of the phenotypic data in each treatment.

4.3.2.6 Number of Locules per Pod

There were some outliers observed in both the wild type and mutagenized population for number of locules per pod (**figure 4.7**). However, there was high phenotypic variation in the M_1 population as compared to the wild type. The wild type ranged from 10-14 and the mutagenized population ranged from 6-20 (**figure 4.7**). Fifty percent of the individuals within the mutagenized population had 11 locules and above whiles the median value for the wild type was 12.

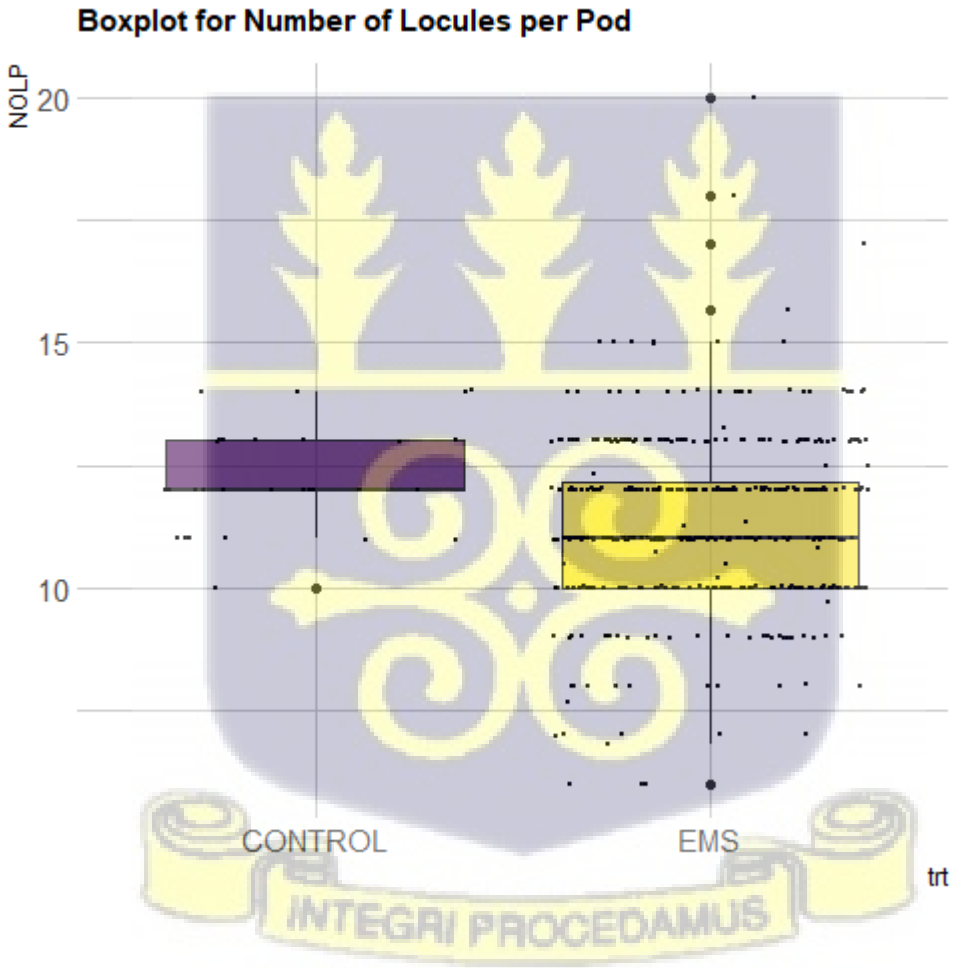

Figure 4.7: Distribution of number of locules per pod into 25th percentile, 50th percentile and 75th percentile in the wild type and mutant population. NOLP=Number of Locules per Pod, trt=Treatment, EMS=Ethyl methane sulfonate. Black dots represent the dispersion and frequency distribution of the phenotypic data in each treatment.

4.3.2.7 Number of Seeds per Pod

In the M_1 generation, there was high phenotypic variation in the mutagenized population whereas low variation was observed in the wild type for number of seeds per pod (**figure 4.8**). The range of the wild type was 5 (from 7-13) and the range of the mutagenized population was 13 (from 1-14) (**figure 4.8**). The individuals in the mutagenized population are widely spread with a median value of **9** whiles the individuals in the wild type were clustered around the median (11).

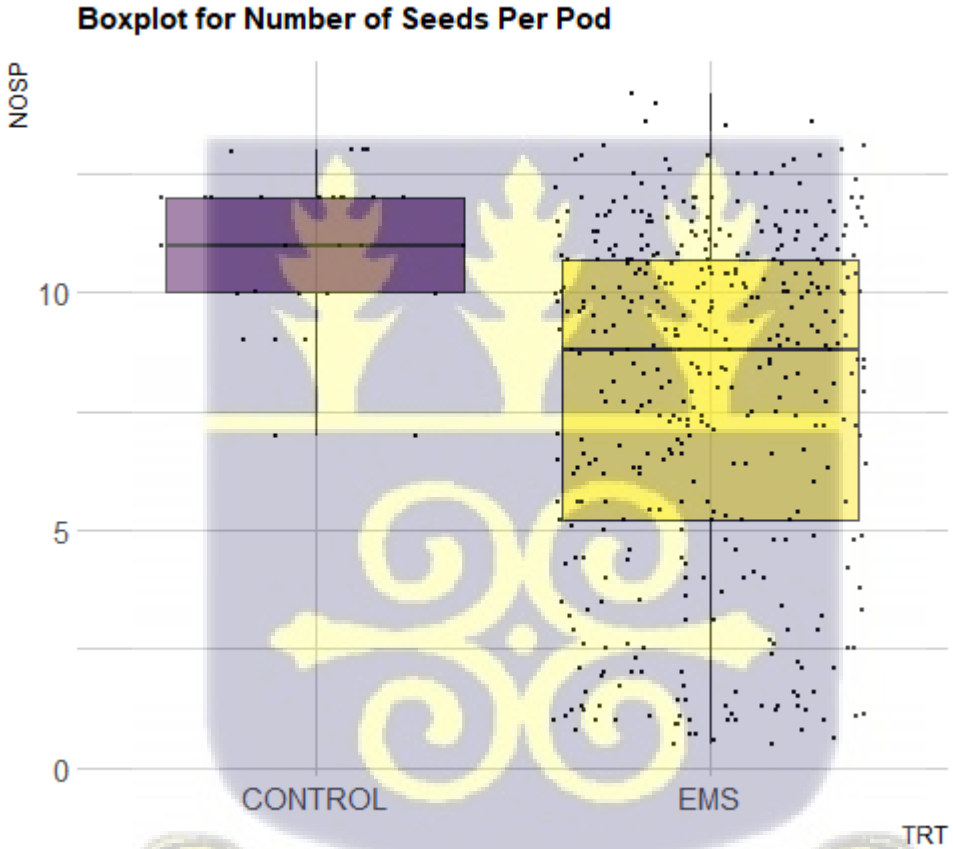

Figure 4.8: Distribution of number of locules per Pod into 25th percentile, 50th percentile and 75th percentile in the wild type and mutant population. NOSP=Number of Seeds per Pod, trt=Treatment, EMS=Ethyl methane sulfonate. Black dots represent the dispersion and frequency distribution of the phenotypic data in each treatment.

4.4 Principal Component Analysis of Quantitative Variables of M_1 population and the Wild Type (WTP)

4.4.1 Principal Component Analysis of Quantitative Traits in the Wild Type (M_1 Generation).

Principal component analysis was done to identify the traits that contributed most to the total variability observed. The eigenvalue for the first principal component was 2.4091 which represent 20.49 % of the total variation in the wild type (**Table 4.3**). The second principal component had an eigenvalue of 2.0034 which represents 20.03 % of the total variation **(Table 4.3)**. The eigenvalues for the third and fourth principal components obtained were 1.08 and 1.02 respectively **(Table 4.3)**. Thus, the first four principal component explains 75.26 % of the total variation in the wild type. Number of locules per pod and number of pods per peduncles were the variables with the highest contribution in dimension 1 and dimension 2 respectively. In dimensions 3 and 4, number of seeds per pod and days to flowering contributed the most to variability.

Table 4.3: Principal component analysis among the wild type showing relative contributions of quantitative variables in the M_1 generation. Dim.1=Dimension 1(PC1), Dim.2= Dimension 2(PC2), Dim.1=Dimension 3(PC3), Dim.4= Dimension 4(PC4)

	Dim.1	Dim.2	Dim.3	Dim.4
Eigenvalue	2.07	1.85	1.08	1.02
Variance percent	25.90	23.19	13.45	12.72
Cumulative variance (%)	25.90	49.09	62.54	75.26
Traits	**Contribution of variables**			
CC	21.86	9.58	5.86	0.58
PH	0.42	19.62	1.24	26.08
DTF	3.04	10.30	1.35	**60.72**
NOPP	6.94	13.02	0.40	8.76
PL	25.56	3.78	13.58	0.51
NPPP	0.60	**24.84**	31.62	0.00
NOLP	**32.27**	8.70	0.93	0.67
NOSP	9.31	10.15	**45.02**	2.68

PH-Plant Height, NOPP-Number of Pods per Plant, NPPP-Number of Pods per Peduncle, CC-Chlorophyll Content, DTF-Days to Flowering, PL-Pod Length, NOLP-Number of Locules per Pod, NOSP-Number of seeds per pod.

From the scree plot (**figure 4.9**), the total variation was explained by all the 8 principal components. The first two principal components explained much of the variability. There was sharp decline of percentage variability after the 2nd principal component (**figure 4.9**). The next 3 principal components after PC2 explained moderate amount of variability and the last 3 dimensions only explained small fraction of the overall variability (**figure 4.9**).

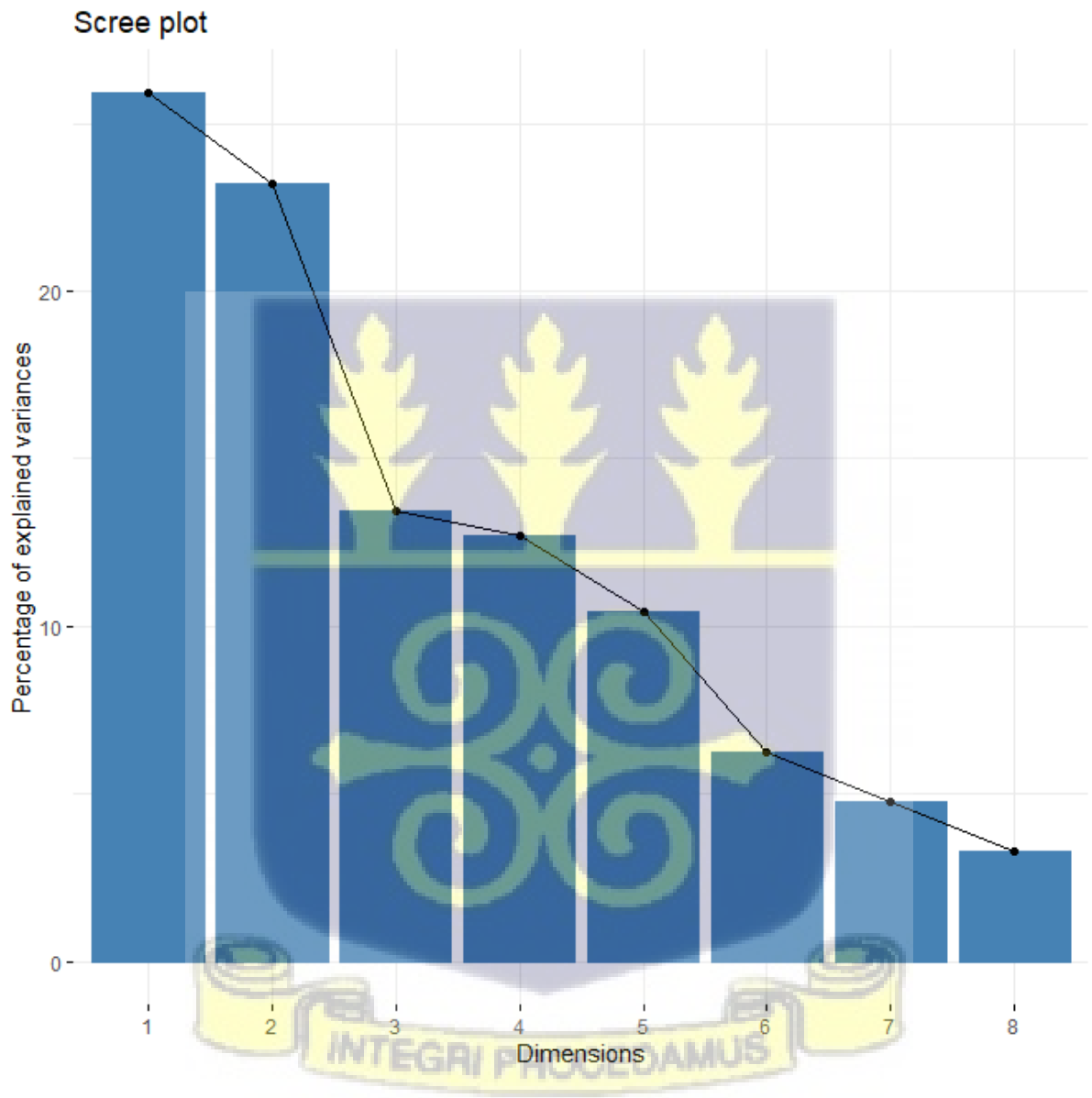

Figure 4.9: Scree plot of percentage of explained variance among dimensions (Principal Components, PC) of quantitative traits in the Wild type.

4.4.2 Principal Component Analysis of Quantitative Traits in the M_1 Population

The eigenvalue obtained in the first principal component was 3.1252 which represents 31.25 % of the total variation in the mutagenized population (**Table 4.4**). The eigenvalue of the second principal component analysis was 1.5028 which represents 15.03 % of the total variation (**Table 4.4**). The eigenvalues obtained for the third and fourth dimensions were 1.00 and 0.75 respectively (**Table 4.4**). Thus, the first four principal component explained 77.22 % of the total variation in the mutant population. Number of pods per plant and Number of locules per pod were the variables with the highest contribution in dimension 1 and dimension 2 respectively. Chlorophyll content and plant height contributed the most in the third and fourth principal components. The sum of eigenvalues was 8 which is the total number of factors involved in the PCA.

Table 4.4: Principal component analysis among the M_1 population showing Relative contributions of quantitative variables. Dim.1=Dimension 1(PC1), Dim.2= Dimension 2(PC2), Dim.1=Dimension 3(PC3), Dim.4= Dimension 4(PC4)

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
Eigenvalue	2.97	1.45	1.00	0.75	0.69
Variance percent	37.09	18.16	12.53	9.44	8.59
Cumulative variance (%)	37.09	55.25	67.78	77.22	85.81
Traits	**Contribution of Variables**				
CC	4.62	0.88	**63.77**	20.25	1.57
PH	11.14	0.82	14.96	**33.46**	32.95
DTF	12.61	3.45	0.20	17.62	**61.48**
NOPP	24.50	0.16	6.11	2.87	1.02
PL	0.04	**47.56**	0.61	15.35	0.78
NPPP	20.75	0.00	11.95	3.72	1.57
NOLP	1.49	46.91	1.40	6.21	0.55
NOSP	**24.86**	0.22	1.00	0.52	0.08

PH-Plant Height, NOPP-Number of Pods per Plant, NPPP-Number of Pods per Peduncle, CC-Chlorophyll Content, DTF-Days to Flowering, PL-Pod Length, NOLP-Number of Locules per Pod, NOSP-Number of seeds per pod.

From the scree plot (**figure 4.10**), the total variation was explained by all the 8 principal components. The level of variability declined sharply from dimension 1 (PC1) and continued to steadily reduce till dimension 8 where 100% variability was obtained (**figure 4.10**). The latter dimensions explained the least proportion of variability in the population and the first two dimensions explained much of the variations in the mutagenized population.

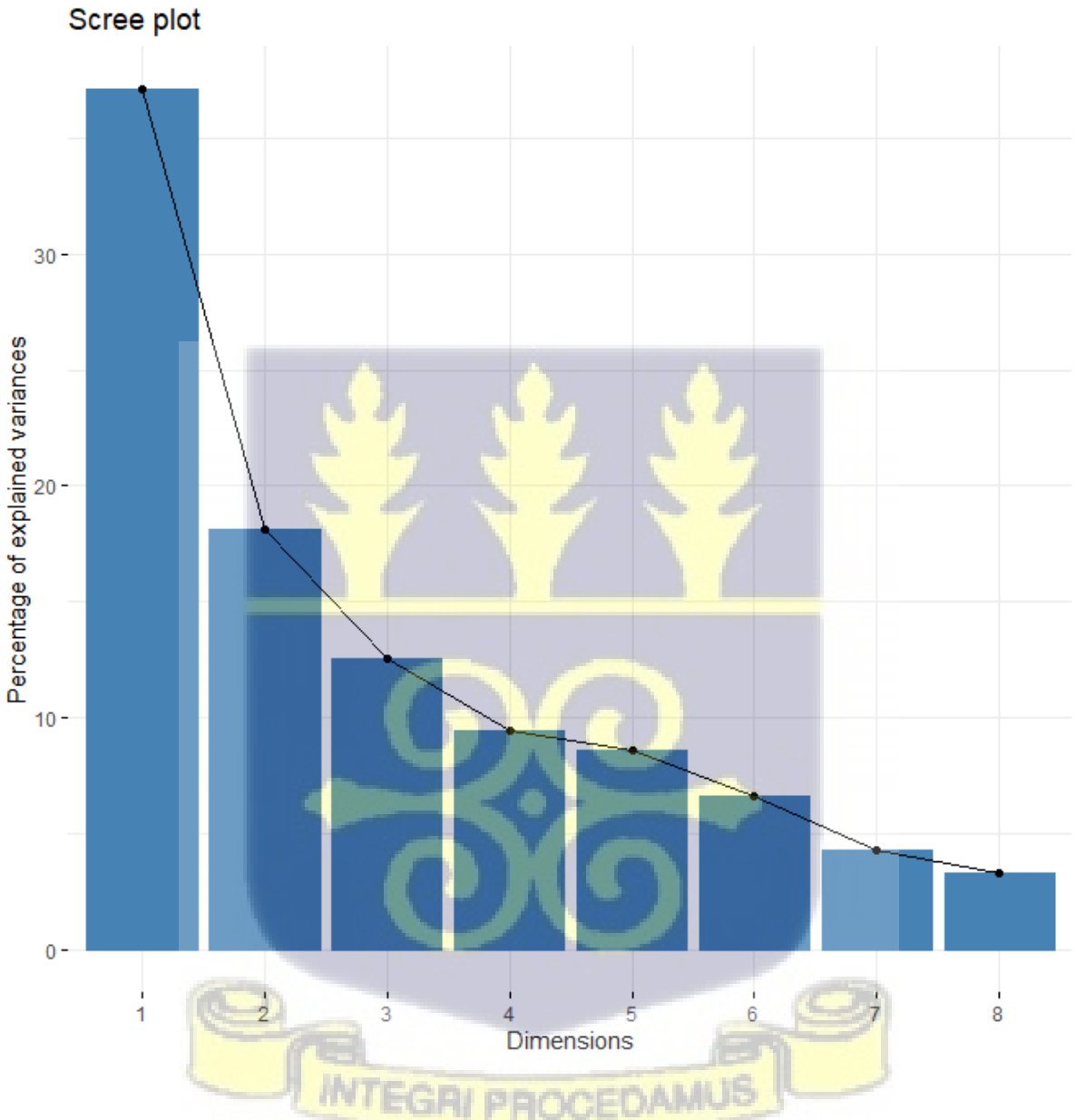

Figure 4.10: Scree plot of percentage of explained variance among dimensions (Principal Components, PC) of quantitative traits in the mutant population.

4.4.3 Correlation Between Quantitative Variables and Principal Components (Dimensions) in the Wild type and Mutagenized Population (M_1 Generation).

The correlation circles were based on the total variability in PC1 and PC2. Traits presented in the same quadrant portrayed a positive correlation whiles negative correlated variables point opposite. In the wild type, percentage variabilities of 25.90% in Dim.1 (PC1) and 23.2% in Dim.2 (PC2) were extracted and used in the construction of the correlation circle with four equal parts which had Dim.1 and Dim.2 represented on both x- axis and y-axis respectively (**figure 4.11**). Pod length (PL), number of locules per pod (NOLP), number of seeds per pod (NOSP) formed an acute angle in the left-bottom quadrant indicating a positive and strong correlation among the parameters. A negative correlation existed between chlorophyll content (CC) and Days to flowering (DTF) represented by an angle of almost 180 (**figure 4.11**). However, plant height (PH) negatively correlated with high yielding variables in the bottom quadrants (number of pods per plants (NOPPP), number of seeds per pod (NOSP), chlorophyll content (CC), and number of locules per pod (NOLP)).

In the mutagenized population, percentage variabilities of 37.1% in Dim.1 (PC1) and 18.2% in Dim.2 (PC2) were extracted and used in the construction of the correlation circle (**figure 4.12**). A positive correlation existed among yield characters (number of pods per plant (NOPP, number of seeds per pods (NOSP), number of locules per pod (NOLP) and pod length (PL). Plant height (PH) formed acute angle with chlorophyll content; however, both negatively correlated with days to flowering (DTF) (**figure 4.12**).

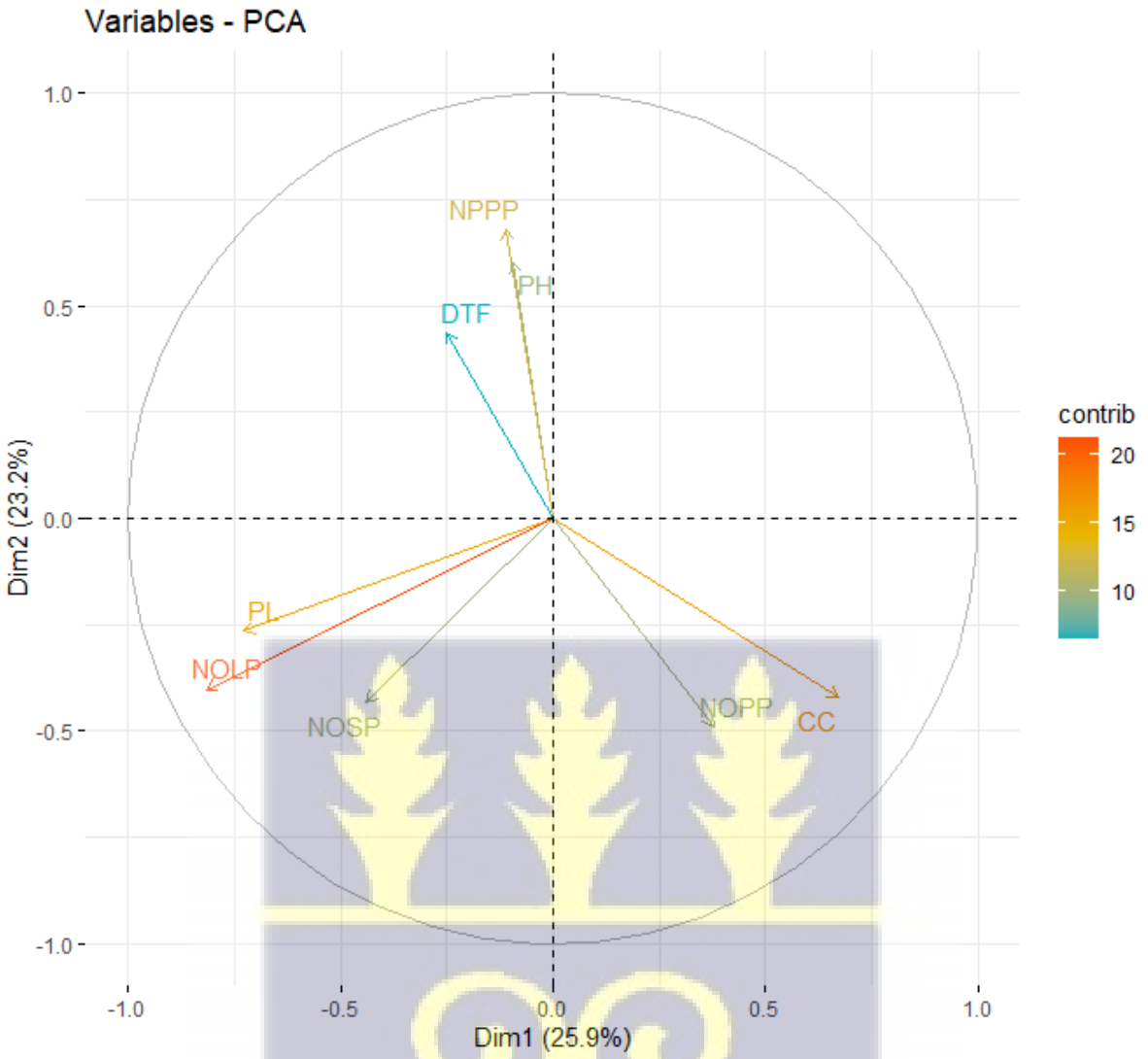

Figure 4.11: Correlation circle of quantitative variables and dimensions (Principal Components, PC) in the wild type. Positive correlated variables point to the same side of the plot. Negative correlated variables point to opposite sides of the graph. PH-Plant Height, NOPP-Number of Pods per Plant, NPPP-Number of Pods per Peduncle, CC-Chlorophyll Content, DTF-Days to Flowering, PL-Pod Length, NOLP-Number of Locules per Pod, NOSP-Number of seeds per pod.

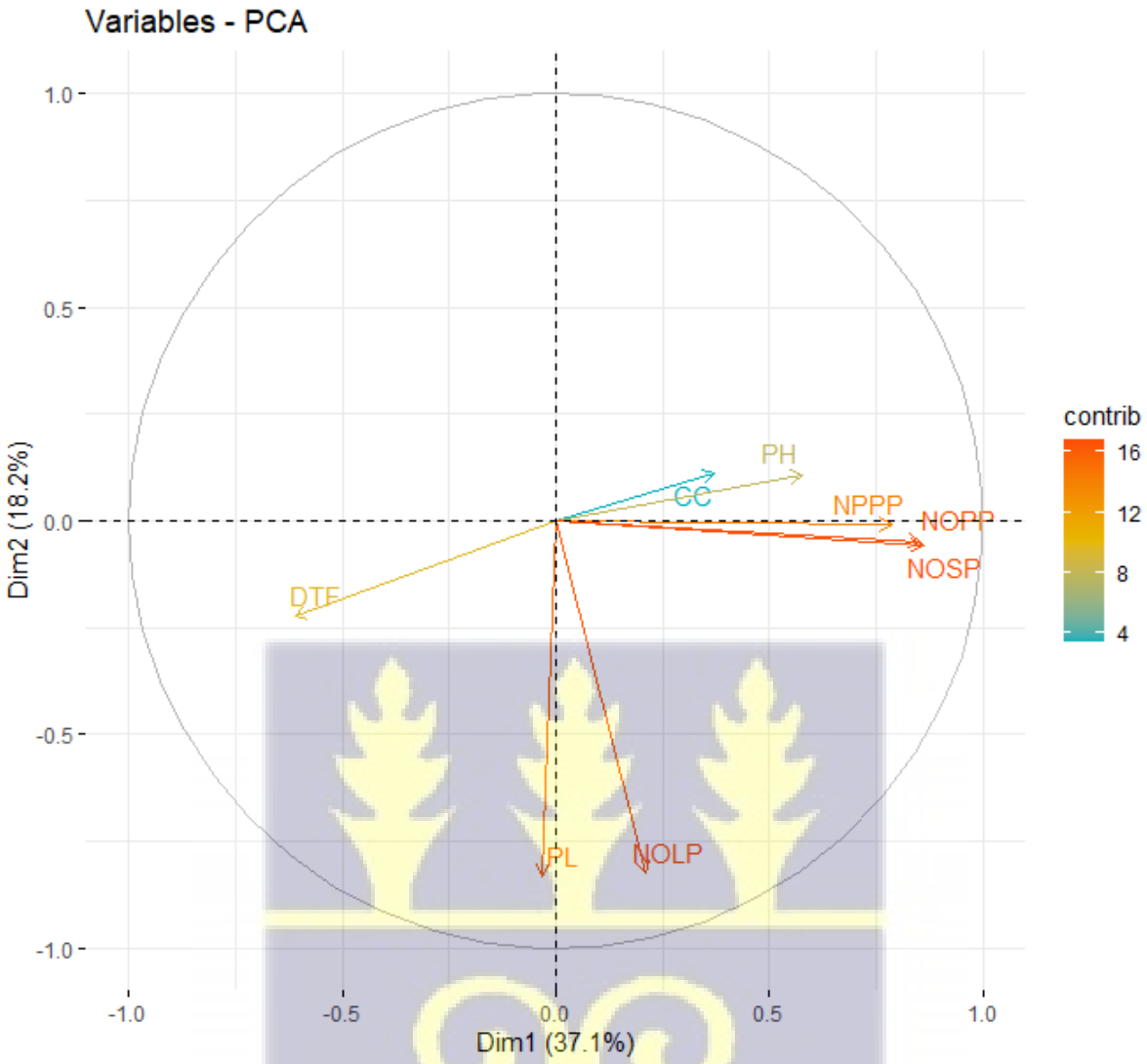

Figure 4.12: Correlation circle of quantitative variables and dimensions (Principal Components, PC) in the mutant population. Positive correlated variables point to the same side of the plot. Negative correlated variables point to opposite sides of the graph. PH-Plant Height, NOPP-Number of Pods per Plant, NPPP-Number of Pods per Peduncle, CC-Chlorophyll Content, DTF-Days to Flowering, PL-Pod Length, NOLP-Number of Locules per Pod, NOSP-Number of seeds per pod.

4.5 Principal Component Analysis of Qualitative Variables of M_1 Mutants and Wild type

4.5.1 Principal Component Analysis of Qualitative Traits in the Wild type (M_1 Generation)

In the M_1 generation, 60.07% variability among the wild type was explained by the first principal component (Dim.1). The eigenvalue for the second principal component (Dim.2) was 2.6015 which explained 32.55% of variation (**Table 4.5**). The level of variability explained by the third dimension (PC3) was 7.38% with an eigenvalue of 0.5900 (**Table 4.5**). The first three principal components therefore accounted for a total of 100% variability (**Table 4.5**). Branch pigmentation (PGB), stem pigmentation (PGS) and petiole pigmentation (PGP) were highly correlated with Dim.1, Dim.2 and Dim.3 respectively. These traits only contributed to the total variability in the wild type.

Table 4.5: Principal component analysis among the wild type showing relative contributions of qualitative variables. Dim.1=Dimension 1(PC1), Dim.2= Dimension 2(PC2), Dim.1=Dimension 3(PC3).

	Dim.1	Dim.2	Dim.3
Eigenvalue	4.8016	2.6015	0.5900
%Total Variance	60.07	32.55	7.38
%Cumulative	60.07	92.62	100.00
Traits	**Contribution of variables**		
PGS	0.06	**99.74**	0.20
PGB	**98.50**	0.09	1.41
PGP	1.44	0.17	**98.39**
LS	0.00	0.00	0.00
GH	0.00	0.00	0.00
FC	0.00	0.00	0.00
PCURV	0.00	0.00	0.00
PC	0.00	0.00	0.00
SS	0.00	0.00	0.00
SC	0.00	0.00	0.00

PGB-Pigmentation of branch, PGP-Pigmentation of Petiole, PGS-Pigmentation of Stem, GH-Growth habit, PCURV- Pod Curvature, FC-Flower color, PC-Pod Colour, SC- Seed Coat Colour, SS- Seed Shape, LS- Leaf Shape

From the scree plot (**figure 4.13**), the total variation was explained by only the first three principal components. There was a sharp decline of level of variability from dimension 1 (PC1) to dimension 3 (PC3) (**figure 4.13**). The curve then flattened from PC4 to PC8 at 0. There was no percentage variability explained by dimensions 4 to 8.

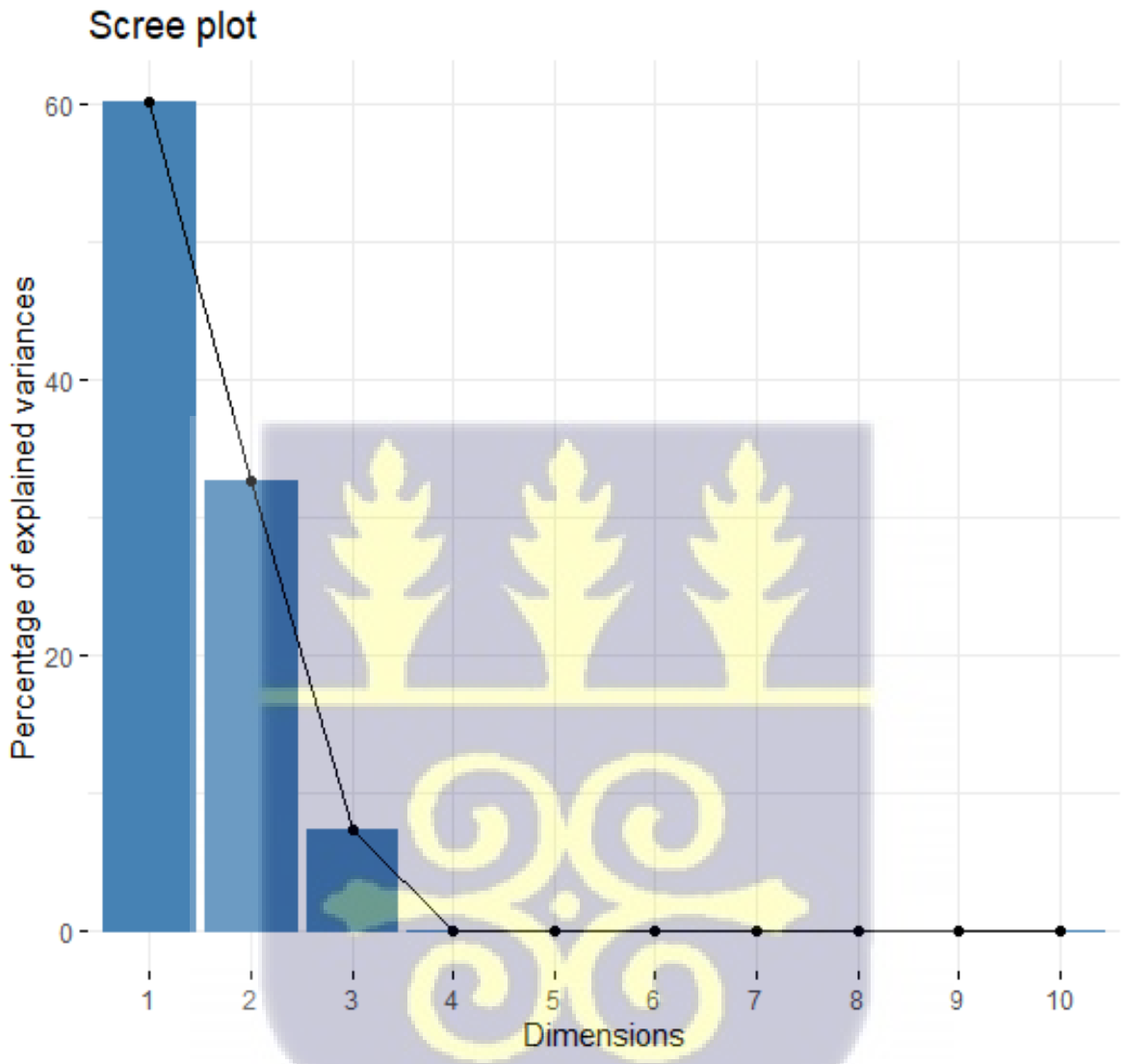

Figure 4.13: Scree plot of percentage of explained variance among dimensions (Principal Components, PC) of qualitative traits in the Wild type.

4.5.2 Principal Component Analysis of Qualitative Traits in the M_1 Population

The eigenvalue for the first principal component was 7.0450 which represent 33.07 % of the total variation in the M_1 population (**Table 4.6**). The second principal component analysis had an eigenvalue of 4.6720 which represents 21.93 % of the total variation (**Table 4.6**). The third and fourth principal components together explained 34.95% variability (**Table 4.6**). Thus, the first four principal components explained 89.9 % of the total variation in the mutagenized population. Branch pigmentation, stem pigmentation, growth habit and pod curvature were the variables with the highest contribution in dimension 1, dimension 2, dimension 3 and dimension 4 respectively.

Table 4.6: Principal component analysis among the M_1 population showing relative contributions of qualitative variables. Dim.1=Dimension 1(PC1), Dim.2= Dimension 2(PC2), Dim.1=Dimension 3(PC3).

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
Eigenvalue	7.0450	4.6720	4.1913	3.2547	1.5248
Variance percent	33.07	21.93	19.68	15.28	7.16
Cumulative variance percent	33.07	55.01	74.68	89.96	97.12
Traits	**Contribution of variables**				
PGS	14.7933	**56.2147**	9.0883	18.8907	0.9702
PGB	**60.1264**	12.7428	20.8000	6.2544	0.0177
PGP	0.2672	0.1526	0.1878	0.1876	0.2693
LS	0.0000	0.0000	0.0000	0.0000	0.0000
GH	23.4492	2.0380	**36.5163**	37.0174	0.2376
FC	0.0000	0.0000	0.0000	0.0000	0.0000
PCURV	1.1403	28.4744	32.7199	**37.5564**	0.0725
PC	0.0000	0.0000	0.0000	0.0000	0.0000
SS	0.2135	0.3535	0.6319	0.0817	**98.3901**
SC	0.0101	0.0241	0.0557	0.0117	0.0426

PGB-Pigmentation of branch, PGP-Pigmentation of Petiole, PGS-Pigmentation of Stem, GH-Growth habit, PCURV- Pod Curvature, FC-Flower color, PC-Pod Colour, SC- Seed Coat Colour, SS- Seed Shape, LS- Leaf Shape

From the scree plot (**figure 4.14**), the total variation was explained by the first seven principal components. The first four principal components explained much of the variability. There was a sharp decline of percentage variability after the fourth principal component (**figure 4.14**). The fifth, sixth and seventh principal components explained moderate amount of variability and the last 3 dimensions explained no fraction of the overall variability.

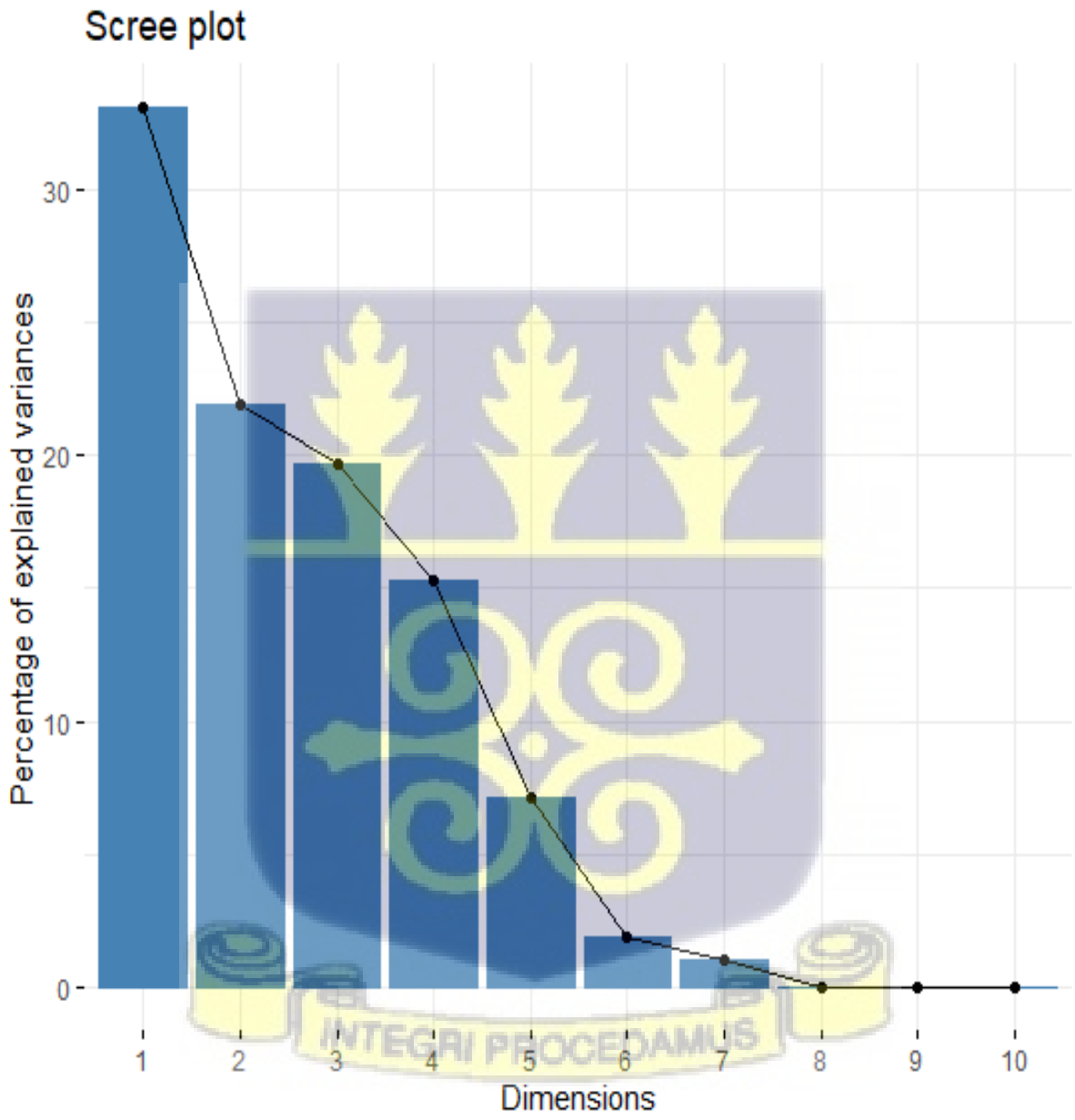

Figure 4.14: Scree plot of percentage of explained variance among dimensions (Principal Components, PC) of qualitative traits in the mutant population.

4.5.3 Correlation Between Qualitative Variables and Principal Components (Dimensions) in the Wild type and Mutagenized Population (M_1 Generation).

The correlation circle in the wild type was constructed based on the 92.6% variability extracted from the first two dimensions; Dim.1 and Dim.2 (**figure 4.15**). There was positive correlation between stem pigmentation (PGS) and petiole pigmentation (PGP). However, branch pigmentation exhibited negative correlation with both petiole pigmentation and stem pigmentation (**figure 4.15**).

In the mutagenized population, the correlation circle was constructed based on the 55.0% variability extracted from the first two dimensions; Dim.1 and Dim.2 (**figure 4.16**). Traits in the left-bottom formed an acute angle indicating strong and positive correlation. A negative correlation existed between stem pigmentation and the traits in the top-left quadrant (**figure 4.16**). Seed coat colour (SC) and petiole pigmentation (PGP) also showed negative correlation with pod colour (PC), flower colour (FC), growth habit (GH) and branch pigmentation (PGB).

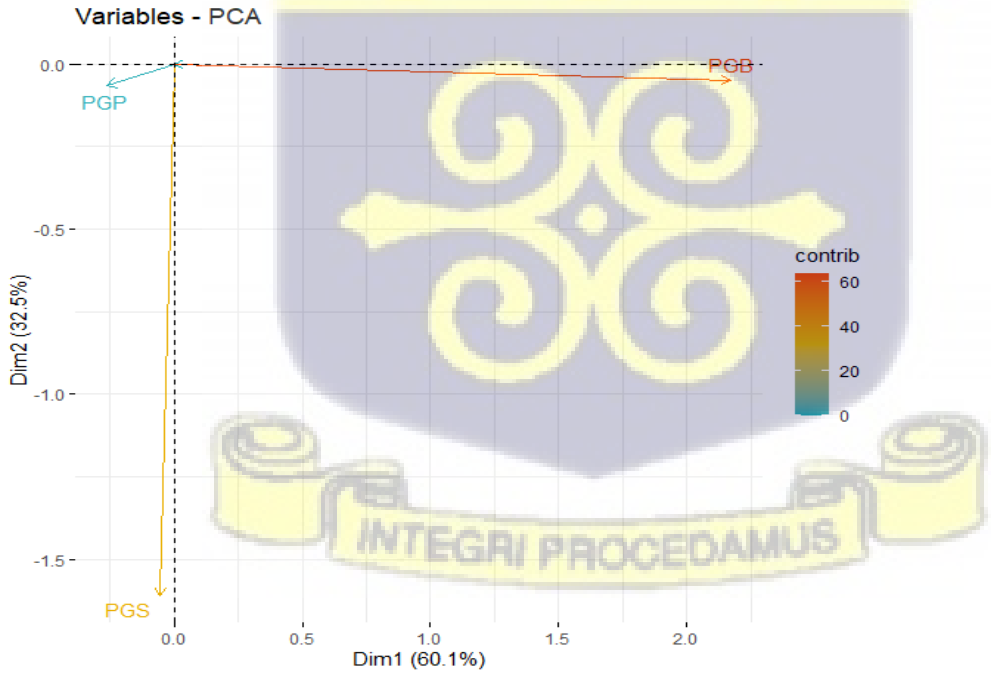

Figure 4.15: Correlation circle of qualitative variables and dimensions (Principal Components, PC) in the wild type. Positive correlated variables point to the same side of the plot. Negative correlated variables point to opposite sides of the graph. PGB-Pigmentation of branch, PGP-Pigmentation of Petiole, PGS-Pigmentation of Stem.

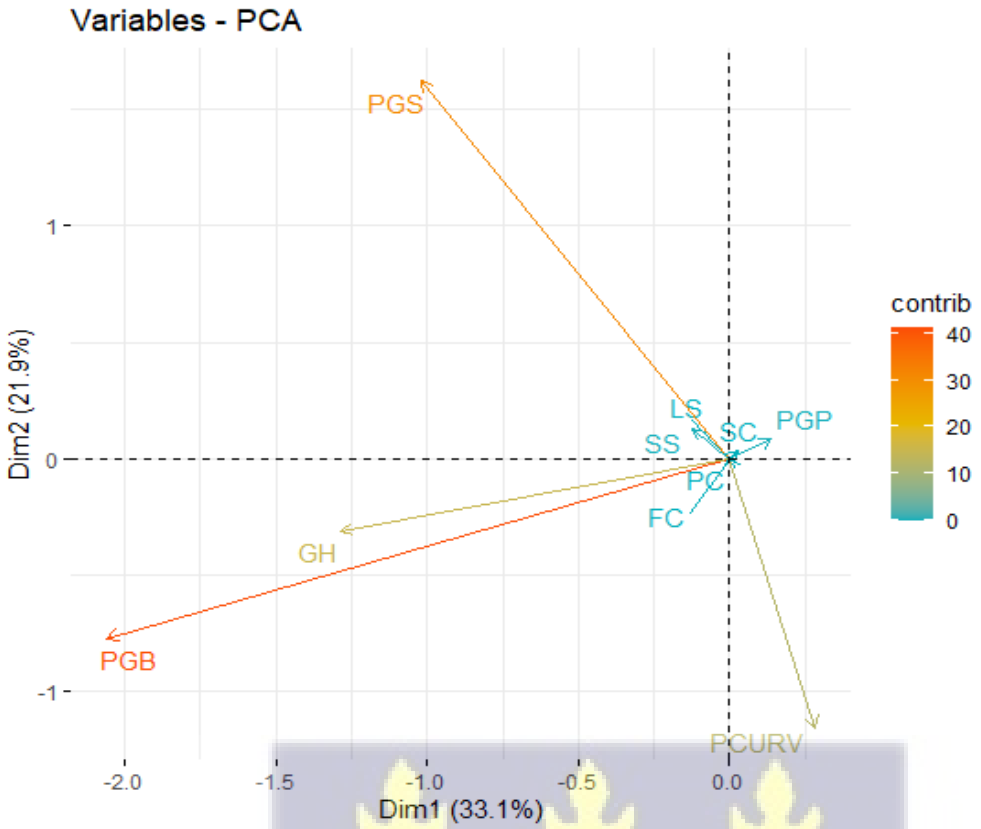

Figure 4.16: Correlation circle of qualitative variables and dimensions (Principal Components, PC) in the mutant population. Positive correlated variables point to the same side of the plot. Negative correlated variables point to opposite sides of the graph. PGB-Pigmentation of branch, PGP-Pigmentation of Petiole, PGS-Pigmentation of Stem, GH-Growth habit, PCURV- Pod Curvature, FC-Flower color, PC-Pod Colour, SC- Seed Coat Colour, SS-Seed Shape, LS- Leaf Shape

4.6 Bi-plot Between Qualitative Variables and M_1 Generation (Mutagenized population and Wild type) based on Dim.1 and Dim.2 values.

From the bi-plot (**figure 4.17**), individuals on the top left and right quadrants correlated positively with stem pigmentation (PGS), while variables on the bottom left quadrant negatively correlated with growth habit (GH), branch pigmentation (PGB), leaf shape (LS) and flower colour (FC). Individuals located further away from the origin in the PCA bi-plot indicated, they were genetically distinct from each other with relation to the eleven qualitative traits observed; the individuals recorded high contribution to variability (cos2). The top twenty (20) genotypes with high cos2 (**Appendix 21**) were all mutagenized plants.

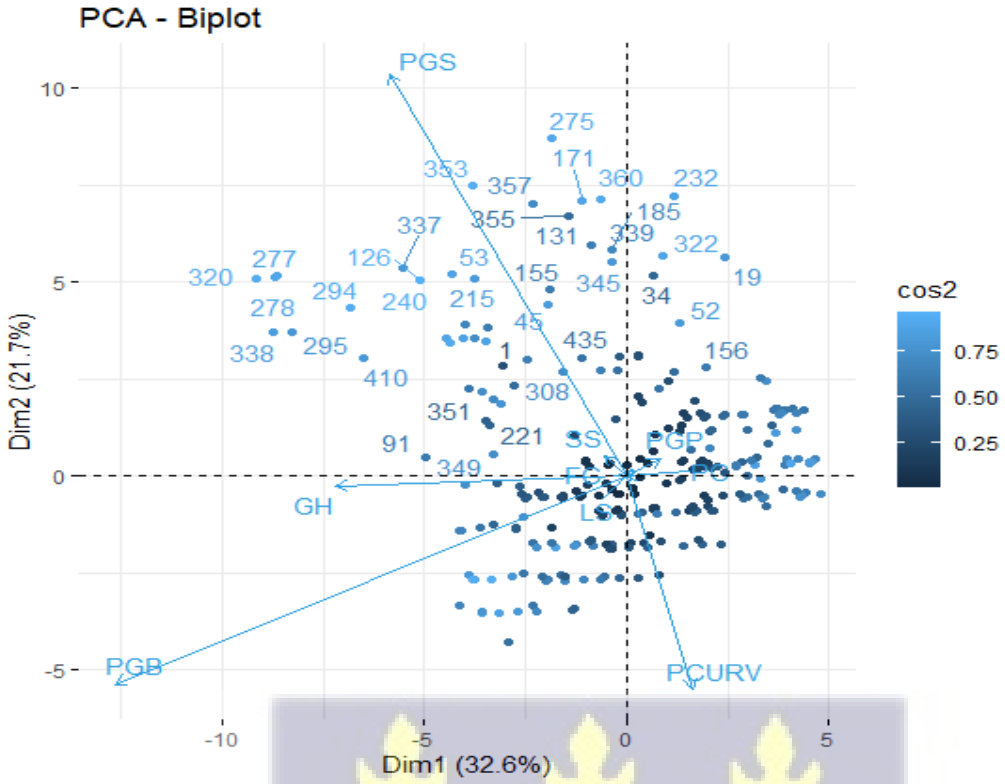

Figure 4.17: Biplot of qualitative variables and individuals in the M_1 generation (wild type and mutagenized population) based on first two dimensions (Dim1 and Dim2). Cos2 (0-1) = individual contributions. Individuals with deep colour have low contribution and individuals with light colour have high contribution. PGB-Pigmentation of branch, PGP-Pigmentation of Petiole, PGS-Pigmentation of Stem, GH-Growth habit, PCURV-Pod Curvature PC, FC-Flower color, PC-Pod Colour, SC- Seed Coat Colour, SS- Seed Shape, LS- Leaf Shape.

4.7 Bi-plot Between Quantitative Variables and M_1 Generation (Mutagenized population and Wild type) based on Dim.1 and Dim.2 values.

From the bi-plot (**figure 4.18**), individuals on the top right and bottom right quadrants correlated positively with the yield characters (number of locules per pod (NOLP), number of pods per peduncle (NPPP), number of pods per plant (NOPP), number of seeds per pod (NOSP) and pod length (PL)), while variables on the bottom left quadrant negatively correlated with days to flowering (DTF) (**figure 4.18**). Individuals that recorded high contribution to variability (cos2) and were genetically distinct from the other individuals with relation to the eight quantitative traits observed were located further away from the origin in the PCA bi-plot. The top twenty (20) genotypes with high cos2 **(Appendix 22**) were all mutagenized plants.

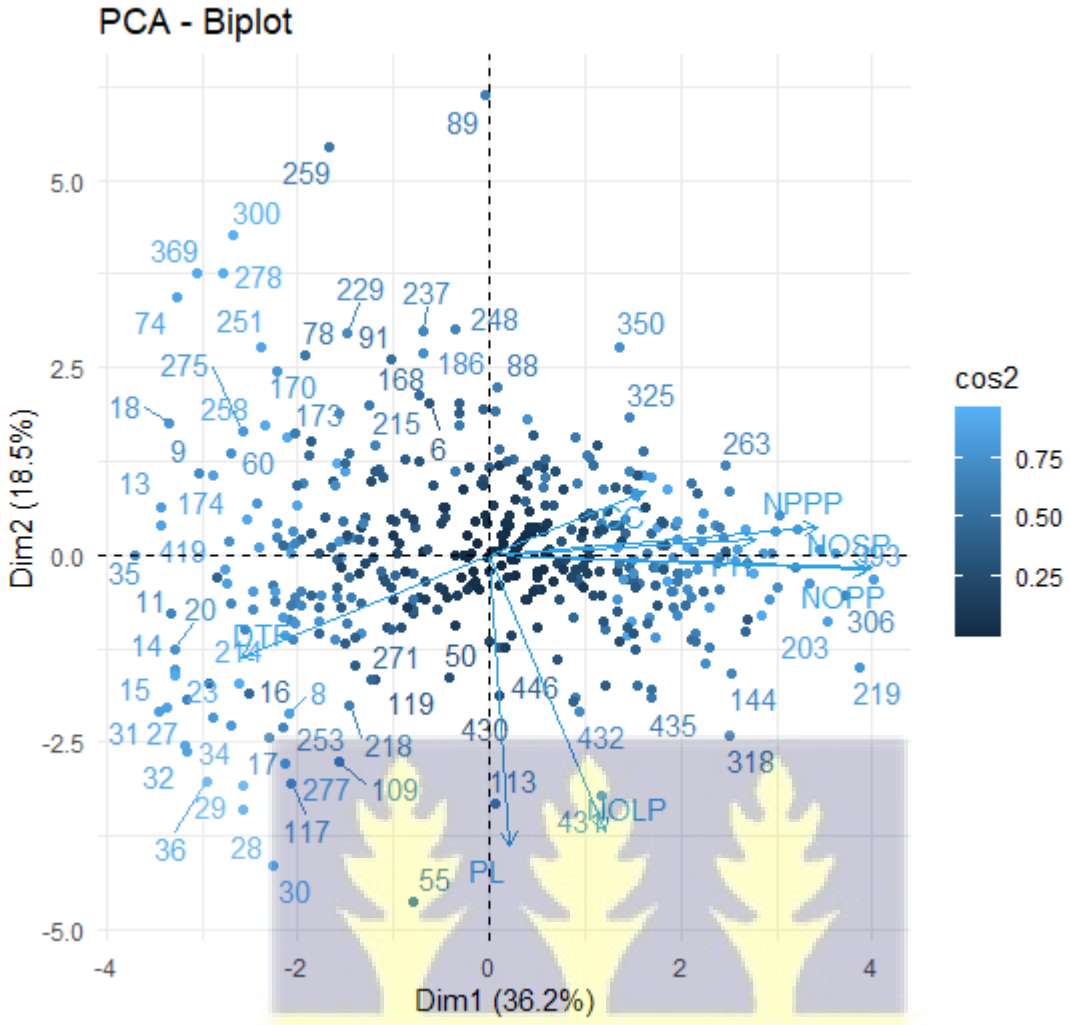

Figure 4.18: Bi-plot of quantitative variables and individuals in the M_1 generation (wild type and mutagenized population) based on first two dimensions (Dim1 and Dim2. Cos2 (0-1) = individual contributions. Individuals with deep colour have low contribution and individuals with light colour have high contribution. PH-Plant Height, NOPP-Number of Pods per Plant, NPPP-Number of Pods per Peduncle, CC-Chlorophyll Content, DTF-Days to Flowering, PL-Pod Length, NOLP-Number of Locules per Pod, NOSP-Number of seeds per pod.

4.8 Phenotyping of M_2 Generation

4.8.1 Percentage Seed Germination

Percentage seed germination ranged from 5% to 100% among the M_2 lines (**Appendix 5**). Total percentage seed germination recorded in the M_2 population was 74.03% and the wild type had 80% (**Table 4.7**). Percentage survival recorded in the M_2 population was 95.80% whiles 100% survival was observed in the wild type.

4.8.2 Germination Speed

Germination speed decreased in the M_2 population as compared with the wild type. The values recorded were 1.43 and 1.56 in the mutagenized population and the wild type respectively (**Table 4.7**).

Table 4.7: Percentage germination, percentage survival and germination speed of the wild type and M_2 population.

	Seeds sown	Germination Count	Percentage Germination	Number of Surviving Plants	Percentage Survival	Germination speed
M_2 Population	6380	4723	74.03	4526	95.8	1.43
Wild type	100	80	80	80	100	1.56

4.8.3 Morphological Mutations in M_2 Population

There were thirty (30) individuals that showed chromosomal mutations in 4,526 individuals observed in the M_2 population (**Table 4.8**). There were eighteen plants that showed variegated leaves (frequency = 0.04), three plants were albino (frequency = 0.07) (**Table 4.8**). There were six plants that showed yellow single leaf (0.13) and three plants were xantha (frequency = 0.07) (**Table 4.8**).

A wide spectrum of leaf mutations was observed in the mutant population with noticeable variation in size, shape, number and arrangement of leaflets (**Table 4.8**). Some mutants have

irregular leaves. These mutants were described by the presence of leaves with serrated leaves, irregular leaf margins, abnormal vernation and irregular shape of lamina.

Individuals with abnormal leaflet numbers were observed in the M_2 population. These mutants produced leaves with less than three or more leaflets. There were mutants with single leaflet, bifoliate (two leaflets), tetrafoliate (four leaflets), pentafoliate (five leaflets), hexafoliate (six leaflets) and septafoliate (seven leaflets).

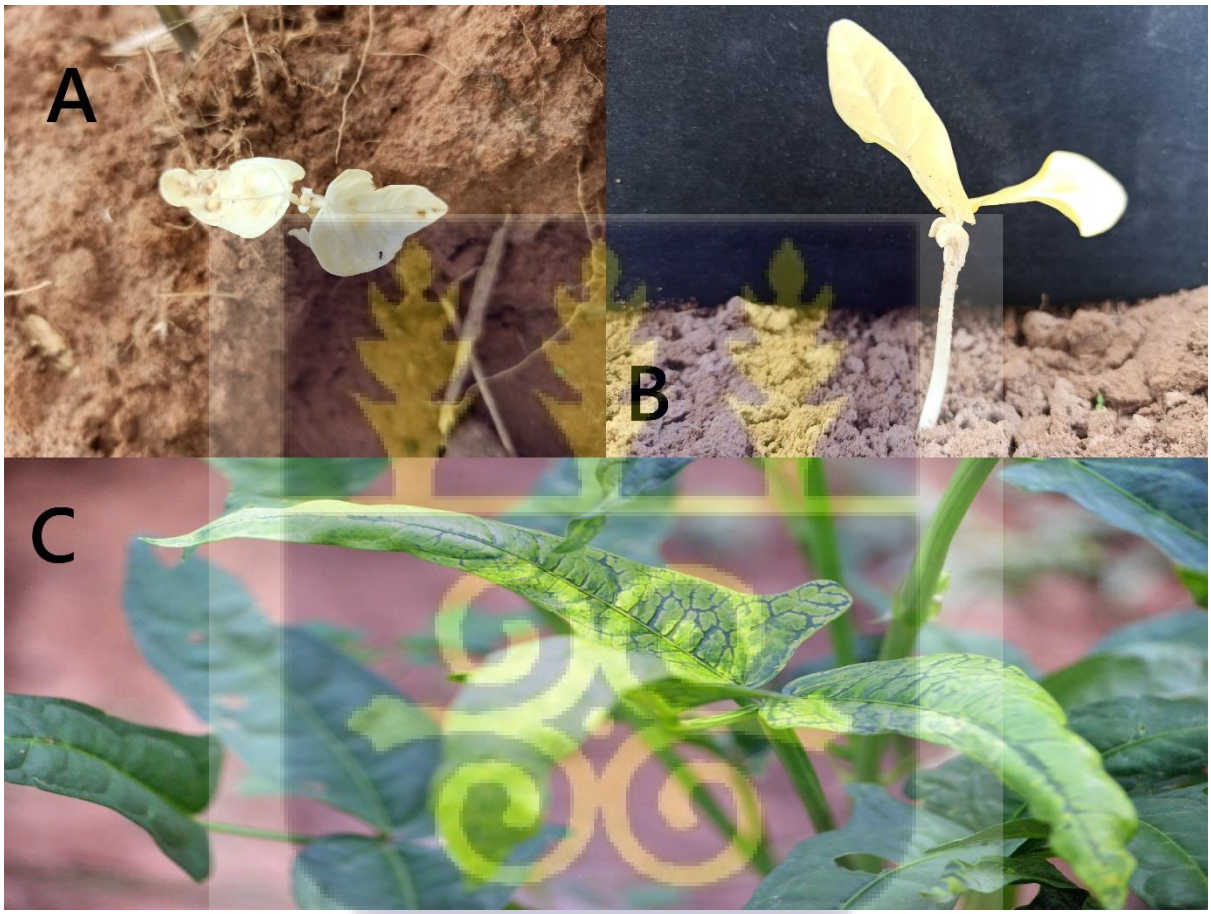

Figure 4.19: Phenotype of chromosomal mutations in M_2 population. A= Albino Plant, B= Xantha Plant, C= Variegated Leaf Plant.

Table 4.8: Frequency of individuals with morphological abnormalities in the mutagenized population (M_2 Generation).

Abnormalities	Number of Plants	Frequency (%)
Monofoliate	3	0.07
Bifoliate	39	0.86
Tetrafoliate	22	0.49
Pentafoliate	6	0.13
Hexafoliate	1	0.02
Septafoliate	1	0.02
Variegated Leaf	18	0.40
Albino	3	0.07
Xantha	3	0.07
Irregular leaves	24	0.53
Single globose leaf	1	0.02
Single yellow leaf	6	0.13
Single pinnate leaf	1	0.02
Triple pinnate leaves	5	0.11

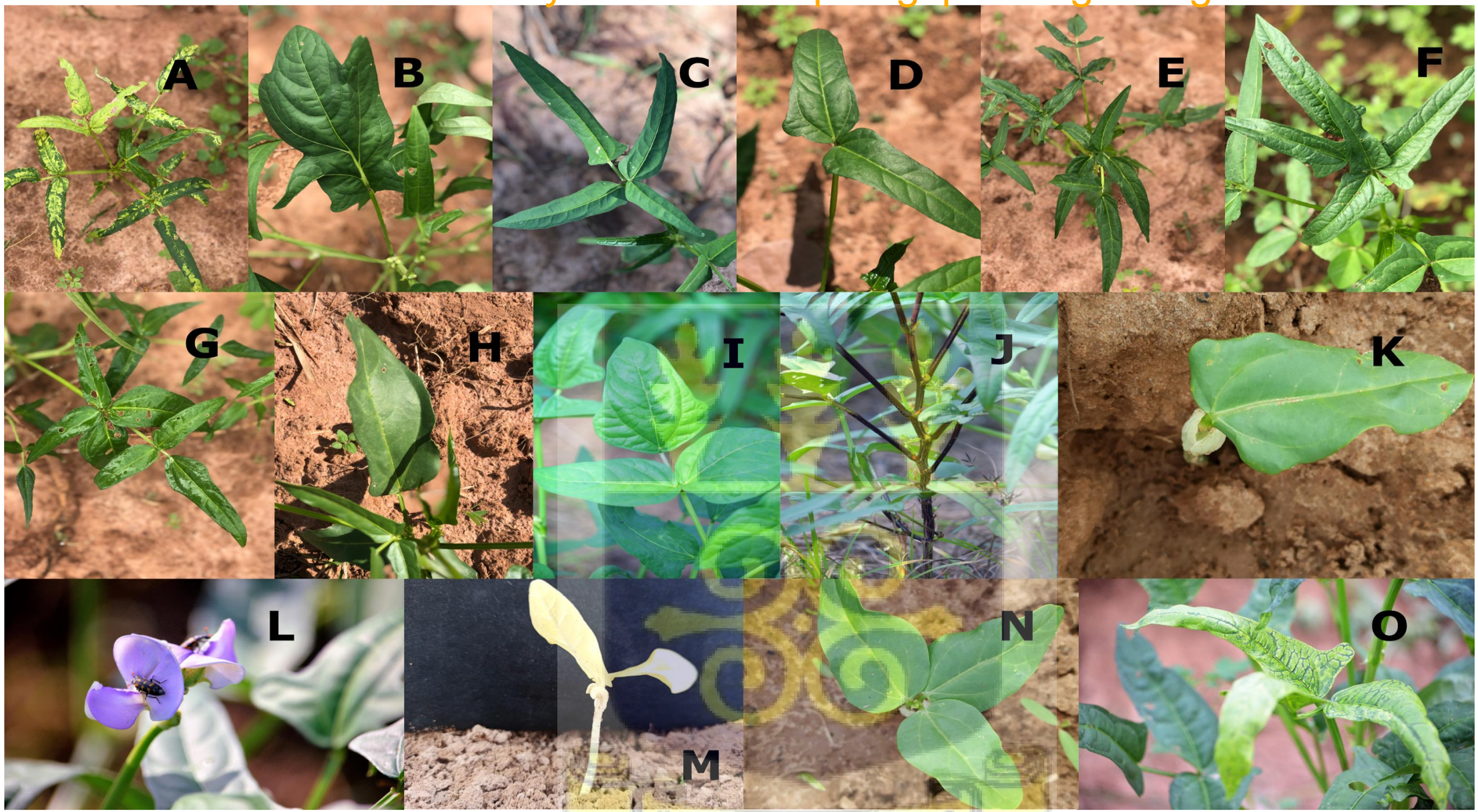

Figure 4.20: Different morphological mutations in the M_2 population. A=variegated leaf plant, B=irregular leaf, C=tetrafoliate leaf, D=bifoliate leaf, E=hexafoliate leaf, F=pentafoliate, G=septafoliate, H=monofoliate, I=sub-hastate leaf, J=solid pigmented plant, K=monopinnate leaf, L=violet flower, M=xantha seedling, N=tripinnate leaf, O=variegated leaf.

4.8.4 Frequency Distribution of Qualitative Traits among Wild type and M_2 Population.

Leaf colour: There was one phenotypic class (*dark green*) of leaf colour in the wild type and three categories in the M_2 population. *Pale green* and *intermediate green* leaf colours were the different classes that were observed only in M_2 population. The frequency ranged from 1.13% (*pale green*) to 78.12% (*dark green*) in the M_2 population (**Table 4.9**).

Leaf shape: one phenotypic class (*Hastate*) was observed in the wild type whiles two classes were observed in the M_2 population. *Sub-hastate* class which was absent from the wild type had the lowest frequency (7.12%) and *Hastate* class had the highest frequency (92.88%) in the M_2 population (**Table 4.9**).

Plant pigmentation: There were four different phenotypic categories in the wild type and six different classes in the M_2 population. The unique class (*solid* pigmentation) which was only present in the M_1 plants was also observed in the M_2 plants. The different category described as *none* was present in the M_2 plants but it was clearly absent in the wild types. The highest frequencies recorded based on treatment were 61.25% (*Moderate*) and 40.41% (*Very slight*) for the wild type and M_2 population respectively. The lowest frequency observed in the wild type was 2.5% (*Extensive*) whiles 0.33% (*Solid*) was the lowest frequency observed in the M_2 population. (**Table 4.9**).

Growth habit: This trait had only one phenotypic class (*Intermediate*) in the wild type and seven categories in the M_2 population. Same classes of growth habit that were observed in the M_1 plants were observed in the M_2 plants. Frequency ranged from 2.69% (*Climbing*) to 38.33% (*Semi-prostrate*) in the M_2 population (**Table 4.9**).

Leaf marking: There was only one phenotypic class (*present*) identified in both the wild type and M_2 population (**Table 4.9**).

Growth pattern: *Indeterminate* growth pattern was the only phenotypic class observed in both the wild type and M_2 population (**Table 4.9**).

Twinning tendency: There were two different phenotypic categories in the wild type and four different classes in the M_2 population. The categories; none and pronounced were absent from the wild type. The highest frequencies recorded based on treatment were 82.50% (*intermediate*) and 53.89% (*None*) for the wild type and M_2 population respectively (**Table 4.9**). The lowest frequency observed in the wild type was 17.50% (*Slight*) whiles 2.69% (*Pronounced*) was the lowest frequency observed in the M_2 population (**Table 4.9**).

Flower colour: There was only one phenotypic class observed for this trait in the wild type and the M_2 population (**Table 4.9**).

Pod colour: There was only one phenotypic class (*pale tan*) observed in both the wild type and M_2 population (**Table 4.9**).

Pod curvature: One phenotypic class (*Slightly curved*) was observed in the wild type whiles three classes were observed in the M_2 population. *Curved* class had the lowest frequency (0.61%) and *Straight* class had the highest frequency (65.39%) in the M_2 population (**Table 4.9**).

Seed shape: Only one phenotypic class (*rhomboid*) was observed in the wild type whiles three classes were observed in the M_2 population as shown in **(Table 4.9)**. The different seed shapes that were observed only in the mutant population were *kidney*, *globose* and *ovoid*. *Globose* class had the lowest frequency (0.05%) and rhomboid class had the highest frequency (50.07%) in the M_2 population.

Seed coat colour: There was one phenotypic category (*walnut*) that occurred in the wild type whiles three different categories were obtained in the M_2 population as shown in **(Table 4.9)**. The different seed coat colours described as *coffee* and *chocolate* were observed in the M_1 plants and as well as the M_2 plants. Frequency distribution ranged from 0.09% (*coffee*) to 85.48% (*walnut*) for the M_2 population.

Table 4.9: Frequency distribution of qualitative Traits in the M_2 Population

		Frequency (%)	
Trait	**Phenotypic class**	**Wild type**	**M2**
Leaf colour	Pale green	0.00	1.13
	Intermediate green	0.00	20.74
	Dark green	100.00	78.12
Terminal leaf shape	Hastate	100.00	92.88
	Sub-hastate	0.00	7.12
Leaf marking	Absent	0.00	0.00
	Present	100.00	100.00
Plant pigmentation	None	0.00	2.55
	Very slight	18.75	40.41
	Moderate	61.25	39.51
	Intermediate	17.50	13.11
	Extensive	2.50	4.1
	Solid	0.00	0.33
Growth pattern	Determinate	0.00	0.00
	Indeterminate	100.00	100.00
Flower colour	Violet	100.00	100.00
	White	0.00	0.00
Growth habit	Acute erect	0.00	32.91
	Erect	0.00	9.34
	Semi-erect	0.00	7.45
	Intermediate	100.00	6.08
	Semi-prostrate	0.00	38.33
	Prostrate	0.00	3.21
	Climbing	0.00	2.69
Twinning tendency	None	0.00	53.89
	Slight	17.50	28.29
	Intermediate	82.50	15.13
	Pronounced	0.00	2.69
Pod curvature	Straight	0.00	65.39
	Slightly curved	100.00	33.99
	Curved	0.00	0.61
Pod colour	Pale tan/Straw	100.00	100.00
Seed shape	Kidney	0.00	2.03
	Ovoid	0.00	47.67
	Crowder	0.00	0
	Globose	0.00	0.05
	Rhomboid	100.00	50.07
Seed coat colour	Walnut	100.00	85.48
	chocolate	0.00	14.43
	Coffee	0.00	0.09

4.8.5 Distribution of Yield and Sub-Yield Characters

Data was collected on 2,201 individuals in the M_2 generation; 2,121 mutagenized individuals and 80 individuals in the wild type. The other mutants were intertwined and individual plants could not be traced. Pods from such plants were harvested by bulking.

4.8.5.1 Days to Flowering

In the M_2 generation, the wild type and M_2 population showed continuous phenotypic variation for days to flowering (**figure 4.21**). The wild type ranged from 40-62 days and the mutagenized population ranged from 38-63 days (**figure 4.21**). The median value obtained in the wild type was 54 and the mutagenized population's median value was 44. Fifty percent of the individuals in the mutant population flowered within 44 days.

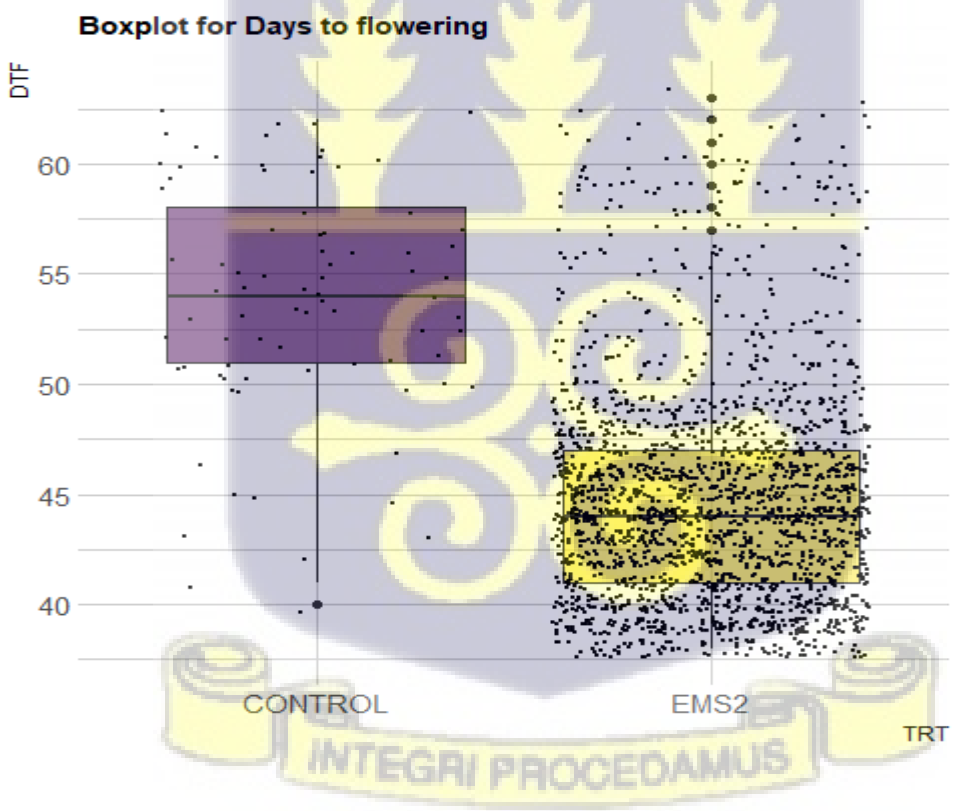

Figure 4.21 Distribution of days to flowering into 25th percentile, 50th percentile and 75th percentile in the wild type and M_2 population. DTF=Days to Flowering, TRT=Treatment, EMS=Ethyl methane sulfonate. Black dots denote the dispersion and frequency distribution of the phenotypic data in each treatment.

4.8.5.2 Number of Pods per Plant

There was high phenotypic variation in the mutagenized population with some outliers whereas low variation was observed in the wild type for number of pods per plant in the M_2 generation. (**figure 4.22**). The range of the mutagenized population was 70 (from 1-71) and the range of the wild type was 14 (from 10-34) (**figure 4.22**). The median value obtained in the wild type was 28 and the median value recorded in the M_2 population was 12. Fifty percent of the mutagenized population had 1-12 pods.

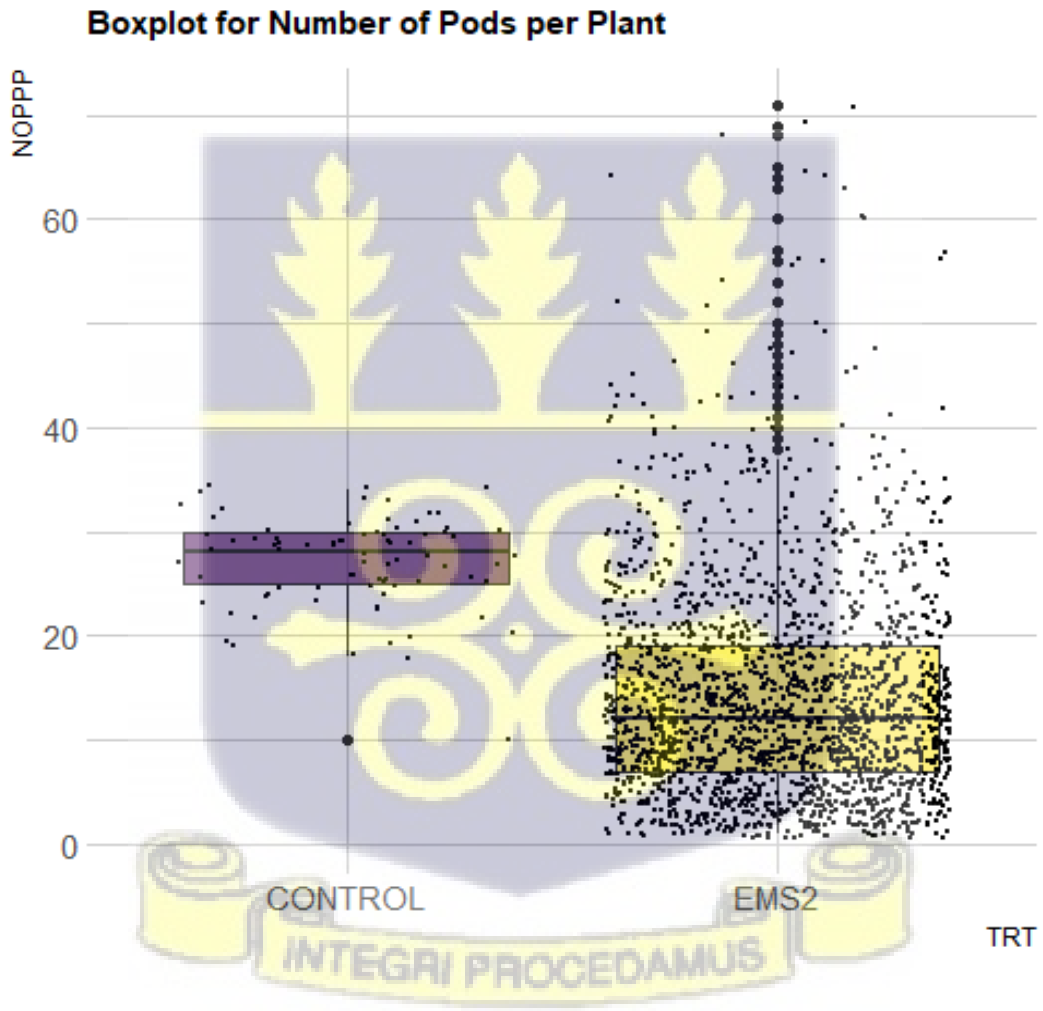

Figure 4.22: Distribution of number of pods per plant into 25th percentile, 50th percentile and 75th percentile in the wild type and M_2 population. NOPPP=Number of Pods per Plant, TRT=Treatment, EMS=Ethyl methane sulfonate. Black dots denote the dispersion and frequency distribution of the phenotypic data in each treatment.

4.8.5.3 Number of Seeds Per Pod

The mutagenized population in the M_2 generation was widely distributed whiles the wild type was narrowly distributed for number of seeds per pod as shown in **figure 4.23**. There were outliers observed in both the wild type and mutant population (**figure 4.23**). The wild type ranged from 9-17 seeds per pod and the mutagenized population ranged from 0-19 seeds per pod (**figure 4.23**). The median value obtained in both the wild type and the M_2 population was 14.

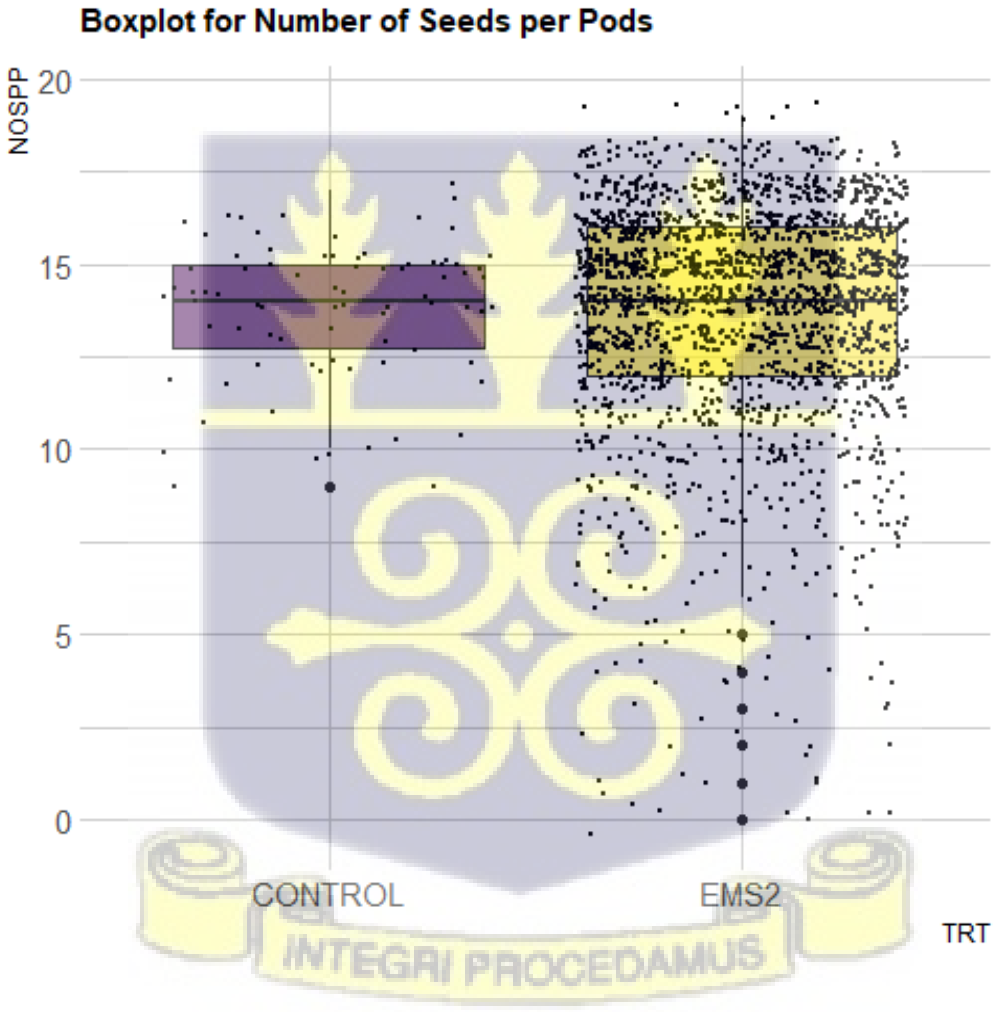

Figure 4.23: Distribution of number of seeds per pods per plant into 25th percentile, 50th percentile and 75th percentile in the wild type and M_2 population. NOSPP=Number of Seeds per Pod per Plant, TRT=Treatment, EMS=Ethyl methane sulfonate. Black dots denote the dispersion and frequency distribution of the phenotypic data in each treatment.

4.8.5.4 Number of Locules Per Pod

In the M_2 generation, there were some outliers observed in both the wild type and mutagenized population for number of locules per pod (**figure 4.24**). However, there was high phenotypic variation in the mutagenized population as compared to the wild type for this trait. The wild type ranged from 10-17 and the mutagenized population recorded a range of 4-20 (**figure 4.24**). The median value obtained in the M_2 population (median=16) was higher as compared to the wild type (median=15). Fifty percent of the mutant population had 16 and more locules in a pod.

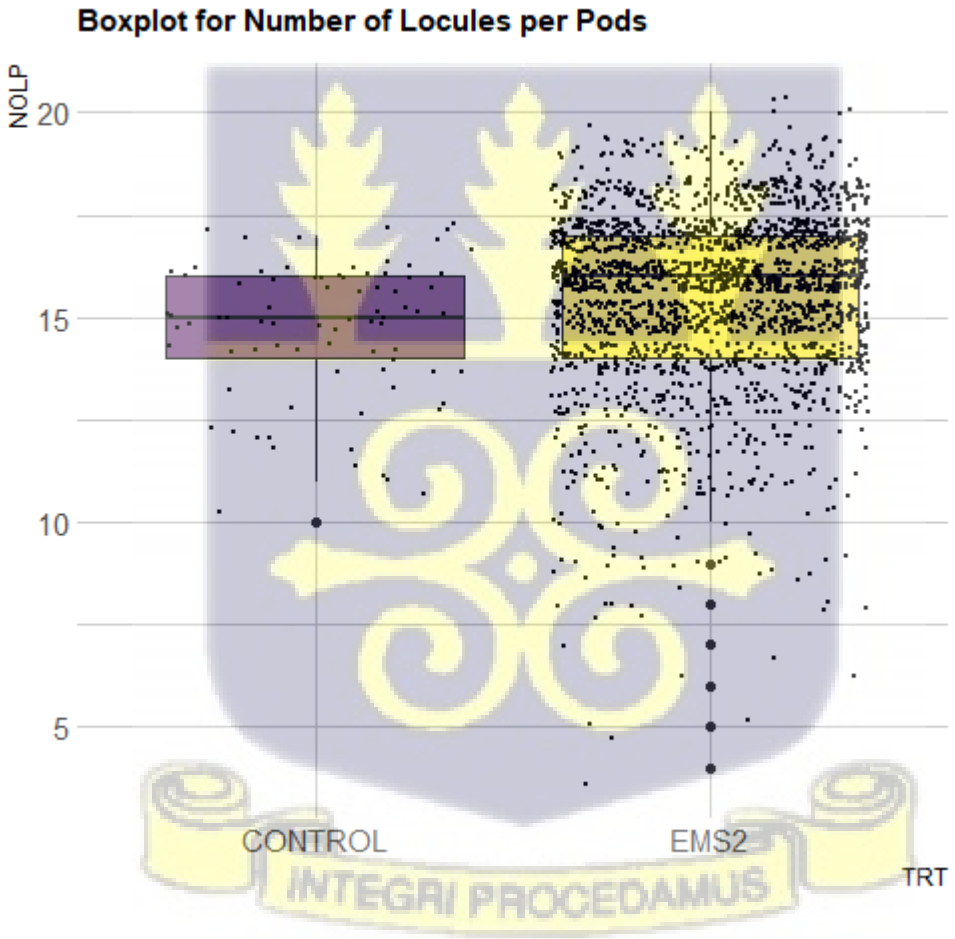

Figure 4.24: Distribution of number of loculess per pods per plant into 25th percentile, 50th percentile and 75th percentile in the wild type and M_2 population. NOLPP=Number of Locules per Pod per Plant, TRT=Treatment, EMS=Ethyl methane sulfonate. Black dots denote the dispersion and frequency distribution of the phenotypic data in each treatment.

4.8.5.5 Percentage Seed Set Per Pod

The mutagenized population had high phenotypic variation with many outliers for percentage seed set per pod (**figure 4.25**). A range of 81.82-100% was observed in the wild type and the mutagenized population had a range of 0.00-100% (**figure 4.25**). The median value obtained for both the wild type and the M_2 population was 93.33%. Fifty percent of the mutagenized individuals in the M_2 generation had 0 to 93.33% seed set.

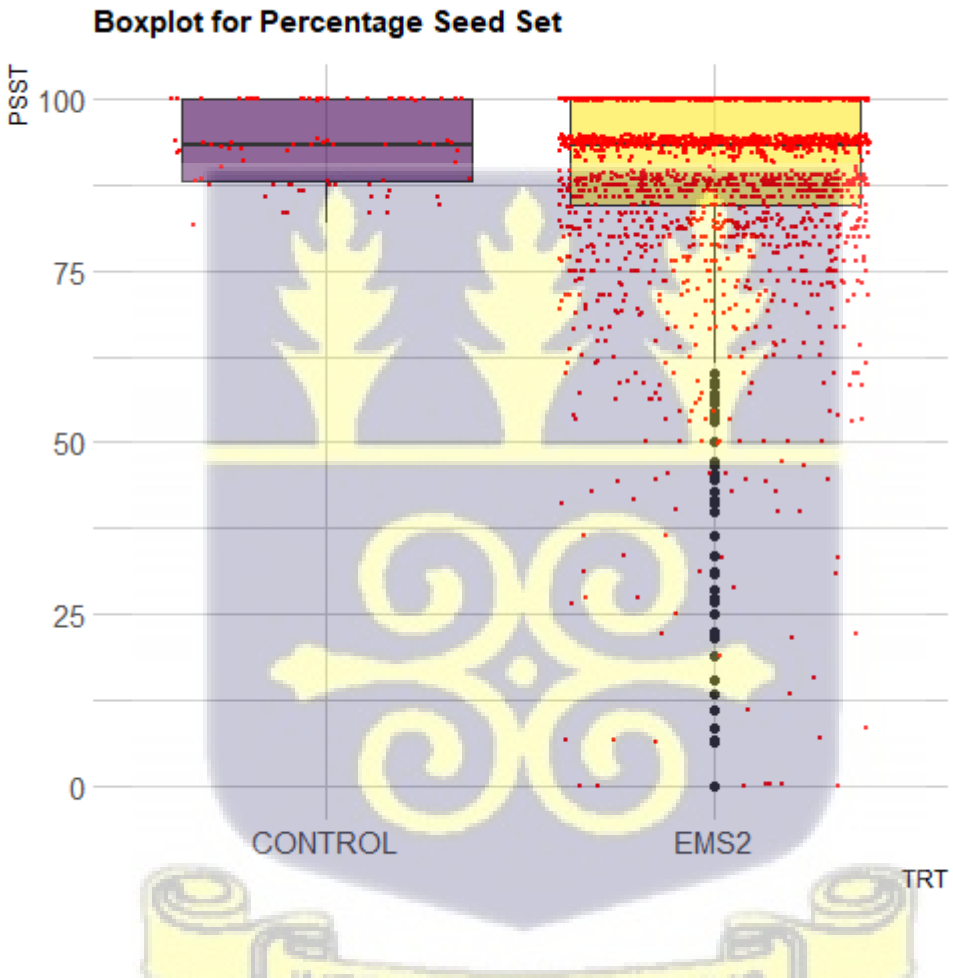

Figure 4.25: Distribution of percentage seed set per pod per plant into 25th percentile, 50th percentile and 75th percentile in the wild type and M_2 population. PSST=Percentage Seed Set, TRT=Treatment, EMS=Ethyl methane sulfonate. Red dots denote the dispersion and frequency distribution of the phenotypic data in each treatment.

4.8.5.6 Pod Length

The M_2 population was widely distributed with many outliers for pod length as shown in **figure 4.26**. The wild type was however narrowly distributed with a range of 12.1-18.3 cm. The mutagenized population had a range of 8.3-20.4 cm (**figure 4.26**). Fifty percent of the individuals in the M_2 population had pod length of 17.1 cm and above. The median value recorded in the wild type was 15.7 cm.

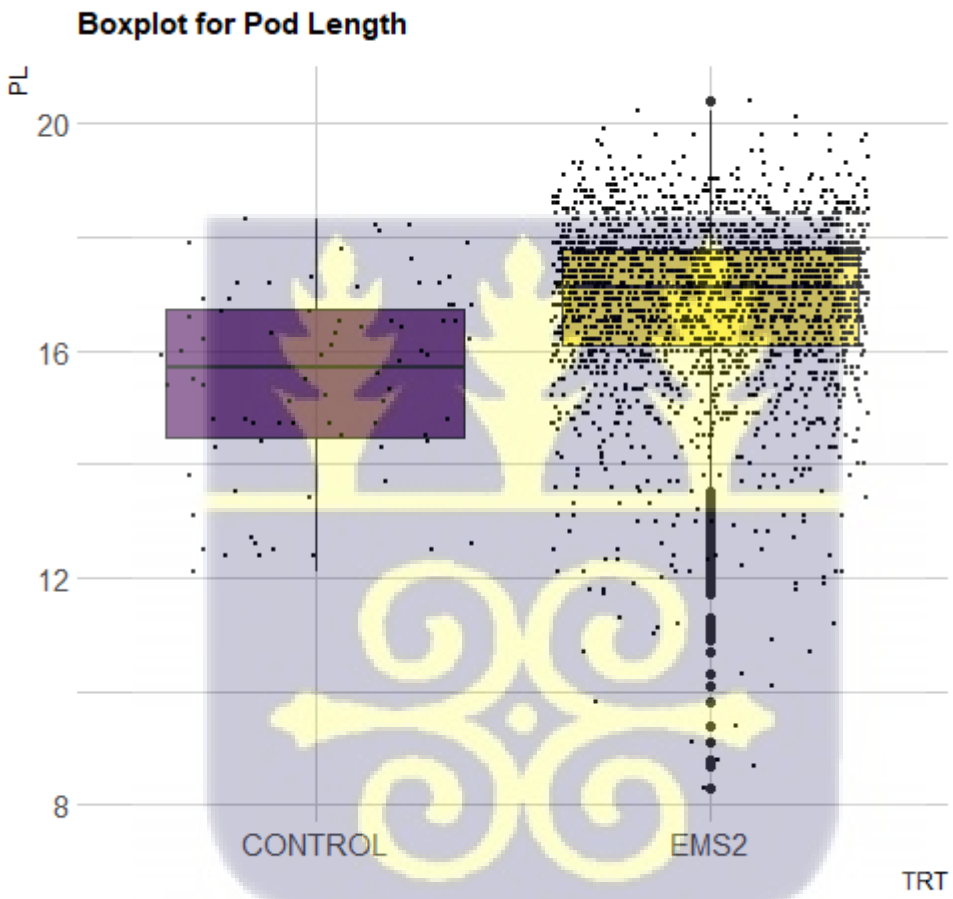

Figure 4.26: Distribution of pod length into 25th percentile, 50th percentile and 75th percentile in the wild type and M_2 population. PL=Pod Length, TRT=Treatment, EMS=Ethyl methane sulfonate. Black dots denote the dispersion and frequency distribution of the phenotypic data in each treatment.

4.8.5.7 Number of Seeds Per Plant

In the M_2 generation, high phenotypic variation was observed in the mutagenized population with many outliers for number of seeds per plant (**figure 4.27**). The mutagenized population ranged from 0-1156 and the wild type ranged from 150-544 (**figure 4.27**). The variation in the wild type was low. However, the median value obtained in the wild type was higher than median value recorded for the mutant population. Fifty percent of individuals in the M_2 population had 0 to 165 number of seeds.

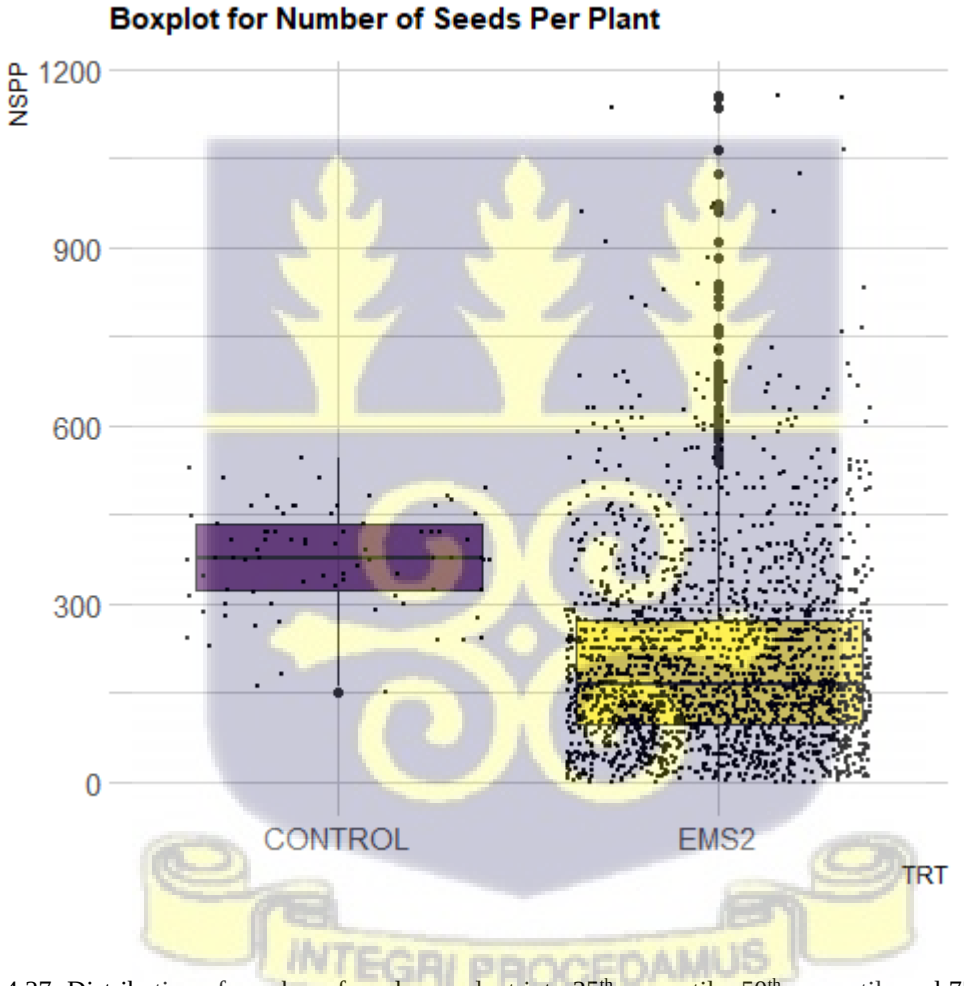

Figure 4.27: Distribution of number of seeds per plant into 25th percentile, 50th percentile and 75th percentile in the wild type and M_2 population. NSSP=Number of Seeds per Plant, TRT=Treatment, EMS=Ethyl methane sulfonate. Black dots denote the dispersion and frequency distribution of the phenotypic data in each treatment.

4.9 Pearson's Chi-square Test of Associations Among Qualitative Traits.

There was only one association observed in the wild type which was not significant. Plant pigmentation associated with twinning tendency ($\chi^2 = 3.654$, P = 0.301).

A total of twenty-seven (27) associations were observed in the mutagenized population (M_2 generation). There were thirteen significant associations ($p < 0.05$) observed (**Table 4.10**). Leaf colour was significantly associated with terminal leaf shape, growth habit and twinning tendency (**Table 4.10**). There were significant association between terminal leaf shape and plant pigmentation and growth habit (**Table 4.10**). Growth habit was significantly associated with plant pigmentation, twinning tendency, seed shape and seed coat colour (**Table 4.10**). There was significant association between plant pigmentation and twinning tendency and twinning tendency also correlated with seed shape and seed coat colour (**Table 4.10**). There was no significant association between pod curvature and other qualitative traits.

Table 4.10: Person's chi-square test of associations among qualitative traits in the mutagenized population.

Traits	Associations	Pearson's Chi-square value	P-value
Leaf colour	Terminal leaf shape	15.072	**0.001**
	Growth habit	104.925	**0.000**
	Plant pigmentation	6.910	0.734
	Twinning tendency	98.806	**0.000**
	Pod curvature	5.165	0.271
	Seed shape	11.738	0.163
	Seed coat colour	18.263	**0.001**
Terminal leaf shape	Growth habit	14.671	**0.023**
	Plant pigmentation	16.396	**0.006**
	Twinning tendency	3.016	0.389
	Pod curvature	2.766	0.251
	Seed shape	0.846	0.932
	Seed coat colour	0.191	0.909
Growth habit	Plant pigmentation	113.249	**0.000**
	Twinning tendency	816.844	**0.000**
	Pod curvature	20.805	0.053
	Seed shape	46.408	**0.004**
	Seed coat colour	25.007	**0.015**
Plant pigmentation	Twinning tendency	92.835	**0.000**
	Pod curvature	8.196	0.610
	Seed shape	21.365	0.376
	Seed coat colour	15.359	0.119
Twinning tendency	Pod curvature	3.871	0.694
	Seed shape	30.363	**0.002**
	Seed coat colour	17.007	**0.009**
Pod curvature	Seed shape	2.138	0.976
	Seed coat colour	1.483	0.830

4.10 Principal Component Analysis of Quantitative Variables of M_2 population and Wild type

4.10.1 Principal Component Analysis of Quantitative Traits in the Wild type (M_2 Generation)

Principal component analysis was done to identify the traits that contributed most to the total variability observed in the M_2 generation. The eigenvalue for the first principal component was 3.3190 which represents 47.41 % of the total variation in the wild type (**Table 4.11**). The second principal component analysis had an eigenvalue of 1.3447 which represents 19.21 % of the total variation (**Table 4.11**). The eigenvalues for the third and fourth principal components were 1.0765 and 0.7141 (**Table 4.11**). Thus, the first four principal components explained 92.20 % of the total variation in the wild type. Number of seeds per pod, number of pods per plant, days to flowering and number of locules per pod were the variables with the highest contribution in dimension 1, dimension 2, dimension 3 and dimension 4 respectively.

Table 4.11: Principal component analysis among the wild type showing relative contributions of quantitative variables in the M_2 generation. Dim.1=Dimension 1(PC1), Dim.2= Dimension 2(PC2), Dim.1=Dimension 3(PC3), Dim.4= Dimension 4(PC4)

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
Eigenvalue	3.3190	1.3447	1.0765	0.7141	0.5375
%Total Variance	47.41	19.21	15.38	10.20	7.68
%Cumulative	47.41	66.62	82.00	92.20	99.88
Traits	**Contribution of Variables**				
NOPPP	7.429	**44.665**	10.461	0.159	-0.114
NOSPP	**25.562**	4.432	4.738	0.010	0.200
NOLP	19.274	13.058	0.094	**0.352**	**0.243**
PSST	10.480	4.102	19.469	-0.622	0.020
PL	12.209	14.643	0.858	-0.029	-0.623
NSPP	23.774	13.344	1.335	0.111	0.026
DTF	1.271	5.755	**63.045**	-0.407	0.191

NOPPP-Number of Pods per Plant, NOSPP-Number of Seeds per Pods, DTF-Days to Flowering, PL-Pod Length, NOLP-Number of Locules per Pod, NSPP-Number of seeds per pod, PSST-Percentage seed set.

From the scree plot (**figure 4.28**), the total variation was explained by all the principal components. There was a sharp decline of percentage variability from dimension1 to dimension 2. Dimensions 2 to 5 explained moderate amount of variability (**figure 4.28**). The curve flattened at 0% variability from dimension 6 to7. The latter two dimensions explained no proportion of variability in the wild type.

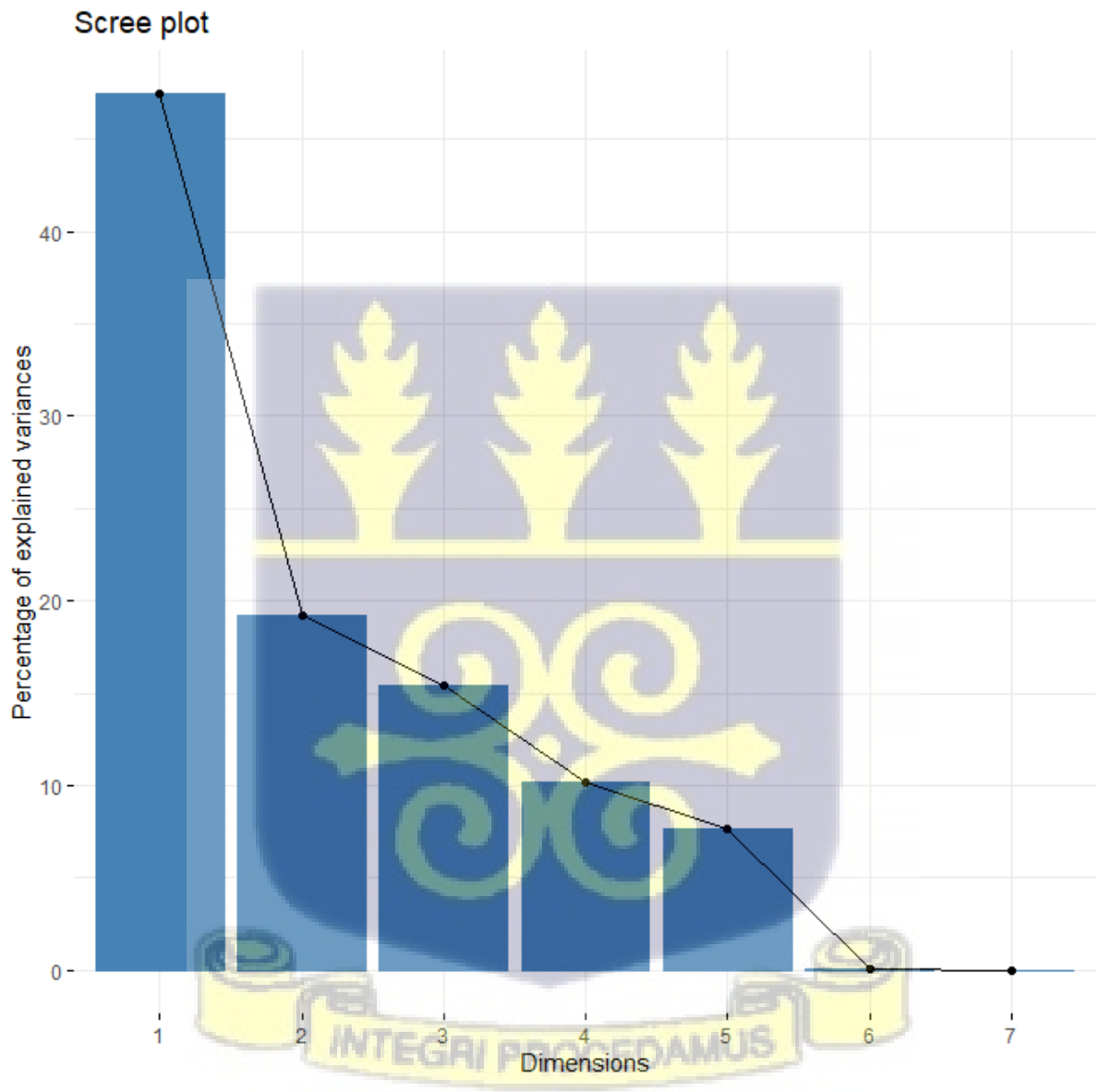

Figure 4.28: Scree plot of percentage of explained variance among dimensions (Principal Components, PC) of quantitative traits in the wild type (M_2 Generation).

4.10.2 Principal Component Analysis of Quantitative Traits in the Mutagenized Population (M_2 Generation)

The first principal component had an eigenvalue of 3.4359 which represents 49.08 % of the total variation in the M_2 population (**Table 4.12**). The eigenvalue of the second principal component analysis was 1.5918 which represents 22.74 % of the total variation (**Table 4.12**). The third and fourth dimensions had eigenvalues of 0.8675 and 0.7955 respectively (**Table 4.12**). Thus, the first four principal component explained 95.58 % of the total variation in the M_2 population. Number of Seeds per Pod and Number of Pods per Plants were the variables with the highest contribution in dimension 1 and dimension 2 respectively.

Table 4.12: Principal component analysis among the M_2 population showing relative contributions of quantitative variables. Dim.1=Dimension 1(PC1), Dim.2= Dimension 2(PC2), Dim.1=Dimension 3(PC3), Dim.4= Dimension 4(PC4)

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
Eigenvalue	3.4359	1.5918	0.8675	0.7955	0.2803
%Total Variance	49.08	22.74	12.39	11.36	4.00
%Cumulative	49.08	71.82	84.22	95.58	99.59
Traits	**contributions of variables**				
NOPPP	11.126	**33.690**	3.765	5.123	0.002
NOSPP	**22.257**	11.416	2.344	1.282	6.048
NOLP	17.057	7.627	9.420	15.731	29.544
PSST	12.164	7.621	26.328	28.201	1.794
PL	18.755	2.820	4.780	12.217	**61.382**
NSPP	16.457	22.716	2.970	4.484	0.881
DTF	2.183	14.111	**50.393**	**32.963**	0.350

NOPPP-Number of Pods per Plant, NOSPP-Number of Seeds per Pods, DTF-Days to Flowering, PL-Pod Length, NOLP-Number of Locules per Pod, NSPP-Number of seeds per pod, PSST-Percentage seed set.

The total variation was explained by all the principal components as shown in **Figure 4.29.** The curve declined sharply from dimension 1 to 2 (**Figure 4.29**). Much of the total variability was explained by the first 2 dimensions and dimensions 3, 4, and 5 explained moderate variability. The last two dimensions explained small fraction of the total variability.

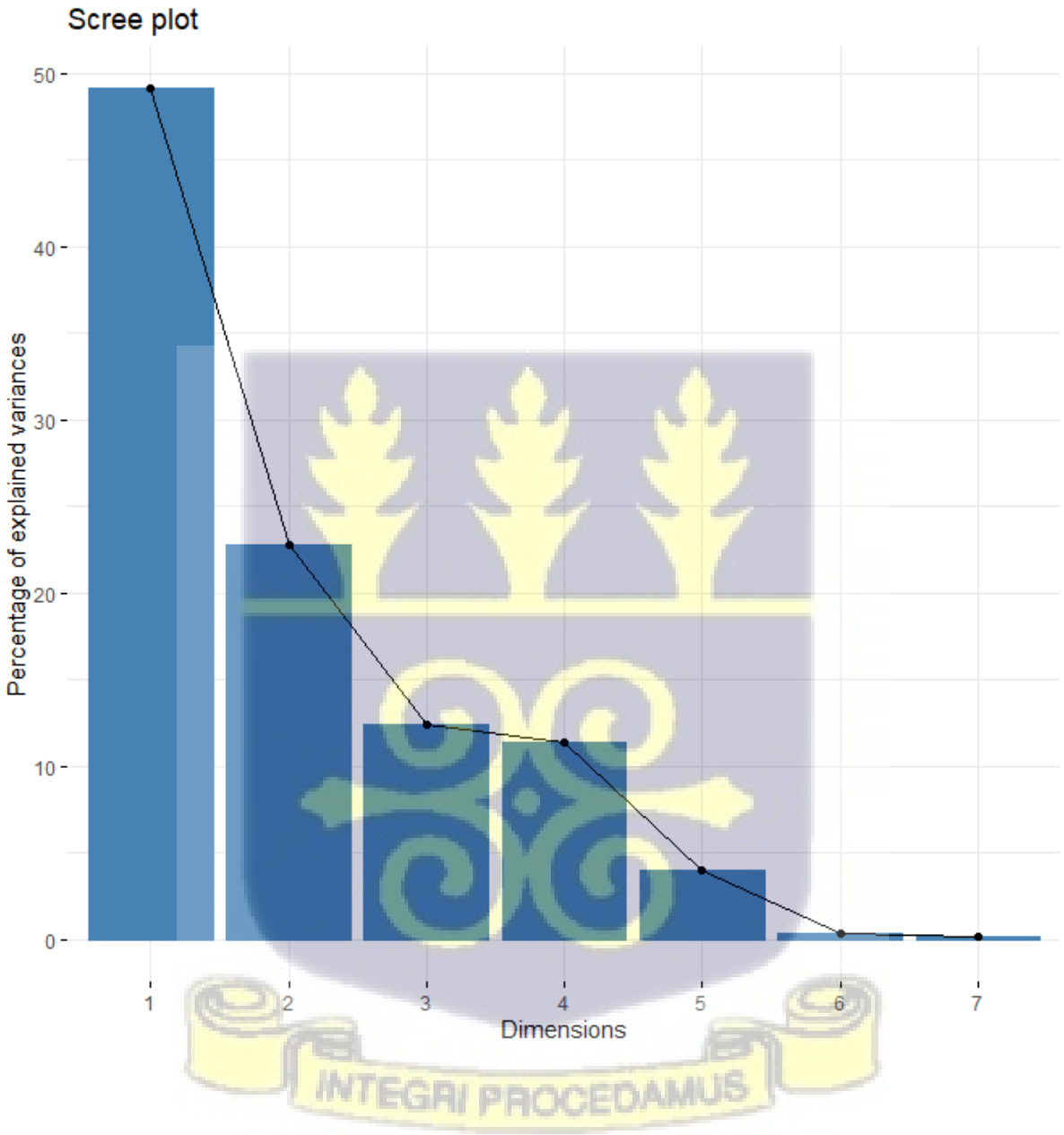

Figure 4.29: Scree plot of percentage of explained variance among dimensions (Principal Components, PC) of quantitative traits in the mutagenized population (M_2 Generation).

4.10.3 Correlation Between Quantitative Variables and Principal Components (Dimensions) in the Wild type and Mutagenized Population (M_2 Generation).

There were positive and negative correlations among the seven quantitative variables as shown in **figure 4.30 and figure 4.31** by the first two principal components in the M_2 generation. The correlation circles were based on the total variability in PC1 and PC2. Traits presented in the same quadrant depicted a positive correlation whiles negative correlated variables point opposite.

In the wild type, percentage variabilities of 47.40% in Dim.1 (PC1) and 19.20% in Dim.2 (PC2) were extracted and used in the construction of the correlation circle with four equal parts which had Dim.1 and Dim.2 represented on both x- axis and y-axis respectively (**figure 4.30**). Percentage seed set (PSST), number of pods per plant (NOPPP), number of seeds per plant (NSPP) formed an acute angel in the right-bottom quadrant indicating a positive and strong correlation among the parameters. A positive correlation existed among days to flowering (DTF), number of locules per pod (NOLP), pod length (PL) and number of seeds per pod (NOSP) represented by an acute angle **(figure 4.30**).

In the M_2 population, percentage variabilities of 49.1% in Dim.1 (PC1) and 22.7% in Dim.2 (PC2) were extracted and used in the construction of the correlation circle (**figure 4.31**). A negative correlation existed among days to flowering and the yield characters (number of pods per plant (NOPPP, number of seeds per pods (NOSP), number of locules per pod (NOLP), percentage seed set (PSST), number of seeds per plant (NSPP) and pod length (PL)). Number of seeds per plant (NSPP) formed acute angle with number of pods per plant; however, both negatively correlated with days to flowering (DTF). Number of locules per pod, number of seeds per pod, pod length and percentage seed set indicating strong positive correlation among these traits.

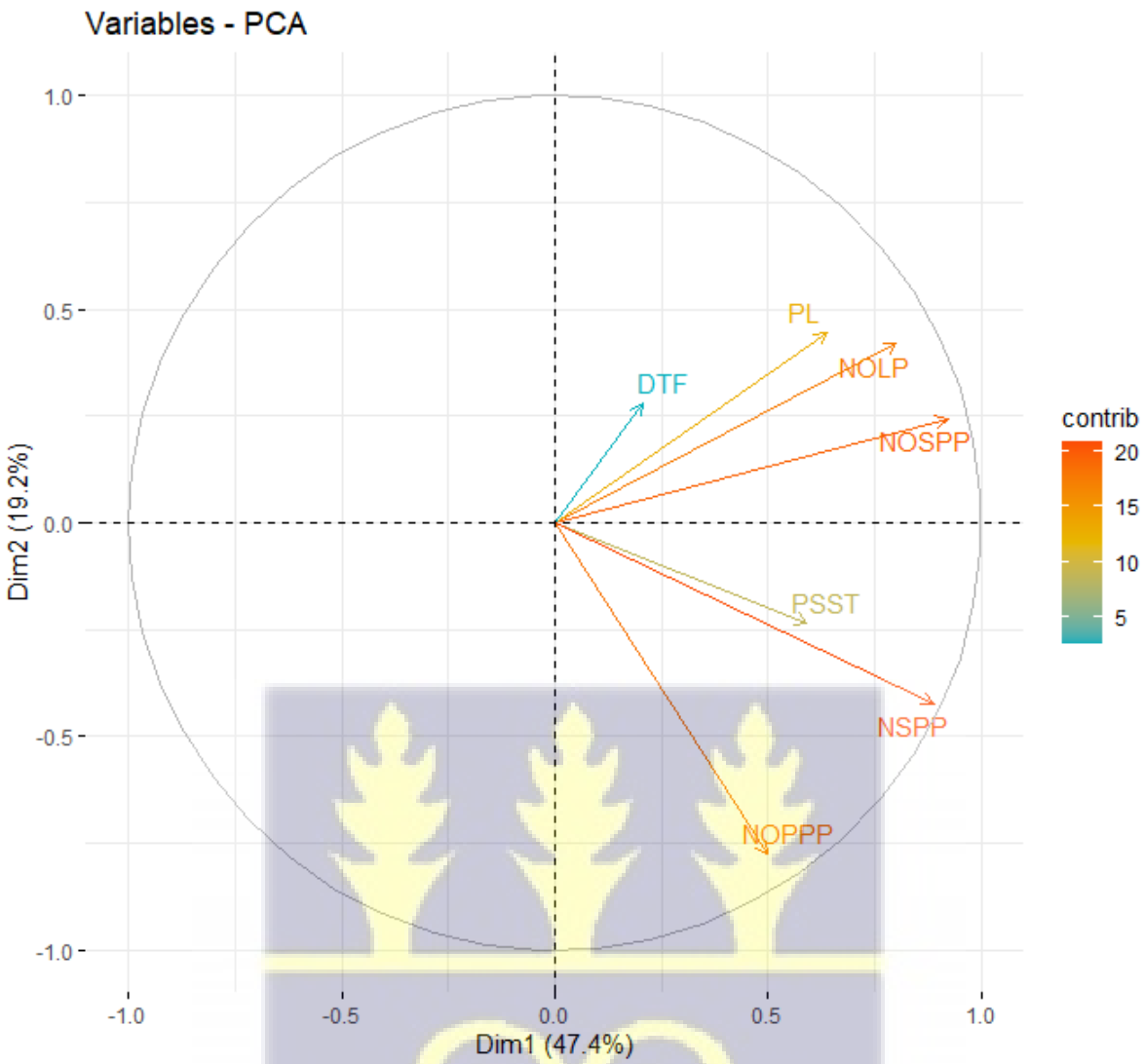

Figure 4.30: Correlation circle of quantitative variables and dimensions (Principal Components, PC) in the wild type (M_2 Generation). Positive correlated variables point to the same side of the plot. Negative correlated variables point to opposite sides of the graph. NOPP-Number of Pods per Plant, NOPPP-Number of Pods per Peduncle, DTF-Days to Flowering, PL-Pod Length, NOLP-Number of Locules per Pod, NOSP-Number of Seeds per Pods, NOSPP- Number of Seeds per Plant, PSST- Percentage Seed Set.

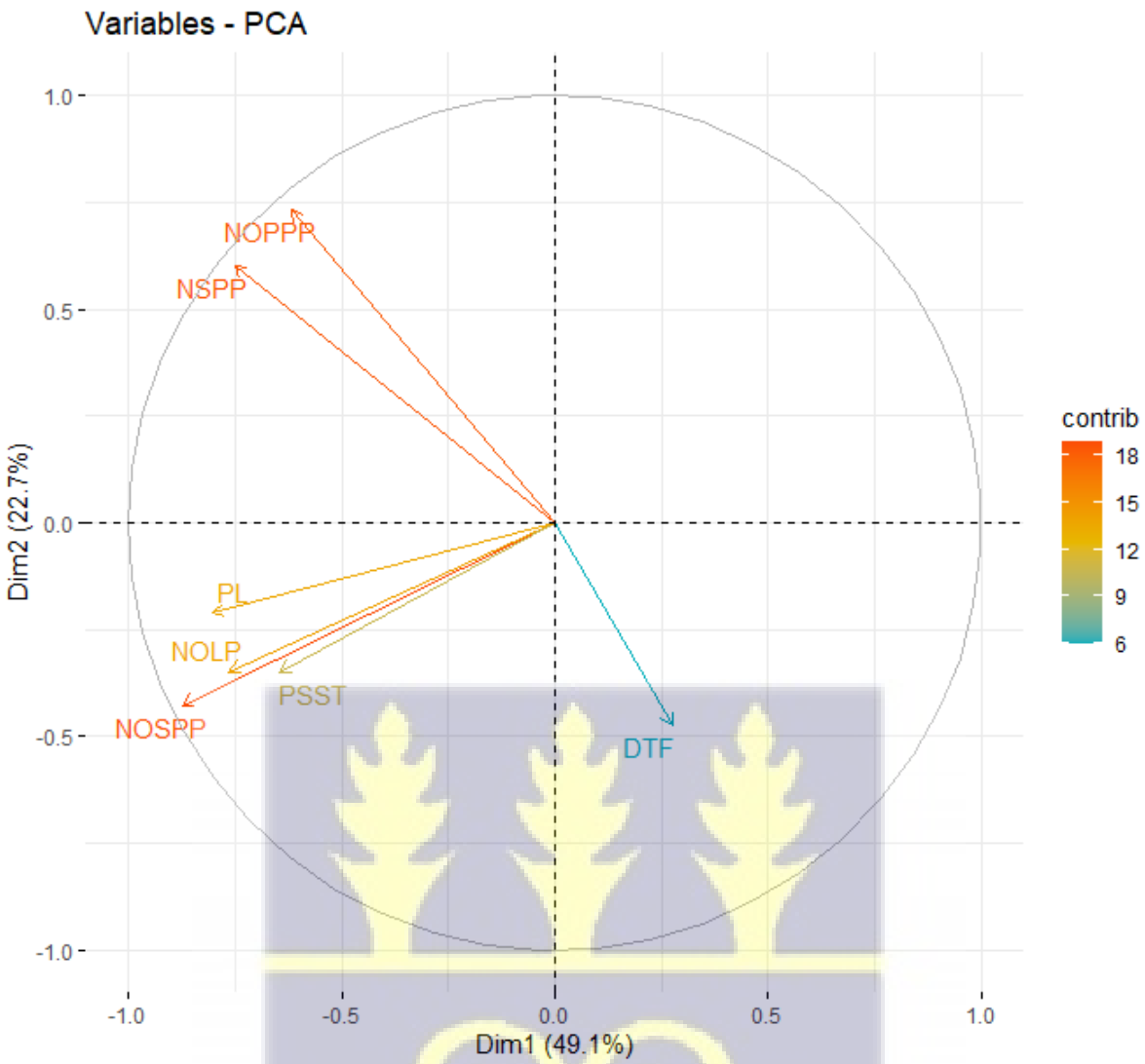

Figure 4.31: Correlation circle of quantitative variables and dimensions (Principal Components, PC) in the mutagenized population (M_2 Generation). Positive correlated variables point to the same side of the plot. Negative correlated variables point to opposite sides of the graph. NOPP-Number of Pods per Plant, NOPPP-Number of Pods per Peduncle, DTF-Days to Flowering, PL-Pod Length, NOLP-Number of Locules per Pod, NOSP-Number of Seeds per Pods, NOSPP- Number of Seeds per Plant, PSST- Percentage Seed Set.

4.10.4 Principal Component Analysis of Qualitative Traits in the Wild type (M_2 Generation)

The eigenvalue of the first principal component (Dim.1) was 1.880 which accounted for 76.65% variability among the qualitative traits in the wild type in the M_2 generation (**Table 4.13)**. The second principal component (Dim.2) had an eigenvalue of 0.573 and accounted 23.35% variation (**Table 4.13)**. The first two principal components therefore accounted for a total of 100.00% variability. Plant pigmentation (PP) and twinning tendency (TT) highly contributed to Dim.1 and Dim.2 respectively **(Table 4.13)**.

Table 4.13: Principal component analysis among the wild type showing relative contributions of qualitative variables in the M_2 generation. Dim.1=Dimension 1(PC1), Dim.2= Dimension 2(PC2), Dim.1=Dimension 3(PC3).

	Dim.1	Dim.2	Dim.3	Dim.4
Eigenvalue	1.880	0.573	0.000	0.000
%Total Variance	76.65	23.35	0.00	0.00
%Cumulative	76.65	100.00	100.00	100.00
Traits	**Contribution of variables**			
LC	0.00	0.00	0.00	0.00
TLS	0.00	0.00	0.00	0.00
LM	0.00	0.00	0.00	0.00
GP	0.00	0.00	0.00	0.00
FC	0.00	0.00	0.00	0.00
GH	0.00	0.00	0.00	0.00
PP	**99.07**	0.93	0.00	0.00
TT	0.93	**99.07**	0.00	0.00
PCURV	0.00	0.00	0.00	0.00
PC	0.00	0.00	0.00	0.00
SS	0.00	0.00	0.00	0.00
SCC	0.00	0.00	0.00	0.00

Leaf colour, TLS-terminal leaf shape, Leaf marking, Growth pattern, FC-Flower color, GH-Growth habit, PP-Plant Pigmentation, TT-twinning tendency, PCURV- Pod Curvature, PC-Pod Colour, SC- Seed Coat Colour, SS-Seed Shape.

From the scree plot (**figure 4.32**), the total variation was explained by the first two principal components. The first dimension explained much of the variability in the wild type. The curve declined sharply from dimension 1 to dimension 2 (**figure 4.32**). The second dimension explained moderate variability. The curve flattened at 0% from dimension 3 to 10. The latter 7 components did not account for variability in the wild type.

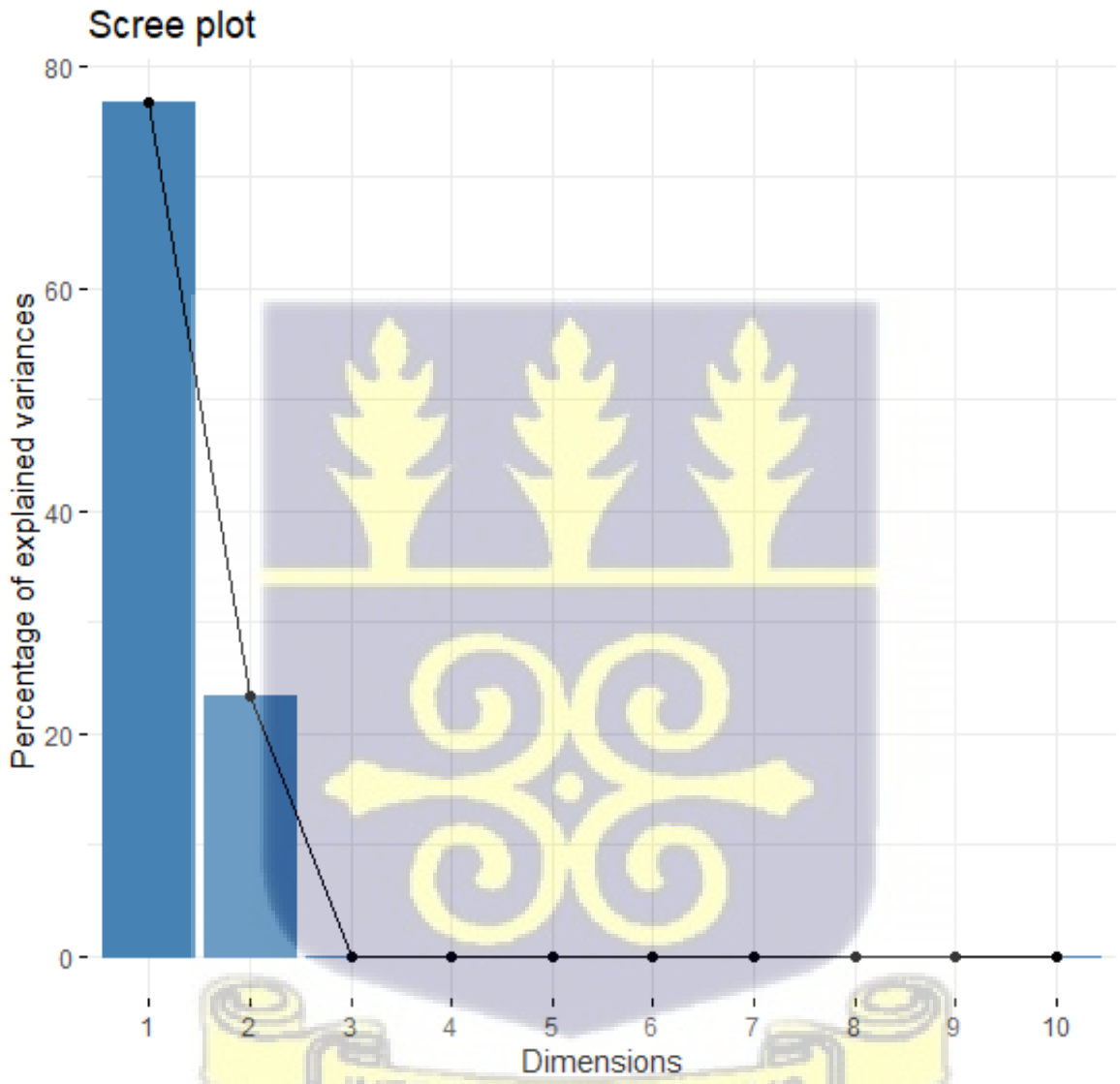

Figure 4.32: Scree plot of percentage of explained variance among dimensions (Principal Components, PC) of qualitative traits in the wild type (M_2 Generation).

4.10.5 Principal Component Analysis of Qualitative Traits in the Mutagenized Population (M_2 Generation)

The eigenvalue for the first principal component was 6.4304 which represents 41.99 % of the total variation among the qualitative traits in the M_2 population (**Table 4.14**). The second principal component analysis had an eigenvalue of 3.0171which represents 19.70 % of the total variation (**Table 4.14**). The eigenvalues for the third and fourth principal components were 2.3087 and 1.6492 respectively (**Table 4.14**). The first four principal components therefore explained 87.54 % of the total variation in the M_2 population. Twinning tendency (TT), plant pigmentation (PP), seed shape (SS) and growth habit were the variables with the highest contribution in dimension 1, dimension 2, dimension 3 and dimension 4 respectively (**Table 4.14**).

Table 4.14: Principal component analysis among the M_2 population showing relative contributions of qualitative variables. Dim.1=Dimension 1(PC1), Dim.2= Dimension 2(PC2), Dim.2=Dimension 3(PC3), Dim.4=Dimension 4(PC4).

	Dim.1	Dim.2	Dim.3	Dim.4
Eigenvalue	6.4304	3.0171	2.3087	1.6492
%Total variance	41.99	19.70	15.08	10.77
%Cumulative	41.99	61.69	76.77	87.54
	contribution of variables			
LC	0.745	0.000	0.001	0.014
TLS	0.002	0.009	0.000	0.000
LM	0.000	0.000	0.000	0.000
GP	0.000	0.000	0.000	0.000
FC	0.000	0.000	0.000	0.000
GH	40.215	5.668	0.000	**53.710**
PP	0.683	**92.666**	0.790	5.763
TT	**57.855**	1.021	0.957	39.732
PCURV	0.018	0.047	0.014	0.076
PC	0.000	0.000	0.000	0.000
SS	0.466	0.587	**98.237**	0.703
SCC	0.017	0.002	0.001	0.002

Leaf colour, TLS-terminal leaf shape, Leaf marking, Growth pattern, FC-Flower color, GH-Growth habit, PP-Plant Pigmentation, TT-twinning tendency, PCURV- Pod Curvature, PC-Pod Colour, SC- Seed Coat Colour, SS-Seed Shape.

The total variation was explained by the first eight principal components as shown in **figure 4.33.** The first dimension explained much of the variability. The curve declined sharply from dimension 1 to dimension 5. Dimensions 2, 3 and 4 explained moderate amount of variability in the M_2 population. The latter dimensions accounted for small fraction of overall variability in the M_2 population.

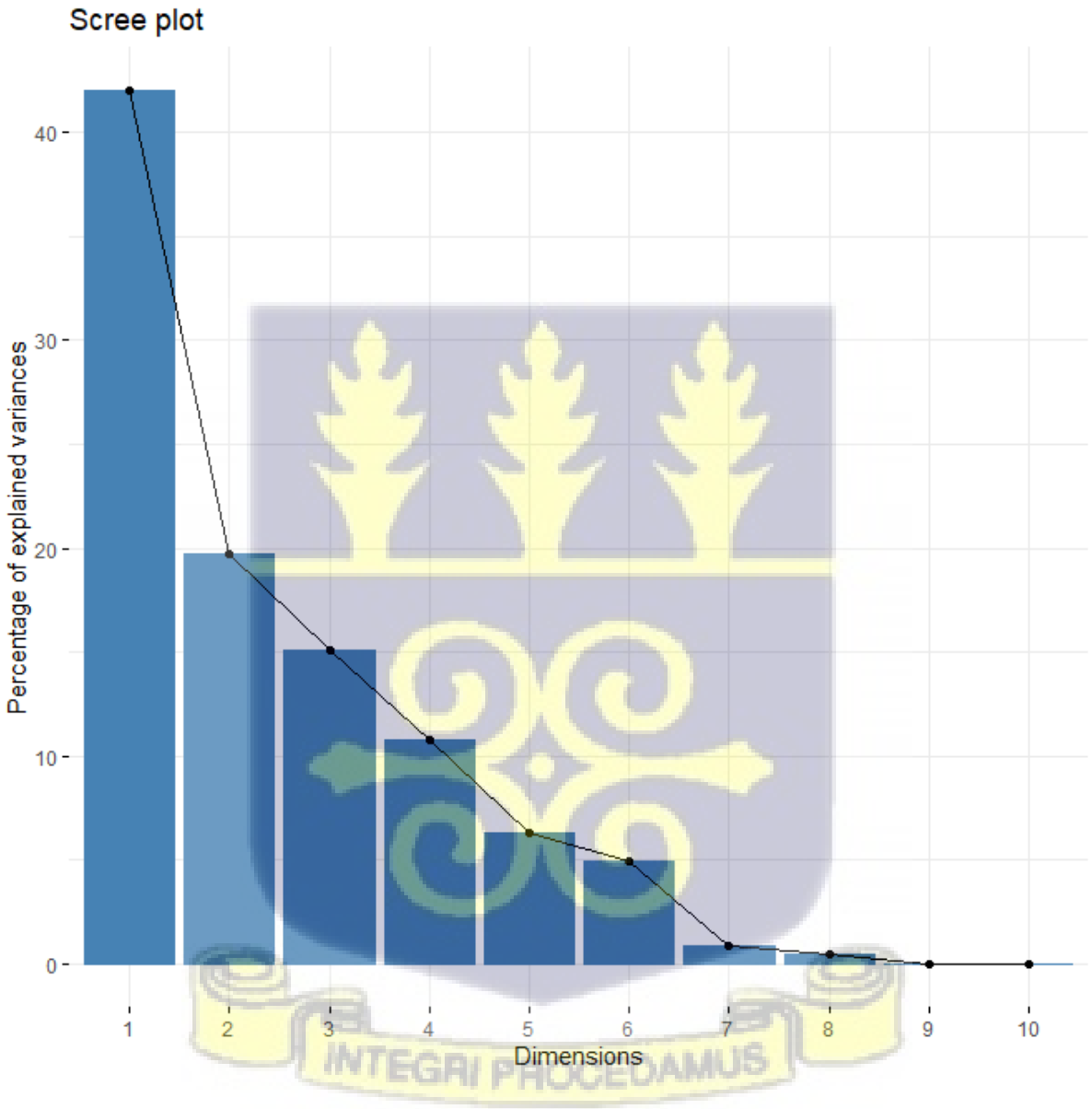

Figure 4.33: Scree plot of percentage of explained variance among dimensions (Principal Components, PC) of qualitative traits in the mutagenized population (M_2 Generation).

4.10.6 Correlation Between Qualitative Variables and Principal Components (Dimensions) in the Wild type and Mutagenized Population (M_2 Generation).

In the M_2 generation, the correlation circle in the wild type was constructed based on the 100% variability extracted from the first two dimensions; Dim.1 and Dim.2 (**figure 4.34**). Only plant pigmentation (PP) and twinning tendency (TT) contributed to the total variability in the wild type.

In the M_2 population, the correlation circle was constructed based on the 61.69% variability extracted from the first two dimensions; Dim.1 and Dim.2 (**figure 4.35**). Traits in the top-right quadrant were positively correlated. Plant pigmentation (PP) and twinning tendency (TT) formed an acute angle indicating strong and positive correlation. A positive correlation existed between seed shape (SS) and growth habit (GH) in the bottom-left quadrant.

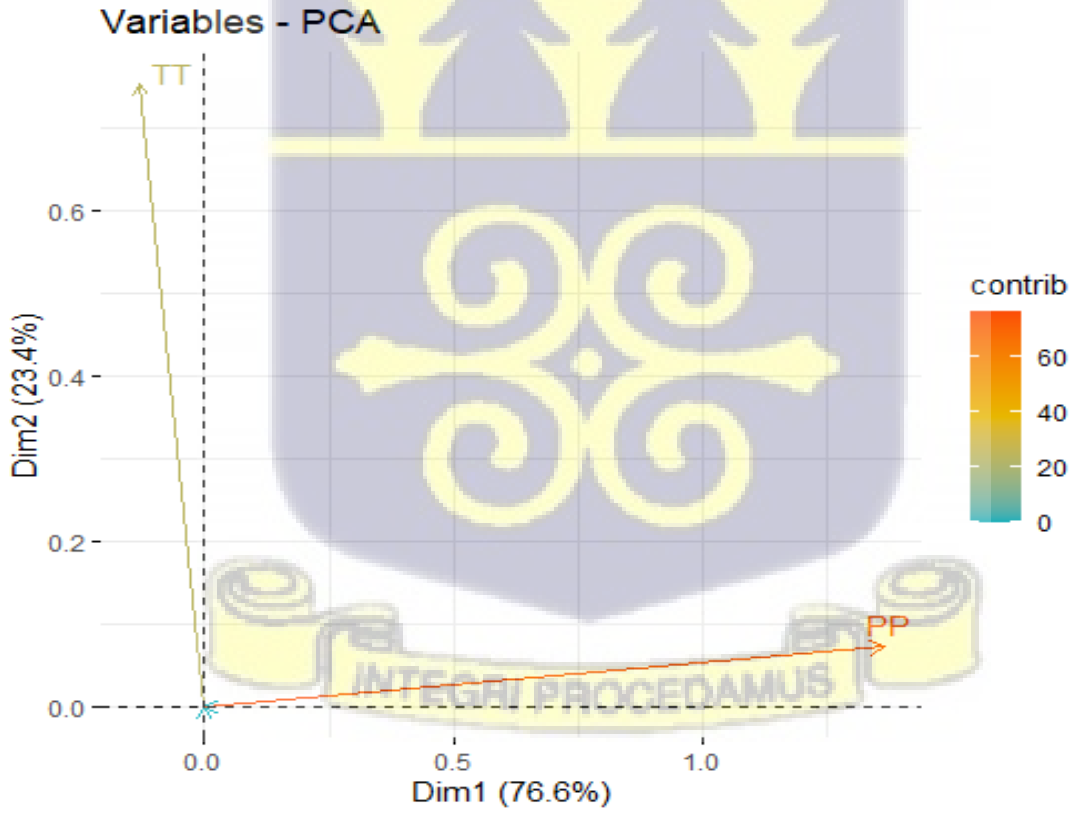

Figure 4.34: Correlation circle of qualitative variables and dimensions (Principal Components, PC) in the wild type (M_2 Generation). Positive correlated variables point to the same side of the plot. Negative correlated variables point to opposite sides of the graph. PP-plant pigmentation, TT-twinning tendency.

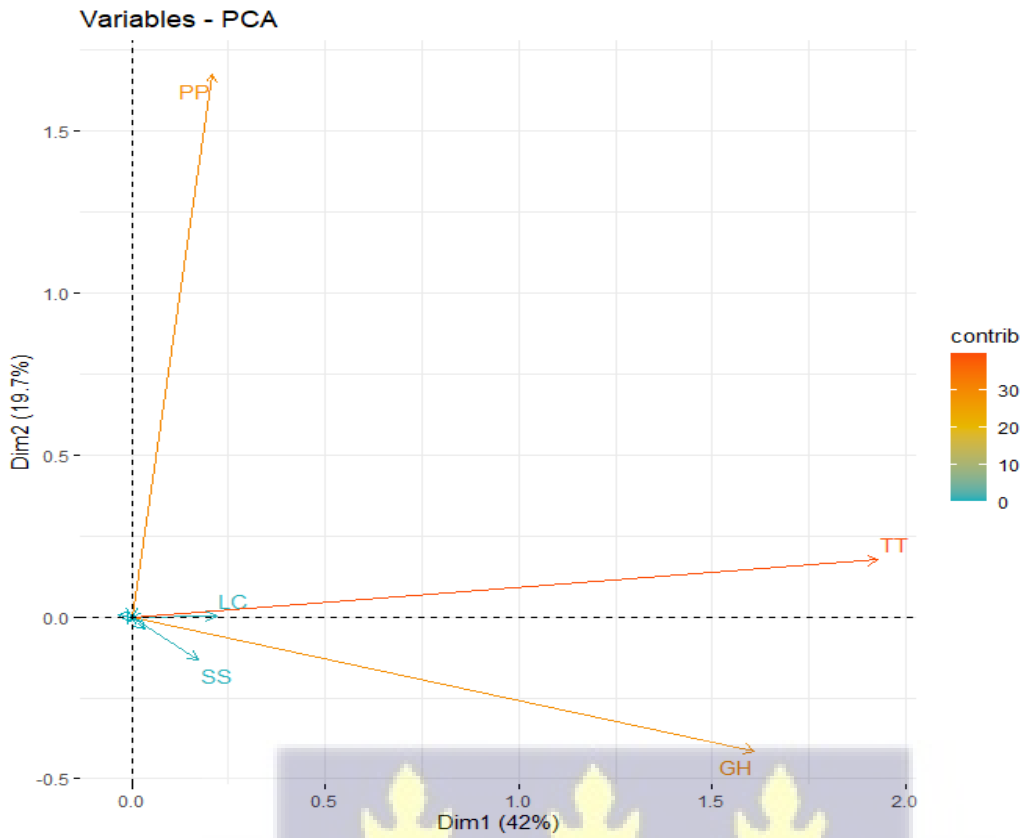

Figure 4.35: Correlation circle of qualitative variables and dimensions (Principal Components, PC) in the mutagenized population (M_2 Generation). Positive correlated variables point to the same side of the plot. Negative correlated variables point to opposite sides of the graph. PP-plant pigmentation, TT-twinning tendency, LC-leaf colour, SS-seed shape, GH-growth habit.

4.11 Bi-plot Between Qualitative Variables and M_2 Generation (Mutagenized population and Wild type) based on Dim.1 and Dim.2 values.

There was positive correlation between individuals on the top left quadrant and the variables; growth habit (GH), seed shape (SS) and leaf colour (LC) (**figure 4.36**). Individuals on the bottom left quadrant negatively correlated with twinning tendency and plant pigmentation. Individuals located further away from the origin in the PCA bi-plot indicated, they were genetically distinct from each other based on the qualitative traits observed; the individuals exhibited high contribution to variability (cos2). The top twenty (20) genotypes that contributed a lot to variability (**Appendix 23**) were all mutagenized plants.

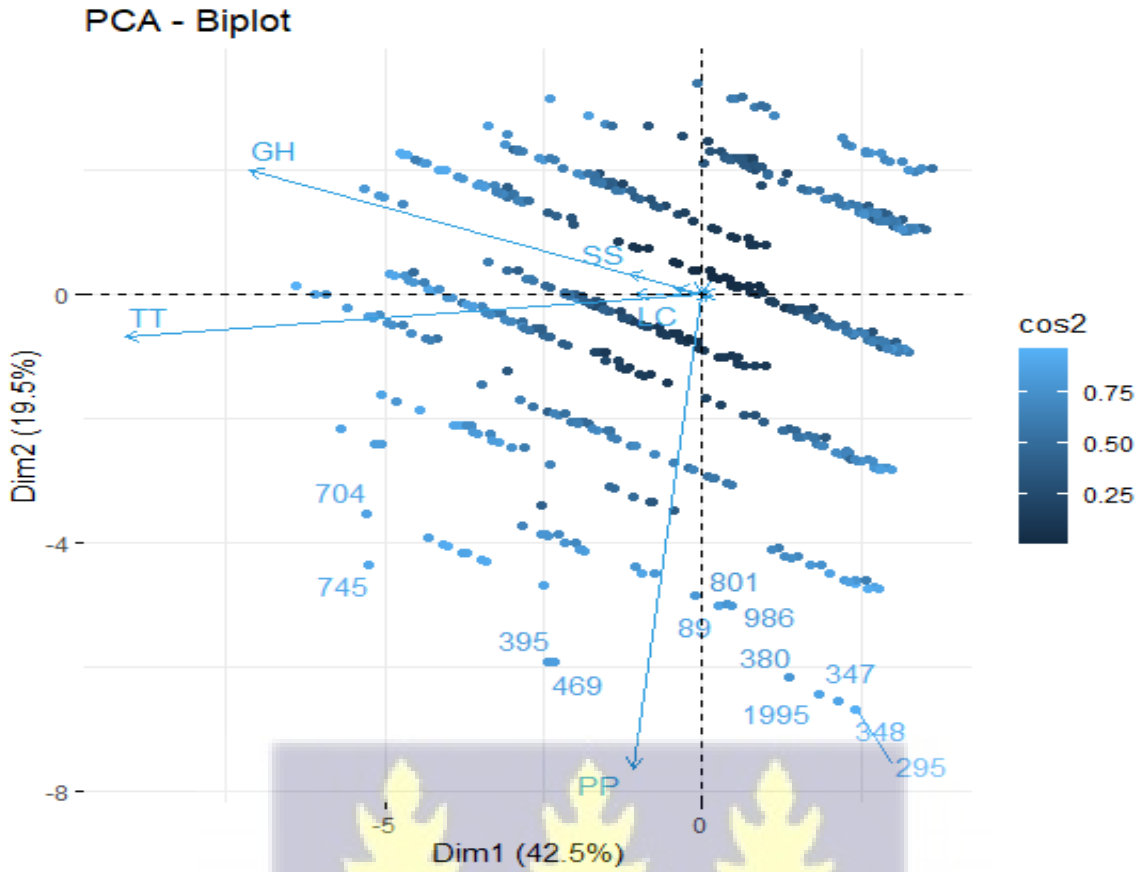

Figure 4.36: Biplot of qualitative variables and individuals in the M_2 generation (wild type and mutagenized population) based on first two dimensions (Dim1 and Dim2). Cos2 (0-1) = individual contributions. Individuals with deep colour have low contribution and individuals with light colour have high contribution. PP-Plant Pigmentation, GH-Growth habit, TT-Twinning Tendency, PCURV- Pod Curvature, FC-Flower color, PC-Pod Colour, SC- Seed Coat Colour, SS- Seed Shape, LS- Leaf Shape.

4.12 Bi-plot Between Quantitative Variables and M_2 Generation (Mutagenized population and Wild type) based on Dim.1 and Dim.2 values.

From the bi-plot (**figure 4.37**), individuals on the top left quadrant exhibited positive correlation with yield variables; number of seeds per plant (NSPP) and number of pods per plant (NOPPP), whiles observations on the bottom left quadrant negatively correlated with number of locules per pod (NOLP), percentage seed set (PSST), number of seeds per pod (NOSP) and pod length (PL). Individuals that recorded high contribution to variability (cos2) and were genetically distinct from the other individuals based on the seven quantitative traits observed were located further away from the origin in the PCA bi-plot. The top twenty (20) genotypes with high cos2 (**Appendix 24**) were all mutagenized plants.

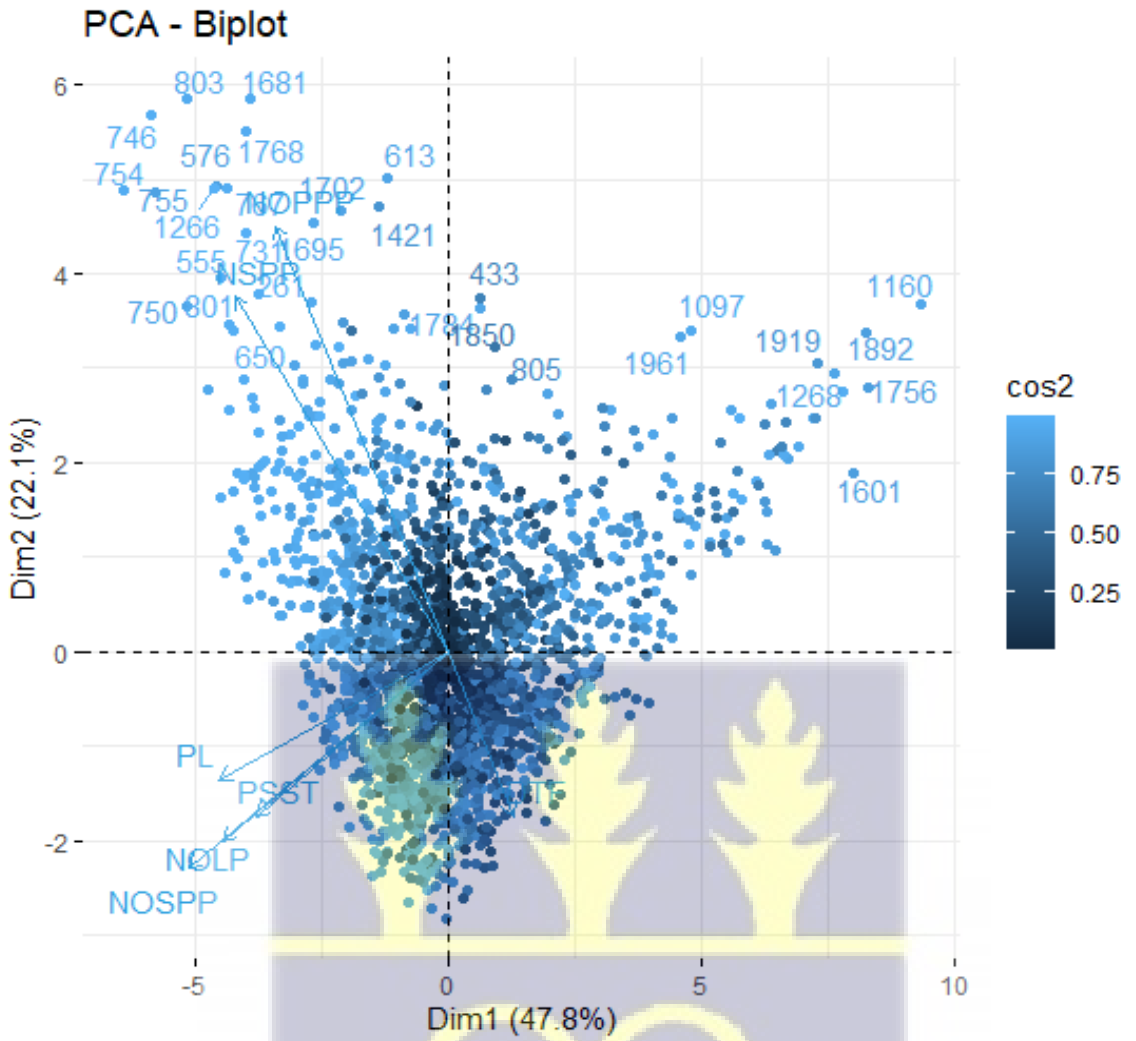

Figure 4.37: Biplot of quantitative variables and individuals in the M_2 Generation (wild type and mutagenized population) based on first two dimensions (Dim1 and Dim2). Cos2 (0-1) = individual contributions. Individuals with deep colour have low contribution and individuals with light colour have high contribution. NOPPP-Number of Pod per Plant, NSPP-Number of Seeds per Plant, PL-Pod Length, NOLP-Number of Locules per Pods, NOSPP-Number of Seeds per Pod, PSST-Percentage Seed Set, DTF-Days to Flowering

4.13 Pearson's Pairwise Correlation among Seven (7) Quantitative Traits in the M_2 Generation.

4.13.1 Pearson's Pairwise Correlation among Seven (7) Quantitative Traits in the Wild type (M_2 Generation).

In the M_2 generation, a total of twenty-eight (28) pairwise correlations were estimated among the seven (7) quantitative traits in the wild type. Out of the total coefficient estimated, seventeen were significantly ($p< 0.05$) correlated (**figure 4.38**). All the significant correlations observed in the wild type were positive. Number of pods per plant significantly correlated with number of seeds per plant ($p<0.05$, $r=0.821$, $r^2=0.674$). Number of seeds per pod significantly correlated with number of locules per pod ($p=0.000$, $r = 0.898$, $r^2 = 0.806$), percentage seed set ($p = 0.000$, $r = 0.587$, $r^2 = 0.345$), pod length ($p = 0.000$, $r = 0.548$, $r^2 = 0.300$) and number of seeds per plant ($p = 0.000$, $r = 0.694$, $r^2 = 0.482$).

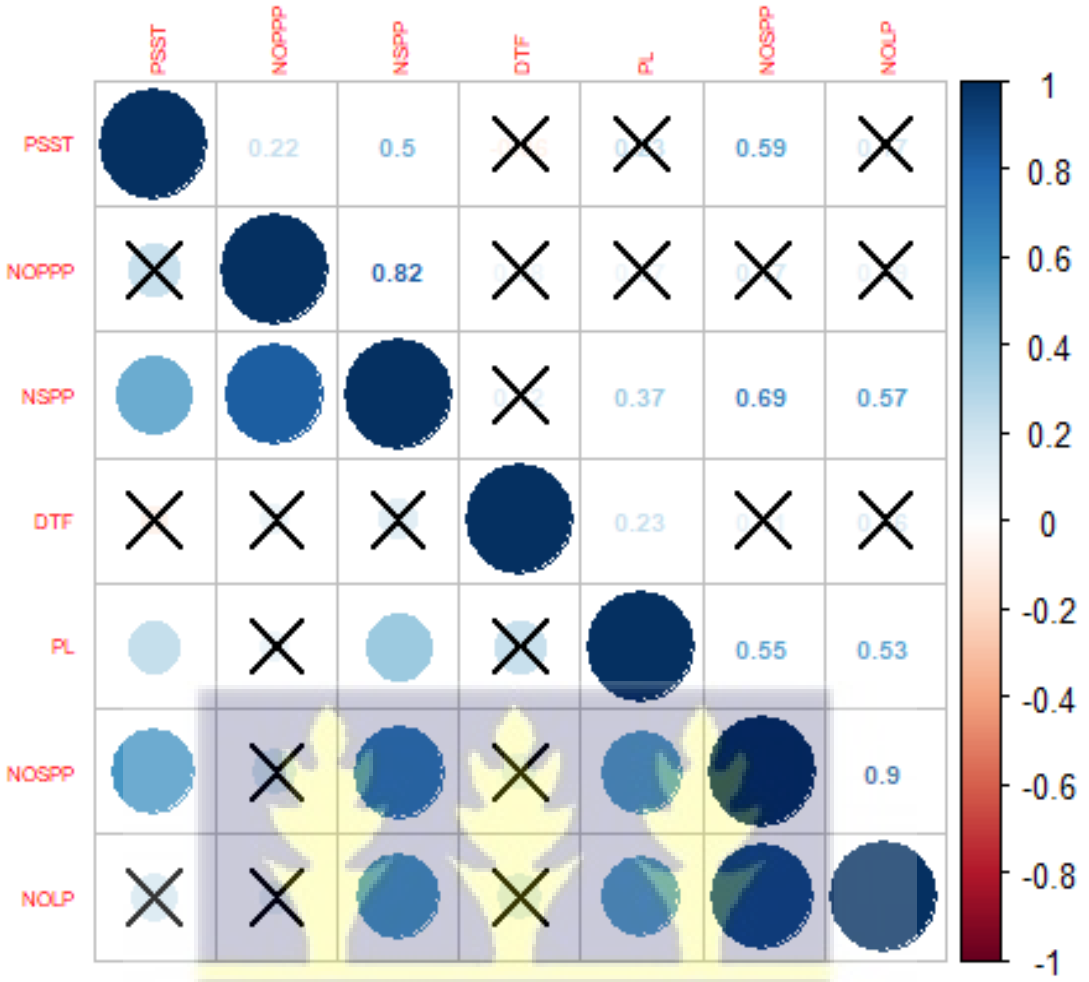

Figure 4.38: Correlogram of pairwise correlations among the quantitative variables in the wild type. Positive correlations are shown in blue and negative correlations are shown in red colour. Colour intensity and size of the circle are proportional to the correlation coefficient. Insignificant correlations are indicated with the times sign ('×'). NOPPP-Number of Pod per Plant, NSPP-Number of Seeds per Plant, PL-Pod Length, NOLP-Number of Locules per Pods, NOSPP-Number of Seeds per Pod, PSST-Percentage Seed Set, DTF-Days to Flowering.

4.13.2 Pearson's Pairwise Correlation among Seven (7) Quantitative Traits in the Mutagenized Population (M_2 Generation).

A total of twenty-eight (28) pairwise correlations were estimated among the seven (7) quantitative traits in the M_2 population and all the correlations were significant ($p<0.05$). Out of the total coefficient correlations estimated, there were six negative correlations (**figure 4.39**). Days to flowering was negatively correlated ($p<0.01$) with all the other six variables in the population. Strong positive correlations existed between number of pods per plant and number of seeds per plant ($r = 0.964$, $r^2 = 0.929$, $p<0.01$). Number of seeds per pod positively correlated with number of locules per pod ($r = 0.775$, $r^2 = 0.601$, $p<0.01$), percentage seed set ($r = 0.816$, $r^2 = 0.666$, $p<0.01$) and pod length ($r = 0.678$, $r^2 = 0.460$, $p<0.01$). Number of locules per pod positively correlated with pod length ($r = 0.737$, $r^2 = 0.543$, $p<0.01$).

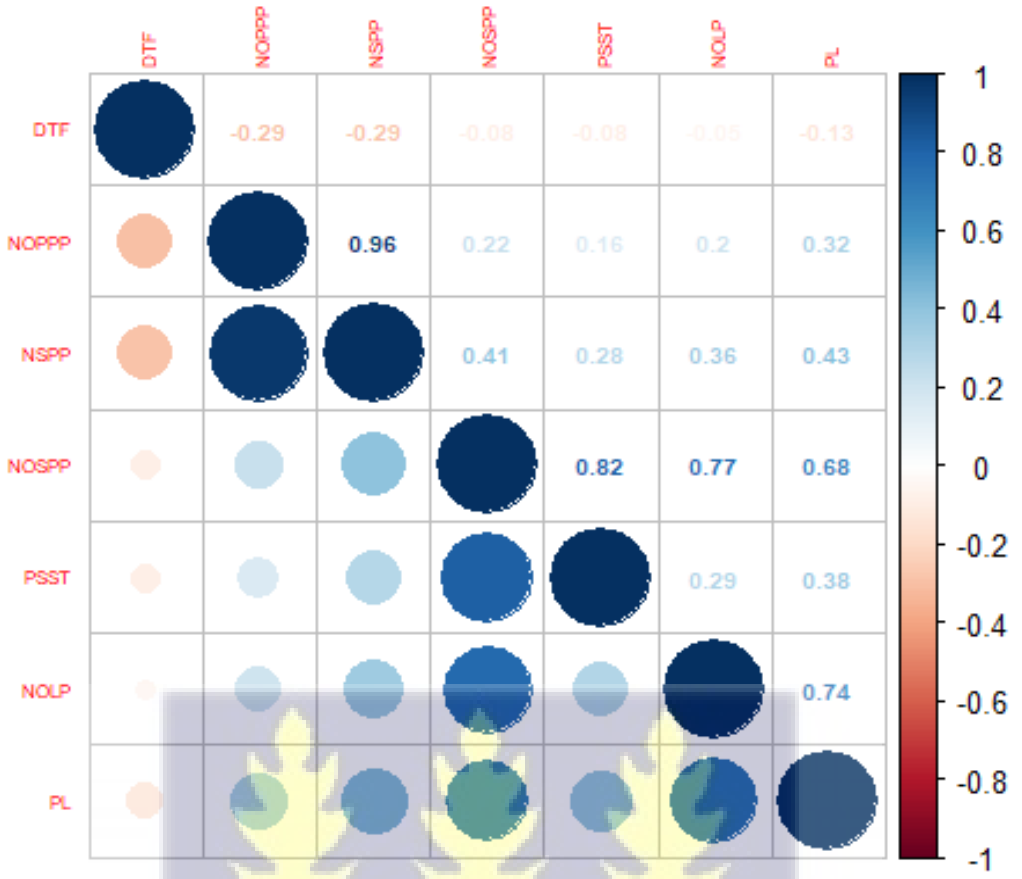

Figure 4.39: Correlogram of pairwise correlations among the quantitative variables in the mutagenized population. Positive correlations are shown in blue and negative correlations are shown in red colour. Colour intensity and size of the circle are proportional to the correlation coefficient. NOPPP-Number of Pod per Plant, NSPP-Number of Seeds per Plant, PL-Pod Length, NOLP-Number of Locules per Pods, NOSPP-Number of Seeds per Pod, PSST-Percentage Seed Set, DTF-Days to Flowering.

4.14 Diversity of the M_2 Generation

Assessment of diversity using quantitative traits clustered the M_2 generation (2201 individuals) into six (6) major clusters at dissimilarity distance 8 **(figure 4.40)**. Cluster 1 comprised 2088 individuals which is the largest cluster; all the wild type individuals and most of the mutant genotypes. Individuals in cluster 1 ranged from 7 to 756 for number of seeds per plant. Cluster 2 was made up of 72 individuals from the mutagenized population with low performance of the quantitative traits studied. There were 3 mutant genotypes in cluster 3 (A61P9, D44P2 and E45P8) with low percentage seed set and low number of pods per plant. Cluster 4 comprised high yielding mutant genotypes (33 individuals). The top five individuals (C37P12, C37P9, C37P8, C41P8 and C21P8) with highest number of seeds belonged to cluster 4. Cluster 5 and 6 had 4 and 5 genotypes respectively. Individuals in cluster 6 were the lowest performing mutant genotypes for number of pods per plant but they had 100% seed set in their pods.

The dendogram grouped the 2201 genotypes observed into four major clusters; 1, 2, 3 and 4 with results obtained on qualitative traits with cluster 1 having 2047 genotypes made up of all the wild type and most of the mutant genotypes (**figure 4.41**). Cluster 2 comprised 2 mutant genotypes (A46P14 and E45P8) which had pale green leaves. There were 150 mutant genotypes grouped under cluster 3 and 2 mutant genotypes in cluster 4.

Assessment of diversity using both quantitative and qualitative traits clustered the M_2 generation (2201 individuals) into seven (7) major clusters at dissimilarity distance 8 (**figure 4.42**). Cluster 1 comprised 2086 with all the wild type and most of the mutagenized population. Cluster 2 had 2 individuals (A46P14 and D62P4). There was only 1 genotype in cluster 7 and 3 in cluster 4. Cluster 3 was made up of 72 mutants genotypes which were low yielding mutant genotypes and cluster 5 comprised 33 genotypes that were yielding including the top 10 high performing mutants for number of seeds per plant.

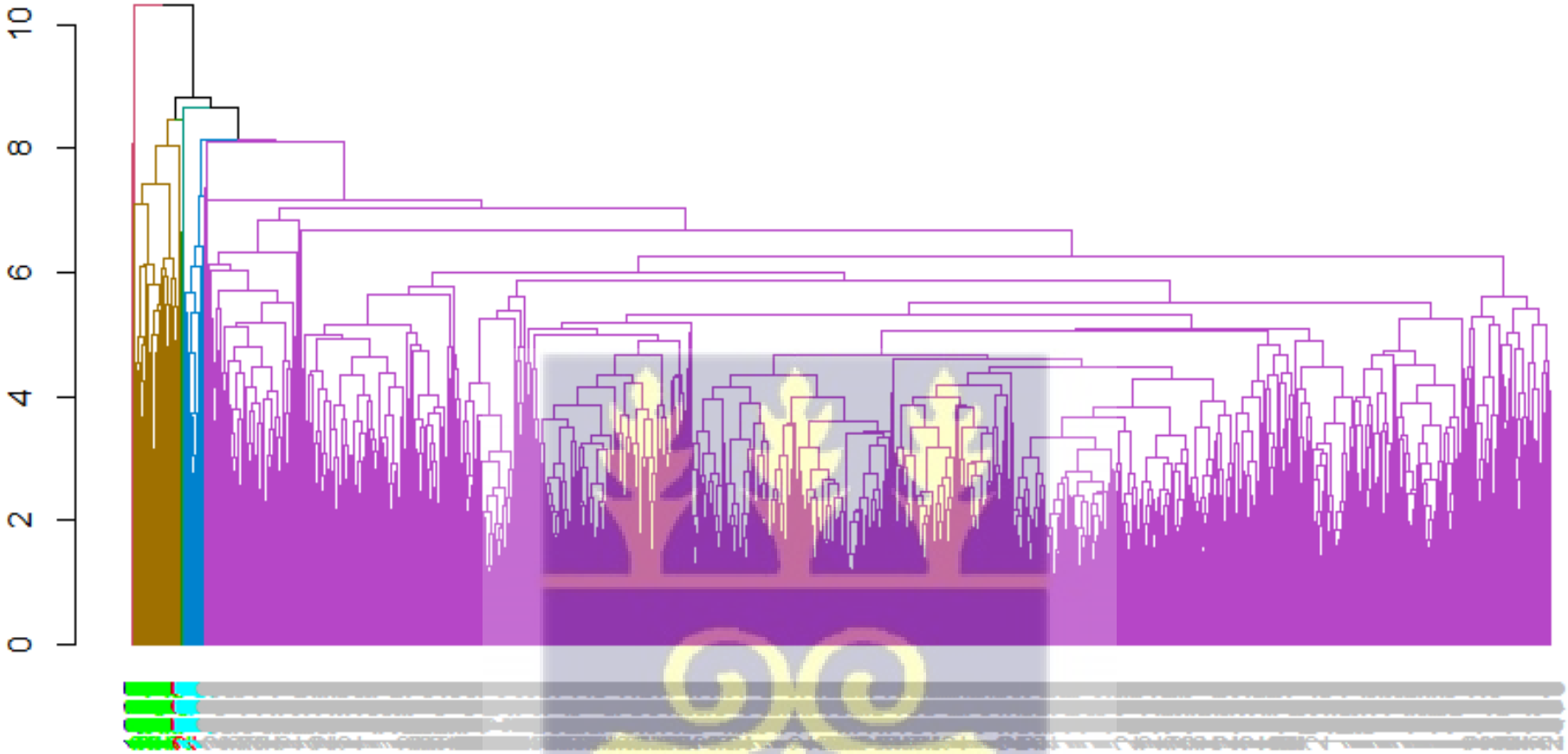

Figure 4.40: Dendogram of hierarchical cluster analysis based on quantitative traits using average linkage method showing the genetic relationships among 2201 individuals in the M_2 generation based on average distance. Cluster 1= grey, cluster 2=green, cluster 3=cyan, cluster 4=red, cluster 5=blue, cluster 6=magenta.

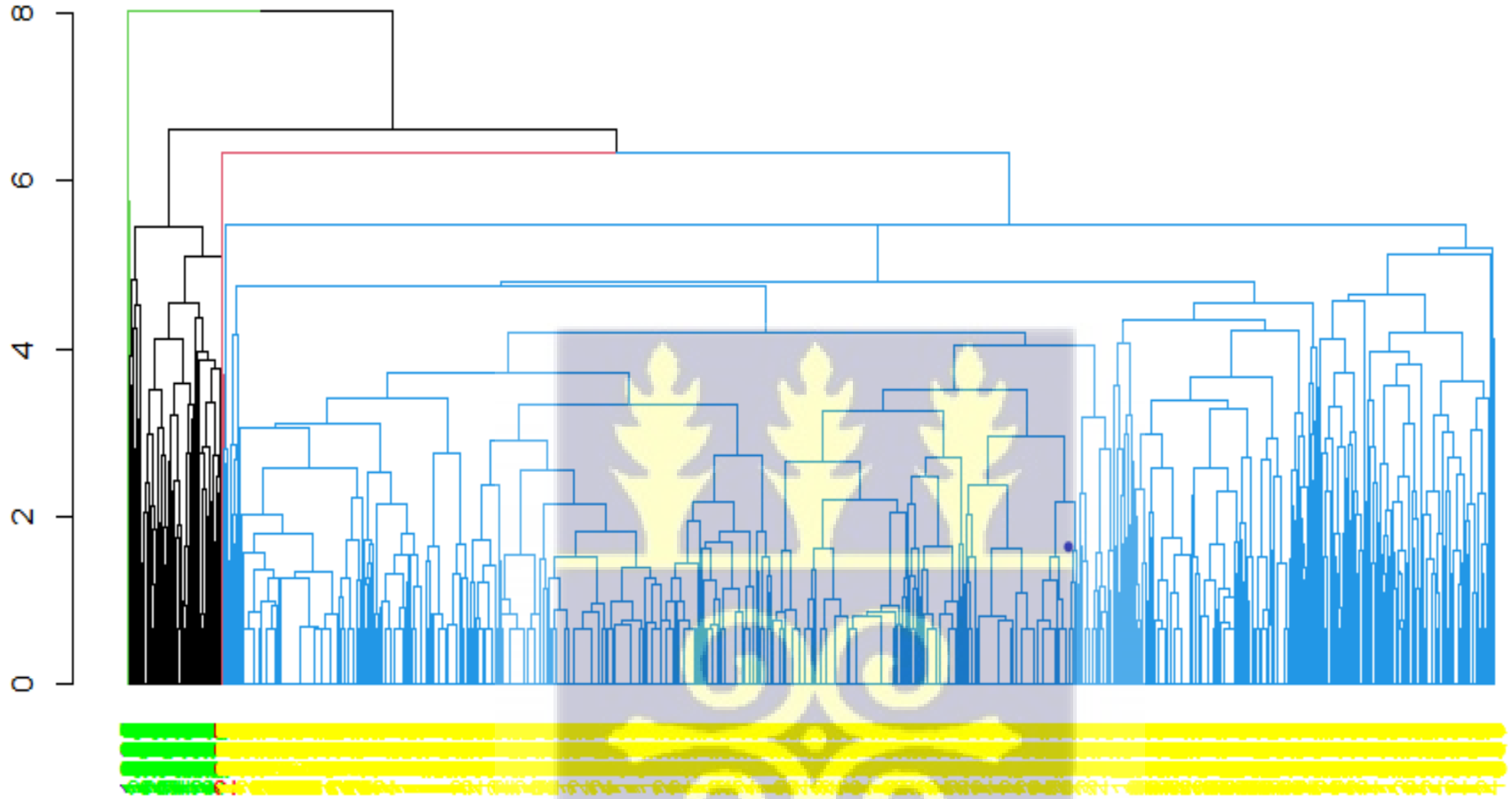

Figure 4.41: Dendogram of hierarchical cluster analysis based on qualitative traits using average linkage method showing the genetic relationships among 2201 individuals in the M_2 generation based on average distance. Cluster 1= yellow, cluster 2=blue, cluster 3=green, cluster 4=red.

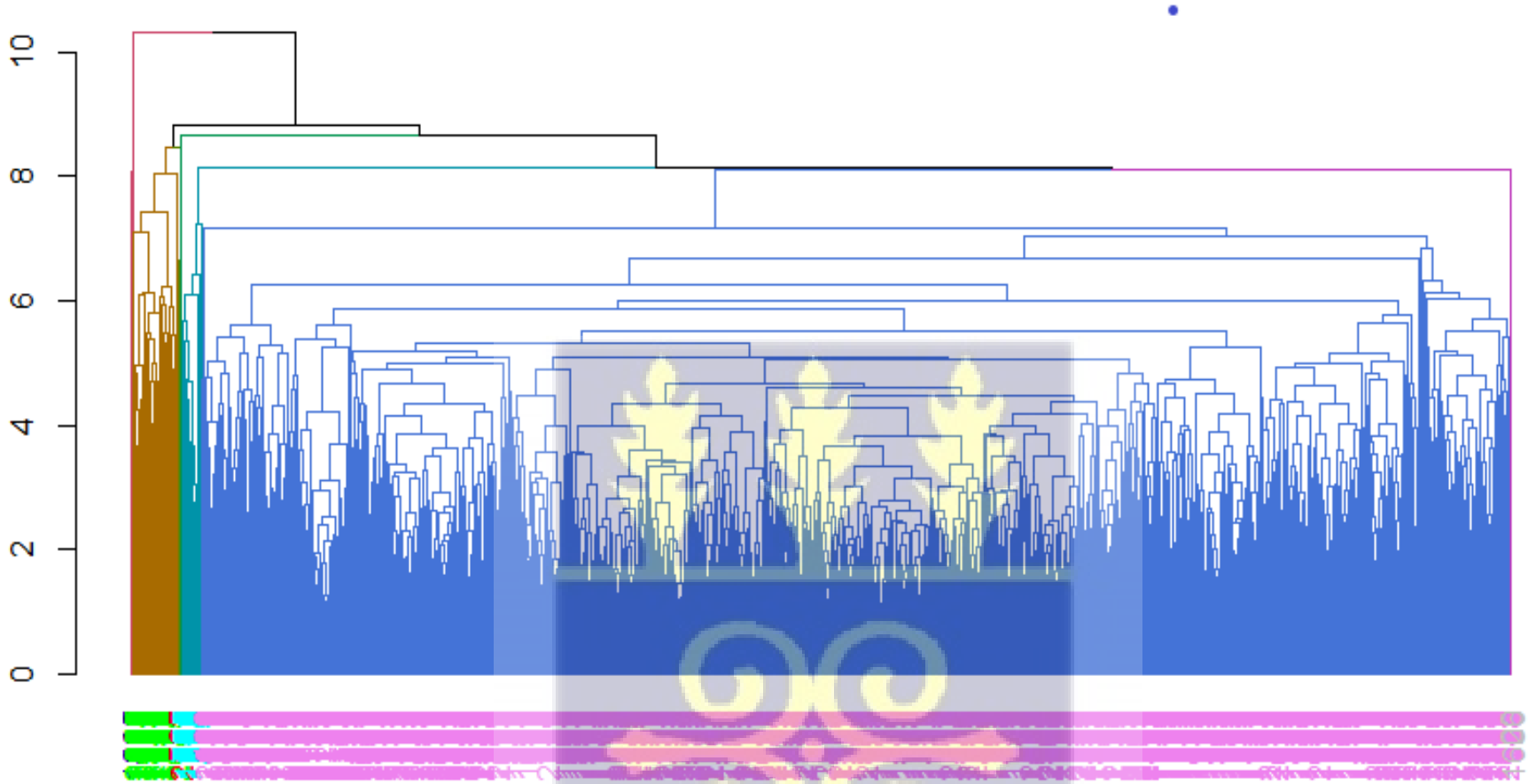

Figure 4.42: Dendogram of hierarchical cluster analysis based on 19 traits studied (both qualitative and quantitative traits) using average linkage method showing the genetic relationships among 2201 individuals in the M_2 generation based on average distance. Cluster 1= violet, cluster 2=brown, cluster 3=green, cluster 4=blue, cluster 5=cyan, cluster 6=red, cluster 7=grey.

4.15 Selected Putative Mutants among the Quantitative Traits in the M_2 Population

4.15.1 Putative Mutants That Showed High Performance Above the Wild type in the M_2 Generation

Individuals in the M_2 population that showed higher performance for the quantitative traits than the wild type were selected as putative mutants. Days to flowering had the highest number of individuals that showed high performance above the wild type ($z = 19.82$, $p<0.05$) with frequency of 27.06% (**Table 4.15**). There were eighty-five (85) individuals in the mutagenized population that exhibited higher performance above the wild type ($z = 16.88$, $p<0.05$) with the least frequency (4.01%) for number of seeds per plant (**Table 4.15**).

Table 4.15: Frequency of Selected Putative Mutants from 2,121 M_2 Individuals Showing High Performance Above the Wild type for Six (6) Quantitative Traits

Traits	Number of individuals	Frequency (%)	z-statistic
NOPPP	101	4.8	**15.58**
NOSPP	86	4.05	**20.3**
NOLP	290	13.67	**18.64**
PL	231	10.89	**17.31**
DTF	574	27.06	**19.85**
NSPP	85	4.01	**16.88**

NOPPP-Number of Pod per Plant, NSPP-Number of Seeds per Plant, PL-Pod Length, NOLP-Number of Locules per Pods, NOSPP-Number of Seeds per Pod, PSST-Percentage Seed Set, DTF-Days to Flowering.

4.15.1 Putative Mutants That Showed High Performance Below the Wild type in the M_2 Generation.

There were 797 individuals out of the 2,121 individuals observed that exhibited low performance below the wild type ($z = 15.58$, $p<0.05$) for number of pods per plant (**Table 4.16**). The highest frequency of putative mutants was observed in number of pods per plant. Days to flowering had the least frequency (0.80%) of individuals that performed significantly low than the wild type ($z = 23.39$, $p<0.05$) (**Table 4.16**).

Table 4.16: Frequency of Selected Putative Mutants from 2,121 M_2 Individuals Showing Low Performance Below the Wild type for Six (6) Quantitative Traits

Traits	Number of Individuals	Frequency (%)	z-statistic
NOPPP	797	37.58	**42.84**
NOSPP	125	5.89	**26.59**
NOLP	38	1.79	**22.47**
PSST	426	20.08	**24.57**
PL	25	1.18	**15.62**
DTF	17	0.80	**23.39**

NOPPP-Number of Pod per Plant, NSPP-Number of Seeds per Plant, PL-Pod Length, NOLP-Number of Locules per Pods, NOSPP-Number of Seeds per Pod, PSST-Percentage Seed Set, DTF-Days to Flowering.

4.15.3 Contrast Analysis

Some of the selected individuals in the mutagenized population showed significant difference ($p<0.01$) for seven (7) quantitative traits observed when compared with wild type mean. For individuals that performed higher than the wild type, there were 55 that were significantly different from the wild type for number of pods per plant; 8 were significantly different from wild type for number of seeds per pod (**Table 4.17**). There was no individual that showed significant different from the wild type mean for percentage seed set. Individual mutants that showed higher performance above the wild type ($p<0.01$) and their f-statistic and p-values are presented in **appendix 26**.

For individuals that performed lower below the wild type range, there were 795 that showed significant difference from the wild type mean for number of pods per plant; 3 were significantly different from the wild type mean for pod length. There was no individual in the mutagenized population that showed low significant difference for days to flowering (**Table 4.18**). Individual mutants that showed lower performance below the wild type ($p<0.01$) and their f-statistic and p-values are presented in **appendix 27**.

Table 4.17: Frequency of Highly Significant Mutants from 2,121 M_2 Individuals Showing High Performance Above the Wild type for Six (6) Quantitative Traits

Traits	Number of plants	Frequency	P-value
NOPPP	55	2.59	<0.01
NOSPP	8	0.38	<0.01
NSPP	61	2.88	<0.01
NOLP	38	1.79	<0.01
PSST	0	0	<0.01
PL	11	0.52	<0.01
DTF	251	11.83	<0.01

NOPPP-Number of Pod per Plant, NSPP-Number of Seeds per Plant, PL-Pod Length, NOLP-Number of Locules per Pods, NOSPP-Number of Seeds per Pod, PSST-Percentage Seed Set, DTF-Days to Flowering.

Table 4.18: Frequency of Highly Significant Mutants from 2,121 M_2 Individuals Showing Low Performance Below the Wild type for Six (6) Quantitative Traits

Traits	Number of plants	Frequency	P-value
NOPPP	795	37.48	<0.01
NOSPP	125	5.89	<0.01
NSPP	950	44.79	<0.01
NOLP	7	0.33	<0.01
PSST	296	13.96	<0.01
PL	3	0.14	<0.01
DTF	0	0.00	<0.01

NOPPP-Number of Pod per Plant, NSPP-Number of Seeds per Plant, PL-Pod Length, NOLP-Number of Locules per Pods, NOSPP-Number of Seeds per Pod, PSST-Percentage Seed Set, DTF-Days to Flowering

Individuals that showed high performance for number of seeds per plants also exhibited high performance in other yield traits (**Table 4.19**). Most of the high performing individuals presented in **Table 4.19** were early maturing mutants with DTF ranging from 38-47. The mutants also exhibited low seed abortion. The pods' percentage seed set (formation) for the top yielding mutants ranged from 76.47 to 100%. There were variations in the qualitative traits for the high yielding mutants (**Table 4.19**). Different growth habit, plant pigmentation, seed coat colour, seed shape and pod curvature were observed in the high yielding mutants (**Table 4.19**). The low performing mutants for number of seeds per plant also exhibited very low performance in other yield traits (**Table 4.20**). Seed formation in the pods of these mutants were very poor. Percentage seed set ranged from 0.00 to 100 with most individuals having less than 20% seed set. The low yielding mutants showed variations in the qualitative traits. Different growth habit, plant pigmentation, seed coat colour, seed shape and pod curvature were observed in the low yielding mutants (**Table 4.20**).

Table 4.19: Catalogue of high performing mutants for NSPP in the M_2 generation.

PLANT	NOPPP	NOSPP	NOLP	PSST	PL	NSPP	DTF	LC	TLS	LM	GP	FC	GH	PP	TT	PCURV	PC	SS	SCC
CONTROL	28	12	14	93.59	15.5	374	54	Dark green	Hastate	Present	Indeterminate	Violet	Intermediate	Intermediate	Intermediate	Slightly-curved	Pale tan	Rhomboid	Brown
C37P12	68	17	18	94.44	18.4	1156	39	Dark green	Hastate	Present	Indeterminate	Violet	Erect	Intermediate	None	Slightly-curved	Pale tan	Rhomboid	Brown
C37P9	64	18	18	100.00	18.2	1152	47	Dark green	Hastate	Present	Indeterminate	Violet	Erect	Moderate	Slight	Slightly-curved	Pale tan	Ovoid	Brown
C37P8	63	18	19	94.74	19.8	1134	39	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Intermediate	Straight	Pale tan	Rhomboid	Brown
C41P8	71	15	18	83.33	18.7	1065	41	Intermediate green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Pronounced	Straight	Pale tan	Ovoid	Brown
C21P8	64	16	16	100.00	17.8	1024	48	Dark green	Hastate	Present	Indeterminate	Violet	Acute Erect	None	None	Straight	Pale tan	Rhomboid	Brown
C37P4	54	18	18	100.00	18.7	972	46	Dark green	Hastate	Present	Indeterminate	Violet	Intermediate	Moderate	Intermediate	Straight	Pale tan	Rhomboid	Brown
E26P3	69	14	15	93.33	17.4	966	42	Dark green	Hastate	Present	Indeterminate	Violet	Acute Erect	Moderate	Intermediate	Straight	Pale tan	Rhomboid	Firebrick
C39P1	60	16	17	94.12	17.2	960	40	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Intermediate	Slightly-curved	Pale tan	Rhomboid	Brown
D32P18	60	16	17	94.12	17.9	960	38	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Slight	Straight	Pale tan	Ovoid	Brown
E32P6	65	14	14	100.00	18.3	910	38	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Straight	Pale tan	Ovoid	Brown
C20P2	52	17	17	100.00	17.9	884	38	Dark green	Hastate	Present	Indeterminate	Violet	Prostrate	Moderate	Slight	Straight	Pale tan	Rhomboid	Brown
C36P12	56	15	17	88.24	18.3	840	38	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Intermediate	Slightly-curved	Pale tan	Rhomboid	Brown
C41P6	49	17	18	94.44	18.0	833	40	Intermediate green	Hastate	Present	Indeterminate	Violet	Acute Erect	Extensive	Slight	Slightly-curved	Pale tan	Ovoid	Brown
E26P8	46	18	19	94.74	18.8	828	43	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Intermediate	Intermediate	Straight	Pale tan	Rhomboid	Brown
C29P10	48	17	18	94.44	17.9	816	39	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Slight	Slightly-curved	Pale tan	Ovoid	Firebrick
B26P6	50	16	16	100.00	17.6	800	39	Dark green	Hastate	Present	Indeterminate	Violet	Climbing	Very slight	Intermediate	Slightly-curved	Pale tan	Rhomboid	Firebrick
C20P3	45	17	17	100.00	18.4	765	42	Dark green	Hastate	Present	Indeterminate	Violet	Prostrate	Very slight	Slight	Straight	Pale tan	Rhomboid	Brown
C3P11	42	18	18	100.00	18.4	756	39	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Slight	Slightly-curved	Pale tan	Rhomboid	Brown
C33P13	43	17	18	94.44	18.1	731	41	Dark green	Hastate	Present	Indeterminate	Violet	Prostrate	Intermediate	Intermediate	Straight	Pale tan	Rhomboid	Brown
E27P8	56	13	17	76.47	17.0	728	40	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Pronounced	Straight	Pale tan	Ovoid	Brown

NOPPP-Number of Pods per Plant, NOSPP-Number of Seeds per Pods, DTF-Days to Flowering, PL-Pod Length, NOLP-Number of Locules per Pod, NSPP-Number of seeds per pod, PSST-Percentage seed set, Leaf colour, TLS-terminal leaf shape, Leaf marking, Growth pattern, FC-Flower color, GH-Growth habit, PP-Plant Pigmentation, TT-twinning tendency, PCURV- Pod Curvature, PC-Pod Colour, SC- Seed Coat Colour, SS- Seed Shape.

Table 4.20: Catalogue of low performing mutants for NSPP in the M_2 generation.

PLANT	NOPPP	NOSPP	NOLP	PSST	PL	NSPP	DTF	LC	TLS	LM	GP	FC	GH	PP	TT	PCURV	PC	SS	SCC
CONTROL	28	12	14	93.59	15.5	374	54	Dark green	Hastate	Present	Indeterminate	Violet	Intermediate	Intermediate	Intermediate	Slightly-curved	Pale tan	Rhomboid	Brown
E57P11	7	1	12	8.33	15.8	7	55	Dark green	Hastate	Present	Indeterminate	Violet	Acute Erect	Moderate	None	Straight	Pale tan	Ovoid	Brown
A64P11	1	6	11	54.55	8.7	6	44	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Intermediate	Straight	Pale tan	Rhomboid	Brown
C61P7	2	3	11	27.27	13.3	6	52	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Slightly Curved	Pale tan	Rhomboid	Brown
D62P1	2	3	9	33.33	8.8	6	59	Dark green	sub-hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Slightly Curved	Pale tan	Ovoid	Brown
E57P2	3	2	9	22.22	17.0	6	44	Intermediate green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Straight	Pale tan	Rhomboid	Brown
B58P13	4	1	15	6.67	16.2	4	43	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Extensive	Slight	Straight	Pale tan	Rhomboid	Firebrick
C20P13	2	2	5	40.00	11.2	4	45	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Slight	Straight	Pale tan	Rhomboid	Firebrick
C21P11	1	4	4	100.00	9.4	4	38	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	None	Straight	Pale tan	Ovoid	Brown
D32P2	2	2	9	22.22	10.1	4	39	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	None	Straight	Pale tan	Ovoid	Brown
A61P9	3	1	15	6.67	16.5	3	53	Dark green	sub-hastate	Present	Indeterminate	Violet	Intermediate	Very slight	Intermediate	Straight	Pale tan	Rhomboid	Brown
D60P5	3	1	15	6.67	15.5	3	48	Dark green	Hastate	Present	Indeterminate	Violet	Acute Erect	None	None	Straight	Pale tan	Rhomboid	Brown
D60P6	3	1	16	6.25	15.8	3	46	Dark green	Hastate	Present	Indeterminate	Violet	Erect	Very slight	None	Straight	Pale tan	Ovoid	Brown
E57P8	3	1	9	11.11	16.5	3	41	Dark green	Hastate	Present	Indeterminate	Violet	Acute Erect	Moderate	None	Straight	Pale tan	Rhomboid	Brown
D24P4	2	0	5	0.00	10.7	0	46	Dark green	Hastate	Present	Indeterminate	Violet	Acute Erect	Very slight	None	Straight	Pale tan	Rhomboid	Brown
D32P1	2	0	13	0.00	14.0	0	41	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	None	Straight	Pale tan	Rhomboid	Brown
E31P5	1	0	10	0.00	11.1	0	48	Dark green	Hastate	Present	Indeterminate	Violet	Acute Erect	Very slight	None	Straight	Pale tan	Rhomboid	Brown
E43P13	4	0	6	0.00	13.4	0	45	Dark green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Slightly Curved	Pale tan	Ovoid	Brown
E46P10	10	0	10	0.00	13.5	0	48	Intermediate green	Hastate	Present	Indeterminate	Violet	Semi-erect	Very slight	None	Straight	Pale tan	Rhomboid	Firebrick
E55P11	2	0	12	0.00	15.3	0	46	Intermediate green	Hastate	Present	Indeterminate	Violet	Acute Erect	Moderate	None	Slightly Curved	Pale tan	Ovoid	Brown
E57P17	4	0	11	0.00	13.8	0	49	Dark green	Hastate	Present	Indeterminate	Violet	Acute Erect	Very slight	None	Slightly Curved	Pale tan	Rhomboid	Brown

NOPPP-Number of Pods per Plant, NOSPP-Number of Seeds per Pods, DTF-Days to Flowering, PL-Pod Length, NOLP-Number of Locules per Pod, NSPP-Number of seeds per pod, PSST-Percentage seed set, Leaf colour, TLS-terminal leaf shape, Leaf marking, Growth pattern, FC-Flower color, GH-Growth habit, PP-Plant Pigmentation, TT-twinning tendency, PCURV- Pod Curvature, PC-Pod Colour, SC- Seed Coat Colour, SS- Seed Shape.

CHAPTER FIVE

5.0DISCUSSION

5.1 Sensitivity Test

Determination of mutagenic sensitivity of germinating seeds constitutes an important aspect in mutation breeding, since healthy crop growth and yield depends upon the seedling establishment (Nair and Mehta, 2014). The selection of an effective and efficient mutagen concentration and growth condition is essential to produce a high frequency of desirable mutations in mutation breeding programs (Wani, 2009; Arisha *et al*., 2014). The current study showed that percentage seed germination decreased as concentration of ethyl methane sulfonate increased for the determination of LD50. The wild type had the highest percentage seed germination (63.0%) whereas mutagenic treatment 0.8% had the lowest percentage seed germination (0.0 %). The decrease in percentage germination in the treated could be due to the effect of EMS. The LD50 for this study was 0.4%. This is in contrast with the report by Sahrish *et al.*, (2019) in cowpea and Arisha *et al*., (2015) in pepper. LD50 is genotype specific and it also depends on the treatment time. The different results obtained in this study could be due to the treatment time and the genotype. Alacantara *et al*. (1996) reported similar results in pepper. The 63% germination obtained in the 0.0% treatment could be as a result of decrease in seed viability of the *Asontem* seeds. This could be due to over storage or seeds not properly stored. In comparison to the wild type, the percent germination was low in all mutant populations (M_1 and M_2 populations). Similar results were reported by Ramya *et al.* (2014), Bind and Dwivedi, (2013), Uma and Salimath, (2001). Significantly reduced germination percentage due to EMS treatment has also been reported in study by Sha *et al.* (2008) in chick pea. Reduction in percentage seed germination in EMS treated M_o seeds in Cucumber (*Cucumis sativus* L.) was reported by Wang *et al*. (2014). The percentage seed germination decreased in EMS treated populations may be resulted from physiological and acute chromosomal damage (Gaur *et al*;

2003 and Nawale *et al*; 2006). According to Bind and Dwivedi, (2013), delay in the one set of mitosis and chromosomal aberration induced enzyme activity such as catalase, lipase and hormonal activity results in reduced germination. Also, the reduction in germination may be due to the seeds absorbing the mutagen, which subsequently reaches the meristemic region and affects the germ cell (Serrat *et al*., 2014).

Germination speed is very important in cowpea cultivation. Germination speed decreased in the mutagenized population in this study. Ariraman *et al*. (2014) reported similar results in pigeon pea. This result is in agreement with earlier report by Baghery *et al*. (2016) in okra. The EMS cause random point mutations as Sikora *et al*. (2011) proposed. As much as the concentration of EMS increases, the probability of point mutation induction would be increased. These mutations may lead to defects in the synthesis of essential compounds (hormones) for the plant. The EMS might have caused changes in the synthesis and regulation of abscisic acid (ABS) and gibberellic acid (GA); hormones responsible for seed dormancy and germination. The decrease in germination speed may be due to decrease in the production of GA and increase in ABS. According to Baghery *et al*. (2016), the higher doses of EMS probably would cause more genetic injuries on treated plants which may explain why survival rates and germination speed are lower among them.

Percentage plant survival (at flowering) in the M_1 and M_2 generations varied within the wild type and the mutagenized populations. The wild type had the highest percentage plant survival and the M_1 population had the lowest. These outcomes are in close agreement with the earlier reports of Nawale *et al*; (2006) Ugorji *et al*. (2012), Dhanavel and Girija, (2009). Bind and Dwivedi, (2013), also reported similar findings in cowpea. The decrease in plant survival within the M_1 population may be due to the effect of the EMS. The chemical mutagen might have caused damages in the genes responsible for growth hormone synthesis pathways.

5.2 Morphological Variations in Qualitative Traits of Mutant Populations

Mutation induction with chemical mutagen like EMS produces functional mutations with a high probability of producing dominant traits in the M_1 generation which can be inherited in the next mutant generation (Arisha *et al.,* 2015a; Shu *et al.,* 2012). Induced mutation has shown impressive results in crop improvement of diverse crops. Mutation induction is the possible means to provide increased levels of variability among crop species thus obtaining variability in limited traits of a particular genotype (Nair *et al.*, 2014). In the study, variations in qualitative and quantitative traits were observed in the mutant populations.

According to Khan *et al.,* (2015), the use of morphological markers plays an important role in germplasm characterization and evaluation, though some morphological traits are influenced by environmental conditions and variation in development stages. Morphological markers can be grouped into two categories; qualitative and quantitative markers. Many cowpea accessions and varieties have been characterized by most researchers by using these markers. In this current research, the qualitative morphological markers used were leaf marking, leaf shape, leaf colour, flower colour, twinning tendency, growth habit, growth pattern, plant pigmentation, pod colour, pod curvature, seed shape and seed coat colour.

Morphological mutants are very important in mutation breeding programs. They play a crucial role in changing the features of a cultivar to improve on it. In the present study some individuals in the M_1 and M_2 populations showed morphological abnormalities in growth habit, leaf, flower, pod and seed coat and seed shape. This research had one category of leaf colour (dark green) in both the wild type and mutant population in the M_1 generation; however, three categories were present in the M_2 population. Whilst all the wild type plants (wild type) had dark green leaves in the second generation, there were individuals with dark green leaves, intermediate green leaves and some individuals with pale green leaves in the M_2 population. Similar results were observed in the studies conducted by Arisha *et al.,* (2015) when pepper was treated with chemical mutagen (EMS). This may be that the chemical mutagen induced

mutation in the DNA sequences of the genes responsible for this trait which did not express at the first generation. Transposable elements could be the reason for the expression and visibility of this variability in leaf colour in the M_2 population.

Leaf v-markings were present in all individuals in both the wild type and mutagenized population. This may due to the fact that the chemical did not cause changes in the genes of this trait.

From the results obtained in the current study, all the plants in the wild type and mutagenized population in the M_1 generation had *hastate* leaf shapes. However, some individuals in the M_2 population had different leaf shapes (*sub-hastate*). This is incongruent with the report by Arisha *et al.*, (2015) in pepper. Nair and Mehta (2014) also reported similar results in cowpea treated with EMS. This could be as a result of EMS induced mutations at the M_2 population.

During the M_1 generation, there was no physiological changes in flower colour and pod colour. This is in disagreement with the reports by Gnanamurthy and Dhanavel (2014), Nair and Mehta (2014) in cowpea. Similar observations occurred in the M_2 generation also. Genetically, during the M_1 generation the probability of the occurrence of phenotypic mutation is extremely low and only dominant mutations can be identified (Roychowdhury and Tah, 2013). The results obtained in this research may be due to the fact that the genes responsible for the synthesis of flower and pod pigments may be dominant or oligogene and that EMS did not induce mutations in these genes in this case.

According to Egbadzor *et al.*, (2017), growth habit exhibited by a plant would help identify the suitable planting system to use during production. The current investigation showed variations in growth habit in the M_1 population with *climbing* occurring the most and *semi-erect* occurring the least. During the M_2 generation too, there was variability in growth habit. Similar results were reported by Nair and Mehta (2014) in cowpea, Arisha *et al.* (2015a) in pepper. According to Matos Filho *et al.* (2014), Ribeiro *et al.* (2014) and Lackyan and Dalvi (2015), growth habit in cowpea is monogenic. The results from this study indicates that EMS might have induced mutation in M_0 seeds when subjected to the chemical mutagen. Mutation induction with

chemical mutagen like EMS produces functional mutations with a high probability of producing dominant traits in the M_1 generation which can be inherited in the next mutant generation (Arisha *et al.*, 2015; Shu *et al.*, 2012).

Although the breeder may not be interested in traits such as plant pigmentation, morphological markers like this play important role in the characterization and evaluation of cowpea germplasm. From the results obtained in the current investigation, varying forms of plant pigmentation were observed in both the wild type and the mutagenized populations. However, there were only five classes of this trait observed in the wild type and all the phenotypic classes were observed in the mutant population. The frequency ranged from 2.5% (*extensive* plant pigmentation) to 61.25% (*moderate* pigmentation) in the wild type whiles phenotypic class *solid* had 0.32% (lowest) and *very slight* had 40.41% (highest) in the mutant population. This research also showed different classes of twinning tendency in the wild type and the mutagenized population during the M_2 generation. All the phenotypic classes for twinning tendency were observed in the mutant population whereas only two classes were observed in the wild type. The occurrence of different phenotypic classes in the mutagenized population and the difference in frequencies may be due to the effect of the chemical mutagen. Alkylating agents such as EMS induce chemical modification of nucleotides, which result in mispairing and base changes which leads to the alteration of gene codons thus, expressing some physical changes.

In the current work, there were different forms of pod curvature observed in the M_1 generation which continued in the M_2 generation. This is in concordance with the earlier report by Nair and Mehta (2014) in cowpea. This may be as a result of EMS causing mutations in gene responsible in this trait. The mutation induced in this trait may be dominant mutation.

Seed coat colour and seed coat pattern are consumer traits which are consciously or unconsciously under selection by either farmers or consumers. Moh, (1971) reported that people in different locations have different preferences in relation to seed coat colour of

legumes thus, seed coat colour is seen as a very essential agronomic character that determines the marketability of a grain legume including cowpea. In the present study, it was observed that different forms of seed coat colour were observed in the M_1 and M_2 populations. Brown, fire-brick and cream seed coats were isolated in the mutant populations. Gnanamurthy and Dhanavel (2014) reported similar results by isolating brown and white seed coat colours in cowpea treated with EMS. This may be due to the effect of the chemical mutagen causing mutation in the gene resulting in the changes from dominant pigmentation factor to its recessive form.

During the M_1 generation, varying forms of seed shapes (*kidney and* ovoid) were observed. This mutation was also observed in the M_2 population with kidney, ovoid and globose seed shapes observed. A study performed earlier affirms that different forms of seed shape were found in chick pea mutagenized with EMS (Wani and Amis, 2008). Chemical mutagens induce physiological damages, gene mutations and chromosomal aberration in mutagenized individuals which can be observed and measured from seed germination or emergence of seedlings, survival reduction, plant height reduction and fertility reduction or sterility (reduction in pod and seed formation) (Kumar *et al*., 2009).

5.3 Morphological Abnormalities and Chlorophyll Mutations in M_2 Population

Leaf abnormalities and chlorophyll mutations were observed in the present research. There were mutants with abnormal leaflet number, irregular leaves and leaf variegation. Leaf variegation is a common mutation which can be either a nuclear or cytoplasmic mutation. EMS may have a high specificity for mitochondrial and plastid genomes (Miller *et al*., 1984). Redei *et al.* (1984) reported that many plastid mutations interfere with the development of the photosynthetic apparatus. Girija and Dhanavel (2009), Nair and Mehta (2014) reported leaf mutant such as tetrafoliate leaf and pentafoliate leaf in cowpea treated with EMS. Tetrafoliate leaf was also observed in studies conducted by Auti and Apparao (2009) in mung bean. Gunckel and Sparrow (1961) explained that the leaf abnormalities might be attributed to

chromosomal breakage, disrupted auxin synthesis and transport, disruption of mineral metabolism.

Chlorophyll mutations usually shows different forms of leaf coloration at seedling stage, also referred to as leaf colouration. It serves as the point of reference for measuring how effective and efficient a mutagen is capable of inducing different forms of mutations resulting in the formation of either desirable or undesirable traits in a particular plant (Devmani *et al*., 2016). In the current investigation, both lethal and non-lethal chromosome mutations were observed. This is in concordance with earlier reports by Nair and Mehta (2014), Gnanamurthy and Dhanavel (2014), Sahrish *et al.,* (2019) in cowpea treated with EMS. Similar results were obtained in the studies conducted by Arisha *et al.,* (2015a) when pepper was subjected to EMS treatment and Thilagavathi and Mullainathan (2009) in black gram. Previous studies reported that chlorophyll development seems to be wild type led by many genes that are located on different chromosomes (Larkin and Scowcroft, 1981; Wang *et al.,* 2013). EMS may have caused mutations in these genes. Mutations affecting the production of chlorophyll are important for identifying gene function and the clarification of chlorophyll metabolism and its regulation (Wu *et al.,* 2007).

5.4 Putative Mutants of Quantitative Traits

Quantitative traits vary in degree and can be attributed to polygenic effects (product of two or more genes), and their environment. Hence, individuals with varying forms of these traits can be regarded as putative mutants (not necessarily true mutants) (Forster and Shu, 2011). Some individuals in the M_1 population were observed to have high chlorophyll contents in the current work. Chloroplast mutagenesis with EMS has been reported in *Capsicum annum* L. (Alacantara *et al.,* 1996) and Pea (Singh *et al.,* 2000). Thus, the increment in chlorophyll content in the mutagenic treatments might be result of the mutagenic effects of EMS.

The application of a mutagen may cause genetic variations through breaking of linkage present in the genetic material resulting in the production of useful traits in crop species (Shah *et al*., 2008). Induction of mutation with EMS has resulted in the acquisition of important morpho-agronomic traits in cowpea production. Selection of putative mutants for quantitative traits is an important step for mutant development. Early mutants with 38-41 DTF were isolated in the M_2 population in the present investigation. Early maturing mutants are very important. This is because they are able to escape drought or tolerate insect or pest damage because of their short duration of reproductive phase. Previous studies by Gnanamurthy and Dhanavel (2014), Nair and Mehta, (2014) isolated early maturing mutants from cowpea mutagenized with EMS. This indicates that EMS caused mutation in the gene responsible for this trait.

The current research revealed plants with high yield and low yield performance in the M_2 population. Highly significant mutants that showed high performance for number of pods per plant, number of seeds per pod per plant, number of seeds per plant, number of locules per pod per plant and pod length were obtained in the M_2 population. Top twenty mutants with high yield performance in number of seeds per plant were selected. Traits including higher number of pods per plant, increased protein content, plant height and high seed weights have been obtained in earlier works by Odeigah *et al*., (1998); Singh *et al*., (2006) in cowpea mutagenesis with EMS. High number of pods per plant was reported by Gnanamurthy and Dhanavel (2014) in cowpea. The high yield performance may be as result of EMS altering the gene(s) for this trait. The higher performance in other yield traits exhibited by mutants presented in **Table 4.19** may be as a result of pleiotropy and mutation in one trait affected the other.

There were highly significant mutants that showed low performance for number of pods per plant, number of seeds per pod per plant, number of seeds per plant, number of locules per pod per plant and pod length. Lowest performing mutants were selected in the current study (**Table 4.20**). Reduction of number of yield characters were observed in the earlier studies by Jagajanantham *et al.*, (2013) in *Abelmoschus esculentus* L. Moench. Previous studies by

Berenschot *et al.,* (2009) reported a reduction in number of seeds per pod and plant in *Petunia hybrid.* According to Alan (2007), induced mutations play prominent role in altering the genetic make-up of genotypes not only at a chromosomal but even at a molecular level. This alteration may improve on economically important trait. However, frequency of desirable mutations is very low, about 0.1 per cent of the total mutations. Mutations in quantitative traits are normally in the direction away from the selection history of the parent variety, example yield.

5.5 Associations and Pairwise Correlations of Traits Studied.

In breeding programmes and identification of traits, knowledge of association and pairwise correlation is very useful. The association of qualitative traits and pairwise correlation of quantitative traits may be due to genetic linkage or pleiotropy. Shu *et al.,* (2012) postulated that, pleiotropy or a pleiotropic effect is the phenomenon whereby a gene influences multiple phenotypic traits; hence a mutation in a certain gene may have an effect on some or all traits simultaneously. From the current investigation, Pearson's Chi-test of association revealed only one association (non-significant) among the qualitative traits within the wild type. There were twenty-seven (27) associations among qualitative traits within the mutant population with thirteen (13) being significant. Growth habit showed significant association with twinning tendency and plant pigmentation. There were significant associations among twinning tendency with two traits, leaf colour with four traits, terminal leaf shape with two traits and plant pigmentation with one trait. Correlation analysis provides important information on interrelationship between important agronomic traits (Owusu *et al*. 2018b; Ajayi *et al*., 2020; Mofokeng *et al*., 2020). There were six negative pairwise correlations and twenty-two positive pairwise correlations obtained among the quantitative traits in the M_2 population for this current research. Days to flowering negatively correlated with all the yield characters observed. There were significant positive correlations among the six yield characters. This result is in agreement with the reports by Owusu *et al.,* (2021) when sixteen (16) breeding lines of cowpea were

studied. Similar results were reported by Sharma *et al.*, 2017; Venkatesan *et al*., 2003; Kutty *et al.*, 2003; Kumawat and Raje, 2005; Chauhan *et al*., 2003; Kumari *et al*., 2010 in different genotypes of cowpea. This implies that EMS induced variability in cowpea and the selection of high yielding mutants would come with earliness. Improving on one yield trait will also affect the other five yield character. The associations among the qualitative traits also implies that the traits may be linked or influenced by one gene and the selection of one affects the other.

5.6 Variability in Mutant Population

Application of PCA is one of the strategies breeders adopt to identify influential traits for effective selection in cultivar development (Mofokeng *et al*., 2020). In the current work, the principal component analysis of the quantitative traits revealed a total percentage variability of 75.26% in the wild type and 77.22% in the mutants for the first four principal components in the M_1 generation. Chlorophyll content, pod length and number of locules per pod contributed the most in the wild type and number of seeds per pods and number of pods per plant contributed the most in the mutants. NOSP, PL, CC and PH mainly contributed for the 77.22% variation explained by the first 4 principal components. In the M_2 population, NOSPP, NOPPP and DTF mainly contributed for the variability explained by the first 4 principal components. Biplot of quantitative trait showed some mutants positively correlated with yield characters. Principal component analysis of the qualitative traits showed 100% variability explained by PP and TT in the wild type and 87.54% variability in the mutants mainly contributed by TT, PP, GH and SS. The contribution of these traits in percentage variability implies that EMS may have induced mutations in the gene(s) responsible for these traits. The mutants that are positively correlated with the yield characters could be targeted for further screening for these economic important traits.

Cluster analysis is used to group accessions or individuals with similar traits into one group. In breeding programs, cluster analysis is very useful for effective selection of individuals from a

common cluster. The present work had all the individuals in the M_2 generation grouped into six (6) clusters based on the quantitative traits with high yielding mutants belonging to cluster 4. Cluster 1 had all the wild types and most of the mutants. Cluster 2 had 72 individuals with the traits observed in this study. The variability in the M_2 population indicates that EMS might have caused mutations in these genes. Individuals in the cluster 4 can be selected for further screening for these economically important traits. The dendogram depicted 4 major groups based on the qualitative data. The level of variability showed by the qualitative traits was moderate since very few mutants were distinct from the population.

The variability among the accessions was generally high as observed, and the improvement of this crop is possible through the conventional breeding techniques (selection and hybridization). High level of variability among the traits will make room for selection as revealed by the PCA (Gana *et al.*, 2013). The mutagenized population can be used in genomics studies for the identification of candidate genes. Genes for the selected traits can be isolated and studied for point mutations induced by EMS. It was also observed that the morphological traits had different discriminatory capacity, as some efficiently discriminated than others, making those better traits of choice in plant diversity programs. Grouping of individuals in biplots had much more conformity with the results from dendrogram and revealed more importance of the first two principal components in both qualitative and quantitative traits, which justifies much of total variance. The cluster analysis and principal component analysis confirm each other.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

In the current study, EMS was used to induce genetic variability in the "*Asontem*" cultivar of *Vigna unguiculata*. The EMS induced mutations were observed in the cultivar as significant differences when putative mutants are compared with the wildtype. Based on germination data, the lethal dose 50% was estimated to be at 0.4% concentration of EMS solution. There was decrease in percentage germination and survival due to the EMS mutagenesis.

During the M_1 generation there were differences between the mutant and the wild type populations. Visible mutations of the morphological traits were observed in the M_1 populations. There were differences in growth habit, plant pigmentation, pod curvature, seed shape and seed coat colour. Distribution of quantitative traits studied showed wide ranges in the mutant population as compared with the wild type. High frequency was observed for farmer preferred traits: erect growth habit and low frequency was observed for consumer preferred trait: cream seed coat colour. During the M_2 generation, the variations in the mutant populations continued. There was decrease in percentage germination and survival. A chlorophyll defect was observed at the seedling stage; albino, xantha and pale green plants were observed in the M_2 population. There were visible mutations observed in leaf architecture in which some plants had different leaflet arrangement and number and irregular leaf structure. There were different levels of variation among the cowpea morphological traits observed. There were variations in leaf colour, leaf shape, plant pigmentation, growth habit, twinning tendency, pod curvature, seed shape and seed coat colour. There was high frequency for erect growth habit in the mutant population as compared with the wild type. There was wider distribution of quantitative traits in the mutant population.

During the association studies, Pearson's Chi-test of association revealed significant associations among the qualitative traits in the mutant population which was not observed in the wild type.

Contrast analysis showed individuals that are significantly different ($p<0.01$) from the wild type. High performing individuals and low performing individuals for the quantitative traits studied were selected from the mutant population. The EMS mutagenesis was effective in inducing the variations that will be useful for breeding and development of new farmer preferred varieties.

6.2 Recommendations

- Molecular work is required to analyze these mutants and determine the genetic reasons underlying the visible changes in order to genetically improve "*Asontem"* cultivar of cowpea.
- High yielding mutants that are identified and selected in this study can be further evaluated and incorporated into breeding programs to ensure food security in Ghana and also in the world. Poor yielding mutants should be maintained and replanted to further evaluate their yield performance.
- Planting distance must be increased during evaluation of the subsequent generations to prevent plants clustering together.

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APPENDICES

Appendix 1: Protocol for Ethyl Methane Sulfonate (EMS) Mutagenesis of Cowpea Seeds

Materials

1. Seeds or germ containing plant part
2. Ethyl methane sulfonate, EMS
3. Sodium thiosulfate
4. Distilled water

A. Prepare up to 5 liters of 1M Sodium thiosulphate

1. In 1 liter add 248.18g of sodium thiosulfate and then heat and stir until dissolution.
2. Repeat for 3 times
3. Move rotating vortex into a cleaned fume hood
4. Get googles, nose mask, and protection clothing ready for everyone
5. Move 1000µl pipette and tips into fume hood.

B. Prepare 0.4% of EMS solution into 50ml of H_20

1. Place 50ml bottle under the hood and
2. Add exactly 49.80ml of H_20
3. Add the cowpea seeds (about 100 seeds = 50ml)
4. Add 200 µl of EMS solution
5. Close bottle tightly and mix thoroughly
6. Put on rotating vertical vortex at 200rpm for 12 – 16 hours
7. Clean hood with Sodium thiosulfate

C. Terminating the experiment

1. Add 100ml sodium thiosulfate solution to neutralize and stop the reaction by inactivating the EMS
2. Keep for 5 minutes on the shaker and dispense the solution into a well labelled restricted container.
3. Add 100ml of H_2O to make the seeds sink
4. Wash 3 times with 100ml H_2o for 5-7 minutes each by gently turning the tube upside down for a couple of times.
5. Clean everything with 1M sodium thiosulfate and rinse with water.

*Note liquid waste should be restricted to and contained in a clearly labeled bottle with inscriptions "EMS treated water" for disposal.

E. Intermediate sowing

Sow in a well-watered soil.

Preparation of EMS concentrations, Total volume = 50ml

1. For 0.2%, add 100 µl of EMS solution in a total volume 50 ml (H_2O=49.9ml volume)
2. For 0.4%, add 200 µl of EMS solution in a total volume 50 ml (H_2O=49.8ml volume)
3. For 0.6%, add 300 µl of EMS solution in a total volume 50 ml (H_2O=49.7ml volume)
4. For 0.8%, add 400 µl of EMS solution in a total volume 50 ml (H_2O=49.6ml volume)

Appendix 2 Morphological Characterization of M_1 and M_2 Populations

Qualitative data

Leaf Colour: The intensity of green colour of the leaflet for each plant was observed and recorded using colour chart.

Leaf markings: Leaves with 'v' markings were observed and recorded at the sixth weeks after planting.

Leaf shape: The leaflet shape for each plant was obtained and recorded (IBPGR, 1983).

Plant pigmentation: The pigmentation of the petiole, branch, stem, peduncle for each plant was determined.

Flower colour: The colour of the flowers of the individual plants was observed and recorded.

Growth habit: The growth habits for each plant within the wild type and treatments were determined.

Growth pattern: With the help of the descriptor, plants with different growth pattern were observed and recorded

Twinning tendency: The twinning tendency for each individual plant within the wild type and mutagenized population was observed and recorded.

Seed coat colour: Seed coat colour of individual plants was determined and recorded.

Seed shape: The shapes of seeds from individual plants within each treatment were determined.

Pod colour: Colour of the matured pod was observed and recorded using the cowpea descriptor and colour chart.

Pod curvature:

Quantitative data

Chlorophyll content of leaves: Chlorophyll content was measured using the chlorophyll meter. The median leaflet of the trifoliate was measured and an average was taken to record the chlorophyll content for each of the individual plants within the wild type and treatments.

Leaf mutant: Plants with different compound leave aside the trifoliate leaves were observed within the wild type and treatments. The number of leaflets (monofoliate, bifoliate, tetrafoliate, pentofoliate etc.) for the mutant leaves was recorded.

Days to flowering: This was observed by counting the number of days it took an individual to form the first flower after planting.

Plant height: The plant height measurement was taken as the distance between the first node and apex of the plant. This measurement was done by using measuring tape.

Pod Length: Three (3) pods of each plant in the wild type and mutant population were measured using a string from the stylar end to the point of attachment of the pod to the stalk. The string was stretched on a 30 cm rule and the pod length was read. The mean pod length calculated and recorded.

Number of seed per pod: Three pods were opened by the hand and the number of seeds in each pod per individual plants within wild type and mutant population was counted. Total number of seeds was recorded and the mean was calculated.

Number of pods per plant: Total number of pods from each plant was counted after harvesting. Harvesting was done once when 80% of the pods were matured.

Percentage germination: The number of seeds germinated among the wild type and treatments were observed. The percentage seed germination was calculated and recorded.

Appendix 3: Summary Statistics of Quantitative Traits in the M_1 Mutagenized Population.

CC=Chlorophyll content, PH= Plant height, NOB=Number of branches, DTF= Days to flowering, DTFMP=Days to first maturing pod, NOPP= number of pods per plant, PL= Pod length, NPPP=number of peduncles per plant, SW=seed weight, NOLP=Number of Locules per plant.

	CC	PH	DTF	NOPP	PL	NPPP	NOSP	NOLP
Minimum	10.4	20.0	60	1	3.0	1	1	6
1st Quimum	31.9	82.25	61	6	13.6	1	5	10
Median	36.45	113.0	62	12	14.3	1	9	11
Mean	36.86	110.38	62	18	14.2	2	8	11
3rd Quimum	41.9	139.0	62	25	15.0	2	11	12
Maximum	56.9	200.0	66	80	18.2	3	14	20
NA's	0	0.0	0	59	59	59	59	59
Total Observed	478	478.0	478	419	419	419	419	419

Appendix 4: Summary Statistics of Quantitative Traits in the Wild type (M_1 Generation).

CC=Chlorophyll content, PH= Plant height, NOB=Number of branches, DTF= Days to flowering, DTFMP=Days to first maturing pod, NOPP= number of pods per plant, PL= Pod length, NPPP=number of peduncles per plant, SW=seed weight, NOLP=Number of Locules per plant.

	CC	PH	DTF	NOPP	PL	NPPP	NOSP	NOLP
Minimum	20.90	100.0	59	18	12.9	1	7	10
1st Quimum	33.15	136.9	62	37	14.4	1	10	12
Median	35.70	160.5	62	40	15.4	2	11	12
Mean	37.04	159.7	62	39	15.4	2	11	12
3rd Quimum	41.77	184.2	62	43	16.3	2	12	13
Maximum	51.00	200.0	65	46	18.1	2	13	14
Total Observed	30	30	30	30	30	30	30	30

Appendix 5: Germination count and Percentage Germination of Mutant Lines and Wild type.

Lines	Germination count	Percentage Germination	Germination speed
A1	16	80	1.6
A2	16	80	1.6
A3	15	75	1.5
A4	15	75	1.5
A5	15	75	1.5
A6	16	80	1.6
A7	16	80	1.6
A8	18	90	1.8
A9	20	100	2
A10	17	85	1.7
A11	17	85	1.7
A12	20	100	2
A13	17	85	1.7
A14	3	15	0.3
A15	18	90	1.8
A16	19	95	1.9
A17	19	95	1.8
A18	18	90	1.8
A19	19	95	1.9
A20	11	55	1.1
A21	16	80	1.6
A22	19	95	1.9
A23	6	30	0.6
A24	19	95	1.9
A25	17	85	1.7
A26	19	95	1.9
A27	18	90	1.8
A28	20	100	2
A29	19	95	1.9
A30	15	75	1.5
A31	16	80	1.6
A32	18	90	1.8
A33	12	60	1.2
A34	18	90	1.8
A35	12	60	1.2
A36	17	85	1.7
A37	15	75	1.5
A38	14	70	1.4
A39	17	85	1.7
A40	18	90	1.8
A41	17	85	1.7
A42	14	70	1.4
A43	17	85	1.7
A44	18	90	1.8

Appendix 6 (cont'd)

Lines	Germination count	Percentage Germination	Germination speed
A45	14	70	1.4
A46	12	60	1.2
A47	19	95	1.9
A48	19	95	1.9
A49	9	45	0.9
A50	17	85	1.7
A51	19	95	1.9
A52	15	75	1.5
A53	18	90	1.8
A54	16	80	1.3
A55	9	45	0.9
A56	11	55	1.1
A57	10	50	0.9
A58	6	30	0.5
A59	14	70	1.4
A60	15	75	1.5
A61	16	80	1.5
A62	12	60	1.2
A63	16	80	1.3
A64	15	75	1.4
B1	13	65	1.3
B2	15	75	1.4
B3	13	65	1.2
B4	14	70	1.4
B5	16	80	1.6
B6	17	85	1.7
B7	17	85	1.7
B8	13	65	1.2
B9	14	70	1.3
B10	13	65	1.3
B11	13	65	1.3
B12	15	75	1.5
B13	17	85	1.7
B14	16	80	1.6
B15	18	90	1.8
B16	16	80	1.6
B17	18	90	1.8
B18	16	80	1.5
B19	18	90	1.8
B20	20	100	2
B21	18	90	1.7
B22	17	85	1.7
B23	17	85	1.7
B24	16	80	1.6
B25	16	80	1.6

Appendix 6 (cont'd)

Lines	Germination count	Percentage Germination	Germination speed
B26	13	65	1.3
B27	14	70	1.4
B28	10	50	1
B29	18	90	1.8
B30	19	95	1.9
B31	16	80	1.6
B32	16	80	1.6
B33	12	60	1.2
B34	17	85	1.7
B35	10	50	0.9
B36	14	70	1.4
B37	17	85	1.7
B38	12	60	1.2
B39	15	75	1.5
B40	12	60	1.2
B41	19	95	1.8
B42	18	90	1.8
B43	9	45	0.9
B44	15	75	1.5
B45	16	80	1.5
B46	18	90	1.7
B47	11	55	0.9
B48	14	70	1.3
B49	19	95	1.7
B50	18	90	1.8
B51	18	90	1.8
B52	14	70	1.2
B53	16	80	1.6
B54	17	85	1.7
B55	12	60	1.2
B56	19	95	0.9
B57	17	85	1.6
B58	13	65	1.3
B59	15	75	1.5
B60	11	55	1.1
B61	10	50	1
B62	13	65	1.3
B63	13	65	1.2
B64	12	60	1.2
C1	17	85	1.7
C2	16	80	1.6
C3	18	90	1.7
C4	16	80	1.4
C5	14	70	1.3
C6	15	75	1.5

Appendix 6 cont'd

Lines	Germination count	Percentage Germination	Germination speed
C7	18	90	1.7
C8	15	75	1.5
C9	15	75	1.5
C10	12	60	1.2
C11	14	70	1.4
C12	17	85	1.6
C13	14	70	1.3
C14	14	70	1.1
C15	15	75	1.5
C16	13	65	1.3
C17	17	85	1.7
C18	16	80	1.5
C19	15	75	1.4
C20	19	95	1.8
C21	17	85	1.6
C22	14	70	1.4
C23	11	55	1.1
C24	9	45	0.7
C25	15	75	1.4
C26	12	60	1.2
C27	12	60	1.2
C28	7	35	0.7
C29	14	70	1.4
C30	4	20	0.3
C31	17	85	1.6
C32	11	55	0.9
C33	13	65	1.3
C34	17	85	1.7
C35	16	80	1.6
C36	15	75	1.5
C37	13	65	1.3
C38	12	60	1.2
C39	14	70	1.3
C40	12	60	1.2
C41	12	60	1.2
C42	9	45	0.9
C43	12	60	1.2
C44	13	65	1.3
C45	15	75	1.4
C46	15	75	1.5
C47	15	75	1.4
C48	14	70	1.4
C49	14	70	1.4
C50	15	75	1.4
C51	16	80	1.5

Appendix 6 (cont'd)

Lines	Germination count	Percentage Germination	Germination speed
C52	16	80	1.6
C53	13	65	1.3
C54	12	60	1.3
C55	11	55	1.1
C56	12	60	1.1
C57	12	60	1.2
C58	15	75	1.5
C59	14	70	1.4
C60	14	70	1.4
C61	15	75	1.4
C62	14	70	1.4
C63	14	70	1.3
C64	10	50	0.9
D1	17	85	1.6
D2	20	100	2
D3	18	90	1.7
D4	11	55	1.1
D5	17	85	1.6
D6	14	70	1.4
D7	11	55	1.1
D8	15	75	1.4
D9	16	80	1.4
D10	15	75	1.4
D11	17	85	1.6
D12	13	65	1.3
D13	13	65	1.3
D14	17	85	1.6
D15	16	80	1.4
D16	15	75	1.4
D17	17	85	1.6
D18	16	80	1.5
D19	11	55	1.1
D20	19	95	1.9
D21	17	85	1.7
D22	19	95	1.9
D23	16	80	1.5
D24	19	95	1.9
D25	16	80	1.5
D26	17	85	1.7
D27	13	65	1.2
D28	16	80	1.6
D29	18	90	1.7
D30	15	75	1.4
D31	16	80	1.6
D32	19	95	1.9
D33	13	65	1.2
D34	14	70	1.5
D35	16	80	1.6

Appendix 6 (cont'd)

Lines	Germination count	Percentage Germination	Germination speed
D36	17	85	1.7
D37	12	60	1.2
D38	15	75	1.5
D39	16	80	1.6
D40	13	65	1.3
D41	15	75	1.4
D42	8	40	0.8
D43	18	90	1.7
D44	16	80	1.6
D45	14	70	1.3
D46	6	30	0.6
D47	17	85	1.6
D48	17	85	1.6
D49	1	5	0.1
D50	19	95	1.8
D51	12	60	1.2
D52	15	75	1.4
D53	11	55	1
D54	9	45	0.7
D55	15	75	1.5
D56	1	5	0.1
D57	17	85	1.8
D58	4	20	0.2
D59	15	75	1.4
D60	12	60	1
D61	11	55	1.1
D62	15	75	1.4
D63	12	60	1.2
D64	17	85	1.7
E1	14	70	1.1
E2	17	85	1.7
E3	16	80	1.5
E4	18	90	1.8
E5	15	75	1.4
E6	14	70	1.1
E7	16	80	1.5
E8	17	85	1.6
E9	13	65	1.3
E10	14	70	1.3
E11	14	70	1.3
E12	16	80	1.4
E13	12	60	1
E14	19	95	1.8
E15	4	20	0.3
E16	15	75	1.4
E17	15	75	1.5
E18	17	85	1.5
E19	15	75	1.3
E20	5	25	0.5
E21	7	35	0.7
E22	13	65	1.3
E23	17	85	1.7

Appendix 6 cont'd

Lines	Germination count	Percentage Germination	Germination speed
E25	14	70	1.4
E26	11	55	1.1
E27	12	60	1.1
E28	18	90	1.7
E29	19	95	1.8
E30	15	75	1.5
E31	18	90	1.8
E32	13	65	1.3
E33	20	100	2
E34	17	85	1.7
E35	18	90	1.8
E36	20	100	2
E37	16	80	1.6
E38	13	65	1.3
E39	17	85	1.7
E40	19	95	1.7
E41	14	70	1.4
E42	17	85	1.7
E43	18	90	1.8
E44	18	90	1.8
E45	13	65	1.2
E46	15	75	1.5
E47	16	80	1.6
E48	17	85	1.5
E49	17	85	1.6
E50	16	80	1.5
E51	18	90	1.8
E52	18	90	1.6
E53	17	85	1.7
E54	12	60	1.2
E55	11	55	1
E56	14	70	1.4
E57	20	100	2
E58	16	80	1.6
E59	11	55	1.1
E60	12	60	0
E61	10	50	1
E62	13	65	0.8
E63	13	65	1.3
Wild type-1	15	75	1.5
Wild type-2	18	90	1.8
Wild type-3	15	75	1.5
Wild type-4	13	65	1.3
Wild type-5	17	85	1.7
Total			
Mutagenized population	4723	74.03	1.43
Wild type	80	80	1.56

Appendix 6: Summary Statistics of Quantitative Traits in the Wild type (M_2 Generation).

DTF= Days to flowering, NOPPP= number of pods per plant, NOSPP= Number of seeds per pod, PL= Pod length, NOLP=Number of Locules per plant, PSST=Percentage Seed Set, NSPP=Number of seeds per Plant

	DTF	NOPPP	NOSPP	PL	NOLP	PSST	NSPP
Minimum	40	10	9	12.1	10	81.82	150
1st quartile	51	25	13	14.5	14	88.65	321
Median	54	28	14	15.7	15	93.33	377
Mean	54	28	12	15.5	14	93.59	374
3rdquartile	58	30	15	16.7	16	100	435
Maximum	62	40	17	18.3	17	100	544

Appendix 7: Summary Statistics of Quantitative Traits in the M_2 Population.

DTF= Days to flowering, NOPPP= number of pods per plant, NOSPP= Number of seeds per pod, PL= Pod length, NOLP=Number of Locules per plant, PSST=Percentage Seed Set, NSPP=Number of seeds per Plant

	DTF	NOPPP	NOSPP	PL	NOLP	PSST	NSPP
Minimum	38	1	0	8.3	4	0	0
1st quartile	41	7	12	16.1	14	84.63	96
Median	44	12	14	17.1	16	93.33	165
Mean	45	11	14	16.8	16	88.31	203
3rdquartile	47	19	16	17.8	17	100	272
Maximum	63	71	19	20.4	20	100	1156

Appendix 5: Z-Test of Means of Number of Pods per Plant (NOPPP) Between the Wild type and Selected High Performing Putative Mutants Assuming Unequal Variances

	NOPPP	*NOPPMUT*
Mean	27.075	42.93069307
Known Variance	19.56392405	79.96514851
Observations	80	101
Hypothesized Mean Difference	0	
z	**-15.57564366**	
P(Z<=z) one-tail	0	
z Critical one-tail	1.644853627	
P(Z<=z) two-tail	0	
z Critical two-tail	1.959963985	

Appendix 6: Z-Test of Means of Number of Seeds per Pod (NOSPP) Between the Wild type and Selected High Performing Putative Mutants Assuming Unequal Variances

	NOSPP	*NOSPPMUT*
Mean	13.75	18.093023
Known Variance	3.582278	0.085363
Observations	80	86
Hypothesized Mean Difference	0	
z	**-20.300028**	
P(Z<=z) one-tail	0	
z Critical one-tail	1.6448536	
P(Z<=z) two-tail	0	
z Critical two-tail	1.959964	

Appendix 7: Z-Test of Means of Number of Locules per Pod (NOLPP) Between the Wild type and Selected High Performing Putative Mutants Assuming Unequal Variances

	NOLP	*NOLPMUT*
Mean	14.6875	18.262069
Known Variance	2.875791	0.242501
Observations	80	290
Hypothesized Mean Difference	0	
z	**-18.637883**	
P(Z<=z) one-tail	0	
z Critical one-tail	1.6448536	
P(Z<=z) two-tail	0	
z Critical two-tail	1.959964	

Appendix 8: T-Test of Means of Pod Length (PL) Between the Wild type and Selected High Performing Putative Mutants Assuming Unequal Variances

	PL	*PLMUT*
Mean	15.45875	18.77316
Known Variance	2.881948	0.147885
Observations	80	231
Hypothesized Mean Difference	0	
z	**-17.309443**	
P(Z<=z) one-tail	0	
z Critical one-tail	1.6448536	
P(Z<=z) two-tail	0	
z Critical two-tail	1.959964	

Appendix 9: T-Test of Means of Days to Flowering (DTF) Between the Wild type and Selected Early Flowering Performing Putative Mutants Assuming Unequal Variances

	DTF	*DTFMUT*
Mean	49.5125	39.66899
Known Variance	19.51883	1.042073
Observations	80	574
Hypothesized Mean Difference	0	
z	**19.854472**	
P(Z<=z) one-tail	0	
z Critical one-tail	1.6448536	
P(Z<=z) two-tail	0	
z Critical two-tail	1.959964	

Appendix 10: T-Test of Means of Number of Seeds per Plant (NSPP) Between the Wild type and Selected High Performing Putative Mutants Assuming Unequal Variances

	NSPP	*NSPPMUT*
Mean	373.7	687.89412
Known Variance	7270.441	21731.24
Observations	80	85
Hypothesized Mean Difference	0	
z	**-16.877962**	
P(Z<=z) one-tail	0	
z Critical one-tail	1.6448536	
P(Z<=z) two-tail	0	
z Critical two-tail	1.959964	

Appendix 11: T-Test of Means of Number of Pods per Plant (NOPPP) Between the Wild type and Selected Low Performing Putative Mutants Assuming Unequal Variances

	NOPPP	*NOPPPMUT*
Mean	27.075	5.562972292
Known Variance	19.56392405	6.35374173
Observations	80	794
Hypothesized Mean Difference	0	
z	**42.80619173**	
P(Z<=z) one-tail	0	
z Critical one-tail	1.644853627	
P(Z<=z) two-tail	0	
z Critical two-tail	1.959963985	

Appendix 12: T-Test of Means of Number of Seeds per Pod (NOSPP) Between the Wild type and Selected Low Performing Putative Mutants Assuming Unequal Variances

	NOSPP	*NOSPPMUT*
Mean	13.75	5.584
Known Variance	3.582278	6.19651613
Observations	80	125
Hypothesized Mean Difference	0	
z	**26.58502**	
P(Z<=z) one-tail	0	
z Critical one-tail	1.644854	
P(Z<=z) two-tail	0	
z Critical two-tail	1.959964	

Appendix 13: T-Test of Means of Number of Locules per Pod (NOLP) Between the Wild type and Selected Low Performing Putative Mutants Assuming Unequal Variances

	NOLP	*NOLPMUT*
Mean	14.6875	8.026316
Known Variance	2.875791	1.972262
Observations	80	38
Hypothesized Mean Difference	0	
z	**22.47414**	
P(Z<=z) one-tail	0	
z Critical one-tail	1.644854	
P(Z<=z) two-tail	0	
z Critical two-tail	1.959964	

Appendix 14: T-Test of Means of Percentage Seed Set (PSST) Between the Wild type and Selected Low Performing Putative Mutants Assuming Unequal Variances

	PSST	*PSSTMUT*
Mean	93.50048	67.10391
Known Variance	34.21689	309.3106
Observations	80	426
Hypothesized Mean Difference	0	
z	**24.57444**	
P(Z<=z) one-tail	0	
z Critical one-tail	1.644854	
P(Z<=z) two-tail	0	
z Critical two-tail	1.959964	

Appendix 15: T-Test of Means of Pod Length (PL) Between the Wild type and Selected Low Performing Putative Mutants Assuming Unequal Variances

	PL	*PLMUT*
Mean	15.45875	10.776
Known Variance	2.881948	1.345233
Observations	80	25
Hypothesized Mean Difference	0	
z	**15.62361**	
P(Z<=z) one-tail	0	
z Critical one-tail	1.644854	
P(Z<=z) two-tail	0	
z Critical two-tail	1.959964	

Appendix 16: T-Test of Means of Days to Flowering (DTF) Between the Wild type and Selected Late Flowering Putative Mutants Assuming Unequal Variances

	DTF	*DTFMUT*
Mean	49.5125	61.70588
Known Variance	19.51883	0.470588
Observations	80	17
Hypothesized Mean Difference	0	
z	**-23.3941**	
P(Z<=z) one-tail	0	
z Critical one-tail	1.644854	
P(Z<=z) two-tail	0	
z Critical two-tail	1.959964	

Appendix 17: Pairwise correlation coefficients for Quantitative Traits in the Wild type (M_2 Generation)

	NOPPP	NOSPP	NOLP	PSST	PL	NSPP	DTF
NOPPP	1	0.172	0.091	0.221	0.071	0.821	0.077
NOSPP	0.172	1	0.898	0.587	0.548	0.694	0.105
NOLP	0.091	0.898	1	0.175	0.531	0.573	0.158
PSST	0.221	0.587	0.175	1	0.232	0.499	-0.065
PL	0.071	0.548	0.531	0.232	1	0.37	0.226
NSPP	0.821	0.694	0.573	0.499	0.37	1	0.123
DTF	0.077	0.105	0.158	-0.065	0.226	0.123	1

Appendix 18: Pairwise correlation p-values for Quantitative Traits in the Wild type (M_2 Generation)

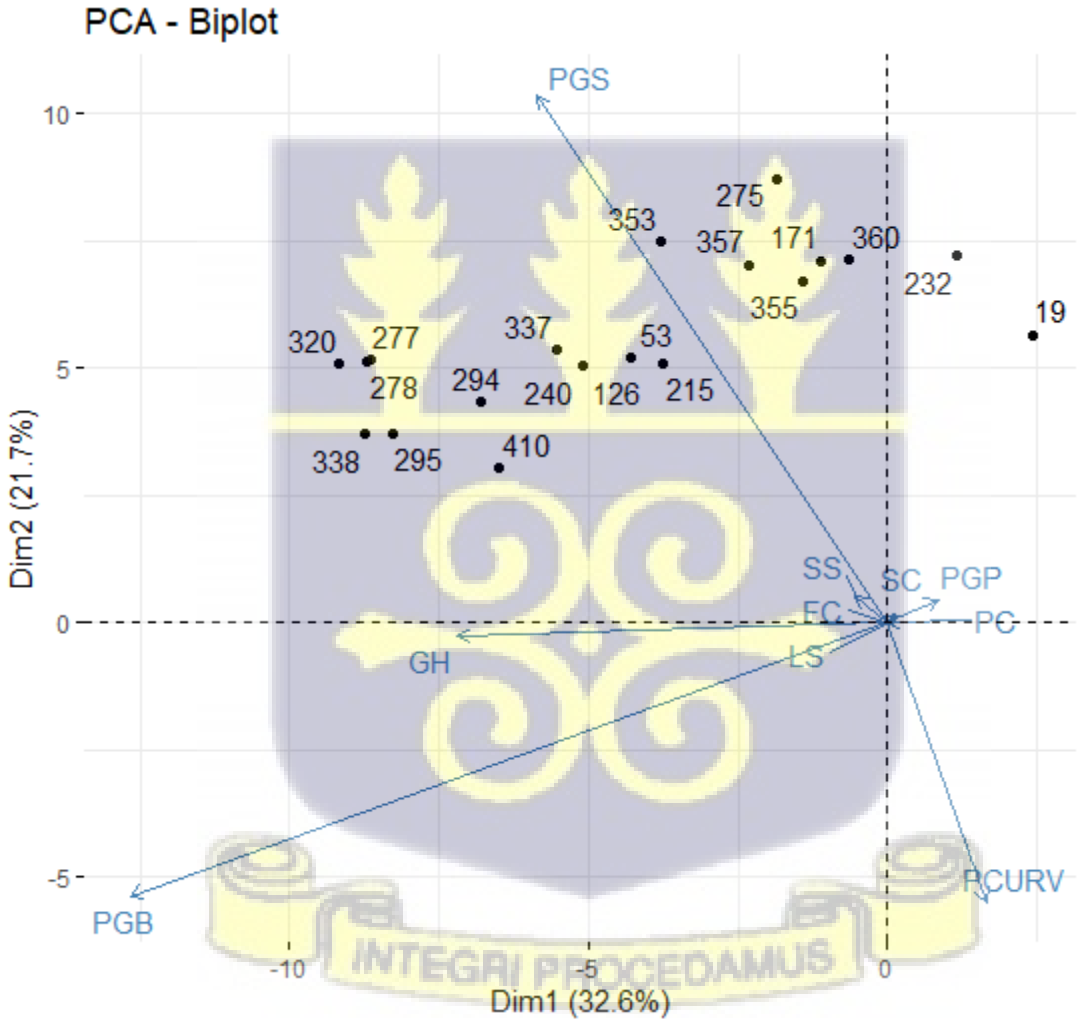
	NOPPP	NOSPP	NOLP	PSST	PL	NSPP	DTF
NOPPP	0	1	1	0.489	1	0	1
NOSPP	0.128	0	0	0	0	0	1
NOLP	0.423	0	0	1	0	0	1
PSST	0.049	0	0.121	0	0.456	0	1
PL	0.533	0	0	0.038	0	0.01	0.48
NSPP	0	0	0	0	0.001	0	1
DTF	0.499	0.354	0.161	0.569	0.044	0.277	0

Appendix 19: Pairwise correlation coefficients for Quantitative Traits in the M_2 Population

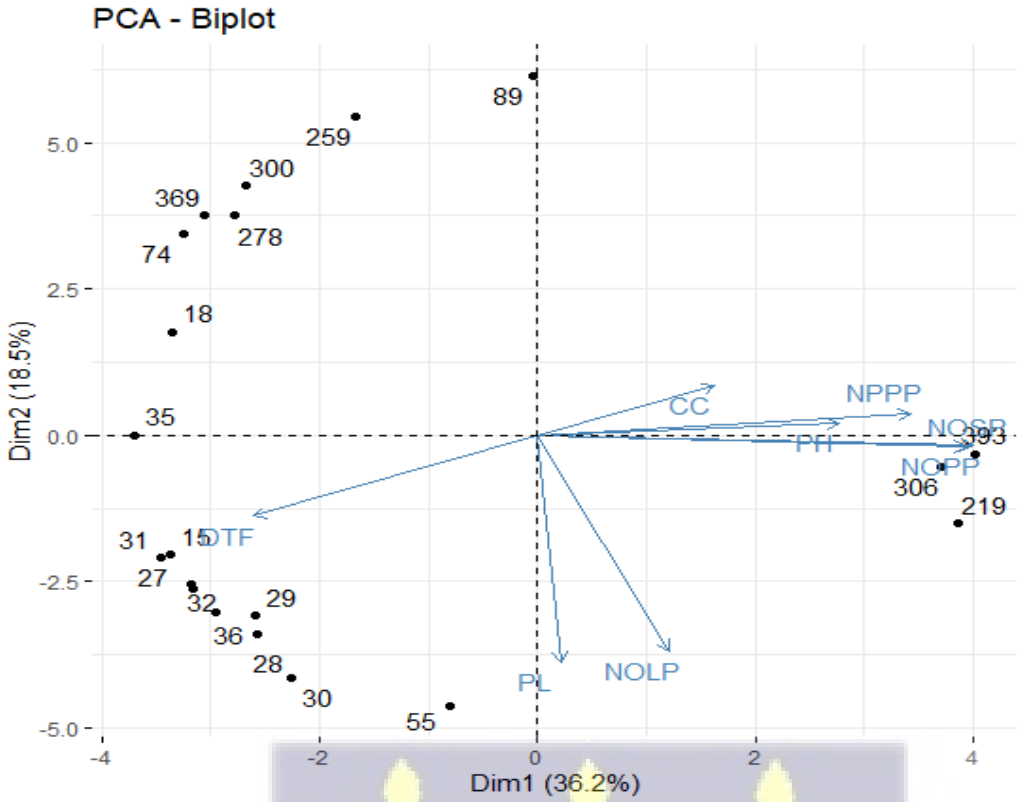
	NOPPP	NOSPP	NOLP	PSST	PL	NSPP	DTF
NOPPP	1	0.221	0.201	0.156	0.316	0.964	-0.294
NOSPP	0.221	1	0.775	0.816	0.678	0.406	-0.084
NOLP	0.201	0.775	1	0.295	0.737	0.357	-0.046
PSST	0.156	0.816	0.295	1	0.377	0.283	-0.083
PL	0.316	0.678	0.737	0.377	1	0.43	-0.131
NSPP	0.964	0.406	0.357	0.283	0.43	1	-0.29
DTF	-0.294	-0.084	-0.046	-0.083	-0.131	-0.29	1

Appendix 20: Pairwise correlation p-values for Quantitative Traits in the M_2 Population

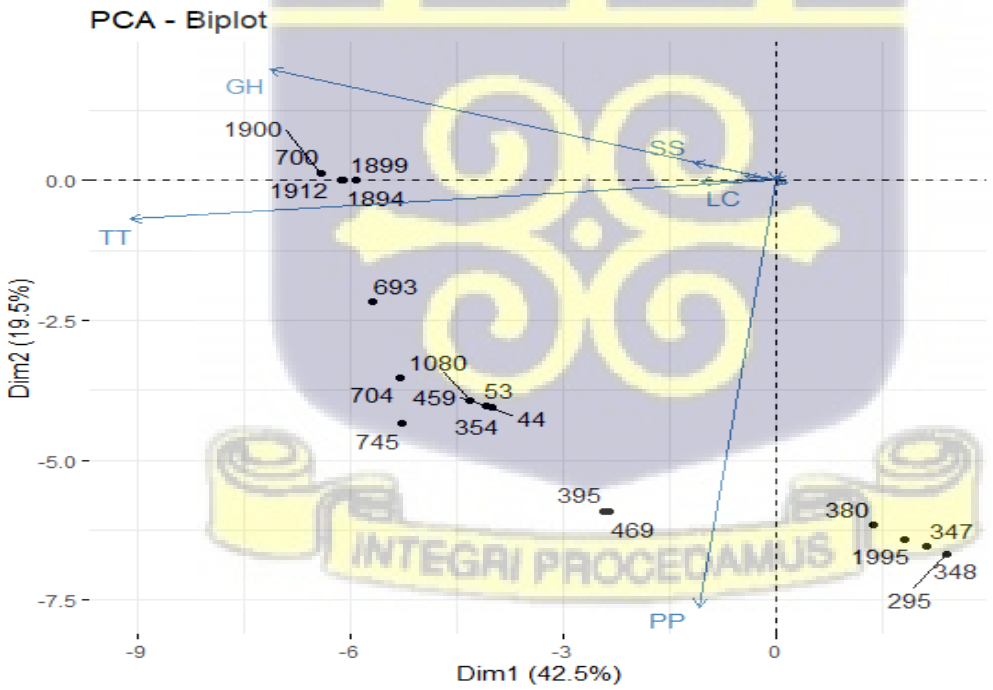
	NOPPP	NOSPP	NOLP	PSST	PL	NSPP	DTF
NOPPP	0	0	0	0	0	0	0
NOSPP	0	0	0	0	0	0	0
NOLP	0	0	0	0	0	0	0.035
PSST	0	0	0	0	0	0	0
PL	0	0	0	0	0	0	0
NSPP	0	0	0	0	0	0	0
DTF	0	0	0.035	0	0	0	0



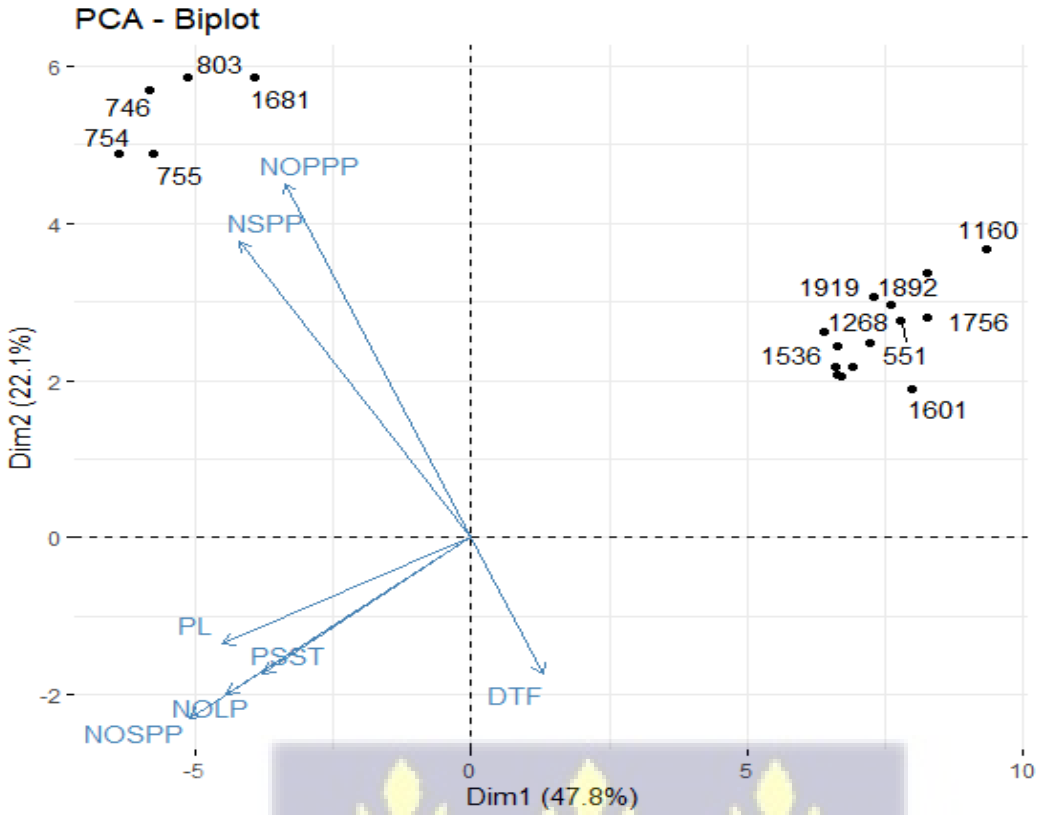
Appendix 21: Biplot of Qualitative Traits and Top 20 Individuals in the M_1 Generation Showing Highest Contribution to Variability.



Appendix 22: Biplot of Quantitative Traits and Top 20 Individuals in the M_1 Generation Showing Highest Contribution to Variability.



Appendix 23: Biplot of Qualitative Traits and Top 20 Individuals in the M_2 Generation Showing Highest Contribution to Variability.



Appendix 24: Biplot of Quantitative Traits and Top 20 Individuals in the M_2 Generation Showing Highest Contribution to Variability.

Appendix 25: Cluster groupings (7, 6, 5, 4, 3, 2) of individuals with both quantitative and qualitative traits.

PLANT	TRT	NOPPP	NOSPP	NOLP	PSST	PL	NSPP	DTF	LC	TLS	LM	GP	FC	GH	PP	TT	PCURV	PC	SS	SCC	Cluster
C37P1	EMS2	33	17	19	89.47	9.1	561	41	Darkgreen	Hastate	Present	Indeterminate	Violet	Intermediate	Moderate	Intermediate	Slightly curved	Pale tan	Kidney	Brown	7
C21P11	EMS2	1	4	4	100.00	9.4	4	38	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	None	Straight	Pale tan	Ovoid	Brown	6
C58P12	EMS2	1	8	8	100.00	12.5	8	44	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Intermediate	None	Straight	Pale tan	Ovoid	Brown	6
D28P10	EMS2	8	6	6	100.00	11.8	48	55	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Very slight	None	Straight	Pale tan	Ovoid	Brown	6
E55P1	EMS2	12	13	13	100.00	8.3	156	45	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Intermediate	Slight	Straight	Pale tan	Rhomboid	Brown	6
B26P6	EMS2	50	16	16	100.00	17.6	800	39	Darkgreen	Hastate	Present	Indeterminate	Violet	Climbing	Very slight	Intermediate	Slightly curved	Pale tan	Rhomboid	Firebrick	5
B52P13	EMS2	25	16	17	94.12	18	400	39	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Solid	Slight	Slightly curved	Pale tan	Ovoid	Brown	5
B55P10	EMS2	43	10	10	100.00	14.4	430	44	Intermediate green	Hastate	Present	Indeterminate	Violet	Acute erect	Extensive	None	Slightly curved	Pale tan	Rhomboid	Brown	5
C20P2	EMS2	52	17	17	100.00	17.9	884	38	Darkgreen	Hastate	Present	Indeterminate	Violet	Prostrate	Moderate	Slight	Straight	Pale tan	Rhomboid	Brown	5
C20P3	EMS2	45	17	17	100.00	18.4	765	42	Darkgreen	Hastate	Present	Indeterminate	Violet	Prostrate	Very slight	Slight	Straight	Pale tan	Rhomboid	Brown	5
C21P8	EMS2	64	16	16	100.00	17.8	1024	48	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	None	None	Straight	Pale tan	Rhomboid	Brown	5
C25P2	EMS2	57	11	12	91.67	16	627	40	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Intermediate	Slightly curved	Pale tan	Rhomboid	Brown	5
C27P10	EMS2	46	15	17	88.24	18	690	45	Darkgreen	Hastate	Present	Indeterminate	Violet	Intermediate	Moderate	Intermediate	Slightly curved	Pale tan	Rhomboid	Brown	5
C29P10	EMS2	48	17	18	94.44	17.9	816	39	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Slight	Slightly curved	Pale tan	Ovoid	Firebrick	5
C33P13	EMS2	43	17	18	94.44	18.1	731	41	Darkgreen	Hastate	Present	Indeterminate	Violet	Prostrate	Intermediate	Intermediate	Straight	Pale tan	Rhomboid	Brown	5
C35P5	EMS2	38	18	19	94.74	17.4	684	43	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Intermediate	Straight	Pale tan	Rhomboid	Brown	5
C36P12	EMS2	56	15	17	88.24	18.3	840	38	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Intermediate	Slightly curved	Pale tan	Rhomboid	Brown	5
C37P12	EMS2	68	17	18	94.44	18.4	1156	39	Darkgreen	Hastate	Present	Indeterminate	Violet	Erect	Intermediate	None	Slightly curved	Pale tan	Rhomboid	Brown	5
C37P4	EMS2	54	18	18	100.00	18.7	972	46	Darkgreen	Hastate	Present	Indeterminate	Violet	Intermediate	Moderate	Intermediate	Straight	Pale tan	Rhomboid	Brown	5
C37P7	EMS2	36	18	19	94.74	18.1	648	41	Darkgreen	Hastate	Present	Indeterminate	Violet	Intermediate	Extensive	Slight	Straight	Pale tan	Rhomboid	Brown	5
C37P8	EMS2	63	18	19	94.74	19.8	1134	39	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Intermediate	Straight	Pale tan	Rhomboid	Brown	5
C37P9	EMS2	64	18	18	100.00	18.2	1152	47	Darkgreen	Hastate	Present	Indeterminate	Violet	Erect	Moderate	Slight	Slightly curved	Pale tan	Ovoid	Brown	5
C39P1	EMS2	60	16	17	94.12	17.2	960	40	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Intermediate	Slightly curved	Pale tan	Rhomboid	Brown	5
C39P6	EMS2	36	19	19	100.00	19	684	39	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Extensive	Intermediate	Slightly curved	Pale tan	Ovoid	Brown	5
C41P6	EMS2	49	17	18	94.44	18	833	40	Intermediate green	Hastate	Present	Indeterminate	Violet	Acute erect	Extensive	Slight	Slightly curved	Pale tan	Ovoid	Brown	5
C41P8	EMS2	71	15	18	83.33	18.7	1065	41	Intermediate green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Pronounced	Straight	Pale tan	Ovoid	Brown	5
C42P7	EMS2	36	18	18	100.00	18.3	648	44	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Intermediate	Straight	Pale tan	Rhomboid	Brown	5
D32P18	EMS2	60	16	17	94.12	17.9	960	38	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Slight	Straight	Pale tan	Ovoid	Brown	5
D37P5	EMS2	52	13	15	86.67	17.2	676	57	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Straight	Pale tan	Ovoid	Brown	5

Appendix 25 cont'd

D44P15	EMS2	56	12	12	100.00	15.3	672	45	Intermediate green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	None	Straight	Pale tan	Ovoid	Brown	5
E26P3	EMS2	69	14	15	93.33	17.4	966	42	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Moderate	Intermediate	Straight	Pale tan	Rhomboid	Firebrick	5
E26P8	EMS2	46	18	19	94.74	18.8	828	43	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Intermediate	Intermediate	Straight	Pale tan	Rhomboid	Brown	5
E27P8	EMS2	56	13	17	76.47	17	728	40	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Pronounced	Straight	Pale tan	Ovoid	Brown	5
E28P14	EMS2	56	12	17	70.59	16.6	672	39	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Intermediate	Pronounced	Straight	Pale tan	Ovoid	Brown	5
E29P8	EMS2	32	16	16	100.00	18.6	512	38	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Extensive	Intermediate	Slightly curved	Pale tan	Ovoid	Brown	5
E32P6	EMS2	65	14	14	100.00	18.3	910	38	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Straight	Pale tan	Ovoid	Brown	5
E33P6	EMS2	41	9	13	69.23	16.1	369	40	Darkgreen	Hastate	Present	Indeterminate	Violet	Prostrate	Extensive	None	Slightly curved	Pale tan	Rhomboid	Brown	5
E43P3	EMS2	38	16	17	94.12	17.6	608	39	Intermediate green	Hastate	Present	Indeterminate	Violet	Climbing	Moderate	Pronounced	Straight	Pale tan	Ovoid	Firebrick	5
A61P9	EMS2	3	1	15	6.67	16.5	3	53	Darkgreen	Sub-hastate	Present	Indeterminate	Violet	Intermediate	Very slight	Intermediate	Straight	Pale tan	Rhomboid	Brown	4
D44P2	EMS2	11	5	16	31.25	15.7	55	59	Pale green	Sub-hastate	Present	Indeterminate	Violet	Erect	Very slight	None	Straight	Pale tan	Rhomboid	Brown	4
E45P8	EMS2	6	6	12	50.00	15.7	36	40	Pale green	Sub-hastate	Present	Indeterminate	Violet	Climbing	Moderate	Intermediate	Slightly curved	Pale tan	Rhomboid	Firebrick	4
A48P12	EMS2	10	9	16	56.25	16	90	60	Darkgreen	Hastate	Present	Indeterminate	Violet	Erect	Intermediate	None	Straight	Pale tan	Ovoid	Firebrick	3
A64P11	EMS2	1	6	11	54.55	8.7	6	44	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Intermediate	Straight	Pale tan	Rhomboid	Brown	3
B51P8	EMS2	2	9	14	64.29	14.4	18	60	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Intermediate	None	Slightly curved	Pale tan	Rhomboid	Brown	3
B58P13	EMS2	4	1	15	6.67	16.2	4	43	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Extensive	Slight	Straight	Pale tan	Rhomboid	Firebrick	3
B58P5	EMS2	4	6	12	50.00	12.1	24	48	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Intermediate	None	Straight	Pale tan	Ovoid	Firebrick	3
B62P6	EMS2	2	13	18	72.22	16.8	26	48	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	None	Straight	Pale tan	Ovoid	Firebrick	3
B63P3	EMS2	3	12	15	80.00	15.9	36	63	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Slight	Slightly curved	Pale tan	Ovoid	Firebrick	3
B63P6	EMS2	3	3	11	27.27	11.3	9	47	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Intermediate	Slight	Straight	Pale tan	Ovoid	Brown	3
B64P5	EMS2	1	8	10	80.00	13.1	8	52	Darkgreen	Hastate	Present	Indeterminate	Violet	Intermediate	Moderate	None	Slightly curved	Pale tan	Ovoid	Firebrick	3
B64P7	EMS2	2	4	13	30.77	12.8	8	51	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Intermediate	Slight	Slightly curved	Pale tan	Ovoid	Brown	3
B64P9	EMS2	5	8	12	66.67	14.3	40	51	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-erect	Intermediate	None	Slightly curved	Pale tan	Ovoid	Firebrick	3
B64P12	EMS2	3	9	14	64.29	15	27	56	Darkgreen	Hastate	Present	Indeterminate	Violet	Intermediate	Moderate	Intermediate	Straight	Pale tan	Ovoid	Firebrick	3
C20P13	EMS2	2	2	5	40.00	11.2	4	45	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Slight	Straight	Pale tan	Rhomboid	Firebrick	3
C25P12	EMS2	4	4	11	36.36	14.1	16	49	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Moderate	Slight	Straight	Pale tan	Rhomboid	Brown	3
C48P2	EMS2	3	8	13	61.54	12.8	24	44	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Intermediate	None	Straight	Pale tan	Ovoid	Brown	3
C50P2	EMS2	2	8	11	72.73	10.9	16	45	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Intermediate	None	Straight	Pale tan	Ovoid	Firebrick	3
C54P7	EMS2	2	4	9	44.44	9.8	8	44	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Moderate	None	Straight	Pale tan	Ovoid	Brown	3
C60P6	EMS2	2	8	13	61.54	14.8	16	62	Intermediate green	Hastate	Present	Indeterminate	Violet	Acute erect	Intermediate	None	Straight	Pale tan	Rhomboid	Firebrick	3
C60P7	EMS2	1	8	8	100.00	13.6	8	51	Intermediate green	Hastate	Present	Indeterminate	Violet	Acute erect	Intermediate	None	Straight	Pale tan	Rhomboid	Firebrick	3

Appendix 25 cont'd

C61P11	EMS2	1	10	17	58.82	16.9	10	47	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Moderate	None	Straight	Pale tan	Ovoid
C61P7	EMS2	2	3	11	27.27	13.3	6	52	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Slightly curved	Pale tan	Rhomboid
D20P8	EMS2	24	5	8	62.50	12.2	120	42	Darkgreen	Hastate	Present	Indeterminate	Violet	Climbing	Very slight	Slight	Straight	Pale tan	Rhomboid
D21P7	EMS2	4	4	14	28.57	15.4	16	40	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Very slight	None	Straight	Pale tan	Ovoid
D22P10	EMS2	9	8	18	44.44	17.5	72	46	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Intermediate	Slightly curved	Pale tan	Ovoid
D22P11	EMS2	8	5	12	41.67	17.2	40	40	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Intermediate	Straight	Pale tan	Kidney
D22P15	EMS2	5	5	11	45.45	14.5	25	46	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Intermediate	Slightly curved	Pale tan	Rhomboid
D22P8	EMS2	6	7	10	70.00	14.8	42	46	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Intermediate	Slightly curved	Pale tan	Ovoid
D24P4	EMS2	2	0	5	0.00	10.7	0	46	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Very slight	None	Straight	Pale tan	Rhomboid
D25P10	EMS2	2	4	10	40.00	11.2	8	41	Darkgreen	Hastate	Present	Indeterminate	Violet	Prostrate	Very slight	Slight	Straight	Pale tan	Ovoid
D32P1	EMS2	2	0	13	0.00	14	0	41	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	None	Straight	Pale tan	Rhomboid
D32P2	EMS2	2	2	9	22.22	10.1	4	39	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	None	Straight	Pale tan	Ovoid
D39P12	EMS2	1	8	12	66.67	14.7	8	42	Intermediate green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Slightly curved	Pale tan	Rhomboid
D39P5	EMS2	9	11	13	84.62	14.6	99	46	Intermediate green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	None	Slightly curved	Pale tan	Rhomboid
D43P12	EMS2	4	5	11	45.45	11.9	20	44	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	None	Slightly curved	Pale tan	Rhomboid
D43P4	EMS2	13	10	14	71.43	15.4	130	61	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	None	Straight	Pale tan	Ovoid
D44P18	EMS2	3	3	14	21.43	14.3	9	43	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	None	Straight	Pale tan	Rhomboid
D44P3	EMS2	19	4	15	26.67	16.9	76	45	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Very slight	None	Straight	Pale tan	Ovoid
D46P6	EMS2	2	4	10	40.00	12.3	8	44	Darkgreen	Hastate	Present	Indeterminate	Violet	Erect	None	None	Straight	Pale tan	Rhomboid
D50P14	EMS2	2	7	14	50.00	15	14	48	Intermediate green	Hastate	Present	Indeterminate	Violet	Acute erect	Very slight	None	Slightly curved	Pale tan	Ovoid
D54P10	EMS2	16	4	8	50.00	10.7	64	55	Intermediate green	Sub-hastate	Present	Indeterminate	Violet	Erect	Very slight	None	Slightly curved	Pale tan	Ovoid
D60P5	EMS2	3	1	15	6.67	15.5	3	48	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	None	None	Straight	Pale tan	Rhomboid
D60P6	EMS2	3	1	16	6.25	15.8	3	46	Darkgreen	Hastate	Present	Indeterminate	Violet	Erect	Very slight	None	Straight	Pale tan	Ovoid
D61P6	EMS2	7	2	15	13.33	16.1	14	48	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Intermediate	Straight	Pale tan	Ovoid
D62P1	EMS2	2	3	9	33.33	8.8	6	59	Darkgreen	Sub-hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Slightly curved	Pale tan	Ovoid
E23P2	EMS2	3	4	9	44.44	13	12	59	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	Slight	Slightly curved	Pale tan	Rhomboid
E24P15	EMS2	7	7	12	58.33	13.8	49	59	Darkgreen	Hastate	Present	Indeterminate	Violet	Intermediate	Moderate	Pronounced	Straight	Pale tan	Rhomboid
E31P5	EMS2	1	0	10	0.00	11.1	0	48	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Very slight	None	Straight	Pale tan	Rhomboid
E40P4	EMS2	6	3	7	42.86	13	18	52	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Moderate	None	Slightly curved	Pale tan	Ovoid
E41P14	EMS2	3	5	8	62.50	11	15	60	Darkgreen	Hastate	Present	Indeterminate	Violet	Erect	None	None	Slightly curved	Pale tan	Ovoid

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E41P8	EMS2	12	7	15	46.67	16.2	84	52	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Moderate	None	Slightly curved	Pale tan	Ovoid	Brown	3
E42P13	EMS2	8	5	16	31.25	16.6	40	54	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-erect	Very slight	Slight	Straight	Pale tan	Rhomboid	Brown	3
E43P13	EMS2	4	0	6	0.00	13.4	0	45	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Slightly curved	Pale tan	Ovoid	Brown	3
E43P16	EMS2	10	2	13	15.38	15.1	20	44	Intermediate green	Hastate	Present	Indeterminate	Violet	Intermediate	Very slight	Slight	Straight	Pale tan	Ovoid	Brown	3
E43P2	EMS2	6	3	16	18.75	16.4	18	50	Intermediate green	Hastate	Present	Indeterminate	Violet	Intermediate	Very slight	Slight	Straight	Pale tan	Rhomboid	Brown	3
E43P8	EMS2	16	8	11	72.73	11.7	128	40	Intermediate green	Hastate	Present	Indeterminate	Violet	Intermediate	Moderate	Intermediate	Straight	Pale tan	Ovoid	Firebrick	3
E43P9	EMS2	20	6	14	42.86	16.1	120	48	Intermediate green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Straight	Pale tan	Rhomboid	Brown	3
E45P11	EMS2	4	6	8	75.00	13.6	24	41	Darkgreen	Sub-hastate	Present	Indeterminate	Violet	Semi-erect	Moderate	Slight	Straight	Pale tan	Rhomboid	Brown	3
E46P10	EMS2	10	0	10	0.00	13.5	0	48	Intermediate green	Hastate	Present	Indeterminate	Violet	Semi-erect	Very slight	None	Straight	Pale tan	Rhomboid	Firebrick	3
E48P10	EMS2	12	4	9	44.44	12.6	48	46	Darkgreen	Sub-hastate	Present	Indeterminate	Violet	Climbing	Moderate	Intermediate	Slightly curved	Pale tan	Rhomboid	Brown	3
E48P9	EMS2	4	2	8	25.00	12.4	8	48	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Moderate	None	Slightly curved	Pale tan	Ovoid	Brown	3
E49P10	EMS2	28	5	11	45.45	12.1	140	48	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-erect	Moderate	None	Straight	Pale tan	Rhomboid	Brown	3
E49P14	EMS2	16	4	11	36.36	12.1	64	49	Intermediate green	Hastate	Present	Indeterminate	Violet	Semi-erect	Moderate	None	Straight	Pale tan	Ovoid	Firebrick	3
E49P4	EMS2	8	4	12	33.33	14.7	32	45	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-erect	Moderate	None	Straight	Pale tan	Ovoid	Brown	3
E51P12	EMS2	2	6	9	66.67	11.8	12	46	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Very slight	None	Straight	Pale tan	Ovoid	Brown	3
E51P7	EMS2	3	5	10	50.00	11.8	15	45	Darkgreen	Hastate	Present	Indeterminate	Violet	Erect	None	None	Straight	Pale tan	Ovoid	Brown	3
E55P11	EMS2	2	0	12	0.00	15.3	0	46	Intermediate green	Hastate	Present	Indeterminate	Violet	Acute erect	Moderate	None	Slightly curved	Pale tan	Ovoid	Brown	3
E57P11	EMS2	7	1	12	8.33	15.8	7	55	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Moderate	None	Straight	Pale tan	Ovoid	Brown	3
E57P17	EMS2	4	0	11	0.00	13.8	0	49	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Very slight	None	Slightly curved	Pale tan	Rhomboid	Brown	3
E57P2	EMS2	3	2	9	22.22	17	6	44	Intermediate green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Slight	Straight	Pale tan	Rhomboid	Brown	3
E57P8	EMS2	3	1	9	11.11	16.5	3	41	Darkgreen	Hastate	Present	Indeterminate	Violet	Acute erect	Moderate	None	Straight	Pale tan	Rhomboid	Brown	3
E59P3	EMS2	4	9	15	60.00	15.3	36	49	Darkgreen	Hastate	Present	Indeterminate	Violet	Climbing	Very slight	Intermediate	Curved	Pale tan	Ovoid	Firebrick	3
E59P7	EMS2	5	8	18	44.44	17.3	40	48	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-erect	Moderate	Slight	Slightly curved	Pale tan	Ovoid	Firebrick	3
A46P14	EMS2	16	13	14	92.86	15.8	208	59	Pale green	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	Intermediate	Straight	Pale tan	Rhomboid	Firebrick	2
D62P4	EMS2	1	17	17	100.00	17.1	17	57	Darkgreen	Hastate	Present	Indeterminate	Violet	Semi-prostrate	Very slight	None	Straight	Pale tan	Rhomboid	Cream	2

Appendix 26: Contrast analysis of high yielding mutants versus the wild type.

Traits	Plants			df	F	P>F
Days to flowering	C37P8	vs	wild type	1	7.64	0.0071
	C48P5	vs	wild type	1	7.64	0.0071
	C35P16	vs	wild type	1	7.64	0.0071
	C38P7	vs	wild type	1	7.64	0.0071
	B26P4	vs	wild type	1	7.64	0.0071
	D38P6	vs	wild type	1	7.64	0.0071
	C39P6	vs	wild type	1	7.64	0.0071
	D22P14	vs	wild type	1	7.64	0.0071
	C22P6	vs	wild type	1	7.64	0.0071
	C9P2	vs	wild type	1	7.64	0.0071
	E34P8	vs	wild type	1	7.64	0.0071
	B45P16	vs	wild type	1	7.64	0.0071
	C42P3	vs	wild type	1	7.64	0.0071
	D36P4	vs	wild type	1	7.64	0.0071
	D32P10	vs	wild type	1	7.64	0.0071
	B26P14	vs	wild type	1	7.64	0.0071
	B48P14	vs	wild type	1	7.64	0.0071
	C45P4	vs	wild type	1	7.64	0.0071
	C36P11	vs	wild type	1	7.64	0.0071
	C41P4	vs	wild type	1	7.64	0.0071
	C48P3	vs	wild type	1	7.64	0.0071
	C37P12	vs	wild type	1	7.64	0.0071
	C3P11	vs	wild type	1	7.64	0.0071
	D21P16	vs	wild type	1	7.64	0.0071
	C46P8	vs	wild type	1	7.64	0.0071
	C40P8	vs	wild type	1	7.64	0.0071
	C45P11	vs	wild type	1	7.64	0.0071

Appendix 26 cont'd

B41P5	vs	wild type	1	7.64	0.0071
D37P2	vs	wild type	1	7.64	0.0071
C40P9	vs	wild type	1	7.64	0.0071
E26P2	vs	wild type	1	7.64	0.0071
C45P1	vs	wild type	1	7.64	0.0071
C21P9	vs	wild type	1	7.64	0.0071
D32P14	vs	wild type	1	7.64	0.0071
C42P8	vs	wild type	1	7.64	0.0071
E33P14	vs	wild type	1	7.64	0.0071
B50P9	vs	wild type	1	7.64	0.0071
D28P15	vs	wild type	1	7.64	0.0071
C33P4	vs	wild type	1	7.64	0.0071
B52P13	vs	wild type	1	7.64	0.0071
B44P9	vs	wild type	1	7.64	0.0071
C39P5	vs	wild type	1	7.64	0.0071
C19P5	vs	wild type	1	7.64	0.0071
B26P16	vs	wild type	1	7.64	0.0071
E31P9	vs	wild type	1	7.64	0.0071
C29P10	vs	wild type	1	7.64	0.0071
E33P15	vs	wild type	1	7.64	0.0071
D26P16	vs	wild type	1	7.64	0.0071
D36P3	vs	wild type	1	7.64	0.0071
B40P3	vs	wild type	1	7.64	0.0071
B55P15	vs	wild type	1	7.64	0.0071
E49P7	vs	wild type	1	7.64	0.0071
B44P14	vs	wild type	1	7.64	0.0071
E33P1	vs	wild type	1	7.64	0.0071
D41P2	vs	wild type	1	7.64	0.0071
B48P12	vs	wild type	1	7.64	0.0071
E34P1	vs	wild type	1	7.64	0.0071
E34P2	vs	wild type	1	7.64	0.0071

Appendix 26 cont'd

B48P10	vs	wild type	1	7.64	0.0071
D33P9	vs	wild type	1	7.64	0.0071
C45P14	vs	wild type	1	7.64	0.0071
C58P5	vs	wild type	1	7.64	0.0071
D38P13	vs	wild type	1	7.64	0.0071
E33P13	vs	wild type	1	7.64	0.0071
E23P9	vs	wild type	1	7.64	0.0071
E33P8	vs	wild type	1	7.64	0.0071
B44P12	vs	wild type	1	7.64	0.0071
E29P9	vs	wild type	1	7.64	0.0071
E43P3	vs	wild type	1	7.64	0.0071
D32P9	vs	wild type	1	7.64	0.0071
C46P6	vs	wild type	1	7.64	0.0071
B26P6	vs	wild type	1	7.64	0.0071
C29P8	vs	wild type	1	7.64	0.0071
C41P11	vs	wild type	1	7.64	0.0071
C29P2	vs	wild type	1	7.64	0.0071
E30P9	vs	wild type	1	7.64	0.0071
C39P8	vs	wild type	1	7.64	0.0071
D26P11	vs	wild type	1	7.64	0.0071
E23P6	vs	wild type	1	7.64	0.0071
D23P12	vs	wild type	1	7.64	0.0071
B43P7	vs	wild type	1	7.64	0.0071
C47P9	vs	wild type	1	7.64	0.0071
E34P6	vs	wild type	1	7.64	0.0071
B52P12	vs	wild type	1	7.64	0.0071
B40P4	vs	wild type	1	7.64	0.0071
C48P10	vs	wild type	1	7.64	0.0071
D20P2	vs	wild type	1	7.64	0.0071
B45P8	vs	wild type	1	7.64	0.0071
B60P12	vs	wild type	1	7.64	0.0071
C38P5	vs	wild type	1	7.64	0.0071
E34P9	vs	wild type	1	7.64	0.0071
C33P12	vs	wild type	1	7.64	0.0071
B45P9	vs	wild type	1	7.64	0.0071
E33P4	vs	wild type	1	7.64	0.0071
B44P13	vs	wild type	1	7.64	0.0071
C41P7	vs	wild type	1	7.64	0.0071
E35P16	vs	wild type	1	7.64	0.0071
E34P7	vs	wild type	1	7.64	0.0071

Appendix 26 cont'd

C48P6	vs	wild type	1	7.64	0.0071
D48P14	vs	wild type	1	7.64	0.0071
C27P6	vs	wild type	1	7.64	0.0071
B55P3	vs	wild type	1	7.64	0.0071
B57P18	vs	wild type	1	7.64	0.0071
D26P8	vs	wild type	1	7.64	0.0071
C34P16	vs	wild type	1	7.64	0.0071
E33P7	vs	wild type	1	7.64	0.0071
C41P2	vs	wild type	1	7.64	0.0071
C22P14	vs	wild type	1	7.64	0.0071
E29P14	vs	wild type	1	7.64	0.0071
B44P3	vs	wild type	1	7.64	0.0071
E35P3	vs	wild type	1	7.64	0.0071
D34P1	vs	wild type	1	7.64	0.0071
C33P2	vs	wild type	1	7.64	0.0071
C28P3	vs	wild type	1	7.64	0.0071
B41P17	vs	wild type	1	7.64	0.0071
E42P9	vs	wild type	1	7.64	0.0071
D20P4	vs	wild type	1	7.64	0.0071
B41P3	vs	wild type	1	7.64	0.0071
E28P14	vs	wild type	1	7.64	0.0071
C49P11	vs	wild type	1	7.64	0.0071
C57P11	vs	wild type	1	7.64	0.0071
C38P11	vs	wild type	1	7.64	0.0071
B41P10	vs	wild type	1	7.64	0.0071
C48P1	vs	wild type	1	7.64	0.0071
E33P3	vs	wild type	1	7.64	0.0071
C55P9	vs	wild type	1	7.64	0.0071
D45P4	vs	wild type	1	7.64	0.0071
E30P16	vs	wild type	1	7.64	0.0071
D47P4	vs	wild type	1	7.64	0.0071
D38P7	vs	wild type	1	7.64	0.0071
E33P5	vs	wild type	1	7.64	0.0071
D53P4	vs	wild type	1	7.64	0.0071
B41P6	vs	wild type	1	7.64	0.0071
D37P6	vs	wild type	1	7.64	0.0071
D34P6	vs	wild type	1	7.64	0.0071
C49P6	vs	wild type	1	7.64	0.0071
B44P6	vs	wild type	1	7.64	0.0071
C49P1	vs	wild type	1	7.64	0.0071

Appendix 26 cont'd

E38P4	vs	wild type	1	7.64	0.0071
E30P14	vs	wild type	1	7.64	0.0071
E35P5	vs	wild type	1	7.64	0.0071
C47P7	vs	wild type	1	7.64	0.0071
E33P2	vs	wild type	1	7.64	0.0071
B44P2	vs	wild type	1	7.64	0.0071
C9P1	vs	wild type	1	7.64	0.0071
E52P17	vs	wild type	1	7.64	0.0071
C58P1	vs	wild type	1	7.64	0.0071
E44P5	vs	wild type	1	7.64	0.0071
B48P11	vs	wild type	1	7.64	0.0071
C20P9	vs	wild type	1	7.64	0.0071
B57P11	vs	wild type	1	7.64	0.0071
B58P6	vs	wild type	1	7.64	0.0071
E24P18	vs	wild type	1	7.64	0.0071
C42P1	vs	wild type	1	7.64	0.0071
E49P5	vs	wild type	1	7.64	0.0071
E30P12	vs	wild type	1	7.64	0.0071
C32P7	vs	wild type	1	7.64	0.0071
E34P16	vs	wild type	1	7.64	0.0071
E33P11	vs	wild type	1	7.64	0.0071
C35P7	vs	wild type	1	7.64	0.0071
D26P7	vs	wild type	1	7.64	0.0071
E52P12	vs	wild type	1	7.64	0.0071
E36P16	vs	wild type	1	7.64	0.0071
D32P2	vs	wild type	1	7.64	0.0071
E36P7	vs	wild type	1	8.71	0.0042
D32P15	vs	wild type	1	8.71	0.0042
E34P10	vs	wild type	1	8.71	0.0042
D35P12	vs	wild type	1	8.71	0.0042
C36P14	vs	wild type	1	8.71	0.0042
C49P9	vs	wild type	1	8.71	0.0042
E33P9	vs	wild type	1	8.71	0.0042
D35P14	vs	wild type	1	8.71	0.0042
E34P11	vs	wild type	1	8.71	0.0042
C34P12	vs	wild type	1	8.71	0.0042
C47P15	vs	wild type	1	8.71	0.0042
E30P8	vs	wild type	1	8.71	0.0042
C41P1	vs	wild type	1	8.71	0.0042
E29P8	vs	wild type	1	8.71	0.0042
E35P13	vs	wild type	1	8.71	0.0042

Appendix 26 cont'd

D38P4	vs	wild type	1	8.71	0.0042
D38P8	vs	wild type	1	8.71	0.0042
C34P9	vs	wild type	1	8.71	0.0042
C36P12	vs	wild type	1	8.71	0.0042
E32P6	vs	wild type	1	8.71	0.0042
C34P14	vs	wild type	1	8.71	0.0042
C17P4	vs	wild type	1	8.71	0.0042
C44P9	vs	wild type	1	8.71	0.0042
C36P8	vs	wild type	1	8.71	0.0042
C25P9	vs	wild type	1	8.71	0.0042
D37P3	vs	wild type	1	8.71	0.0042
D32P17	vs	wild type	1	8.71	0.0042
C37P5	vs	wild type	1	8.71	0.0042
D31P15	vs	wild type	1	8.71	0.0042
C38P3	vs	wild type	1	8.71	0.0042
C21P7	vs	wild type	1	8.71	0.0042
C36P13	vs	wild type	1	8.71	0.0042
E35P14	vs	wild type	1	8.71	0.0042
D23P10	vs	wild type	1	8.71	0.0042
D32P18	vs	wild type	1	8.71	0.0042
C46P11	vs	wild type	1	8.71	0.0042
C20P2	vs	wild type	1	8.71	0.0042
E36P8	vs	wild type	1	8.71	0.0042
E30P7	vs	wild type	1	8.71	0.0042
D38P2	vs	wild type	1	8.71	0.0042
B41P18	vs	wild type	1	8.71	0.0042
E35P6	vs	wild type	1	8.71	0.0042
C23P3	vs	wild type	1	8.71	0.0042
D38P1	vs	wild type	1	8.71	0.0042
D32P19	vs	wild type	1	8.71	0.0042
D35P9	vs	wild type	1	8.71	0.0042
C37P13	vs	wild type	1	8.71	0.0042
D35P10	vs	wild type	1	8.71	0.0042
C19P15	vs	wild type	1	8.71	0.0042
C25P10	vs	wild type	1	8.71	0.0042
E28P1	vs	wild type	1	8.71	0.0042
B43P2	vs	wild type	1	8.71	0.0042
E38P1	vs	wild type	1	8.71	0.0042
D41P8	vs	wild type	1	8.71	0.0042
C47P6	vs	wild type	1	8.71	0.0042
E32P5	vs	wild type	1	8.71	0.0042

Appendix 26 cont'd

	E29P7	vs	wild type	1	8.71	0.0042
	D34P2	vs	wild type	1	8.71	0.0042
	B43P4	vs	wild type	1	8.71	0.0042
	E35P8	vs	wild type	1	8.71	0.0042
	C59P7	vs	wild type	1	8.71	0.0042
	E32P2	vs	wild type	1	8.71	0.0042
	C34P13	vs	wild type	1	8.71	0.0042
	E32P7	vs	wild type	1	8.71	0.0042
	D36P2	vs	wild type	1	8.71	0.0042
	D38P5	vs	wild type	1	8.71	0.0042
	C29P3	vs	wild type	1	8.71	0.0042
	E36P2	vs	wild type	1	8.71	0.0042
	C37P6	vs	wild type	1	8.71	0.0042
	D41P1	vs	wild type	1	8.71	0.0042
	C38P2	vs	wild type	1	8.71	0.0042
	D28P12	vs	wild type	1	8.71	0.0042
	C46P7	vs	wild type	1	8.71	0.0042
	D35P8	vs	wild type	1	8.71	0.0042
	E36P14	vs	wild type	1	8.71	0.0042
	E37P4	vs	wild type	1	8.71	0.0042
	D41P9	vs	wild type	1	8.71	0.0042
	B57P10	vs	wild type	1	8.71	0.0042
	C36P9	vs	wild type	1	8.71	0.0042
	E35P10	vs	wild type	1	8.71	0.0042
	D41P7	vs	wild type	1	8.71	0.0042
	E33P12	vs	wild type	1	8.71	0.0042
	E35P9	vs	wild type	1	8.71	0.0042
	C35P8	vs	wild type	1	8.71	0.0042
	E33P10	vs	wild type	1	8.71	0.0042
	C36P10	vs	wild type	1	8.71	0.0042
	C21P11	vs	wild type	1	8.71	0.0042
PL	C50P2	vs	wild type	1	7.12	0.0092
	D24P4	vs	wild type	1	7.76	0.0067
	D54P10	vs	wild type	1	7.76	0.0067
	C58P13	vs	wild type	1	9.12	0.0034
	D32P2	vs	wild type	1	9.84	0.0024
	C54P7	vs	wild type	1	10.97	0.0014
	C21P11	vs	wild type	1	12.58	0.0007
	C37P1	vs	wild type	1	13.86	0.0004
	D62P1	vs	wild type	1	15.2	0.0002
	A64P11	vs	wild type	1	15.65	0.0002
	E55P1	vs	wild type	1	17.56	0.0001

Appendix 26 cont'd

NOLP	C36P4	vs	wild type	1	10.37	0.0019
	C36P14	vs	wild type	1	10.37	0.0019
	C33P8	vs	wild type	1	10.37	0.0019
	D29P9	vs	wild type	1	10.37	0.0019
	E50P2	vs	wild type	1	10.37	0.0019
	A64P7	vs	wild type	1	10.37	0.0019
	D33P8	vs	wild type	1	10.37	0.0019
NOSP	E60P4	vs	wild type	1	7.6	0.0072
	D41P12	vs	wild type	1	7.6	0.0072
	B26P8	vs	wild type	1	7.6	0.0072
	C35P8	vs	wild type	1	7.6	0.0072
	C44P5	vs	wild type	1	7.6	0.0072
	C20P6	vs	wild type	1	7.6	0.0072
	C21P12	vs	wild type	1	7.6	0.0072
	C39P6	vs	wild type	1	7.6	0.0072
NSPP	A57P1	vs	wild type	1	7.08	0.0094
	A57P2	vs	wild type	1	7.08	0.0094
	C33P2	vs	wild type	1	7.08	0.0094
	C20P6	vs	wild type	1	7.46	0.0078
	C45P11	vs	wild type	1	7.46	0.0078
	D38P4	vs	wild type	1	7.46	0.0078
	E43P3	vs	wild type	1	7.46	0.0078
	A55P1	vs	wild type	1	7.71	0.0068
	C31P1	vs	wild type	1	7.71	0.0068
	C33P3	vs	wild type	1	7.71	0.0068
	D28P15	vs	wild type	1	7.71	0.0068
	D44P14	vs	wild type	1	7.91	0.0062
	C21P12	vs	wild type	1	8.72	0.0042
	C25P2	vs	wild type	1	8.72	0.0042
	B26P4	vs	wild type	1	8.85	0.0039
	C38P6	vs	wild type	1	8.85	0.0039
	D36P4	vs	wild type	1	8.85	0.0039
	D38P6	vs	wild type	1	8.85	0.0039
	D20P11	vs	wild type	1	8.92	0.0037
	D33P4	vs	wild type	1	8.92	0.0037
	E35P12	vs	wild type	1	8.92	0.0037
	C37P7	vs	wild type	1	10.22	0.0020
	C42P7	vs	wild type	1	10.22	0.0020
	D35P12	vs	wild type	1	10.22	0.0020
	C28P1	vs	wild type	1	10.83	0.0015
	D24P3	vs	wild type	1	11.13	0.0013
	D26P1	vs	wild type	1	11.13	0.0013
	D35P14	vs	wild type	1	11.37	0.0012

Appendix 26 cont'd

	D28P2	vs	wild type	1	11.61	0.0010
	D24P1	vs	wild type	1	12.09	0.0008
	D44P15	vs	wild type	1	12.09	0.0008
	E28P14	vs	wild type	1	12.09	0.0008
	D37P5	vs	wild type	1	12.41	0.0007
	C39P6	vs	wild type	1	13.08	0.0005
	C35P5	vs	wild type	1	13.08	0.0005
	C38P4	vs	wild type	1	13.08	0.0005
	C46P12	vs	wild type	1	13.08	0.0005
	E36P10	vs	wild type	1	13.25	0.0005
	C27P10	vs	wild type	1	13.59	0.0004
	C36P8	vs	wild type	1	14.2	0.0003
	D37P3	vs	wild type	1	14.91	0.0002
	E27P8	vs	wild type	1	17.05	0.0001
	C33P13	vs	wild type	1	17.34	0.0001
	C3P11	vs	wild type	1	19.85	0.0000
	C20P3	vs	wild type	1	20.8	0.0000
	B26P6	vs	wild type	1	24.69	0.0000
	C29P10	vs	wild type	1	26.58	0.0000
	E26P8	vs	wild type	1	28.04	0.0000
	C41P6	vs	wild type	1	28.66	0.0000
	C36P12	vs	wild type	1	29.54	0.0000
	C20P2	vs	wild type	1	35.37	0.0000
	E32P6	vs	wild type	1	39.07	0.0000
	C39P1	vs	wild type	1	46.7	0.0000
	D32P18	vs	wild type	1	46.7	0.0000
	E26P3	vs	wild type	1	47.66	0.0000
	C37P4	vs	wild type	1	48.63	0.0000
	C21P8	vs	wild type	1	57.45	0.0000
	C41P8	vs	wild type	1	64.92	0.0000
	C37P8	vs	wild type	1	78.53	0.0000
	C37P9	vs	wild type	1	82.29	0.0000
	C37P12	vs	wild type	1	83.14	0.0000
NOPPP	D35P14	vs	wild type	1	7.18	0.0090
	D36P1	vs	wild type	1	7.18	0.0090
	D36P15	vs	wild type	1	7.18	0.0090
	E25P5	vs	wild type	1	7.18	0.0090
	D33P1	vs	wild type	1	8.43	0.0048
	E28P9	vs	wild type	1	8.43	0.0048
	E32P10	vs	wild type	1	8.43	0.0048
	E32P11	vs	wild type	1	8.43	0.0048
	E33P1	vs	wild type	1	8.43	0.0048
	C28P1	vs	wild type	1	9.79	0.0025

Appendix 26 cont'd

C36P8	vs	wild type	1	9.79	0.0025
D44P14	vs	wild type	1	9.79	0.0025
D55P2	vs	wild type	1	9.79	0.0025
E33P6	vs	wild type	1	9.79	0.0025
C3P11	vs	wild type	1	11.25	0.0012
C41P11	vs	wild type	1	11.25	0.0012
D44P6	vs	wild type	1	11.25	0.0012
E36P11	vs	wild type	1	11.25	0.0012
A57P1	vs	wild type	1	12.8	0.0006
A57P2	vs	wild type	1	12.8	0.0006
B55P10	vs	wild type	1	12.8	0.0006
C33P13	vs	wild type	1	12.8	0.0006
C33P2	vs	wild type	1	12.8	0.0006
D36P13	vs	wild type	1	12.8	0.0006
D24P3	vs	wild type	1	14.46	0.0003
D26P1	vs	wild type	1	14.46	0.0003
C20P3	vs	wild type	1	16.22	0.0001
C25P3	vs	wild type	1	16.22	0.0001
E35P12	vs	wild type	1	16.22	0.0001
C27P10	vs	wild type	1	18.08	0.0001
D34P10	vs	wild type	1	18.08	0.0001
E26P8	vs	wild type	1	18.08	0.0001
D37P3	vs	wild type	1	20.04	0.0000
C29P10	vs	wild type	1	22.1	0.0000
D24P1	vs	wild type	1	22.1	0.0000
C41P6	vs	wild type	1	24.27	0.0000
E36P10	vs	wild type	1	24.27	0.0000
B26P6	vs	wild type	1	26.53	0.0000
C20P2	vs	wild type	1	31.36	0.0000
D37P5	vs	wild type	1	31.36	0.0000
C37P4	vs	wild type	1	36.6	0.0000
C36P12	vs	wild type	1	42.24	0.0000
D44P15	vs	wild type	1	42.24	0.0000
E27P8	vs	wild type	1	42.24	0.0000
E28P14	vs	wild type	1	42.24	0.0000
C25P2	vs	wild type	1	45.21	0.0000
C39P1	vs	wild type	1	54.73	0.0000
D32P18	vs	wild type	1	54.73	0.0000
C37P8	vs	wild type	1	65.15	0.0000
C21P8	vs	wild type	1	68.83	0.0000
C37P9	vs	wild type	1	68.83	0.0000
E32P6	vs	wild type	1	72.61	0.0000
C37P12	vs	wild type	1	84.55	0.0000
E26P3	vs	wild type	1	88.74	0.0000
C41P8	vs	wild type	1	97.4	0.0000

Appendix 27: Contrast analysis of low yielding mutants versus the wild type.

Traits				df	F	P>F
PL	C47P2	vs	wild type	1	8.37	0.0049
	C37P2	vs	wild type	1	7.7	0.0069
	C44P5	vs	wild type	1	7.38	0.0081
PSST	D32P1	vs	wild type	1	252.34	0.0000
	E55P11	vs	wild type	1	252.34	0.0000
	E57P17	vs	wild type	1	252.34	0.0000
	E31P5	vs	wild type	1	252.34	0.0000
	E46P10	vs	wild type	1	252.34	0.0000
	E43P13	vs	wild type	1	252.34	0.0000
	D24P4	vs	wild type	1	252.34	0.0000
	D60P6	vs	wild type	1	219.74	0.0000
	A61P9	vs	wild type	1	217.63	0.0000
	D60P5	vs	wild type	1	217.63	0.0000
	B58P13	vs	wild type	1	217.63	0.0000
	E57P11	vs	wild type	1	209.38	0.0000
	E57P8	vs	wild type	1	195.94	0.0000
	D61P6	vs	wild type	1	185.52	0.0000
	E43P16	vs	wild type	1	176.16	0.0000
	E43P2	vs	wild type	1	161.29	0.0000
	D44P18	vs	wild type	1	149.93	0.0000
	D32P2	vs	wild type	1	146.66	0.0000
	E57P2	vs	wild type	1	146.66	0.0000
	E48P9	vs	wild type	1	135.44	0.0000
	D44P3	vs	wild type	1	128.92	0.0000
	C61P7	vs	wild type	1	126.61	0.0000
	B63P6	vs	wild type	1	126.61	0.0000
	D21P7	vs	wild type	1	121.69	0.0000
	B64P7	vs	wild type	1	113.59	0.0000
	E42P13	vs	wild type	1	111.85	0.0000
	D44P2	vs	wild type	1	111.85	0.0000
	D38P7	vs	wild type	1	104.5	0.0000
	E49P4	vs	wild type	1	104.5	0.0000
	D62P1	vs	wild type	1	104.5	0.0000
	C25P12	vs	wild type	1	94.24	0.0000
	E49P14	vs	wild type	1	94.24	0.0000
	D25P10	vs	wild type	1	82.62	0.0000
	D46P6	vs	wild type	1	82.62	0.0000
	C20P13	vs	wild type	1	82.62	0.0000

Appendix 27 cont'd

D26P5	vs	wild type	1	79.02	0.0000
D22P11	vs	wild type	1	77.54	0.0000
E43P9	vs	wild type	1	74.02	0.0000
E35P9	vs	wild type	1	74.02	0.0000
E40P4	vs	wild type	1	74.02	0.0000
E59P7	vs	wild type	1	69.48	0.0000
D22P10	vs	wild type	1	69.48	0.0000
C54P7	vs	wild type	1	69.48	0.0000
E23P2	vs	wild type	1	69.48	0.0000
E48P10	vs	wild type	1	69.48	0.0000
D43P12	vs	wild type	1	66.64	0.0000
D22P15	vs	wild type	1	66.64	0.0000
E49P10	vs	wild type	1	66.64	0.0000
E41P8	vs	wild type	1	63.3	0.0000
D25P5	vs	wild type	1	62.25	0.0000
A64P8	vs	wild type	1	54.62	0.0000
A59P7	vs	wild type	1	54.62	0.0000
D50P14	vs	wild type	1	54.62	0.0000
E58P7	vs	wild type	1	54.62	0.0000
B58P5	vs	wild type	1	54.62	0.0000
E45P8	vs	wild type	1	54.62	0.0000
E46P1	vs	wild type	1	54.62	0.0000
E51P7	vs	wild type	1	54.62	0.0000
D54P10	vs	wild type	1	54.62	0.0000
A59P6	vs	wild type	1	47.49	0.0000
B52P10	vs	wild type	1	47.49	0.0000
C22P13	vs	wild type	1	46.58	0.0000
B54P7	vs	wild type	1	46.58	0.0000
E55P4	vs	wild type	1	46.58	0.0000
E36P9	vs	wild type	1	46.58	0.0000
E36P12	vs	wild type	1	46.58	0.0000
E53P12	vs	wild type	1	45.38	0.0000
E42P8	vs	wild type	1	45.38	0.0000
A64P11	vs	wild type	1	43.79	0.0000
E51P16	vs	wild type	1	43.79	0.0000
E24P12	vs	wild type	1	43.79	0.0000
E34P16	vs	wild type	1	43.79	0.0000
E37P3	vs	wild type	1	41.55	0.0000
D63P9	vs	wild type	1	40.05	0.0000
A48P12	vs	wild type	1	40.05	0.0000
D22P2	vs	wild type	1	40.05	0.0000

Appendix 27 cont'd

A48P13	vs	wild type	1	40.05	0.0000
E31P1	vs	wild type	1	40.05	0.0000
D61P1	vs	wild type	1	38.16	0.0000
E43P15	vs	wild type	1	38.16	0.0000
E51P13	vs	wild type	1	38.16	0.0000
E49P5	vs	wild type	1	38.16	0.0000
D28P7	vs	wild type	1	35.7	0.0000
E57P16	vs	wild type	1	35.7	0.0000
E24P15	vs	wild type	1	35.7	0.0000
C20P5	vs	wild type	1	35.7	0.0000
C61P11	vs	wild type	1	34.72	0.0000
E59P1	vs	wild type	1	34.72	0.0000
D21P17	vs	wild type	1	34.72	0.0000
D46P3	vs	wild type	1	34.72	0.0000
E39P2	vs	wild type	1	34.72	0.0000
A64P7	vs	wild type	1	32.39	0.0000
D33P8	vs	wild type	1	32.39	0.0000
C56P9	vs	wild type	1	32.39	0.0000
D37P6	vs	wild type	1	32.39	0.0000
E59P3	vs	wild type	1	32.39	0.0000
E45P2	vs	wild type	1	32.39	0.0000
E49P3	vs	wild type	1	32.39	0.0000
E53P14	vs	wild type	1	29.48	0.0000
C60P6	vs	wild type	1	29.48	0.0000
D1P6	vs	wild type	1	29.48	0.0000
C48P2	vs	wild type	1	29.48	0.0000
D30P8	vs	wild type	1	29.48	0.0000
E41P3	vs	wild type	1	27.74	0.0000
E43P14	vs	wild type	1	27.74	0.0000
C32P5	vs	wild type	1	27.74	0.0000
D31P14	vs	wild type	1	27.74	0.0000
D44P4	vs	wild type	1	27.74	0.0000
E49P15	vs	wild type	1	27.74	0.0000
D35P4	vs	wild type	1	27.74	0.0000
E41P14	vs	wild type	1	27.74	0.0000
D20P8	vs	wild type	1	27.74	0.0000
C64P3	vs	wild type	1	25.74	0.0000
E50P14	vs	wild type	1	25.74	0.0000
E30P12	vs	wild type	1	25.74	0.0000
B51P8	vs	wild type	1	24.63	0.0000

Appendix 27 cont'd

B64P12	vs	wild type	1	24.63	0.0000
E41P9	vs	wild type	1	24.63	0.0000
E56P5	vs	wild type	1	24.63	0.0000
E35P10	vs	wild type	1	24.63	0.0000
B41P9	vs	wild type	1	24.63	0.0000
E26P1	vs	wild type	1	24.63	0.0000
B47P6	vs	wild type	1	23.93	0.0000
D29P1	vs	wild type	1	23.93	0.0000
E24P14	vs	wild type	1	23.93	0.0000
E36P7	vs	wild type	1	23.93	0.0000
E59P5	vs	wild type	1	20.78	0.0000
A52P6	vs	wild type	1	20.78	0.0000
A63P10	vs	wild type	1	20.78	0.0000
A47P12	vs	wild type	1	20.78	0.0000
E49P12	vs	wild type	1	20.78	0.0000
E49P2	vs	wild type	1	20.78	0.0000
E42P4	vs	wild type	1	20.78	0.0000
D24P2	vs	wild type	1	20.78	0.0000
D39P12	vs	wild type	1	20.78	0.0000
E21P2	vs	wild type	1	20.78	0.0000
B64P9	vs	wild type	1	20.78	0.0000
E23P5	vs	wild type	1	20.78	0.0000
E41P10	vs	wild type	1	20.78	0.0000
C44P11	vs	wild type	1	20.78	0.0000
E33P2	vs	wild type	1	20.78	0.0000
E51P12	vs	wild type	1	20.78	0.0000
E28P15	vs	wild type	1	20.78	0.0000
E35P11	vs	wild type	1	18.16	0.0001
C31P12	vs	wild type	1	18.16	0.0001
E60P6	vs	wild type	1	17.68	0.0001
E47P1	vs	wild type	1	17.68	0.0001
D50P5	vs	wild type	1	17.68	0.0001
D26P3	vs	wild type	1	17.68	0.0001
A47P16	vs	wild type	1	17.68	0.0001
D41P3	vs	wild type	1	17.68	0.0001
E23P11	vs	wild type	1	17.68	0.0001
E21P5	vs	wild type	1	17	0.0001
D43P2	vs	wild type	1	17	0.0001
E28P5	vs	wild type	1	17	0.0001
E31P8	vs	wild type	1	17	0.0001
E41P7	vs	wild type	1	17	0.0001
E24P17	vs	wild type	1	17	0.0001
E21P1	vs	wild type	1	17	0.0001

Appendix 27 cont'd

E29P12	vs	wild type	1	17	0.0001
E33P6	vs	wild type	1	17	0.0001
D22P8	vs	wild type	1	15.94	0.0001
B47P3	vs	wild type	1	15.94	0.0001
E35P2	vs	wild type	1	15.94	0.0001
C57P1	vs	wild type	1	15.15	0.0002
A48P6	vs	wild type	1	15.15	0.0002
E23P12	vs	wild type	1	15.15	0.0002
E40P7	vs	wild type	1	15.15	0.0002
E30P2	vs	wild type	1	15.15	0.0002
C47P13	vs	wild type	1	15.15	0.0002
E36P13	vs	wild type	1	15.15	0.0002
E27P10	vs	wild type	1	15.15	0.0002
C25P7	vs	wild type	1	15.15	0.0002
E28P14	vs	wild type	1	15.15	0.0002
E48P6	vs	wild type	1	14.06	0.0003
B51P18	vs	wild type	1	14.06	0.0003
E42P1	vs	wild type	1	14.06	0.0003
B51P14	vs	wild type	1	14.06	0.0003
C51P4	vs	wild type	1	14.06	0.0003
C40P7	vs	wild type	1	14.06	0.0003
E37P10	vs	wild type	1	14.06	0.0003
E29P11	vs	wild type	1	14.06	0.0003
D43P4	vs	wild type	1	14.06	0.0003
E24P16	vs	wild type	1	14.06	0.0003
E45P7	vs	wild type	1	14.06	0.0003
E47P2	vs	wild type	1	14.06	0.0003
E30P14	vs	wild type	1	14.06	0.0003
D26P6	vs	wild type	1	14.06	0.0003
D31P9	vs	wild type	1	14.06	0.0003
E25P11	vs	wild type	1	14.06	0.0003
E43P5	vs	wild type	1	14.06	0.0003
A63P11	vs	wild type	1	13.07	0.0005
B51P4	vs	wild type	1	13.07	0.0005
B62P6	vs	wild type	1	13.07	0.0005
A47P1	vs	wild type	1	13.07	0.0005
E36P8	vs	wild type	1	13.07	0.0005
C50P2	vs	wild type	1	12.45	0.0007
C56P3	vs	wild type	1	12.45	0.0007
D21P1	vs	wild type	1	12.45	0.0007
E43P8	vs	wild type	1	12.45	0.0007

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C29P8	vs	wild type	1	12.45	0.0007
D50P10	vs	wild type	1	11.74	0.0010
E40P13	vs	wild type	1	11.74	0.0010
E43P10	vs	wild type	1	11.74	0.0010
E58P6	vs	wild type	1	11.74	0.0010
B61P5	vs	wild type	1	11.74	0.0010
D61P3	vs	wild type	1	11.74	0.0010
D21P9	vs	wild type	1	11.74	0.0010
C3P5	vs	wild type	1	11.74	0.0010
E41P2	vs	wild type	1	11.74	0.0010
B59P8	vs	wild type	1	11.74	0.0010
E25P1	vs	wild type	1	11.74	0.0010
E32P1	vs	wild type	1	11.74	0.0010
E25P6	vs	wild type	1	11.74	0.0010
E46P13	vs	wild type	1	11.74	0.0010
C21P3	vs	wild type	1	11.74	0.0010
C9P1	vs	wild type	1	11.74	0.0010
E41P13	vs	wild type	1	11.74	0.0010
E41P1	vs	wild type	1	11.74	0.0010
D57P2	vs	wild type	1	11.74	0.0010
E36P15	vs	wild type	1	11.74	0.0010
D34P10	vs	wild type	1	11.74	0.0010
E60P2	vs	wild type	1	11.34	0.0012
E30P3	vs	wild type	1	11.34	0.0012
C27P2	vs	wild type	1	11.34	0.0012
D29P9	vs	wild type	1	9.88	0.0024
E50P2	vs	wild type	1	9.88	0.0024
B64P8	vs	wild type	1	9.88	0.0024
C50P10	vs	wild type	1	9.88	0.0024
C59P9	vs	wild type	1	9.88	0.0024
E49P1	vs	wild type	1	9.88	0.0024
C10P10	vs	wild type	1	9.88	0.0024
D41P2	vs	wild type	1	9.88	0.0024
B45P13	vs	wild type	1	9.88	0.0024
C64P10	vs	wild type	1	9.88	0.0024
D24P19	vs	wild type	1	9.88	0.0024
E22P4	vs	wild type	1	9.88	0.0024
E24P11	vs	wild type	1	9.88	0.0024
C37P3	vs	wild type	1	9.88	0.0024
C18P10	vs	wild type	1	9.88	0.0024
C48P1	vs	wild type	1	9.88	0.0024
E47P11	vs	wild type	1	9.88	0.0024
E34P11	vs	wild type	1	9.88	0.0024
A47P3	vs	wild type	1	9.88	0.0024
C26P1	vs	wild type	1	9.88	0.0024

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E26P11	vs	wild type	1	9.88	0.0024
E30P16	vs	wild type	1	9.88	0.0024
C20P9	vs	wild type	1	9.88	0.0024
E48P4	vs	wild type	1	9.88	0.0024
E43P11	vs	wild type	1	9.88	0.0024
E45P11	vs	wild type	1	9.88	0.0024
D26P7	vs	wild type	1	9.88	0.0024
E36P16	vs	wild type	1	9.88	0.0024
D60P1	vs	wild type	1	8.37	0.0049
D35P5	vs	wild type	1	8.37	0.0049
D57P15	vs	wild type	1	8.37	0.0049
E59P6	vs	wild type	1	8.37	0.0049
C63P9	vs	wild type	1	8.37	0.0049
D31P8	vs	wild type	1	8.37	0.0049
E31P15	vs	wild type	1	8.37	0.0049
A47P14	vs	wild type	1	8.37	0.0049
A47P17	vs	wild type	1	8.37	0.0049
A49P7	vs	wild type	1	8.37	0.0049
C40P1	vs	wild type	1	8.37	0.0049
D20P7	vs	wild type	1	8.37	0.0049
D31P11	vs	wild type	1	8.37	0.0049
C29P3	vs	wild type	1	8.37	0.0049
C40P12	vs	wild type	1	8.37	0.0049
A48P17	vs	wild type	1	8.37	0.0049
C37P13	vs	wild type	1	8.37	0.0049
D33P10	vs	wild type	1	8.37	0.0049
C40P11	vs	wild type	1	8.37	0.0049
C41P11	vs	wild type	1	8.37	0.0049
E27P8	vs	wild type	1	8.37	0.0049
E53P16	vs	wild type	1	7.94	0.0061
E40P6	vs	wild type	1	7.94	0.0061
D23P1	vs	wild type	1	7.94	0.0061
B62P5	vs	wild type	1	7.94	0.0061
E24P18	vs	wild type	1	7.94	0.0061
E45P5	vs	wild type	1	7.94	0.0061
E30P17	vs	wild type	1	7.94	0.0061
E47P4	vs	wild type	1	7.94	0.0061
E36P3	vs	wild type	1	7.94	0.0061
D25P9	vs	wild type	1	7.94	0.0061
C29P13	vs	wild type	1	7.94	0.0061
C15P10	vs	wild type	1	7.13	0.0092

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	C26P11	vs	wild type	1	7.13	0.0092
	D23P13	vs	wild type	1	7.13	0.0092
	C25P13	vs	wild type	1	7.13	0.0092
	D43P13	vs	wild type	1	7.13	0.0092
	D51P1	vs	wild type	1	7.13	0.0092
	A47P8	vs	wild type	1	7.13	0.0092
	C58P13	vs	wild type	1	7.13	0.0092
	A51P3	vs	wild type	1	7.13	0.0092
	E41P5	vs	wild type	1	7.13	0.0092
	E37P2	vs	wild type	1	7.13	0.0092
NOLP	C36P4	vs	wild type	1	10.37	0.0019
	C36P14	vs	wild type	1	10.37	0.0019
	C33P8	vs	wild type	1	10.37	0.0019
	D29P9	vs	wild type	1	10.37	0.0019
	E50P2	vs	wild type	1	10.37	0.0019
	A64P7	vs	wild type	1	10.37	0.0019
	D33P8	vs	wild type	1	10.37	0.0019
NOSP	B64P5	vs	wild type	1	9.12	0.0034
	C22P13	vs	wild type	1	9.12	0.0034
	C58P12	vs	wild type	1	9.12	0.0034
	C60P7	vs	wild type	1	9.12	0.0034
	D39P12	vs	wild type	1	9.12	0.0034
	E53P14	vs	wild type	1	9.12	0.0034
	C50P2	vs	wild type	1	9.12	0.0034
	C56P3	vs	wild type	1	9.12	0.0034
	C60P6	vs	wild type	1	9.12	0.0034
	D1P6	vs	wild type	1	9.12	0.0034
	D61P1	vs	wild type	1	9.12	0.0034
	C48P2	vs	wild type	1	9.12	0.0034
	E21P2	vs	wild type	1	9.12	0.0034
	B54P7	vs	wild type	1	9.12	0.0034
	B64P9	vs	wild type	1	9.12	0.0034
	D22P12	vs	wild type	1	9.12	0.0034
	E59P7	vs	wild type	1	9.12	0.0034
	D30P8	vs	wild type	1	9.12	0.0034
	D61P5	vs	wild type	1	9.12	0.0034
	E33P11	vs	wild type	1	9.12	0.0034
	E55P4	vs	wild type	1	9.12	0.0034
	D22P10	vs	wild type	1	9.12	0.0034

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B54P5	vs	wild type	1	9.12	0.0034
E43P15	vs	wild type	1	9.12	0.0034
E23P5	vs	wild type	1	9.12	0.0034
E41P10	vs	wild type	1	9.12	0.0034
E56P9	vs	wild type	1	9.12	0.0034
E36P9	vs	wild type	1	9.12	0.0034
E51P13	vs	wild type	1	9.12	0.0034
C44P11	vs	wild type	1	9.12	0.0034
D21P1	vs	wild type	1	9.12	0.0034
D25P5	vs	wild type	1	9.12	0.0034
E49P5	vs	wild type	1	9.12	0.0034
E43P8	vs	wild type	1	9.12	0.0034
E36P12	vs	wild type	1	9.12	0.0034
E52P12	vs	wild type	1	9.12	0.0034
C29P8	vs	wild type	1	9.12	0.0034
E33P2	vs	wild type	1	9.12	0.0034
A59P7	vs	wild type	1	12.56	0.0007
C58P13	vs	wild type	1	12.56	0.0007
D28P7	vs	wild type	1	12.56	0.0007
D26P5	vs	wild type	1	12.56	0.0007
D50P14	vs	wild type	1	12.56	0.0007
E53P12	vs	wild type	1	12.56	0.0007
E42P8	vs	wild type	1	12.56	0.0007
E57P16	vs	wild type	1	12.56	0.0007
E58P7	vs	wild type	1	12.56	0.0007
C64P3	vs	wild type	1	12.56	0.0007
D22P8	vs	wild type	1	12.56	0.0007
A51P3	vs	wild type	1	12.56	0.0007
B47P3	vs	wild type	1	12.56	0.0007
E24P15	vs	wild type	1	12.56	0.0007
C20P5	vs	wild type	1	12.56	0.0007
E41P5	vs	wild type	1	12.56	0.0007
E31P4	vs	wild type	1	12.56	0.0007

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E35P2	vs	wild type	1	12.56	0.0007
E37P2	vs	wild type	1	12.56	0.0007
E41P8	vs	wild type	1	12.56	0.0007
E50P14	vs	wild type	1	12.56	0.0007
D44P17	vs	wild type	1	12.56	0.0007
E30P12	vs	wild type	1	12.56	0.0007
A64P11	vs	wild type	1	16.56	0.0001
E51P12	vs	wild type	1	16.56	0.0001
E51P16	vs	wild type	1	16.56	0.0001
B58P5	vs	wild type	1	16.56	0.0001
E45P11	vs	wild type	1	16.56	0.0001
E24P12	vs	wild type	1	16.56	0.0001
E45P8	vs	wild type	1	16.56	0.0001
D28P10	vs	wild type	1	16.56	0.0001
E46P1	vs	wild type	1	16.56	0.0001
D26P7	vs	wild type	1	16.56	0.0001
E28P15	vs	wild type	1	16.56	0.0001
E34P16	vs	wild type	1	16.56	0.0001
E36P16	vs	wild type	1	16.56	0.0001
E43P9	vs	wild type	1	16.56	0.0001
E35P9	vs	wild type	1	16.56	0.0001
E41P14	vs	wild type	1	21.11	0.0000
E51P7	vs	wild type	1	21.11	0.0000
D43P12	vs	wild type	1	21.11	0.0000
D22P15	vs	wild type	1	21.11	0.0000
D22P11	vs	wild type	1	21.11	0.0000
E42P13	vs	wild type	1	21.11	0.0000
B59P1	vs	wild type	1	21.11	0.0000
D44P2	vs	wild type	1	21.11	0.0000
D38P7	vs	wild type	1	21.11	0.0000
D20P8	vs	wild type	1	21.11	0.0000
E49P10	vs	wild type	1	21.11	0.0000
C21P11	vs	wild type	1	26.21	0.0000
B64P7	vs	wild type	1	26.21	0.0000
C54P7	vs	wild type	1	26.21	0.0000
D25P10	vs	wild type	1	26.21	0.0000
D46P6	vs	wild type	1	26.21	0.0000
E23P2	vs	wild type	1	26.21	0.0000
C25P12	vs	wild type	1	26.21	0.0000
D21P7	vs	wild type	1	26.21	0.0000
E49P4	vs	wild type	1	26.21	0.0000
E48P10	vs	wild type	1	26.21	0.0000
D54P10	vs	wild type	1	26.21	0.0000
E49P14	vs	wild type	1	26.21	0.0000
D44P3	vs	wild type	1	26.21	0.0000
C61P7	vs	wild type	1	31.86	0.0000

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D62P1	vs	wild type	1	31.86	0.0000
B63P6	vs	wild type	1	31.86	0.0000
D44P18	vs	wild type	1	31.86	0.0000
E40P4	vs	wild type	1	31.86	0.0000
E43P2	vs	wild type	1	31.86	0.0000
C20P13	vs	wild type	1	38.06	0.0000
D32P2	vs	wild type	1	38.06	0.0000
E57P2	vs	wild type	1	38.06	0.0000
E48P9	vs	wild type	1	38.06	0.0000
D61P6	vs	wild type	1	38.06	0.0000
E43P16	vs	wild type	1	38.06	0.0000
A61P9	vs	wild type	1	44.82	0.0000
D60P5	vs	wild type	1	44.82	0.0000
D60P6	vs	wild type	1	44.82	0.0000
E57P8	vs	wild type	1	44.82	0.0000
B58P13	vs	wild type	1	44.82	0.0000
E57P11	vs	wild type	1	44.82	0.0000
E31P5	vs	wild type	1	52.13	0.0000
D24P4	vs	wild type	1	52.13	0.0000
D32P1	vs	wild type	1	52.13	0.0000
E55P11	vs	wild type	1	52.13	0.0000
E43P13	vs	wild type	1	52.13	0.0000
E57P17	vs	wild type	1	52.13	0.0000
E46P10	vs	wild type	1	52.13	0.0000