EFFECT OF GAMMA IRRADIATION ON PROPAGATION AND CREATION OF VARIABILITY IN *Caesalpinia Pulcherrima* L AND *Canna Indica* L.

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DECLARATION

This thesis is the result of research conducted by EDWARD OWUSU at the Department of Nuclear Agriculture and Radiation Processing of the School of Nuclear And Allied Sciences, University of Ghana, under the supervision of PROFESSOR KENNETH ELLIS DANSO and DR. WILFRED ELEGBA.

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DEDICATION

I dedicate this thesis to my two sons Aseda and Ayeyi, and my lovely wife Selina for their love, support and prayers.

Also, to my parents Mr. Yaw Owusu Wiredu and late Mad. Comfort Bediako for their encouragement.

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2, 4-D	-	2, 4-Dichlorophenoxyacetic acid
ANOVA	-	Analysis of Variance
BAP	-	6-Benzylaminopurine
BNARI	-	Biotechnology and Nuclear Agriculture Research Institute
CRD	-	Completely Randomized Design
DAG	-	Days After Germination
DNA	-	Deoxyribonucleic Acid
EMS	-	Ethyl Methanesulphonate
FAO	-	Food and Agricultural Organization
GAEC	-	Ghana Atomic Energy Commission
HIV-RT	-	Human Immunodeficiency Reverse Transcriptase
IAEA	-	International Atomic Energy Agency
LD50	-	Lethal Dose
MS	-	Murashige and Skoog (1962) basal medium
NAA	-	Naphthalene Acetic Acid
RTC	-	Radiation Technology Centre
SAM	-	Shoot Apical Meristem

SPSS	-	Statistical Packages for Social Sciences
UNCTAD	-	United Nations Conference on Trade and Development
VPCs	-	Vegetatively Propagated Crops

ABSTRACT

A survey conducted in the Greater Accra and some parts of Eastern Region on the floriculture industry indicates that the industry is fast emerging offering employment for majority of the educated youth in Ghana. However, most of the ornamental plants propagated and marketed are imported with few local varieties with no genetic improvement. The survey further shows that the multiplication of both local and imported species are constrained by lack of planting materials, low viability of seeds and narrow genetic base. This study was therefore aimed at using gamma irradiation to improve propagation and creation of variability in *Caesalpinia pulcherrima* and *Canna indica* L. two wild ornamental plants to enhance their aesthetic values. Low dosage of gamma irradiation (100-300 Gy) enhanced germination and stimulated growth in *C. pulcherrima* while high doses (400-1000 Gy) was phytotoxic leading to significant reduction in percentage germination and growth. The lethal dose (LD_{50}) referring to the dose at which 50% of the irradiated propagules did not survive was determined to be 583.33 Gy and 645.39 Gy using germination. Additionally, gamma irradiation had significant influence on height, flower colour and size as well as spine development. At higher doses (400-600 Gy), dwarf plants with distinct morphometric traits were developed compared to the controls indicating creation of morphological variant. Germination in *Canna indica* was achieved by simple scarification on any side of the seed except the micropylar end to break seed coat induced dormancy. Higher frequency of germination (89%) was achieved when scarified seeds were irradiated and cultured *in vitro*. Post-flask survival was influenced by gamma irradiation as higher doses resulted in high survival rate and reduced days to 50% flowering to 117 days compared to the controls (139 days). The study concludes that gamma

irradiation resulted in higher frequency of germination in both *Caesalpina pulcherrima* and *Canna indica* by breaking dormancy and also created variation. The spineless shoot developed in *C. pulcheririma* and dwarf plants in both species can enhance its use as a cut flower in the floriculture industry. However, further investigations are needed to determine whether the variations created are genetical or epigenetic.

CHAPTER ONE

1.1 INTRODUCTION

All over the world, the flower industry is growing at an alarming rate due to both huge local and international demands. Currently, it is an international multibillion-dollar industry (Nemali, 2016), providing huge foreign exchange and job opportunities for the teeming youth in many countries. It is estimated that the increased use of flowers has created a global industry worth US $104 billion as at 2016 (Stratcomm Africa, 2017). Ingels (2010), observed that ornamental horticulture is a multifaceted industry, which offers challenging employment opportunities. Altmann *et al.*, (2016) forecasted that global demand will continue to increase especially in the major industrialized nations in Europe, Asia and America. However, Ghana is not maximizing the benefits in the industry despite favorable conditions in the country for growing of flowering plants (Donkor *et al*., 2017).

The increased demand for flowers can be attributed to the incorporation of flowers into human livelihoods. Global festivities such as Christmas, New Year's, Valentine's Day, and Mother's Day, as well as individual occasions such as marriages, birthdays, and funerals, all use flowers to communicate feelings to one another (Stratcomm Africa, 2017).

The packaging of ornamental plants for marketing takes place in various forms. These are cut flowers which represent the largest segment of the industry followed by potted plants, tree and nursery crops, flower bulbs, and other propagation materials (Jensen and Malter, 1995).

Besides their economic gains, ornamental plants are highly aesthetic and are thus propagated principally for their decorative purposes in gardens and landscape, homes and as avenue plants in major cities and towns. According to Fidler (2017), the world would be a duller place to live in without flowers.

Additionally, flowers are beneficial to both people and animals, offering natural medicines and nourishment as well as creating raw materials for widely eaten meals such as honey produced by bees. The importance of flowers is also noticed in the role they play in the ecosystem. They are a source of food for animals particularly insects and thus aid in a plant's reproduction by enticing pollinators (Fidler, 2017).

In spite of the huge economic importance of flowers, their propagation is constrained by several biotic and abiotic factors, which include water, temperature and light. Other limiting factors such as seed dormancy, pests and diseases are also a major challenge in the cultivation of flowers. Furthermore, since the flower industry is now emerging in Ghana, there is lack of planting materials for large-scale propagation and this coupled with inadequate knowledge on the propagation of some of the species is slowing down the pace of development of the industry in the country. Some of the flowering species in Ghana could also be described as orphans as they grow in the wild and have never seen improvement through research, hence there are no routine protocols for their propagation. Currently, the floriculture industry in Ghana propagates and markets several species of flowers of both foreign and local origin. These include *Heliconia psittacorum*, *Polyalthia longifolia, Celosia argentea, Curcuma longa, Gladiolus palustris, Hibiscus rosa-sinensis, Croton petra, Millettia thonningii, Arecaceae, Ixora coccinea, Euphorbia milii, Allamanda cathartica, Melampodium divaricatum, Thuja orientalis, Lantana camara, Tombeja,* *Ipomoea sp., Araucaria columnaris, Adenium obesum, Ficus benjamina (*Nero *et al.,* 2018). However, this study focuses on the effect of gamma irradiation on propagation and aesthetic values of *Caesalpinia pulcherrima* and *Cana indica* two economically important flowers that grow wild in Ghana.

1.2 *Caesalpinia pulcherrima L.*

The economic importance of *Caesalpinia pulcherrima* is enormous and varied. It is a multi-purpose plant, which grows wild but very useful for local food and medicinal use. It is commonly used in afforestation and as living fence because of its small size and inflorescence diversity (Ferro *et al.*, 2019). In Ghana, it is commonly grown in public places and as avenue plant for decoration along streets and pathways. In addition to its aesthetic value, *C. pulcherrima* is used for the treatment of various ailments such as skin diseases and wounds, gonorrhoea, sleeping sickness and constipation (Opoku, *et al.*, 2018). According to Zanin *et al.,* (2012), *C. pulcherrima* is used to treat ulcers, asthma, fever, skin disorders and tumors. Fern (2019), also reported that *C. pulcherrima* is a remedy for colds and fevers and can also be a strong abortifacient. An infusion of *C. pulcherrima* is used to relieve constipation, treatment for kidney stones and to accelerate delivery (Zofou and Titanji, 2013). Leaves of the yellow-flowered morphotypes are used to alleviate stomach pains, while blooms of the red-flowered morphotypes are also used to treat urinary tract disorders (DeFilipps *et al.*, 2019). Besides, *C. pulcherrima* is a leguminous plant, and thus has a symbiotic relationship with certain soil bacteria, which enable it to fix atmospheric nitrogen into the soil (Huxley, 1992).

A study by Randall (2012) shows that *C. pulcherrima* has escaped from cultivation and behaves as weed. This characteristic feature causes it to invade places where it is not wanted hence it is considered as weed. Consequently, there is very little research on the species to improve on its aesthetic qualities. Hitherto, most available literature or research on this plant has focused mainly on its phytochemical and pharmacogenetic properties.

The lack of interest in *C. pulcherrima* may also be due to several other factors. Firstly, shoots of *C. pulcherrima* plant have thorns, which makes it suitable for use as hedges or fence (Bruno, 2019) but not as a cut flower because the thorny stems pose danger to humans especially children and animals. Consequently, the plant does not attract the interest of floriculturists, especially as a cut flower. Secondly, *C. pulcherrima* and many members of the genus are semi-deciduous and therefore shed their leaves often during adverse weather conditions making the surroundings unpleasant. Thirdly, *C. pulcherrima* is conventionally propagated through seeds, which are not often reliable because of low germination rate and poor seed viability (Nusrat, 2014). According to Ferro *et al.*, (2019) species from the Fabaceae family are known to present integumentary dormancy and this may account for the large disparity in germination of *C. pulcherrima*. Opoku *et al*. (2018) have reported that the seeds of members of the Fabaceae generally have hard seed coats, which hinder imbibition during germination. Furthermore, the seeds can be of different sizes, depending on their position in the pod, thereby influencing their germination (Rocha *et al.*, 2017). Thus, there is the need to develop protocols to overcome the low germination rate to enhance the improvement of the species for the floriculture industry.

1.3 *Canna indica* L.

Canna indica, unlike *C. pulcherrima*, is a member of the Musaceae family and is widely employed as an ornamental in garden and square compositions owing to its aesthetic merits (Gomes *et al.*, 2016). It is a significant plant not just for its aesthetic value, but also for starch production and therapeutic purposes (Tabbicca *et al.*, 2018). According to a recent study by Woradulayapinij *et al.*, (2005), proteins in the aqueous extract of *C. indica* (93% at 200 μg/mL) fresh rhizome have a powerful capacity to inhibit human immunodeficiency reverse transcriptase (HIV-RT) virus *in vitro* and therefore might be utilized for HIV therapy. They also stated that several components of *C. indica* were employed in traditional medicine as a diaphoretic and diuretic in fevers and dropsies, as a demulcent to induce menstruation, cure suppuration and rheumatism, and to recover vitality (Wafa *et al.*, 2016). A decoction of the root combined with fermented rice is used to cure gonorrhoea and amenorrhoea (Magee *et al.*, 2017). Aside from its therapeutic properties, *C. indica* rhizomes, which are high in starch, are consumed as boiled rhizomes and noodles, as well as in the production of alcoholic drinks and flour in South East Asia and Southern China (Tanaka 2004).

C. indica's therapeutic benefits may be linked to the presence of numerous bioactive flavonoids, a class of polyphenolic secondary metabolites found in the plant (Tinoi *et al.*, 2006), and thus commonly consumed in diets. Flavonoids have been shown to inhibit a wide range of oxidation enzymes, including 5–lipoxygenase, cyclooxygenase, monooxygenase, and xanthine oxidase (Mahajan *et al.*, 2008). Some members of the family have been discovered to exhibit anti-ischemic, anti-platelet, anti-inflammatory, and anti-lipoperoxidant properties, and all of these biological properties may be connected to their antioxidative actions. *C. indica* leaf extracts demonstrated significant promise as a botanical molluscicide and may thus be used to control snails and other similar pests in gardens (Mahajan *et al.*, 2008).

Although, *Canna indica* is a seed-bearing plant, sexual propagation is constrained by hard seed coat, which imposes dormancy in the seed. Even though crosses to achieve heterozygosity are possible, the inability of the resultant seeds to germinate readily due to dormancy makes breeding very difficult. Consequently, propagation of *C. indica* has evolved from sexual propagation to asexual propagation via rhizomes.

According to Barbosa *et al.*, (2005), propagation of *C. indica* can be achieved through the division of its rhizome which has buds for regeneration (Venugopal *et al.*, 2009). Propagation by splitting of the rhizome, which occurs throughout the year, is however slow and susceptible to viral transmission from one generation to the other since it is vegetatively propagated. To overcome this limitation, modern micropropagation techniques can be employed. Micropropagation technique does not only free regenerants from virus but can also be employed for rapid multiplication of the plant (Mishra *et al.*, 2015).

Another major hindrance to both propagation and genetic improvement of *C. indica* is its asexual mode of propagation. Sexual propagation enhances genetic improvement through hybrids as well as the production of large number of seedlings without damaging the plant matrix (Barbosa *et al.*, 2005). However, seed propagation in *C. indica* is hindered by impervious seed coat, which imposes physical dormancy. For large scale multiplication and genetic improvement of the plant, this seed coat dormancy should be overcome by pre-sowing treatments such as scarification and soaking of seeds in hot water (Venugopal et *al.*, 2009). Although sexual propagation in *Canna* offers huge advantage as the hard coat prevents insects, fungi or viruses from destruction of the seed (Verchot and Webb, 2017), it does not allow for genetic improvement of the plant. Thus, successfully overcoming the seed coat induce dormancy through scarification is highly recommended.

In spite of the huge economic potential of these two ornamental plants, their propagation by flower growers has reduced significantly in recent years. The major reason for their limited commercial exploitation is the limited variability and diversity in colour of petals and morphometric features which attracts customers. In the flower industry, buyers prefer to pay more for beautiful petals because of its aesthetic value. Flower features, particularly colour and size, are utilized to assess the commercial worth of ornamental species (Rodrigues *et al.,* 2012). However, upon closer study, the form of the leaves, the length of plants, and the colour of their flowers may also be important factors influencing customer desire (Tabbicca *et al.*, 2018). The low genetic diversity of *C. pulcherrima* and *C. indica* can be enhanced by using irradiation to increase genetic variability.

Globally, induced mutation using gamma rays, X-rays or other mutagens has contributed significantly to the improvement of food, ornamentals and tree crops. The International Atomic Energy Agency (IAEA) has documented over 3,200 mutant varieties out of which over 720 are ornamentals (Yamaguchi, 2018). According to Ahloowalia *et al.,* (2004), the primary aim in mutation-based breeding has been to improve well-adapted plant types by modifying one or two main characteristics. Plant height, bloom colour, maturity, breaking of seed dormancy, and disease resistance are all features that lead to enhanced production and quality. Furthermore, low doses of gamma irradiation can overcome seed coat-induced dormancy (Yildiz *et al.,* 2017).

In creation of genetic variability among plants using irradiation, different mutagens are used. These include gamma rays, X-rays and ion beam. Chemical mutagens can also be used to achieve the same effects, but their use is limited due to their highly carcinogenic nature. Of all the reported mutants developed, gamma and X-rays are the most frequently used (Patil and Patil, 2009).

Patil and Patil (2009) have observed that improvement in flowering plants is very important to the development of new morphotypes, new petal colours and their large-scale production to generate the interest of stakeholders in the industry. New varieties developed through sexual breeding provide thrilling results but the process is time consuming while irradiation to create variability could take much less time.

A recent study by Mohammad *et al.,* (2019) has shown that irradiation of *Chrysanthemum* at 25 Gy resulted in purple cultivar with a mutation rate of 54.56%. Also, in the pink cultivar, the highest number of coloured flowers was observed with a change of 32.11 % in the 25 Gy treatments. Based on the results of their study, four mutants of *Chrysanthemum* were introduced to the Iranian flower industry as new cultivars. In spite of the effectiveness of irradiation to produce genetic variability in several ornamental plants, its application to improve the aesthetic values of both *C. pulcherrima* and *C. indica* is not yet exploited. Thus, the effect of gamma irradiation on both propagation and aesthetic values of *C. pulcherrima* and *C. indica* is worth investigating.

1.4 Main objectives

The overarching objective of this research is to study the effect of gamma irradiation on *Caesalpinia pulcherrima* and *Canna indica,* two wild ornamental plants, to enhance their aesthetic value.

1.5 Specific objectives

The specific objectives are to:

a. to determine the economic importance of the flower industry as well as propagation challenges using field survey in greater Accra.
b. improve germination in *Canna indica* species using mechanical scarification
c. improve germination of C. *indica* using *in vitro* technique
d. study the effect of gamma irradiation on germination and morphometric variation in *C. pulcherrima* and *C. indica* to enhance its aesthetic value

CHAPTER TWO

LITERATURE REVIEW

2.1 Economic Importance of the Floriculture Industry

Globally, the floriculture industry produces and markets a wide variety of wild and improved plant species with aesthetic values creating job opportunities in many countries (Uffelen and Degroot, 2005; Donkor *et al.*, 2017). It is a multibillion-dollar industry in the United States with businesses ranging from small neighbourhood flower shops to corporations engaging in international trade (Ingels, 2010). A study by Altmann *et al.*, (2016) shows that the strong global concentration of demand for flowers and ornamental plants are from major industrialized nations in Europe, Asia and America.

The Netherlands is the world's largest exporter of cut flowers and plants, accounting for 52 percent of the worldwide industry and earning USD 3.2 billion in sales annually. According to a 2008 report by the United Nations Conference on Trade and Development (UNCTAD), the floriculture sector is stimulating economic growth and development in African nations such as Kenya, Ethiopia, Egypt, South Africa, Uganda, and Tanzania (United Nations, 2008). The majority of these African nations gain significant foreign exchange from the European market through the sale of horticulture products. For example, in Africa, Kenya is the highest exporter of cut flowers and plants to Europe. According to World's Top Exports, the country's export in 2017 of flowers represented 10.4% of total exports making it the second most exported product from Kenya (Johnson, 2019). In 2017, the country earned $595.6 million from flowers exported to Europe, the United States and some parts of Africa employing more than 500,000 residents (Khan, 2018).

2.2 The Flower Industry in Ghana

The floriculture industry in Ghana has the potential to create long-term job opportunities that would help to reduce the country's high youth unemployment rate, particularly among university graduates (Frimpong, 2019). It is estimated that Ghana exports over 766,090 kg of flowers earning about US$2,326,368 annually (Stratcomm-Africa, 2017). Comparing Ghana's floriculture sector to Kenya and other African nations, clearly shows that the country lags far behind, despite favourable environmental conditions for producing floricultural products. It is estimated that the flower industry has the potential to generate US$ 120 million revenue (Donkor *et al.,* 2017) to support the country's export earnings. A recent report by Frimpong (2019), shows that the flower industry in Ghana is currently under-developed and this may be attributed to very little research and development in the sector, lack of adequate skills and knowledge in the production and propagation of flowers as well as low awareness of the value of the industry. Donkor *et al.* (2017) have reported that Ghanaians are generally not flower-loving people; however, this perception is gradually changing.

2.3 Cultivation of ornamental plants in Ghana

Ghana has not just a great tradition and culture, but it has a great diversity of flowering plants. The country's excellent climate, geography, and other natural factors make it a suitable location for the growth of both indigenous and exotic flower breeds. The annual rainfall in Ghana is approximately 1,000-1,400 mm, with two rainy seasons happening from April to July and September to November, and the temperature is typically between

21 °C and 35 °C (Logah *et al.*, 2013); such climatic circumstances promote the production of flowers. Africa Business Insight (2010) have reported that plant species such as *Heliconia psittacorum*, *Celosia argentea*, *Curcuma longa*, *Gladiolus palustris and Hibiscus rosa-sinensis* have all performed well in Ghana under natural conditions and there is potential for the expansion of areas under cultivation for these and other cultivars which are yet to be introduced into the country. Nbangan (2019) has reported that Ghana is an ideal location for flower gardening because of its very good tropical weather, the amazing source of sunlight and good soils.

2.4 Classification of ornamental plants

Ornamental plants come in a variety of species and may be classified based on stem type, growth cycle, leaf form, usage, and other factors (Huylenbroeck, 2018). Cut flowers, decorative grasses, lawn or turf grasses, potted and indoor plants, bedding plants, trees and shrubs are some examples. Cut flowers are plant parts with an opened flower or buds that have been cut from the plant with the thorns removed and are ready to be used in fresh flower arrangements for decorating. Plants commonly used for cut flowers include *Rosa gallica*, *Dianthus caryophyllus*, *Chrysanthemums*, *Tulips*, *Lilium* and *Gerbera jamesonii*. Ornamental grasses used in the floriculture industry include the rushes, restios, and cat-tails. Lawn or turf grasses, which include perennial grasses or creeping legumes that are used to completely cover private lawns, golf courses and sporting fields are also considered as ornamental plants.

Potted and indoor plants are normally grown in residences and offices for decorative purposes. They also have positive psychological and health as well as environmental effects as they act as indoor air purification. The most common potted plants are *Adenium* *obesum*, *Cacti*, *Dracaena*, *Ficus*, *Poinsettia* and *Guzmania spp*. Bedding plants are grown, usually in pots or flats in greenhouses and are intended to be transplanted to a flower garden, hanging basket, window box or other outdoor planters. Some important bedding plants are impatiens, marigolds, and petunias.

Ornamental trees and shrubs are propagated for gardens and landscaping. Ornamental trees include cherry blossoms, cedar, mulberry and different palms. In the flower industry the most commonly propagated tree crops are *Ivy*, *Lavender*, *Magnolia*, *Hibiscus rosa-sinensis Caesalpinia pulcherrima* and *Ficus* species (Pocket., 2014). One of such ornamental tree crop grown in Ghana is *Caesalpinia pulcherrima.* It is grown in many homes in Ghana but they are generally collected from the wild. Considering their good aesthetic value, there is the need for them to be improved.

2.5 Nomenclature, classification and distribution *Caesalpinia pulcherrima* L.

Taxonomically, *Caesalpinia pulcherrima L.* belongs to the subfamily Caesalpinioideae of the family Fabaceae (Zanin *et al.*, 2012). It is known by several names across the globe as poinciana, peacock flower, red bird of paradise and pride of Barbados. It is native to the tropics and subtropics of the Americas. The exact origin of this plant is unknown due to its widespread cultivation, however, it is believed to be a native of the West Indies (Selvam, 2019). The genus consists of more than 500 species, which are mostly woody species occurring in tropical and subtropical zones. A well-known species of the genus, *C. pulcherrima* is a legume found in several countries of Central America, South America and India (Zanin *et al.*, 2012). In Ghana, it is commonly grown in public places as well as avenue trees for decoration along streets and pathways (Opoku *et al.*, 2018).

It is an evergreen plant in the tropics, especially in cooler climate but frost-free areas. Although, it is a deciduous plant, in areas with occasional frosts, it can survive as a perennial plant. However, they die in the cold season but resume growth during the warmer weather (Huxley, 1992). The deciduous nature of the species limits its usefulness as an ornamental plant. Thus, there is the need to improve it to become an economic ornamental plant.

The seeds of *C. pulcherrima* are dispersed by both mechanical and intentional introduction to other areas by human migration. However, as a legume the major mode of dispersal is by mechanical explosion, which propels the seeds away when they are matured and well dried (Puy *et al.*, 2002). This mode of dispersal limits its spread hence it has been intentionally dispersed by humans across tropical regions for both ornamental and agroforestry purposes. Its rapid spread across the globe may be attributed to its fast-growing nature. Furthermore, the plant is known to have escaped cultivation and sometimes naturalized in non-native habitats (Randall, 2012).

The plant thrives in a variety of soil types, including sand, clay, and loam, as well as acidic or alkaline soils. It is drought tolerant but not flood tolerant. It is also fairly resistant to aerosol salt, allowing it to be planted beside the shore. Although it may grow in partial shade, it needs full sunshine to blossom (Gilman and Watson, 2014).

2.5.1Morphology of *Caesalpinia pulcherrima* plant

Morphologically, *C. pulcherrima* is a perennial shrub or small tree with a woody trunk, which can grow to a height of 4 m (Figure 2.1A) bearing compound bipinnate leaves with 4-8 pairs of sessile pinnae which are about 6 to 12 centimeters long (Selvam, 2019). The pinnae are 7 to 11 pairs, which are oblong in shape, elliptic, and 1 to 2 centimeters long.

Tipping of the branches during the growing season creates a shrub producing more flowers. Consequently, the plant needs pruning to shape it to enhance its aesthetic value (Gilman and Watson, 2014). The shoots occasionally bear pairs of thorns at the node, which explains its usefulness in making hedge (Selvam, 2019). The plant produces flowers profusely throughout the year (Figure 2.0 C), mainly during wet and dry season, which produces legume-like fruits (Rodrigues *et al.*, 2012).

The flowers are borne on terminal, lax racemes, about 4 centimeters in diameter (Hua, 2016). Each flower has five sepals and five coloured petals, which are crisped and clawed with the colour varying from red, yellow or a mixture of red and yellow (mottled) which accounts for its aesthetic value. The fruit is a straight pod, which is bilaterally flattened with 6 to 8 seeds per pod. (Figure 2.1 B) (Godofredo, 2016).

Figure 2.1: *Caesalpinia pulcherrima* showing (A) structure of the plant (B) seeds in pod (C) yellow flower (D) red and yellow flower (mottled). (Bar indicates 3mm).

2.5.2 Economic importance

The existence and well-being of the human race is dependent on trees, ornamentals, and other plants (Bruno, 2019). Some ornamentals are culturally significant, while others are necessary to provide fundamental human necessities such as food, housing, clothes, and employment. They also serve an essential role in preserving the integrity of the ecosystem. *Caesalpinia sp*. is used for a variety of purposes across the world, including decorative and landscape features (Ferreira *et al.*, 2019). It is a multifunctional plant that is widely used in afforestation and as a living fence (Ferro *et al.*, 2019).

2.5.3 Medicinal use

According to Zanin *et al.*, (2012), *C. pulcherrima* contains a variety of therapeutic characteristics that may be used to treat a variety of disorders such as ulcers, asthma, fever, skin problems, and tumors. *C. pulcherrima* is used as indigenous medicine in Asian nations particularly, China and India to cure a variety of illnesses such as bronchitis, diabetes, and malaria (Moteriya and Chanda, 2016; Ferro *et al.*, 2019).

The tree is used for the treatment of various ailments including as skin diseases and wounds, gonorrhoea, sleeping sickness and constipation (Opoku *et al.*, 2018). It may also be used as a mouthwash for teeth or gums and a remedy for colds and fevers, or even as a strong abortifacient (Fern, 2019). For example, the seeds, flowers and roots are reported to be abortifacient (Zanin *et al.*, 2012). The leaves and flowers are used as purgative and emmenagogue Seeds, flowers and roots are reported to be abortifacient. According to Godofredo (2016), the bark is considered a powerful emmenagogue and abortifacient.

2.5.4 Phytochemistry and pharmacology

The medicinal value of *C. pulcherrima* may be attributed to its rich source of phytochemicals. Zanin *et al.*, (2012) reports that the genus *Caesalpinia* contains a virtually inexhaustible source of bioactive metabolites within the more than 500 species distributed worldwide. Several classes of phytochemicals have been isolated from plants of genus *Caesalpinia*, mainly flavonoids, diterpenes and steroids (Viji and Wilson, 2017) and this explains its medicinal properties. The bark of the stem contains saponins, flavonoids, phenols, terpenoids, tannins, and alkaloids. Two known compounds, pulcherrin J and 6-cinnamoyl-7-hydroxyvouacapen-5-ol were isolated from the HEEA fraction. Also, phytochemical screening of leaves revealed the presence of alkaloids, phytosterols, saponins, tannins, phenols, flavonoids, and lignins. A study of pulverized leaves of red and yellow varieties of *C. pulcherrima* showed 0.50 and 0.52% v/w of essential oils using hydro- distillation (Godofredo 2016).

Pharmacologically, species of this genus exhibit analgesic, adaptogenic, antiulcer, anticancer, antidiabetic, anti-inflammatory, antimicrobial, anthelmintic, antibacterial, insecticidal, antifungal, anti-inflammatory, antipyretic, antioxidant, antiproliferative, antiviral, immunomodulatory, and immunosuppressive activities. (Zanin *et al.*, 2012).

2.6 Propagation of *C. pulcherrima*

Seeds are important for propagation of plant rootstocks as well as hybrid development. However, most seeds do not germinate readily due to several factors. Germination of *C. pulcherrima* seeds is influenced by both external and internal factors, which may lead to physiological or physical dormancy. These factors include hard seed coat, undeveloped embryo or chemical inhibitors that induce seed dormancy (Agrawali and Dadlani, 1995;

Hartmann *et al.,* 2002; Opoku *et al.,* 2018). According to Opoku *et al.,* (2018), seed dormancy can be eliminated by different methods including soaking in water, scarification and application of gibberellin.

Sexual propagation of *C. pulcherrima* by seeds is not reliable because of the low germination rate and poor seed viability. Moreover, plants propagated by seeds, show high heterozygosity and variation in growth habit and yield which may negatively affect its aesthetic value (Selvam, 2019). Donkor *et al.*, (2017) reported that to enhance germination, floriculturists soak *C. pulcherrima* seeds in hot water at 65^{o}C for 10 minutes before sowing.

2.6.1 Germination and or emergence of *Caesalpinia pulcherrima* seeds

According to (Ferreira *et al.,* 2019), seedlings of *C. pulcherrima* emerge five days after sowing provided all conditions of germination are present. Rocha *et al.*, (2017), observed that seeds of the family Fabaceae can be of factor to germination (Taiz and Zeiger, 2017). In their study, Ferreira *et al.,* (2019), observed that percentage germination, mean germination time, as well as time for 50% germination, were influenced different sizes depending on their position in the pod, which subsequently influence their emergence. It has also been reported that the position of the seed in the fruit contribute significantly the variations in the content of the proteins reserve present in the seed which in turn influences germination percentage.

Seeds of *C. pulcherrima* show physical and physiological dormancy, according to Opoku *et al.,* (2018). The seed coat, which acts as a barrier to uniform and quick germination, may be responsible for the physical dormancy. As a result, more drastic pre-treatments are required to improve germination.

A study by Ferro *et al.*, (2019) suggests that during storage, the seeds of *C. pulcherrima* developed a possible secondary dormancy, which can be overcome after lengthy period of storage. They further reported that different light qualities and storage periods have significant effect on germination of *C. pulcherrima* seeds (Ferro *et al.,* 2019). For example, freshly harvested seeds exposed to far-red light had a germination percentage of 98%, higher than seeds with 12 months of storage which had 80.5% germination.

Nogueira *et al.,* (2010) have reported that in *Caesalpinia ferrea*, proximally positioned seeds in the pod showed better germination response compared to those at the medial and distal positions. Studying the effect of seed position on germination, Ferreira *et al.,* (2019) reported that seeds in position 4 (P4) in the pod germinated better than the remaining positions.

2.7 Nomenclature, Classification and Distribution of *Canna indica*

Canna indica, also known as Saka siri, Indian shot, Canna, Bandera, Chancle, Coyol or Platanillo, is a species of the genus Canna of the family Cannaceae (Mahajan *et al.*, 2008). It is a large perennial herb of tropical and subtropical regions (Venugopal *et al.,* 2009); However, it is a native of the Caribbean and tropical America (Mahajan *et al.*, 2008). It has been reported that hybridization of the species took place in France during the 1840's with a specific focus on foliage development, hence in England *Cannas* were treated as foliage plants (McIntyre, 2001). Various morphological, cytological and taxonomical characteristics of family Cannaceae show a close relation to other members of Zingiberales, which includes Musaceae, Strelitziaceae, Lowiaceae, Heliconiaceae, Zingiberaceae, Costaceae and Marantaceae. The genus comprises of about 51 species of flowering plants (Mishra *et al.*, 2015) and is commonly found in moist places along streams, springs, ditches, and the margins of woods. It may also be found in wet temperate, mountainous regions.

The Cannaceae family is commonly cultivated in flower gardens (Al-snafi, 2015), in both tropical and temperate regions where they produce some of the worlds' most beautiful and exotic blossoms (Mahajan, 2008).

2.7.1 Economic Importance

The economic importance of *Canna indica* is enormous and varied. It is an important plant propagated not only for its aesthetic value, but also for starch production as well as its medicinal values (Tabbicca, 2018).

A study by Woradulayapinij *et al.,* (2005) discovered that proteins in the water extract of *C. indica* fresh rhizome have the potential to inhibit human immunodeficiency reverse transcriptase (HIV-RT) virus *in vitro*. Furthermore, many parts of *C. indica* are used in traditional medicine as a diaphoretic and diuretic in fevers and dropsy, as a demulcent, to stimulate menstruation, treat suppuration, rheumatism, and to regain energy. Magee *et al.*, (2017) have also reported that a decoction of the root with fermented rice is used in the treatment of gonorrhoea and amenorrhoea.

In addition to its profound medicinal values, rhizomes of *C. indica*, which are rich in starch, have traditionally been consumed as boiled rhizome and noodles and it is used to make alcoholic beverages and flour in southeastern Asia and southern China (Tanaka 2004; Wafa *et al.*, 2016).

The biological activities of flavonoids in *C. indica* have been extensively examined, according to Mahajan *et al.*, (2008), and some of them have been discovered to have anti-ischemic, anti-platelet, anti-inflammatory, and anti-lipoperoxidant effects. Flavonoids have also been discovered to block a variety of oxidation enzymes, including 5–lipoxygenase, cyclooxygenase, monooxygenase, and xanthine oxidase. The biological activities of these compounds are linked to their antioxidative properties. The flower extract of *C. indica* showed abilities as a natural indicator in acid-base titration, and the leaf extracts of *C. indica* showed tremendous potential as botanical molluscicides.

2.7.2 Morphometric features of *Canna indica*

Canna indica is a tropical herb grown from rhizomes and seeds, with banana like leaves and multicoloured flowers Wafa *et al.*, (2016). The plants are perennial with erect, unbranched, leafy shoots (Figure 2.2). The leaves are large with narrowly ovate to elliptic which sheath the stems with varying colours ranging from green to a purple-bronze making it attractive; thus horticulturists have turned it into bright attractive, colourful garden plant.

The plant can reach a height of 3 meters bearing flowers with spathe, which are typically red, orange or yellow or a combination of these colours and are aggregated into inflorescences that are spikes or panicles (Figure 2.2). Despite their beautiful colours, the flowers are non-fragrant. Generally, the plant flowers from September to April and in some regions throughout the year.

The fruit of *C. indica* are generally green, spiny and three-halved capsules (McIntyre, 2001). At the developmental stages, the seeds are white in colour but at maturity are black with chestnut brown spots which are protected with a smooth coat (Figure 2.2C) (Al-snafi, 2015). *C. indica* has rhizomes which are yellowish white or pinkish on the outside and yellowish white within but at maturity turn brownish externally due to a thick outer covering. The roots are thick, cylindrical and creamy white in colour with a diameter of 2-5mm with numerous root hairs while the primary and secondary lateral roots are thin (Al-snafi, 2015).

Figure 2.1: *Canna indica* showing (A) yellow flower (B) red flower and (C) matured pod bearing black seeds. (Bar indicates 4mm).

2.7.3 Propagation of *Canna* species

Canna species are propagated conventionally by division through the rhizomes or by seeds thus, it has both sexual and asexual mode of propagation. Sexual propagation by seed is important in genetic improvement as it leads to the production of productive hybrids as well as large number of seedlings without damaging the plant matrix (Gomes *et al.*, 2016). Another advantage of sexual propagation in *Canna* is that its hard seed coat protects the heterozygous propagule against insects, fungi or viruses, thus it is a better alternative for producing healthy plants (Verchot and Webb, 2017).

However, seed propagation in Canna is limited by its impervious seed coat as they are hard, hence exhibiting physical dormancy. Venugopal *et al.*, (2009) have reported that pre-sowing scarification or soaking of seeds in hot water can be used to overcome seed coat dormancy during germination.

Although asexual vegetative mode propagation using rhizomes has become conventional for multiplication of *Canna sp*, it is associated with transfer of systemic plant diseases from one generation to the other. Thus, breeding in *Canna sp*. are aimed at developing hybrid varieties with viable seeds. Also, rhizomes are often infested by insects and fungi, which require pesticides to control. Consequently, plants grown from rhizomes need more care than those grown from seeds.

2.7.4 Seed Dormancy in *Canna species*

The seed coat of *Canna sp*. includes exotesta composed of palisade malpighian cell layers that offer mechanical strength (Mara, 2012). In addition to lipids, it contains silica beads, callose, and lignin in its top layers. These structures act as a barrier to water absorption, resulting in physical dormancy (Gomes *et al.*, 2016). It also inhibits gaseous flow in and out of the seed while also providing mechanical resistance to embryo protrusion during germination (Mensah & Ekeke, 2016).

Several authors have shown variation in the intensity of dormancy in *C. indica* species (Gilman and Watson, 20014; Oliveira *et al.*, 2010). Independent studies by Ferro *et al.*, (2019) showed that during storage, the seeds developed a possible secondary dormancy, which was overcome after twelve months of storage. They further suggested that one of the survival mechanisms used by some species is, seed dormancy, which delays germination for a long time until favourable climatic conditions occur. Besides dormancy, other determinants of seed germination are environmental factors which include temperature, light and substrate humidity (Ferro *et al.*, 2019). The breaking of seed dormancy in *C. indica* and *C. pulcherrima* will enhance the production of planting materials through increased germination percentage and reduction of germination time. Afshar *et al.,* (2014) has reported that dormant seeds of *Canna indica* showed an optimum germination of 95% after three- to four-hours pretreatment with sulphuric acid.

2.8 Irradiation and breaking of dormancy

Seed dormancy limits germination, making commercial production of ornamental plants, particularly Canna species, unprofitable. Telci *et al.* (2011) and Beyaz *et al.* (2018), for example, discovered that the low frequency of *in vitro* seed germination observed in *Lathyrus chrysanthus* is due to dormancy. To overcome this limitation, various methods such as scarification of the seed coat, temperature and light treatments, application of exogenous, growth regulators, and chemicals, and the use of exogenous, growth regulators, and chemicals have been widely used to break dormancy in seeds. It has been demonstrated that sodium hypochlorite solutions can be used successfully as a dormancy-breaking agent in *Lathyrus chrysanthus Boiss* seeds.

According to Gomes *et al.,* (2016), the germination of Canna seeds in nature is restricted by coat numbness, but when treated with acid, they can reach a high germination percentage. Salehi *et al.*, (2014), observed 95% germination after three to four hours of sulphuric acid scarification prior to sowing. They further reported that highest rate of germination occurred on the fifth day after sowing indicating the efficiency of sulphuric acid.

Similarly, pre-soaking of seeds in hot water has proved to be efficient dormancy breaking treatment. For example, Opoku *et al.,* (2018) observed that seeds of *C. indica* soaked in hot water at 65^{0}C for 10 minutes gave the highest germination percentage, leading to the highest production of leaves and roots per plant as well as the tallest plant.

Besides chemical pretreatment for breaking of dormancy, irradiation of seeds has also been used to break dormancy in buds and seeds (Beyaz *et al.*, 2016). According to Beyaz *et al.*, (2018) gamma irradiation of *Lathyrus chrysanthus* seeds prior to sowing increased percentage of germination of seeds, height of seedling, lengths of roots, fresh weight and dry matter content of seeds as well as total chlorophyll content in the leaves of seedlings. The study showed that the highest percentage (62.4%) seed germination was obtained when the seeds were irradiated at 150 Gy. According to the same study, irradiation of seeds at higher doses stressed the seeds resulting in significant decreases in all the parameters studied (Beyaz *et al.*, 2018). *Cajanus cajan*, when exposed to varying doses of gamma radiation, showed persistent changes in germination, growth, and development under both *in vivo* and *in vitro* conditions, according to Neelam *et al.,* (2014). Asif *et al.,* (2020) discovered that abrasion in *Prosopis juliflora* combined with irradiation improved seed germination in the plant.

2.9 Micropropagation of *Canna sp.*

Since Canna is a seed-bearing plant, its propagation using micropropagation may not be commercially viable. However, micropropagation may be required for its improvement via genetic engineering as well as to overcome seed dormancy in the plant. The term micropropagation refers to the growing of plant tissue or cell on a nutrient medium under sterile conditions *in vitro* often leading to regeneration of whole new plants. *In vitro* cultured cell usually retains its potentiality to grow and establish into a whole plantlet without alterations to the genome of the parent plant. Regeneration of an organism from a single cell or a group of cells makes tissue culture technique advantageous over other asexual mode of propagation (Mishra *et al.*, 2015).

In the last few decades, micropropagation or tissue culture coupled with molecular biotechnology techniques have become important tools for multiplication of large numbers of disease-free planting materials, breeding for diseases and pests resistant crop varieties and crop improvement. Elite and difficult to propagate crops have successfully been propagated rapidly on commercial scale through micropropagation techniques. The technique has also been used for short term conservation of endangered plant genetic resources (Sarasan, 2006).

Pocket (2014) reported that the ornamental industry has relied heavily on micropropagation for large-scale multiplication of elite superior varieties. He also stated that micropropagation is the preferred method for propagating ornamental crops because clonal propagation by this method of cloning is relatively faster and produces exact replicas of the mother plant.

A reliable micropropagation protocol, according to Wafa *et al.*, (2016), is a prerequisite for any genetic alteration and protoplast fusion for the enhancement of Canna varieties. *In vitro* culture methods for the selection of variant types in ornamental plants, particularly for flower colour, plant shape, and physiological features, have been described for many years, according to Hasbullah *et al.*, (2012).

Besides organogenesis for either direct or indirect plantlet production, callus culture can also be used for micropropagation under tissue culture conditions. Callus is defined as an unorganized and undifferentiated mass of parenchyma cells formed from isolated plant cells or tissues under aseptic conditions. Since meristematic cells are totipotent, they have the capacity for vigorous growth and division, and are thus used for the initiation of callus. In plant tissue culture, young and immature parts of plant such as leaf, stem, root, nodes and embryonic tissues are used for callus initiation. According to Ahmad *et al.,* (2010) callus culture offers tools for genetic transformation, induction of somaclonal variation for development of new varieties, induced mutagenesis and genetic engineering which are not much more rapid than conventional breeding.

In *Canna edulis*, embryogenic callus-like structure was obtained by supplementing 1.5 - 2 mg/l BA in Gamborg B5 medium and MS medium (Mishra *et al.*, 2015). In a study by Wafa *et al.,* (2016) complete plantlets were obtained from leaf explant of *C. indica* via indirect regeneration through *in vitro* callus and somatic embryogenesis. Furthermore, they reported that rhizome explants of *C. indica* produced high frequency of shoots (73.3%) and roots (86.7%) which resulted in complete plantlet regeneration on MS medium supplemented with 3.0 mg/L BAP plus 1.5 mg/L NAA two weeks after inoculation of culture. Raihana *et al.*, (2011) also observed that rhizome buds of *Curcuma manga* gave the best response of shoot formation in MS medium containing a high concentration of BAP (9.0 mg/L BAP). Wafa *et al.,* (2016) have reported that among the cytokinins used, BAP was found to be the most suitable in promoting cell division, shoot multiplication, and axillary bud formation, while inhibiting root development. It further suggests that the combinations of BAP plus NAA are substantially important in propagation of various ornamental species; at equal concentration, combination can yield callus production while at other concentrations it can result in direct regeneration and rhizogenesis.

Multiplication of plantlets through *in-vitro* culture will facilitate production of large number of disease-free planting materials for supply to flower growers. The ornamental industry has relied immensely on micropropagation, using it for large-scale plant multiplication of elite superior varieties (Pocket 2014).

2.10 Effect of Gamma Irradiation on *Caesalpinia pulcherrima* and *C. indica*

Since the discovery of X-rays, induced mutation induction has played highly significant role in plant breeding and improvement programmes contributing to food security (Ahloowalia *et al.*, 2004). Its role in the floriculture industry also cannot be over emphasized. According to Huylenbroeck, (2018), induced mutation technique is a valuable tool that has been exploited for ornamental breeding for the past three decades. Miri, (2018) has reported that mutation induction has been more successful in ornamental plants because changes in flower traits such as colour, size, morphology, fragrance, leaf characters (form, size, pigmentation), growth habit (compact, climbing, branching) as well as, physiological traits (photoperiodic reaction, early flowering, free flowering), and tolerance against biotic and abiotic stress factors are easily detected.

In addition, the technique is cheap and offers a rapid method of developing new varieties as compared to hybridization methods which take a long time to develop a new variety (Patil, 2009). In Hasbullah *et al.*, (2012) view, many ornamental species are suitable for mutation breeding, since flower colour and other mutations can be produced without altering other traits of the original ideotype. It is more effective for the improvement of oligogenic characters than polygenic traits (Patil and Patil, 2009).

Mutations occur in cells in two ways. Firstly, by alteration in nuclear deoxyribonucleic acid (DNA) (point mutation) causing addition, deletion, transition and transversion, which when inherited changes the organism's original traits. Secondly, mutagens cause changes in cytoplasmic DNA, phenomenon referred to as cytoplasmic mutation. Patil and Patil, (2009) has reported that cytoplasmic mutant 'male sterility' has been induced in some ornamental crops.

Both physical and chemical mutagens have been used successfully to artificially create the genetic variation for the development of mutant varieties (Andrea and Rownak 2018). Most commonly used chemical mutagens are alkylating agents and azides (Andrea and Rownak 2018) which include ethyl methane sulphonate (EMS), methyl methane sulphate, ethyl ethane sulphonate, ethylene amines, 5-bromouracil, 2-amino purine, acriflavin, proflavin, nitrous acid, hydroxylamine and sodium azide (Patil and Patil, 2009). Although most of these chemical mutagens are effective, their application in vegetatively propagated crops (VPC) is seriously limited. Firstly, penetration of chemical mutagens into multi-cellular or woody plant tissues is often difficult and this may lead to low reproducibility. Secondly, materials or seeds that are dormant or have long germination time, may require special pre-treatments prior to chemical mutagen treatment (FAO/IAEA, 2018). There are serious health and safety concerns due to toxic or carcinogenic properties of chemical mutagens (Kodym, 2018). According to Oladosu *et al.*, (2016) chemical mutagens are generally carcinogenic and the ratio of mutation to undesirable modifications is generally higher than for physical mutagens. They further reported that the major advantage of using physical mutagenesis compared to chemical mutagenesis is the degree of accuracy and sufficient reproducibility, particularly for gamma rays, which have a uniform penetrating power in the tissue.

The response of plant tissues to chemical mutagens differ. Patil and Patil (2009) reported that maximum number of sprouts (62) was obtained when *Rosa* 'Christian Dior' was pretreated with 2% DES while 32 sprouts occurred in Kiss of Fire pretreated with 0.25% ethyl methanesulphonate (EMS). A report by Maluszynski *et al.*,(2000) showed that the application of EMS to *Dianthus caryophyllus* (carnation) have led to the successful development of mutants *Dioon edule*, *Enzett Barther Fruhl*, and *Dianthus caryophyllus L* all with variant flower colour.

Several physical mutagens are used for mutation induction in plants. The most commonly used ones are gamma rays, X-rays, alpha particles, beta particles, ultraviolet rays, fast and thermal neutrons (IAEA 1992). Recently, ion beams are increasing being used for mutation induction in plants with high success rate (Caplin and Willey, 2018).

Physical mutagens such as X-rays and gamma rays have been used successfully to increase diversity in ornamental plants (Mohammed *et al.*, 2019). Among all the physical mutagens, gamma rays are mostly preferable due to its sparsely ionizing, deeply penetrating and its non-particulate nature (Dilta, 2003). Mohammad *et al.*, (2019) observed that about 70% of mutated varieties of ornamental plants are produced by gamma rays. According to Yamaguchi (2018), approximately 720 induced ornamental plants have already been produced using mutagens. Some of the important cut and potted ornamental plants obtained from mutation induction are *Dendranthema grandiflora, Orchid sp., Rosa sp., Pelargonium inquinans C. indica, and Dianthus caryophyllus* (Miri, 2018).

Mutagens can be applied to both micro and macro propagules. These include seed, seedlings, *in vitro* cuttings, somatic embryos and calli (Shobhana and Rajeevan, 2003). Bado *et al.*, (2017), however, have reported that most successes in mutation breeding have been achieved in seed crops as they are easy to treat and handle. A study by Marcu *et al.*, (2013) on the effects of radiation on seeds revealed that the mutagen treatment had effect on germination potential, quality of the germinated seedlings (root and shoots lengths), and the time of germination compared to non-irradiated seeds. IAEA (1992) reported that a mutant of *Canna indica* with improved aesthetic petal colour was developed by irradiation of seeds at 9.2 Gy using gamma rays. According to Xie (2017), radiation not only impacts the probability of seed germination, but it also results in longer-term effects on seedlings and their ultimate rate of survival after germination.

The response of cells of higher plants to physical and chemical mutagens is influenced by a varying degree by numerous biological (nature of seed coat, cell cycle, genetics), environmental (oxygen, water content, temperature) and chemical factors (type of mutagen, dose range, exposure time) (Caplin and Willey 2018). These factors modify the effectiveness and efficiency of mutagens in the cells of higher plants (FAO/IAEA., 2018). A decreasing trend has been observed in number of sprouts as the doses increases.

In an experiment performed by Pallavi *et al.* (2017) and Miri (2018) using 75, 100 or 125 Gy, a novel mutant variety of *Zinnia elegans var. Dreamland* with higher frequency of flower colour mutation was observed in 100Gy from which one dwarf variety and eight varieties with varying desirable floral colours were selected for commercial exploitation. Both Sooch *et al.*, (2007) and Patil (2009) have independently reported that 10 Gy gamma irradiation increased the number of shoots (7.17), shoot length (1.38 cm), number of roots

(18.30) and root length (1.18 cm) but decreased the days to root initiation (6.90) in *Dianthus caryophyllus*.

To create genetic variability in *Chrysanthemum* 'Local Golden,' Patil *et al.*, (2015) reported that irradiation of seeds at 50-300 Gy showed that the LD_{50} was found between 250 and 300 Gy. He further reported that the best treatment for optimal performance was 300 Gy as it resulted in flower changes in diameter and number of ray florets per flower.

Hase *et al.*, (2010) and Yamaguchi, (2018) also demonstrated that when sucrose-treated Petunia seedlings containing a high concentration of anthocyanin were irradiated with ion beams, flower colour mutants were obtained at a higher frequency than when non-treated seedlings were irradiated. According to Danso *et al.*, (2009), while mutation breeding is inexpensive and simple to implement, one major constraint has been the high cost of managing large populations, which may stymie the breeding programme's progress.

2.11 *In vitro* mutagenesis in ornamental plants

Recent advancements in plant breeding, which combine *in vitro* methods with mutation induction, hold tremendous promise for the generation of useful mutants (Danso *et al.*,2009). *In vitro* mutagenesis has proven effective in the generation of novel mutant varieties and might be used to flowering plants. Irradiation has been shown to impact cell differentiation and plant growth *in vitro*, which aids in the development of novel plant types (Hasbullah *et al.*, 2012). *Canna indica* is dormant because to its stiff seed coat. In the past, such dormancy was disrupted by using gamma irradiation.

Datta *et al*., (2005) have demonstrated that *in vitro* mutagenesis through direct regeneration helped in the development of solid mutants of *Chrysanthemum morifolium Ramat. cvs.*

Flirt, Puja, Maghi and *Sunil* without diplontic selection when ray florets were treated with 5 Gy and 10 Gy and cultured on MS media supplemented with different concentrations and combinations of growth regulators. Abou-Dahab *et al.,* (2017) found that doses of 1000 or 1200 Gy of 60 Co gamma rays in *Eustoma gradiflorum* delayed flower senescence for six days and the number of petals per flower increased, in addition to generating a wide range of flower colours through *in vitro* mutagenesis. FAO/IAEA, (2017) reports of improvement of *Dahlia* by mutagenesis using 20-30 Gy of 60 Co gamma rays.

2.12 Dissolution of chimera

In spite of the advantages of mutation induction, one major limitation in its application is the development of chimeras. The occurrence of chimeras after mutagen treatment is of great importance for the implementation of a mutation breeding programme, in particular with regards to the handling of the mutated populations (FAO/IAEA., 2018). Chimera formation makes mutant selection very difficult in vegetatively propagated crops (VPCs) and needs to be dissolved before solid mutants can be selected. Chimera originates from different genetic tissues in the apical meristem which after irradiation leads to the development of variegated plants (Gakpetor *et al.,* 2017). Chimera types are broadly classified as either somatic or reproductive. The most visible indication of somatic chimerism is chlorophyll variegation in leaves with chlorophyll deficient sectors forming longitudinal streaks in monocotyledons and irregular patches in dicotyledons. Chimeras can persist in VPCs but may be dissolved quickly in seed crops. However, even in seed crops chimeras can be transmitted to the next generation at a low frequency (FAO/IAEA, 2018). For varietal development in flower plants, chimera development may be beneficial as it enhances the aesthetic value of the plants. According to Gakpetor *et al.*.(2017), many important selections of foliage, floricultural, and landscape plants are chimeras.

Chimeras are categorized according on the genetic makeup of the shoot apical meristem layers. Periclinal chimeras have a uniform, genetically distinct layer of cells in the shoot apical meristem (SAM), whereas mericlinal chimeras have a heterogenomic population of cells within a single SAM layer; sectorial chimeras have either a heterogenomic population of cells traversing multiple SAM layers or non-patterned heterogenomic patches of cells (Frank *et al.*, 2018).

Chimera formation has been reported in *chrysantemum* and many other ornamental plants. Plants with periclinal chimeric structures caused by natural or artificial mutations, such as flower colour sports in chrysanthemum, have been used as basis for selection in *Chrysanthemum* cultivars (Aida *et al.*, 2016). Although, currently there is no report of chimera formation in *Canna sp.*, its development to this ornamental plant propagated vegetatively using rhizome can lead to development of new traits to enhance its aesthetic value.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Survey of floral industry in Accra and its environs.

A survey on the flower industry in Ghana was conducted mainly in the Greater Accra Region and some parts of Eastern region of Ghana. Greater Accra was chosen for this study because there are both small and large-scale florists growing ornamental plants on commercial scale. In addition, Greater Accra Region has a lot of peri-urban youth engaged in the flower industry for their livelihood. The survey mainly focused on the small-scale florists and buyers of ornamental plants in Accra. A questionnaire consisting of 22 questions (Appendix 1) was administered to these small-scale florists and buyers in Spintex, East Legon, Dzorwulu, Frafraha, Atomic, and Medie, all suburbs of Accra. Although Nsawam is not in the Greater Accra Region, it was included in the study because of its close proximity to Accra and the several florists in that town. Below is map showing the study area.

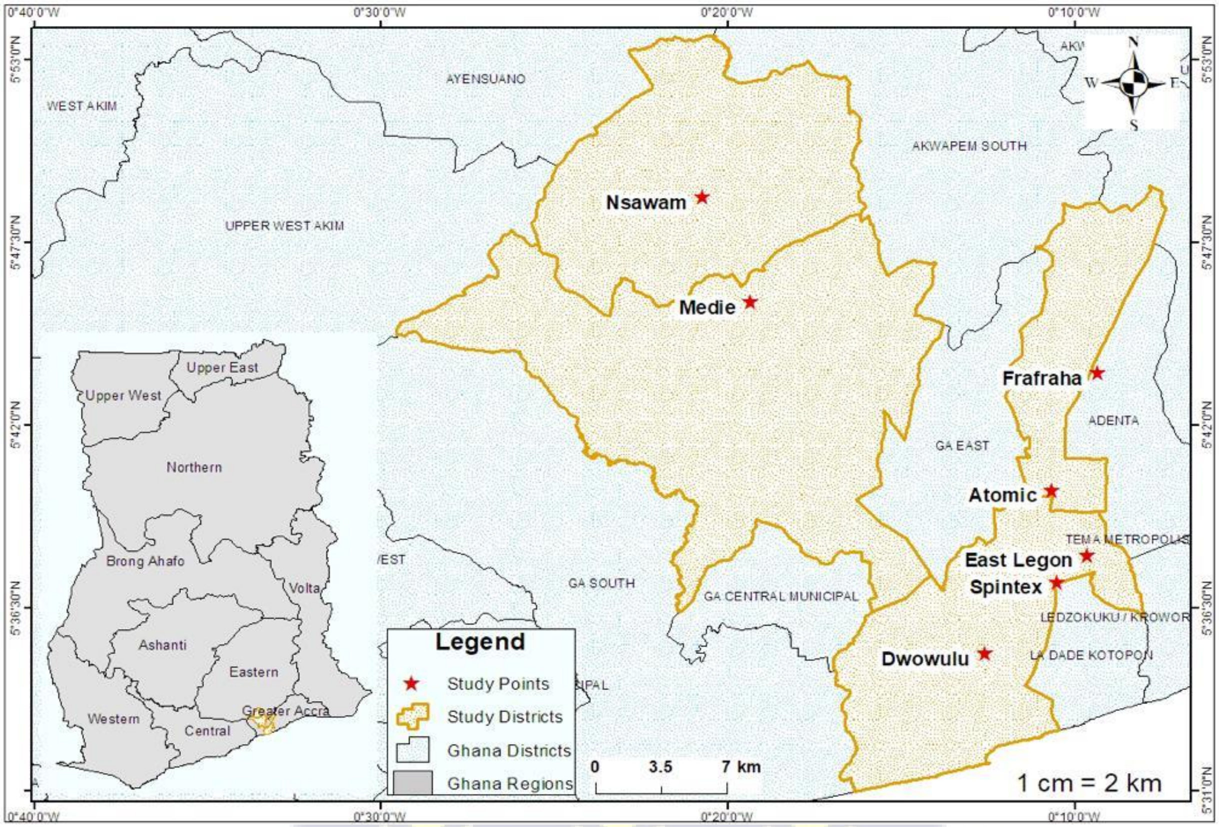

Figure 3.1: Geographical map of Greater Accra and part of Eastern Region showing the areas where the survey was conducted.

Each respondent was visited at his or her site and primary data were collected using structured questionnaire (Appendix 1). The questionnaire was in English, however, where the respondent could not speak the English language, it was translated into vernacular for ease of response; thus an oral interview was used. The questions were administered to 120 respondents. The questionnaire solicited for information on biodata (gender, age, educational background) of respondents, most flower colours preferred by customers, unit price of flowers, lucrativeness of the industry as well as challenges in the floriculture industry. Additionally, the questionnaire sought to find out mode of propagation of *C.*

indica and *C. pulcherrima* as well as challenges involved in the propagation of both species.

3.2 Propagation of *Caesalpinia pulcherrima* and *Canna indica*

3.2.1 Study site

This study was conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Accra between August 2019 and July 2020. However, due to the global corona virus pandemic, data collection was extended beyond July 2020. GAEC is located north-west of the University of Ghana. The study area lies within latitudes 5°6′7’’N to 5°6′9’’N and longitudes 0°21′W to 0°26′W at elevation of 64 m. The maximum and minimum mean temperatures for the period of study were 30.7℃ and 26.0 ℃ respectively with a mean annual rainfall of 830 mm. The lowest and highest relative humidity is between 60 and 75% (Ghana Meteorological Agency, 2020).

3.2.2 Collection of planting materials of *Caesalpinia* and *Canna seeds*

Two species of ornamentals used for this study were *Caesalpinia pulcherrima* and *Canna indica*. For *C. pulcherrima*, seeds from two landrace varieties, which grow wild in Ghana, were collected from BNARI in the Greater Accra and Coaltar in the Eastern Region of Ghana. These species produce yellow, red or (mottled flowers). Seeds of *C. indica* which also grows in the wild were collected from Otoase in the Eastern region of Ghana. Seeds of *C. pulcherrima* and *C. indica* were dried in the sun for 5 days. Healthy looking uniform seeds were then selected for use in the experiments.

3.3 Determination of viability of seeds

Twenty (20) healthy uniform seeds of both *C. pulcherrima* (yellow and mottled flowers) and *C. indica* (red flower) were each selected and place in Petri dish containing moist cotton wool. The Petri dish was covered and kept in the tissue culture laboratory of BNARI at room temperature and observed for seedling emergence 5 days after planting. Seedling emergence was calculated after 5 days of emergence

$$\text{Germination } \% = \frac{Number\ of\ germinated\ seedlings}{Seeds\ sown} \times 100$$

3.4 Irradiation and germination of *C. pulcherrima* seeds

Seeds of *C. pulcherrima* were irradiated using a Cobalt-60 gamma source at the Radiation Technology Centre (RTC) of BNARI, Ghana Atomic Energy Commission. Twenty (20) seeds were bagged in a brown envelope and irradiated at 0 (control), 100, 200, 300, 400, 500, 600 or 700 Gy. Based on the results of the first experiment, irradiation of seeds were repeated using 200 Gy, 400 Gy, 600 Gy, 800 Gy or 1000 Gy including a control to determine the LD_{50}. All seed samples were irradiated at a dose rate of 301 Gy per hour. The experiment was conducted using a completely randomized design (CRD) with each treatment (dose) being replicated 20 times. Irradiated seeds were immediately washed under tap water and sown in black polyethylene pots containing loamy soil with two seeds per pot at BNARI of GAEC. The bags were kept under the plant barn and irrigated with tap water thrice weekly. Number of seeds germinated was counted five days after sowing when the first emergence was observed and continued at weekly interval for 30 days. Germination occurred when the hypocotyl hook or leaves was observed. The plants were allowed to grow in plastic pots for 15 days and the number of seeds that survived was counted. The percentage germination was calculated on the 5th, 10th, and 15th days using the formula below:

$$\text{Germination } \% = \frac{Number\ of\ germinated\ seeds}{Seeds\ sown} \times 100$$

The percentage survival after germination was also calculated on the 30th day using the following formula:

$$\text{Survival (\%) at 30 days} = \frac{Number\ of\ survivals\ at\ 30\ DAG}{Number\ of\ seeds} \text{ X } 100.$$

Lethal dose (LD_{50}) referring to the dose at which 50% of the irradiated propagule did not survive was then calculated using the percentage germination as follows:

$$LD_{50} = \frac{Number\ of\ survival\ plants\ at\ 10\ DAG}{Number\ of\ germinated\ plants} \text{ X } 100.$$

The height of the developing seedlings were measured at 28, 42 and 56 days using a meter rule and the number of leaves were also counted. After 28 days, the healthy seedlings were transplanted into plastic bags filled with loamy soil to enhance their growth. The height of the plants and number of leaves were again measured 42 and 56 days after transplanting. Finally, plant height was measured at first flowering. Also, the number of days when the plants flowered was recorded as well as the number of petals and branches at first flowering. To study the effect of irradiation on *C. pulcherrima*, morphometric data were also recorded and this included the number of dwarf plants. Plants with unique morphometric (qualitative) traits, which were indicative of the effect of radiation, were also tagged and monitored. These traits include flower colour, leaf size and shape as well as plant height.

3.5. Viability of *Canna indica* seeds

The viability of *C. indica* was tested as described above (section 3.3)

3.6. Scarification and germination of *Canna indica* seeds

Sixty (60) seeds of *C. indica* were scarified by scratching the seeds at either the micropylar end or any other side with the aid of a plier and sandpaper (Figure 3.2). Non scratched or scarified seeds served as controls. Each seed served as an experimental unit and was replicated twenty (20) times. All the seeds were sown in trays and watered daily. The number of seeds that germinated were counted and was used to calculate percentage germination five days after sowing.

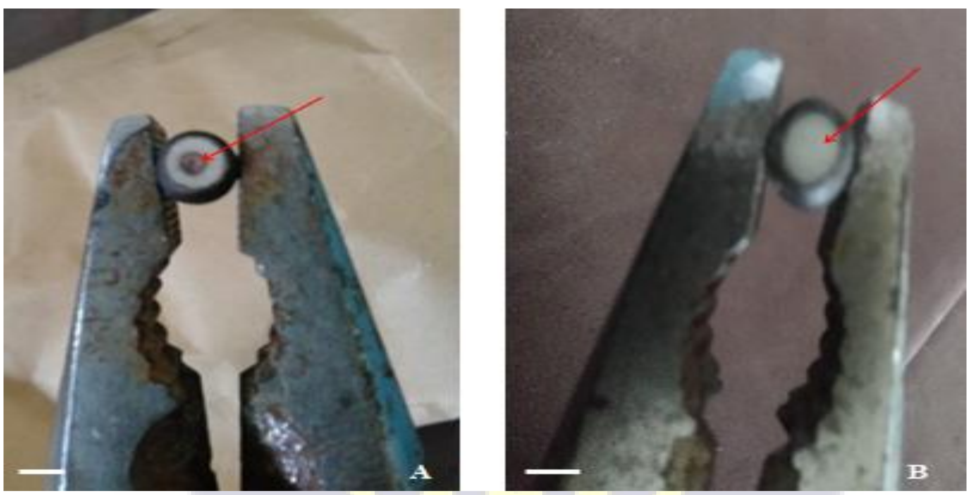

Figure 3.2: Scarification of *C. indica* seeds at (A) micropylar end and (B) other part of the seed coat. Arrow showing embryo at micropylar end. (Bars indicate 4mm).

3.6.1 Irradiation of scarified seeds of *Canna indica*

Three hundred and thirty (330) uniform seeds were irradiated at 0 Gy (control), 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 Gy. Irradiated seeds were scarified on any other part of the seed coat as described in section 3.2.2 and sown *ex vitro* in trays. The experimental design used for this study is Complete Randomized Design with 10 replications per treatment. The number of seeds that germinated were counted and recorded.

3.7 *In vitro* propagation of *C. indica*

3.7.1 *In vitro* germination of *C. indica*

Seeds of *C. indica* were thoroughly washed with Sterlised distilled water. After washing, the seeds were sterilised by immersion in 10% sodium hypochlorite for 5 minutes, followed by immersion in 70% ethanol for 5 minutes under the laminar flowhood (Nuaire Biological Safety Cabinet, UK). Sterlised seeds were cultured on 50 ml of Murashige and Skoog (1962) (MS) basal medium supplemented with vitamins in culture vessels. One seed was cultured in a bottle containing 50ml of the basal medium. The composition of the MS medium is shown in Appendix 2. The culture medium was adjusted to pH 5.8±0.1 using 1M NaOH or 1M HCl and solidified with 3.5g/l phytagel prior to autoclaving at 121 ℃ for 15 minutes. Cultured seeds were transferred to the growth room at a temperature of 27± 2℃, with a photoperiod of 16-hour light and 8-hour darkness and a light intensity of 2700 lux provided by white bulbs in the growth room. The experiment was replicated 10.times using a completely randomized design (CRD). Data were taken on the number of germinated seeds, number of shoots and roots for each dose after incubation in the growth room.

3.8 Acclimatization of *in vitro* plantlets

C. indica plantlets were transferred to loamy soil in black polythene bags when they were 21 days old. The roots of the plantlets were gently washed in tap water before being transferred into bags to remove any adhering media. For three days, the plantlets were covered with clear plastic cups to create a humidity chamber and partial sunlight. The number of plantlets that survived were counted after 14 days. Plant height was measured with a meter rule and number of leaves, number of days to flowering and number of multiple shoot formed were counted.

3.9 Data collection and statistical analysis

All data collected from the respondents of the survey questionnaires in the Greater Accra Region were analysed using Statistical Packages for Social Sciences (SPSS), version 25 software. All quantitative data collected from the various experiments were subjected to Minitab Statistical Software version 20. Analysis of Variance (ANOVA) and Turkey's Least Significant Difference (LSD) was used for the mean separation where significant differences occurred. All data were tested for their normality and where data deviated from normality they were transformed. All graphical presentations were done using Microsoft Excel, version 2010.

CHAPTER FOUR

RESULTS

4.1 Response to survey conducted on flower growers in Greater Accra Region

In Ghana, the floriculture industry is very vibrant in the greater Accra region providing job opportunities for the unemployed youth. One hundred and twenty (120) respondents comprising of flower producers and customers (buyers) responded to the questionnaires, which was administered in both English and the vernacular to allow for ease of response.

4.1.1. Demographic background of the respondents

Of the 120 respondents, 73.3% were males while 26.7% were females (Table 4.1) suggesting that males dominate the floriculture industry in the Greater Accra Region. Forty percent (40%) representing majority of the respondents are in middle age (36-40 years) while 28.3% are between 26 to 35 years. The rest falls within 18 to 25 years (26.7%) and 5% are above 50 years. All the respondents have had some form of formal education ranging from Junior High School (JHS), Senior High School to the University level. Fifty (50%) of the respondents have completed Junior High School while 40.8 and 9.2% have had Senior High School and Tertiary education respectively.

Table 4. 1 Demographic background of the respondents

Category	Demographic of respondents	Frequency	Proportion of respondents (%)
Sex	Male	88	73.3
	Female	32	26.7
Age	18 – 25	32	26.7
	26 – 35	34	28.3
	36 – 50	48	40.0
	Above 50	6	5.0
Level of Education	Basic	60	50.0
	Secondary	49	40.8
	Tertiary	11	9.2
	None	0	0

4.1.2 Reasons for engaging in the floriculture industry by respondents.

Figure 4.1 shows reasons why the respondents were engaged in the floriculture industry. More than 66% of the respondents indicated that it gave them employment or job while almost 30% said it gave them an income (Figure 4.1). Less than 10% of the respondents and as low as 2.5% claimed that they entered the industry as hobby and for beautification of the environment respectively.

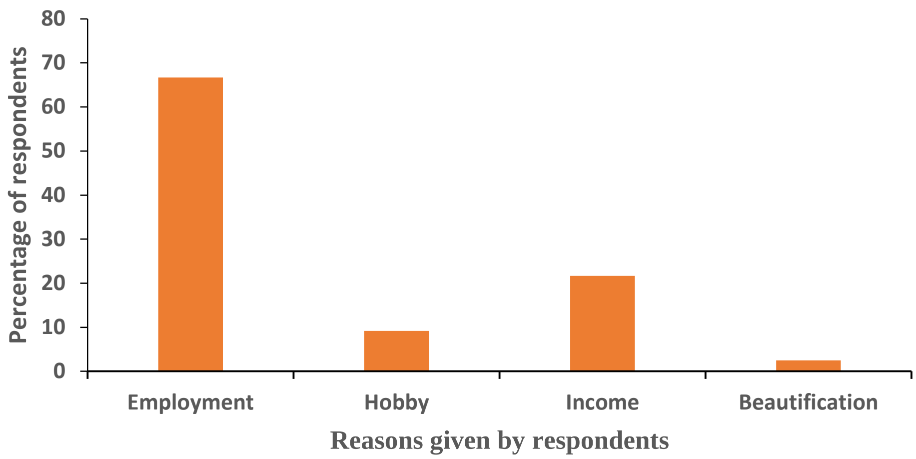

Figure 4.1: Purpose for engaging in the floriculture industry.

4.1.3 Types of Flowers, mode of propagation and challenges encountered by respondents

The survey revealed that the most cultivated ornamental plants propagated by floriculturist in Accra and its environs are *Roystonea regia*, *Ixora coccinea*, *Euphorbia milii*, *Allamanda cathartica*, *Melampodium divaricatum*, *Thuja orientalis*, *Lantana camara*, *Tombeja*, *Ipomoea sp.*, *Araucaria columnaris*, *Adenium obesum* and *Ficus benjamina* (Figure 4.2). Of these ornamental plants, *Roystonea regia*, *Ixora coccinea* and *Euphorbia milii* are the most common flowers propagated and sold by the floriculturists while *Arucaria*, *Adenium and Ficus benjamin* were the least propagated.

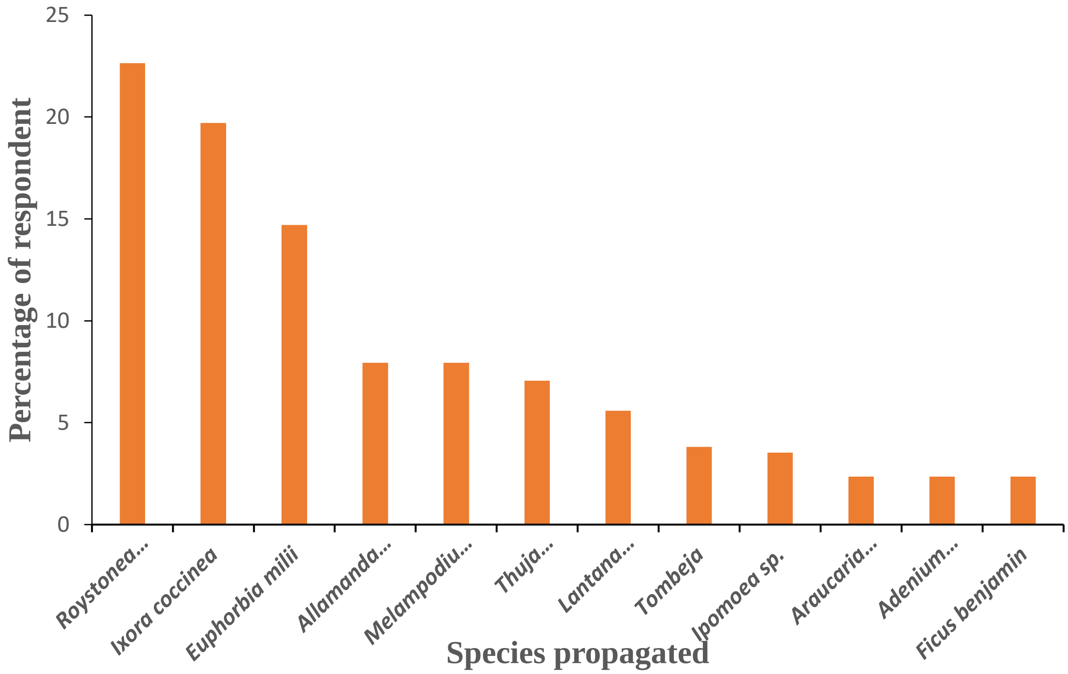

Figure 4.2: Common ornamental plants propagated by respondents.

Table 4.2 shows three varieties of *C. pulcherrima* propagated by the respondents. These three are distinguished by the colour of their petals namely; mottled, yellow or red (Figure 2.1). More than 66% of the respondents indicated that they propagate *C. pulcherrima* with mottled petals while less than 21 and 13% respectively propagate yellow and red varieties respectively.

Both sexual and asexual mode of propagation are used by the propagators in this survey, however, almost all the respondents indicated that *C. pulcherrima* is propagated sexually using seeds while only 1.67% use cuttings for propagation. More than thirty six percent (36.67%), of the respondents said seeds of *C. pulcherrima* are not viable and hence have low germination rate when used as planting material while more than 25% of the respondents report that the ornamental plant sheds too much leaves, 12.50% complain of the plant's susceptibility to pest and disease attack. Eleven (11) and nine (9 %) percent of the respondents said the seeds are dormant and the plants produce thorns respectfully.

Table 4.2: Preferred petal colour, mode of propagation and challenges involved in propagation of *C. pulcherrima* by respondents.

Questions	Category	Proportion of respondents (%)
Preferred petal/flower colour	Mottled	66.70
	Yellow	20.80
	Red	12.50
Mode of propagation	Seeds	98.33
	Cuttings	1.67
	Others	0.00
Propagation challenges	Seed viability	36.67
	Shedding of leaves	25.83
	Pest and disease attack	12.50
	Dormancy	11.67
	Thorns	9.17

4.1.4 Survey on propagation of *C. pulcherrima*

C. pulcherrima bears a variety of flower colours including red, yellow and mottled (red and yellow). More than twelve percent (12.5%) of the respondent preferred red while 20.8 and 66.7% preferred the yellow and mottled colour respectively. The survey revealed that 98.3% of the respondents propagate *C. pulcherrima* by seeds and only 1.7% propagates by cuttings. However, according to the respondents, there are a number of challenges on the mode of propagation of the ornamental plant. These include seed dormancy, pests and diseases, rotten of cuttings and lack of rapid multiplication technique to meet the demand of consumers. While 36.67% have challenge with the viability of the seeds, 11.67% complained of seed dormancy. Almost thirteen percent (that is, 12.5%) are of the view that the plant sheds a lot of leaves which is a nuisance in the environment. Additionally, 25.83% respondents suggested that the seeds, a major mode of propagation are easily attacked by diseases and pest. Less than ten percent (9.17%) of the respondents indicated that the presence of thorns on the shoot or the stem is nuisance and does not allow the plant to be used as a cut flower. With the above challenges there is no denying the fact that consumers would appreciate an improved *C. pulcherrima* with good aesthetic value.

4.1.5 Responses to survey on *Canna indica*

Stakeholders in the flower industry propagate and market three landrace varieties of *C. indica* (Table 4.3). Of these three varieties, more than 83.3% prefer *C. indica* with red petals followed by orange colour (14.2%) and yellow (2.5%) in that order. All the respondents (100%) reported that *C. indica* is mainly propagated by suckers. The responses revealed that there is lack or inadequate knowledge of respondents on the improved methods or tissue culture techniques for rapid multiplication of this ornamental plant. They also indicated that there are several challenges associated with propagation of *C. indica*. More than forty eight percent (48.33%) reported that the major challenge involved in the propagation of *C. indica* is seed dormancy while 13.3 and 10% of the respondents said that there were challenges with pests and diseases and sucker rot respectively. Furthermore, 14.17% of the respondent reported that *C. indica* requires regular watering which makes propagation expensive since they pay for the irrigation of the plants. Almost six percent (6%) of the respondents claim the species multiplies rapidly making it a dominant species and a nuisance in the environment.

Table 4.3: Proportion of respondents on preferred *C. indica* flower colour, mode of propagation and propagation challenges of *C. indica*.

Questions	Category	Proportion of respondents (%)
Flower Colour Preferred	Red	83.30
	Orange	14.20
	Yellow	2.50
Mode of Propagation	Suckers	100.0
	Seeds	0.00
	Others	0.00
Propagation Challenges	Seed dormancy	48.33
	Requires much watering	14.17
	Pest and disease attack	13.33
	Sucker rot/death	10.00
	Rapid shoot multiplication	5.83

4.1.6 Perceptions of the flower industry by respondents

This section of the survey sought to ascertain the lucrativeness of the flower industry and propagation challenges confronting the propagators. When the respondents were asked whether they would prefer modern methods and improved seeds to enhance the propagation of *C. indica*, a significantly high (95.83%) proportionof the respondents affirm that they would like to use improved planting materials if they were made available to them (Figure 4.3). Contrarily, 4.17% and 6.67% respectively claimed that they had used improved materials and methods with not much benefit.

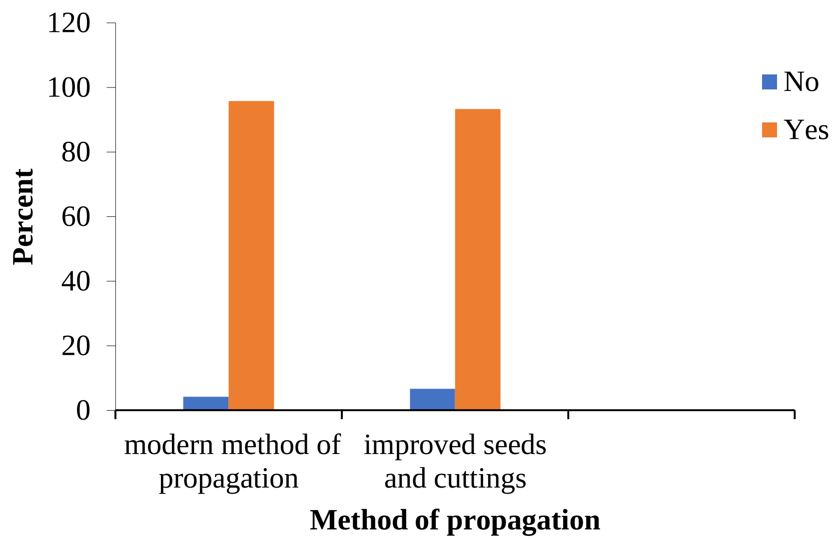

Figure 4.3: Modes of propagation preferred by respondents.

The respondents enumerated several challenges, which limit the potential growth of the flower industry. These challenges range from land acquisition to marketing (Figure 4.4). More than 36% of the respondents indicated that water for irrigation was a major problem. The other major challenges were land acquisition, pests and diseases which were reported by more than 25 and 20% of the respondents respectively. The rest of the challenges are expensive planting materials (8%), costumer preference (4%), marketing (4%) and other challenges which included export (2%).

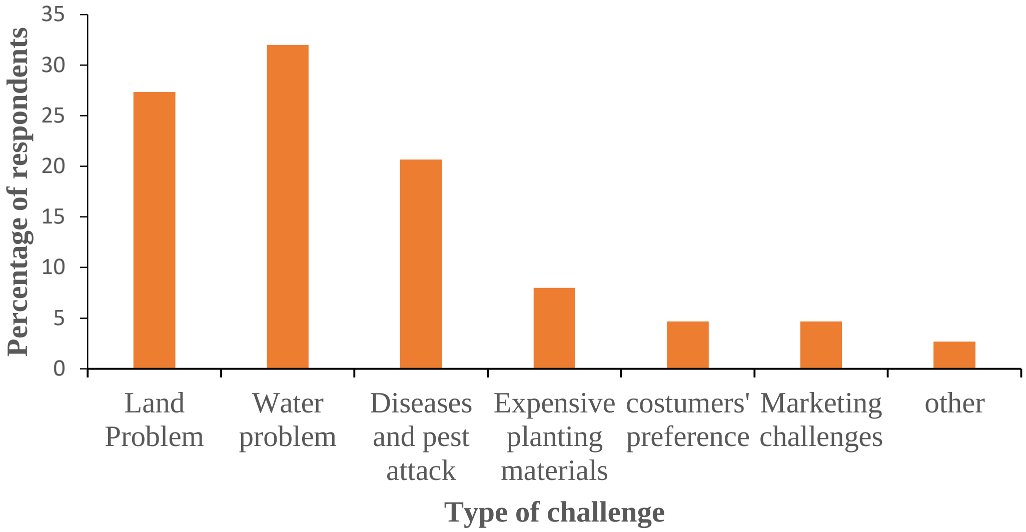

Figure 4.4: Challenges in the flower industry as indicated by the respondents

Almost 80.84% of the stakeholders in the industry indicated that the flower industry is lucrative. Of these 50.84% said the industry is very lucrative while 33.5% indicated the industry is lucrative. Only 10.83% of the respondents said the industry is not lucrative while less than 5% could not indicate whether the industry is lucrative or not (Figure 4.5).

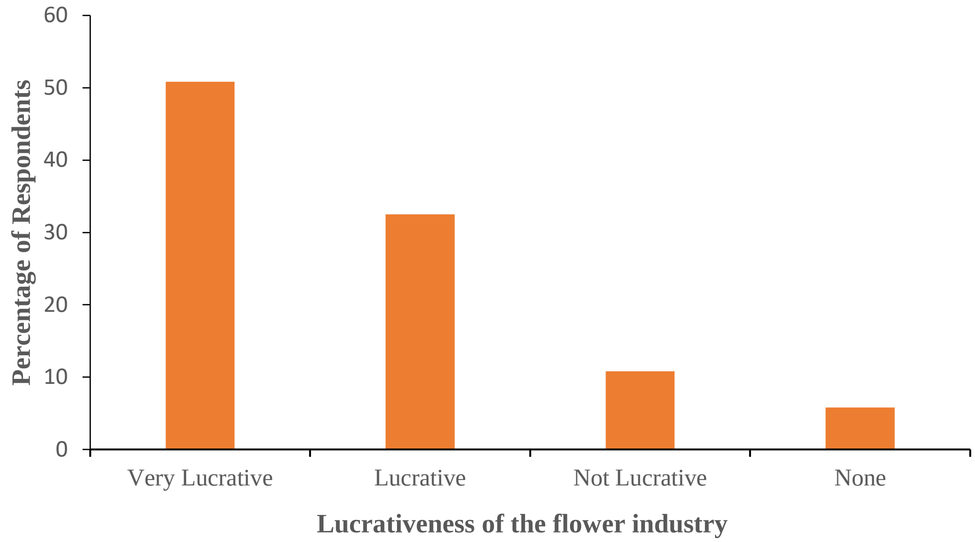

Figure 4.5: Proportion of respondents on lucrativeness of the flower industry.

4.2 Germination test of *Ceasalpinia pulcherrima* and *Canna indica*

The viability of *C. pulcherrima* and *C. indica* was tested by sowing twenty seeds of each variety on moist cotton wool and placed in tissue culture laboratory of BNARI. Seeds of *C. pulcherima* germinated while there was no germination in *C. indica* (Figure 4.6) after 5 days of sowing on moist cotton wool. Eighty percent (80%) of *C. pulcherrima* yellow flower variety germinated compared to 75% germination in mottled flowers. Analysis of Variance (ANOVA) did not show any significant difference (P≥0.5) between the two landrace varieties of *C. pulcherrima* (Appendix 3a).

Table 4. 4: Percentage viability of *C. pulcherrima* and *C. indica* after 5 days of sowing

Species	No. of seeds sown	Germination (%)
C. pulcherrima (yellow flower)	20	16(80)
C. pulcherrima (mottled flower)	20	15(75)
C. indica (red flower)	20	0 (0)

Note: Figures in parenthesis indicate percentage germination.

Figure 4.6: Results of Germination Test of (A) *C. pulcherrima* and (B) *C. indica* after 5 days of sowing. (Bar indicates 4mm).

4.3 Effect of gamma irradiation on germination and survival of yellow flower variety of *C. pulcherrima*

To study the effect of irradiation on germination, seeds of *C. pulcherrima* were irradiated at 100-700 Gy including a control and then sown in black polythene bags. Both the irradiated and non-irradiated controls germinated. However, percentage germination varied. After 5 days of sowing, seeds irradiated at 300 and 400 Gy had the highest germination (50%) (Table 4.5) while seeds irradiated at 200 Gy, 500 Gy and control had a mean of 40% germination, The least percentage germination occurred at 600 Gy (30%) and 700 Gy (20%). Analysis of variance (ANOVA) shows there was no significant difference between the doses (Appendix 3b).

On the tenth day, percentage germination increased and highest germination was obtained at control (0 Gy), 200 Gy and 300 Gy. Germination of seeds irradiated at 200 Gy, 300 Gy and control increased from 40% and 50% respectfully to 70% (highest germination) on the tenth day. Similarly, seeds irradiated at 100 Gy increased from 35% to 65% while those irradiated at 400 Gy and 500 Gy increased to 60 and 50% respectivefully. The least percentage germination (20%) was observed at 700 Gy. Analysis of variance (ANOVA) shows there was significant ($p < 0.05$) decrease in germination after 500 Gy (Appendix 3c).

Germination of seeds continued to increase 15 days after sowing beyond which there was no more germination. The dose that gave the highest percentage of germination (90%) was 100 Gy and 200 Gy followed by 300 Gy (85%), control (75%), 500 Gy (65%) and 400 Gy (60%) in that order. With the exception of seeds irradiated at 500 Gy to 700 Gy, which had the lowest percentage of germination, all the irradiated seeds had higher percentage of germination than the control (75%). Anaysis of variance showed that seeds irradiated at

200-400 Gy had significantly ($p<0.05$) higher percentage germination than the remaining dose treatments (Appendix 3d). There was no increase in germination between 15 and 21 days after sowing. Thus, data on germination was not taken 21 days after sowing but rather the number of surviving plants were recorded 30 days after germination (Table 4.5).

The percentage survival of the seedlings decreased as the dose of irradiation increased. Also survival of seedlings from seeds irradiated at 100 to 400 Gy were significantly different from those irradiated at 500 to 700 Gy. The highest survival of seedlings (90%) occurred at 200 Gy. Seedlings from seeds irradiated at 700 Gy did not survive 30 days after germination while only 10 and 40% of seedlings survived when seeds were irradiated at 600 and 500 Gy respectively. Generally, percent survival declined significantly from 400 Gy to 700 Gy.

Table 4. 4: Effect of gamma irradiation on the germination and survival of seedlings of *C. pulcherrima* (yellow flower variety).

Gamma Dose (Gy)	Germination			Survival at 30 days
	Day 5	Day 10	Day 15	
0	40±0.50^{a}	75±0.51^{a}	75±0.51^{ab}	75±0.47^{ab}
100	35±0.49^{a}	65±0.51^{ab}	90±0.31^{a}	80±0.41^{a}
200	40±0.50^{a}	70±0.47^{a}	90±0.31^{a}	90±0.37^{a}
300	50^{a}±0.51^{a}	70±0.47^{a}	85±0.37^{a}	85±0.41^{a}
400	50±0.51^{a}	60±0.50^{ab}	60±0.50^{ab}	60±0.49^{ab}
500	45±0.51^{a}	50±0.49^{ab}	65±0.49^{ab}	40±0.50^{bc}
600	30±0.47^{a}	30±0.47^{b}	40±0.50^{b}	10±0.31^{cd}
700	20±0.41^{a}	20±0.41^{b}	30±0.47^{b}	00±0.00^{d}

Means followed by different letters are significantly different ($P \leq 0.05$) according to Turkey's pairwise comparisons.

4.3.1 Determination of Lethal Dose (LD_{50})

The lethal dose (LD_{50}) was calculated using the results of percentage germination 10 DAG (Figure 4.7). The LD_{50} using percentage germination was graphically determined to be 583.33 Gy

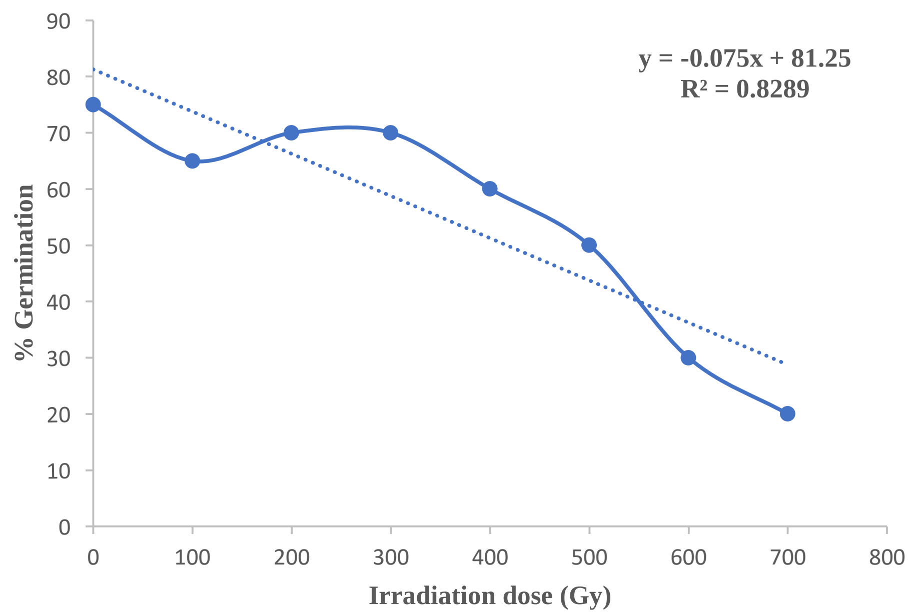

Figure 4.7: Effect of gamma irradiation on germination of seeds of yellow flower *C. pulcherrima* variety.

4.3.2 Effect of Gamma radiation on morphometric features of yellow flower variety- *C. pulcherrima*

The effect of gamma irradiation on *C. pulcherrima* was determined using both morphometric and reproductive traits. The morphometric traits are plant height, number of leaves and branches while the reproductive traits are days to flowering and number of flowers. The results are presented in Tables 4.6 and 4.7. The growth of *C. pulcherrima* seedlings increased gradually as the dose of irradiation increased until it reached the highest at 300 Gy independent of days on which the data was taken and thereafter declined with increasing dose of irradiation (Table 4.6). At 600 Gy the plants were dwarfed (Figure

4.8). There was a significant difference ($p<0.05$) between the growth of non irradiated seed seedlings (control) and the irradiated seeds (Appendix 3f) when the data was taken 28 days after germination. Seedings obtained from 200 Gy irradiated seeds grew faster (10.40 cm), and there was significant differences ($p<0.05$) in height between the doses of irradiation and the controls. The height of the seedlings continued to increase at 42 days after germination. After 42 days of germination, seedlings from 200 and 400 Gy irradiated seeds grew faster 21.01 and 19.52 cm respectively than the controls (18.50 cm) and the remaining treatments suggesting that the irradiation has had stimulatory effect on growth of the seedlings. At highest dose of irradiation (600 Gy), growth of the plant decreased significantly to 10.00 cm and this was due to the adverse effect of irradiation on plant growth resulting in dwarf plants.

All the plants continued to grow and therefore the last set of data were taken 52 days after germination (Table 4.6). The fastest growth was observed on seedlings from seeds irradiated at 200 Gy. At this dose, the height of plant was 55.9 cm which is significantly ($P\leq0.05$) different from the controls (42.52 cm) and the remaining doses. Figure 4.8 shows the extreme effect of irradiation dose on height of plant. At 600 Gy the height of plants produced after 52 days of germination was 30.33cm which is almost half the height of the controlled plants. These plants were described as dwarf or variants and were identified at 400, 500 and 600 Gy. These dwarf plants had reduced height of 32.13cm compared to control (42.52). The leaf area of these dwarfed plants were smaller with shorter petiole length than control. A mean of three (3) branches per plant were oberved in these dwarf.

Figure 4.8: Effect of gamma irradiation on plant height (dwarf plants 32cm) and branches at 56 days. Bar indicates 4mm

4.3.3 Effect of gamma irradiation on number of leaves produced by seedlings

The effect of irradiation on the number of leaves produced was also studied. Similarly, the number of leaves produced by the seedlings also correspondingly decreased with increased irradiation dose. When the number of leaves on seedlings were counted on the 28th day those obtained from 200 Gy irradiated seeds had the highest number (8.50) of leaves followed by 300 Gy (8.30) and 100 Gy (7.70) in that order. However only seedlings obtained from 200 Gy produced significantly ($P \leq 0.05$) higher number of leaves than the controls (Appendix 3i). It was also observed that seedlings from 500 Gy and 600 Gy significantly reduced the number of leaves from 7.70 in the controls to 6.00 and 4.75 respectively.

After 42 days of germination seedlings from 200 Gy produced leaves which were comparatively higher than the controls and statistically significantly different. (Appendix 3j) Similarly, the number of leaves produced at higher doses significantly reduced and were more profond at 100, 500 and 600 Gy where a mean of 2.60, 3.0 and 2.50 leaves respectively were added only after 14 days compared to the controls (1.10 leaves). The number of leaves produced continued to increase at 56 days after planting with the same trend except that the rate of leaf production was higher than between 28 and 42 days. Statistical analysis showed highly significant difference ($P \leq 0.05$) between the mean number of leaves at control, 100 and 200 Gy and the remaining doses and the (Appendix 3k).

Twelve weeks after germination, the number of branches generated per plant was counted. The effect of irradiation dosage on the number of branches generated was comparable to the effect of irradiation dose on the number of leaves produced. The highest number of branches was produced at 400 Gy where a significantly higher ($P \leq 0.05$) mean number of branches (3.10) were produced per plant. The remaining dose teatmented resulted in the production of almost the same number of branches ranging from 1.90 at 100 Gy to 2.50 at 600 Gy.

Table 4. 5: Effect of Gamma irradiation on plant height, number of leaves and branches

Gamma dose	Height(cm) (Days)			Number of Leaves (Days)			Number of branches
	28	42	56	28	42	56	
0 Gy	9.10±1.61^{a}	18.50±3.40^{a}	38.91±13.46ab	7.70±1.64^{abc}	8.80±1.62^{ab}	14.70±1.25^{a}	1.90±0.32^{b}
100 Gy	9.00±1.86^{a}	18.04±5.07^{a}	45.21±19.00ab	4.80±1.03^{e}	7.50±1.18^{b}	14.60±1.71^{a}	1.90±0.99^{b}
200 Gy	10.40±1.78^{a}	21.01±3.58^{a}	55.99±15.48a	8.50±0.85^{a}	9.50±0.53^{a}	14.50±2.41^{a}	2.00±0.82^{b}
300 Gy	9.82±2.17^{a}	18.03±2.97^{a}	45.08±14.09ab	8.30±2.21^{ab}	9.10±1.66^{ab}	13.50±1.35^{ab}	2.00±0.67^{b}
400 Gy	10.17±2.78^{a}	19.52±3.52^{a}	42.52±14.65ab	7.30d±2.41^{abc}	8.70±0.82^{ab}	12.67±1.00^{ab}	3.10±0.74^{a}
500 Gy	7.25±5.14^{ab}	15.36±5.89^{b}	30.01±10.92b	6.00±2.36^{bcde}	8.20±1.03^{ab}	14.00±0.87^{ab}	1.90±0.74^{b}
600 Gy	3.25±5.04^{bc}	6.00±5.29^{c}	30.33±2.89ab	4.75±0.50^{cde}	7.25±0.50^{b}	12.00±1.73^{ab}	2.50±0.71^{ab}
700 Gy	Nd	nd	nd	Nd	nd	nd	nd

Means followerd by different letters are significantly different at (P≤0.05) using Tukey's pairwise comparison.
Note: nd means data not determined.

4.3.4 Effect of gamma irradiation on flower production

The number of days to 50% flowering was also determined to give an idea of how long *C. pulcherrima* plants takes to mature. The number of days to 50% flowering ranged from 147.3 to 160.8 days but it did not follow any particular trend as the mean number of leaves and branches. Plants obtained from seeds irradiated with 300 Gy delayed flowering to 160.8 days. The days to flowering at this dose was almost the same as the controls (159.2) where there was no irradtion. Although plants from 600 Gy flowered almost 10 days earlier (147.3), it was not significantly different from the rest of the remaining doses. In general, Analysis of Variance did not show any significant differences ($P \geq 0.05$) between the irradiated doses and the controls (Appendix 3m). Number of opened flowers produced were counted. Though plants obtained from 200 Gy irradiation produced more flowers (6.50) followed by 500 Gy (6.00), there was no significant difference between plants obtaned from irradiation and non irradiated plants.

Table 4. 7: Effect of gamma irradiation on days to flowering and no. of flowers per plant.

Dose (Gy)	No. of days to 50% flowering	No. of flowers per plant
0 (Control)	159.2±19.23[a]	5.40±2.46[a]
100	154.3±14.53[a]	5.30±2.26[a]
200	150.7±12.87[a]	6.50±3.34[a]
300	160.8±21.43[a]	5.90±1.73[a]
400	152.6±15.71[a]	5.50±2.68[a]
500	159.4±16.67[a]	6.00±3.20[a]
600	147.3±10.50[a]	5.90±1.13[a]
700	Nd	Nd

Means followerd by different letters are significantly different at (P≤0.05) using Tukey's pairwise comparison. Note: nd means not determined.

4.4 Effect of higher doses of gamma irradiation on the germination and survival of seedlings of yellow and mottled flower *C. pulcherrima* variety

Since the radiation effect was not clearly distinguishable 52 days after germination, the experiment was repeated using higher dose interval of 200 Gy and ranging from 0 (controls) to 1000 Gy using yellow and mottled flower variety.

Both yellow and mottled flower varieties responded to irradiation treatment. However, the response varied depending on the variety and the dose of irradiation (Figure 4.9). After five days of sowing, irradiated seeds were lower than the controls. This is with respect to the yellow and the mottle flowered varieties. In yellow flower variety, the decrease is slightly significant while in the mottled flower the decrease is not statistically significant (Table 4.8). As the dose of irradiation increased, the percentage germination increased until it reached a peak (50%) at 600 Gy and thereafter declined in yellow flowered variety. Contrarily, for the mottled flower variety, increasing the dose of irradiation correspondingly decreased percentage germination from 35% to 5% at 1000 Gy. In both varieties, after five days of sowing, percentage germination from seeds irradiated at 1000 Gy was very low (5%).

The percentage germination continued in both varieties ten days after sowing. However, at 200 Gy the percentage germination increased to 65% in yellow flower variety and it also increase from 35% to 65% in mottled flower variety. The highest percentage of germination (65%) was obtained when the seeds were irradiated at control and 200 Gy in yellow flowered variety while for the mottled flower, the highest percentage germination was obtained at 400 Gy indicating that it is more radiosensitive than yellow variety. While the percentage of germination increased from 5% to 30% in yellow variety, in mottled flower variety it increase from 5% to only 10%. Statistically, the increase in germination in mottled flower variety and yellow variety was significant.

At fifteen days after sowing, germination increased in some of the doses while in others it did not. In yellow flower variety, germination increased at 600 Gy from 45% at day 10 to 70% in day 15 and at 800 Gy it increased from 20% to 50% at day 15. Germination percentage also increased from 10% to 30% for seeds irradiated at 1000 Gy. In the remaining doses, there were no more germination as percentage germination remained the same. Similar observation and trend is seen in the mottled flower variety. However, in this variety increased germination was observed at control, 200 Gy 400 Gy and 600 Gy while in the remaining treatment there was no significant increase in germination. Again, in this mottled flower variety, the dose of irradiation significantly influenced germination.

Seedlings were monitored for their growth and development and their survival was recorded 30 days after germination (Table 4.8). Comparatively, the percentage survival was higher in the mottled flower variety than the yellow flower in the irradiated seeds. In mottled flower variety, seeds irradiated at the lowest dose of 200 Gy resulted in the highest percentage of survival (65%) and this was twice that of the yellow flower variety and also comparatively higher than the controls. Also, in both varieties seedling survival significantly differed ($P \leq 0.05$) between the seedlings obtained from doses ranging from 200 to 400 Gy and from 600 to 1000 Gy; while the difference between the yellow flower variety was slightly significant, those of the mottled flower was highly significant (Appendix 4d and 5d). However, in the yellow flower variety survival of seedlings from the irradiated varieties were significantly higher than the controls. In both varieties none of the seedlings obtained from 800 to 1000 Gy survived.

Figure 4.9 Effect of Cobalt-60 gamma irradiation on germination. Records were taken 10 days after sowing. Bar indicates 8mm

4.4.1 Determination of Lethal Dose (LD_{50})

The LD_{50} for germination was calculated using survived seedlings after 10 days of germination over total number of germinated seedlings for both yellow and mottled flower varieties and it was found to be 571.43 and 645.39 Gy respectively for yellow and mottled flower (Figure 4.10) indicating that yellow flower was more sensitive to irradiation than mottled flowers. Similarly, the number of survived seedlings was recorded for both flower varieties (Figure 4.11)

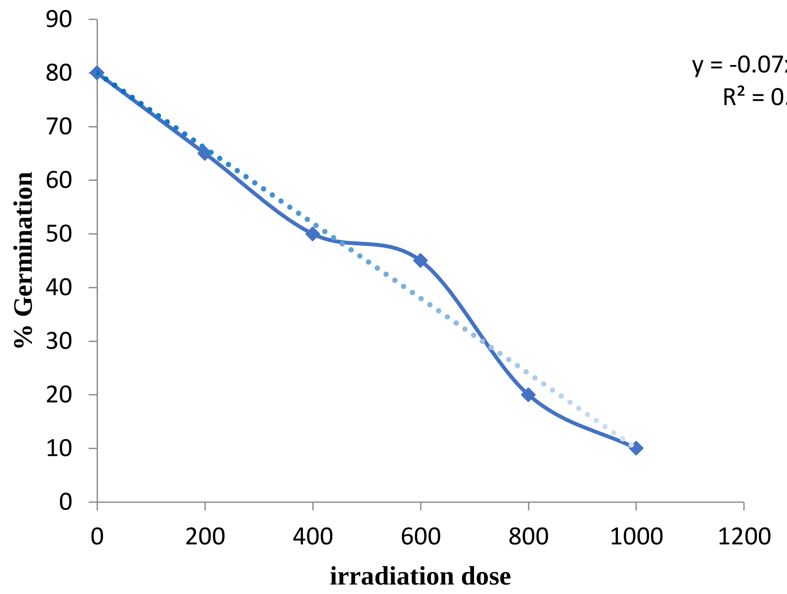

Figure 4.10: Effect of gamma irradiation on the germination of *C. pulcherrima*–Yellow flowered variety

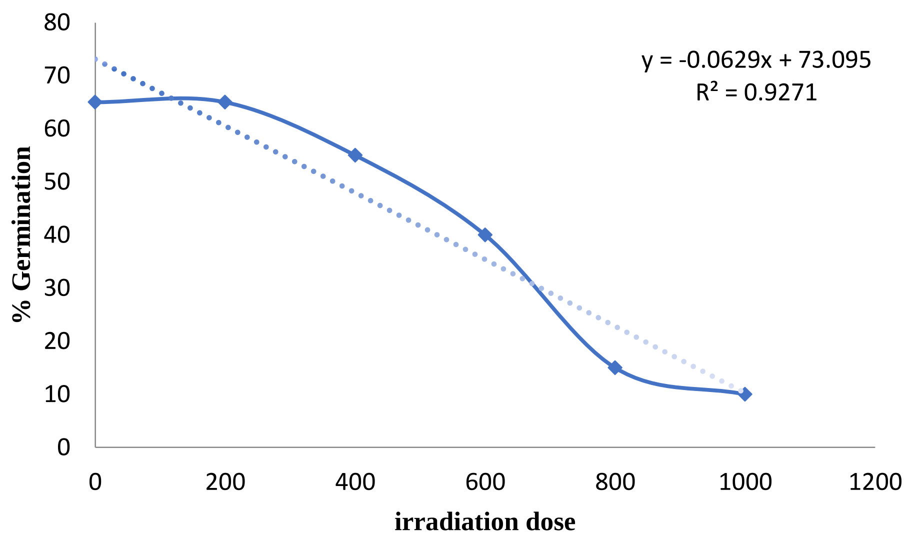

Figure 4.11: Effect of gamma irradiation on the germination of *C. pulcherrima* seedlings – mottle-flowered variety.

Table 4.8: Effect of gamma irradiation on the germination and survival of seedlings of *C. pulcherrima* (yellow flower and mottle variety)

Gamma Dose (Gy)	Yellow flower variety				Mottled flower variety			
	Days after germination (%)			Survival at 30 DAG	Days after germination (%)			Survival at 30 DAG
	Day 5	Day 10	Day 15		Day 5	Day 10	Day 15	
0	55±0.51[a]	80±0.47[a]	80±0.47[a]	80±0.51[a]	35±0.49[a]	65±0.50[a]	65±0.50[ab]	60±0.50[a]
200	30±0.47[ab]	65±0.47[ab]	65±0.50[ab]	30±0.47[ab]	35±0.49[a]	65±0.49[a]	75±0.44[a]	65±0.49[a]
400	40±0.50[ab]	50±0.51[ab]	50±0.51[ab]	25±0.44[ab]	30±0.47[a]	55±0.50[ab]	60±0.50[ab]	55±0.51[a]
600	50±0.51[a]	45±0.47[ab]	70±0.47[a]	5±0.22[b]	10±0.31[a]	40±0.51[ab]	60±0.50[ab]	5±0.22[b]
800	25±0.44[ab]	20±0.51[c]	50±0.51[ab]	00±0.00[b]	10±0.31[a]	15±0.41[bc]	20±0.41[bc]	00±0.00[b]
1000	5±0.22[b]	10±0.47[c]	30±0.47[b]	00±0.00[b]	5±0.22[a]	10±0.31[c]	10±0.31[c]	00±0.00[b]

Means followed by different letters are significantly different (P≤0.05) according to Turkey's pairwise comparisons.

4.4.2 Effect of gamma radiation on morphometric features of yellow and mottled flower varieties of *C. pulcherrima*.

Records on effect of gamma irradiation on number of leaves, height and number of branches as well as flowering were taken on 28, 42 and 56 days after sowing.

The height of shoots significantly ($P \leq 0.05$) decreased from 21.7 cm in the controls to 12.50 cm at 600 Gy in the yellow flower variety (Table 4.9). There was no survival at 800 to 1000 Gy and therefore no measurement was taken. The plants continued to grow and the height almost doubled when records were taken 42 days after germination in all the treatments including the controls. The height of the control plants still differed significantly from the irradiated seeds when statistical analysis using ANOVA was done (Appendix 4d). At 600 Gy, the height of plants was 21.00 cm indicating almost twice the height at 28 days after germination (12.5 cm). The growth of the plant continued resulting in an increase in height when the data was taken at 56 days after germination. Although, the growth of plants was high, the increase was not as high as was recorded on the 42 days after germination. The growth in height within the dose treatments was more profound when seeds were irradiated at 200 Gy where the growth difference was more than 20.0 cm (from 33.62 to 54.12 cm). This stimulation of growth at low doses is known as hormesis effect. The growth rate was low at 600 Gy.

Similar growth pattern was observed in the mottled flower variety (Table 4.10). However, in this variety, even though the plants grew, the height of the control plants was not significantly higher than the treatments. Similarly, the growth of plants from 600 Gy treated seeds were significantly lower (10.17 cm) than the controls and the remaining dose treatments as well as in the yellow flower variety (12.50 cm) indicating that the mottled flower is more sensitive to gamma irradiation. The growth of plants at 42 days after planting was comparatively higher in all the treatments including the controls. It ranged from 44.36 cm at 200 Gy to 30.33 cm at 600 Gy. With the exception of seeds treated at 600 Gy, the growth rate in mottled flower variety was comparatively higher than the yellow flower variety where growth rate ranged from 44.36 cm at 200 Gy to 18.33 cm at 600 Gy. In mottled flower variety, gamma irradiation stimulated shoot growth at 200 Gy, an observation which did not occur in the yellow flower, again confirming that this variety is more sensitive to irradiation. The difference in growth rate between the 28th to 56th days ranged from 85.63 cm at 200 Gy to 37.42 cm at 600 Gy in mottled flower variety compared to 61.38 cm to 33.25 cm at 600 Gy in yellow flower variety. For the controls, growth of the plants between the same period was 60.85 cm and 39.63 cm in mottled and yellow flower respectively. At higher doses of irradiation (400 to 600 Gy), twenty (20%) of plants derived from seeds were dwarfed as a result of high irradiation dose treatment (Figure 4.15).

Figure 4.12: Effect of gamma irradiation on plant height (dwarf plant) at 63 days.
(Bar indicates 2mm).

Table 4. 9: Effect of irradiation on plant height, number of leaves, branches and height at flowering in yellow flower variety

Gamma dose (Gy)	Height (cm)			Number of Leaves			Number of branches at 56 days	Height at flowering
	28	42	56	28	42	56		
0 (control)	21.75±4.83^{a}	40.50± 8.65^{a}	61.38±7.31^{a}	12.13±3.40^{a}	18.00±2.14^{a}	22.50±3.25^{a}	1.75±0.46^{a}	62.75±15.79^{a}
200	18.88±6.22^{ab}	33.62±7.95^{ab}	54.12±12.05^{ab}	13.63±2.83^{ab}	17.75±2.12^{ab}	23.38±3.58^{a}	1.63±0.74^{ab}	61.38±10.74^{a}
400	15.25±3.06^{bc}	27.88± 5.22^{b}	39.00±8.83^{c}	9.75±3.77^{ab}	13.63±3.62^{abc}	18.25±4.10^{ab}	2.50±0.93^{ab}	44.75±9.72^{b}
600	12.50±2.20^{c}	21.00±4.91^{b}	33.25±6.77^{c}	7.50±3.54^{ab}	10.17±2.93^{c}	16.50±4.95^{ab}	1.33±0.52^{b}	39.62±6.00^{b}
800	Nd	Nd	nd	nd	nd	nd	nd	Nd
1000	Nd	Nd	nd	nd	nd	nd	nd	Nd

Means followed by the same letter are not significantly different at ($P \leq 0.05$) according to Tukey's pairwise comparisons.

Note: nd indicates not determined.

Table . 4.10: Effect of irradiation of plant height, number of leaves, branches, flowers and number of days to flowering in mottled flower.

Gamma dose (Gy)	Height (cm)			Number of leaves			Number of branches at 56 days	Height at flowering
	28	42	56	28	42	56		
0 (control)	18.40±3.38[a]	39.97±9.21[a]	79.25±17.73[ab]	9.17±2.33[a]	17.25±3.22[a]	19.33±2.19 [a]	1.58±0.52[b]	89.58±10.58[a]
200	20.83±4.90[a]	44.36±11.78[a]	85.63±15.71[a]	10.33±4.62[a]	18.50±3.61[a]	20.42±3.15[a]	1.92±0.79[ab]	98.96±7.25[a]
400	17.50±4.83[a]	39.95±12.50[a]	68.83±24.59[b]	9.667±3.03[a]	16.58±5.00[a]	18.83±5.04 [a]	2.50±0.80[a]	79.33±21.65[a]
600	13.75±6.42[b]	18.33±12.46[b]	37.42±21.18[c]	4.50±2.36[ab]	5.84±2.50[b]	7.62±5.00[b]	0.42±0.52[c]	58.80±12.33[b]
800	nd	Nd	nd	nd	nd	nd	nd	nd
1000	Nd	Nd	nd	nd	nd	nd	nd	nd

Means followed by the same letter are not significantly different at (P≤0.05) according to Tukey's pairwise comparisons.Note: nd indicates not determined.

4.4.3 Effect of higher gamma irradiation dose on number of leaves and branches produced by seedlings

The number of leaves produced by each plant was counted on 28, 42 and 56 days after sowing to ascertain the effect of gamma irradiation on the leaf production. The numbers of leaves developed were also counted on the same days the heights of shoots were measured. The mean number of leaves was high per plant in both varieties. In yellow mottled flower variety, it ranged from 13.63 at 200 Gy to 7.50 at 600 Gy. Plants obtained from seeds irradiated at 200 Gy produced significantly more leaves than the remaining treatments as well as the controls (Appendix 4h). As the plants grew, more leaves were produced at 42 days after germination. The number of leaves produced from control (non-irradiated) seeds was 17.25 as compared to those irradiated at 200 Gy (18.50). The least number of leaves 10.17 was observed at 600 Gy which is significantly different from control and the remaining doses (Appendix 4i).

Additionally, the number of leaves (22.50) produced at the controls and the 200 Gy (20.42) were significantly different from seeds irradiated at 600 Gy. After 56 days of germination the number of leaves increased from 9.17 to 19.33 in the controls while for those seeds irradiated at 200 Gy, the number of leaves increased from 13.63 to 23.38 higher than the controls (Table 4.9).

Comparatively, the number of leaves produced by the mottled flowered plants was significantly higher than the yellow flowered variety. The highest number of leaves (10.33) were produced when seeds were irradiated at 200 Gy followed by those irradiated at 400 Gy (9.67) (Table 4.10). However, these were not significantly different from the controls.

In mottled flowers, significant differences in leaf production occurred between controls (0 Gy) to 400 Gy and 600 Gy. At day 28, 4.50 leaves were produced at 600 Gy. The number of leaves increased rapidly after 42 days after sowing. In all the treatments including the controls, the number of leaves produced almost doubled. At 200 Gy, the number of leaves increased from 10.33 at day 28 to 18.50 at day 42 while those produced by the controls increased from 9.17 to 17.25.

The increase in number of leaves marginally increased at 56 days after planting in all doses including controls. The stimulatory effect of low dose of 200 Gy was still observed as the number of leaves was comparatively higher than the controls. Morphologically the leaves produced by plants irradiated at higher doses showed great variation in length and size of pinnate leaves (Figure 4.13)

Figure 4.13 Effect of gamma irradiation on leaf morphology on 56th day.

The number of branches produced by the plants as well as the height at which the first flowers were produced was also recorded on day 56 after germination (Table 4.9). In both plants, the number of branches produced was high at 400 Gy with a mean of 2.50 branches per plant. The number of branches produced by plants at this dose was significantly higher than the controls and the remaining treatments (Appendix 3l and 4l). The least number of branches was produced at 600 Gy. At this dose the number of branches produced by the yellow flower variety was comparatively higher than the mottled flower variety. The height at flowering was also significantly influenced by the gamma irradiation decreasing as the dose of irradiation increased (Table 4.9 and 4.10).

Gamma irradiation also influenced the number of spines produced on the shoot of *C. pulcherrima.* About fifteen percent (15.4%) of mottled flower plants irradiated at 400Gy had spineless stem (Figure 4.15A) while 7.7% plants obtained from 400 Gy of the mottled flower formed semi deciduous plants and did not shed their leaves (Figure 4.11C).

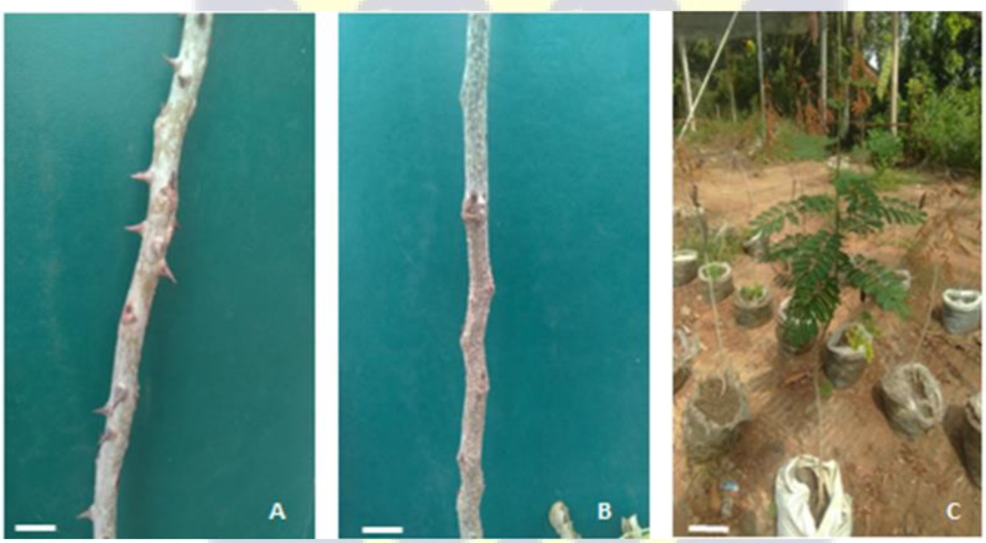

Figure 4. 14: Effect of irradiation on morphometric features of *C. pulcherrima* – mottle flower (A) Thorny stem (control), (B) Thornless stem at 400 Gy and (C) Non shedding bushy variant at 400 Gy. (Bar indicates 4mm).

4.4.4 Effect of gamma irradiation on flower production

The number of days to 50% of plants to flower was also influenced by gamma irradiation in both species. In yellow flowered variety, 200 Gy resulted in early flowering (154 DAG) while in the controls it took 159 days to flower (Table 4.11). Similarly, in mottled flower variety irradiation reduced flowering to 184 days compared to 207 days in the controls. Of the two varieties, gamma irradiation stimulated flowering 30 days earlier in yellow flower variety (154 days) than mottled flower (184 days). However, there were no significant differences on the effect of gamma irradiation to days of flowering in both varieties. While in yellow flowered variety 400 Gy delayed flowering (170 days), in mottled flower variety 600 Gy delayed flowering (199 days).

Similarly, the number of flowers produced in both plant varieties followed the same trend. Gamma irradiation dose of 200 Gy produced comparatively more flowers than the controls in both varieties. The yellow variety produced significantly more flowers (6.13) than yellow flowered variety (5.17). Also, the number of flowers produced by mottled flower variety differed significantly between 400 and 600 Gy (Appendix 5n).

The flowers produced showed morphological variation compared to the controls (Figure 4.11) in flower colour, size and shape. More than seven percent (7.7%) of the mottle flower variety produced from seeds irradiated at 200 Gy produced flowers with reduced petal size while 7.7% had faded mottled flowers (Figure 4.15). Also, at 400 Gy, 7.7% of the flowers produced had abnormal flowers with short stamens (Figure 4.16).

Figure 4.15. Effect of gamma irradiation on the morphological traits of flowers in *C. pulcherrima*. Bar indicates 3mm

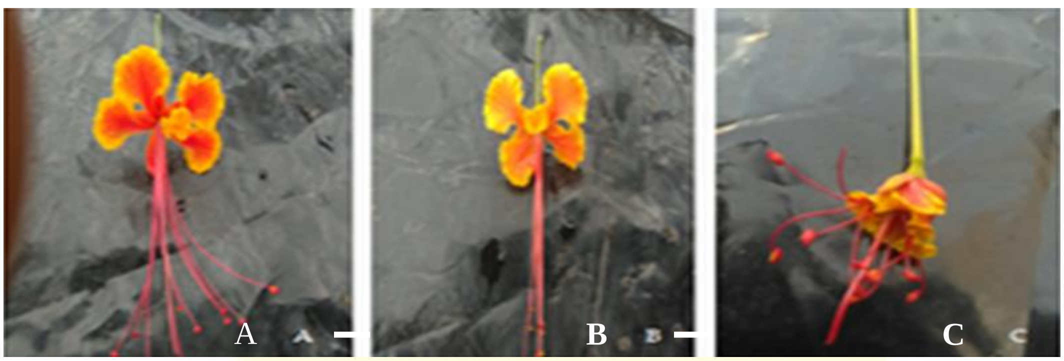

Figure 4.16: Effect of gamma irradiation on petal colour and shape of mottled flower variety (A) Normal shape and colour (B) faded colour 200Gy (C) abnormal petal shape 400Gy. Bar indicates 2mm.

Table 4.11: Effect of gamma irradiation on days to flowering and number of flowers per plant.

	Yellow flowers		Mottled flowers	
Gamma dose (Gy)	Number of days to 50% flowering	Number of opened flowers	Number of days to 50% flowering	Number of opened flowers
0 (control)	159.4±11.80[a]	5.75±2.49[a]	207.7±20.87[a]	4.67±2.23[a]
200	154.2±12.41[a]	6.13±1.96[a]	184.7±10.50[a]	5.17±1.90[a]
400	170.4±17.45[a]	5.25±2.38[a]	193.2±25.27[a]	4.50±2.39[a]
600	174.0±14.10[a]	5.00±2.83[a]	199.5±70.20[a]	1.83±1.53[b]
800	nd	nd	nd	nd
1000	nd	nd	nd	nd

Means followed by the same letter are not significantly different at ($P \leq 0.05$) according to Tukey's pairwise comparisons.

4.5 Propagation of *Canna indica*

4.5.1 Effect of site of scarification on germination of *C. indica* seeds

The effect of site of scarification on germination of *C. indica* seeds was determined. Scarification of seeds at either micropylar end or any side of the seeds enhanced germination (Figure 3.2) and (Figure 4.3) while non-scarified seed did not germinate. Of the two scarified treatments, those with scarification at any part led to significantly higher percentage germination (70%) than those scarified at the micropylar end (10%) (Figure 4.17)

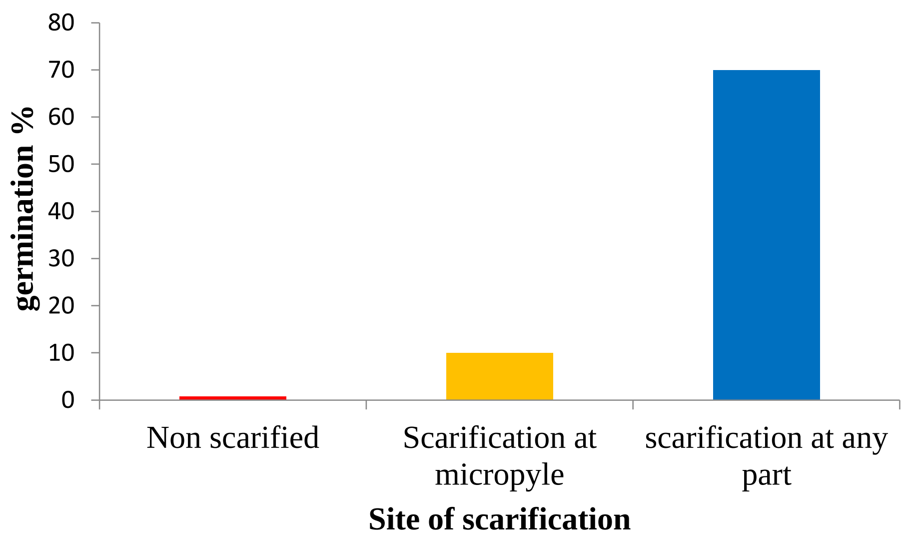

Figure 4.17: Effect of site of scarification on germination of *C. indica* seeds

4.5.2 Effect of scarification pre-treatment on germination of *C. indica* seeds under *ex-vitro* and *in vitro* conditions

In this study, an experiment was designed to find the effect of two scarification treatments followed by sowing under *ex-vitro* and *in vitro* conditions on germination. The scarification treatment and medium in which they were sown had effect on germination of *C. indica* seeds (Table 4.12) (Figure 4.17) Seeds scarified and sown under *in vitro* condition significantly ($P \leq 0.05$) increased germination (89%) compared to seeds scarified and sown *ex-vitro* (30%). Similarly, the percentage germination of seeds scarified with warm water and sown *ex-vitro* was higher (45%) than the controls suggesting that both scarification and the medium in which the seeds were sown had positive influence on *C. indica* seeds.

Table 4.12: Effect of scarification pre-treatment on germination of *C. indica* seeds under *ex-vitro* and *in vitro* conditions

Scarification treatment	Germination (%)
Control	0.0
Scarified and sown *ex vitro*	30
Scarified with warm water (45°C) and sown *ex vitro*	45
Scarified and sown *in vitro*	89

4.5.3 Effect of irradiation and scarification on *C. indica* seeds

To further improve germination, scarified seeds (scratched) were irradiated and cultured on Murashige and Skoog (1962) basal medium under *in vitro* and *ex vitro* conditions. After 21 days of culture, all irradiated seeds cultured under *in vitro* conditions independent of the dose germinated (Figure 4.18) while seeds sown under *ex vitro*, germination occurred only at 200 Gy, 300 Gy and 400 Gy. However, percentage germination did not follow any particular trend and ranged from 70% to 100%. All seeds irradiated at 100 Gy, 300 Gy, 400 Gy, 700 Gy, 800 Gy, 900 Gy and 1000 Gy produced 100% germination after 21 days of culture while for the remaining dose treatments the percentage germination was 70% for seeds irradiated at 200 and 500 Gy and 90% for 600 Gy. Only 50% of the non-irradiated seeds (controls) germinated suggesting that irradiation had positive effect on germination of *C. indica* seeds. While germination *ex vitro* ended on the 10th day beyond which there was no more germination, *in vitro* germination continued up to the 21st day. All the *ex vitro* seedlings died as a result of pest attack.

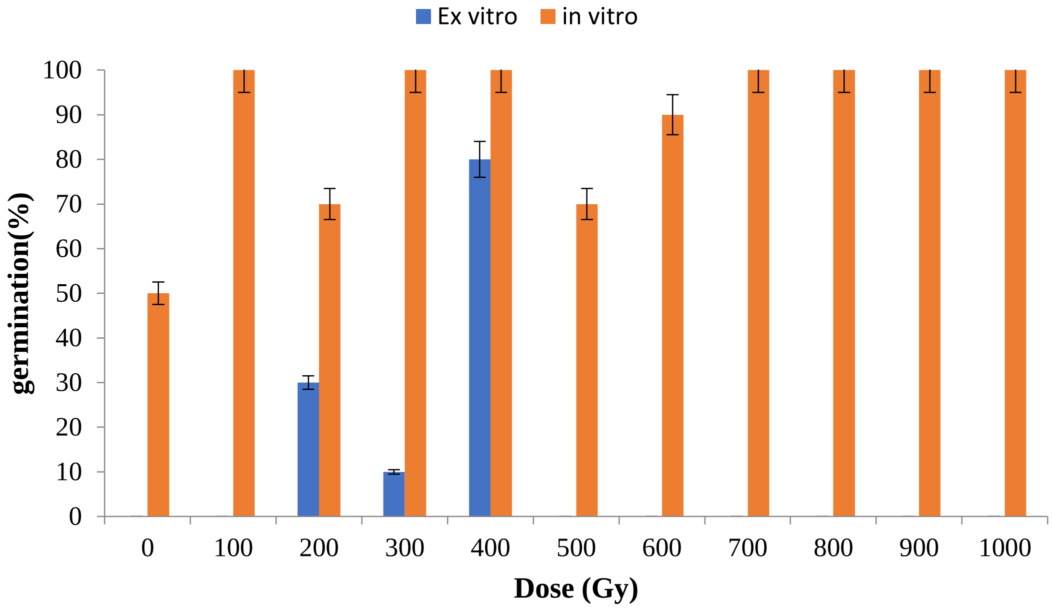

Figure 4.18: Effect of *in vitro* and *ex vitro* conditions on germination of *C. indica* seeds. Data were taken 21 days after culture.

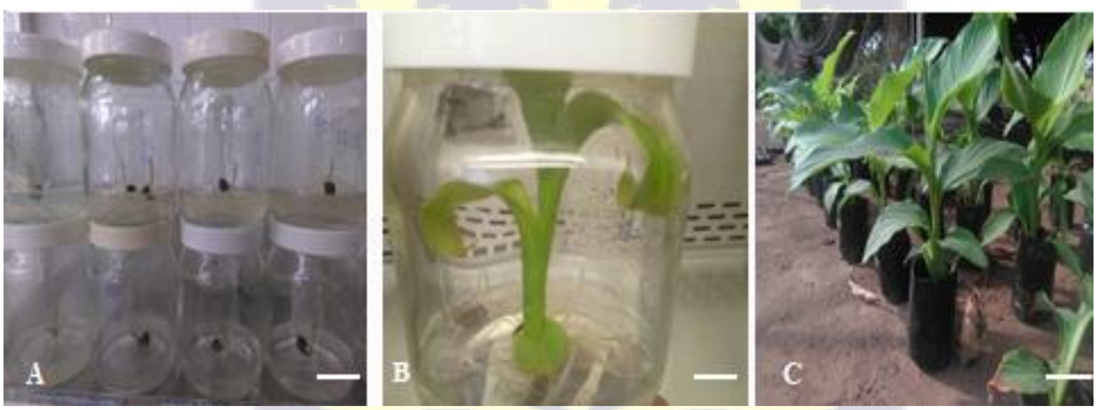

Figure 4.19: Effect of pre-treatment on germination of *C. indica* seeds (A) scarified seeds germinating under *in vitro* conditions, (B) plantlet development 21 days after germination, (C)weaned plantlets growing under plant barn 6weeks after acclimatization. (Bars; A=8mm, B=5mm, C= 3mm)

4.5.4 Effect of irradiation on germination, number of leaves and roots of *C. indica* under *in vitro* conditions.

The number of leaves and roots were counted on the 15th day after culture. The number of roots and number of leaves were significantly different among different treatments ($p \leq 0.05$) (Appendix 6b and c among treatments applied). Seeds irradiated at 300 Gy produced the highest number of leaves (1.90) and roots (14.10), followed by 800 Gy with 1.50 number of leaves and 12.80 numbers of roots. The least number of leaves 0.20, and roots (1.30) were produced at 200 Gy (Table 4.13).

Table 4.13: Effect of irradiation on germination, number of leaves and number of roots of *C. indica* under *in vitro* conditions.

Gamma Doses	% Germination after 15 days	Number of leaves Day 15	Number of roots Day 15
0 Gy	50±0.53[b]	0.40±0.84[b]	4.20±6.37[bc]
100 Gy	100±0.00[a]	0.80±0.79[ab]	6.60±7.09[abc]
200 Gy	70±0.48[ab]	0.20±0.63[b]	1.30±4.11[c]
300 Gy	100±0.00[a]	1.90±0.32[a]	14.10±2.47[a]
400 Gy	100±0.00[a]	1.20±0.92[ab]	12.80±5.20[ab]
500 Gy	70±48[ab]	1.00±1.05[ab]	8.10±8.69[abc]
600 Gy	90±0.32[ab]	1.30±1.16[ab]	10.40±7.44[abc]
700 Gy	100±0.00[a]	0.90±0.99[ab]	9.50±7.00[abc]
800 Gy	100±0.00[a]	1.50±1.18[ab]	12.80±3.16[ab]
900 Gy	100±0.00[a]	0.70±0.95[ab]	7.50±6.92[abc]
1000 Gy	100±0.00[a]	0.80±1.03[ab]	6.80±6.44[abc]

Means followed by the same letter are not significantly different at ($P \leq 0.05$) according to Tukey's pairwise comparisons

4.5.5 Effect of irradiation on post-flask plantlet survival, plant height, number of leaves and suckers and days to flowering *ex vitro*.

Plantlets cultured under *in vitro* conditions were weaned 21 days after culture. After 3 days of weaning, all the controlled plantlets as well as those irradiated at 200 Gy and 1000 Gy survived (Table 4.14). For the remaining treatments, plantlet survival ranged from 50% in 500, 60% in 300, 400, 600, and 700 Gy, 67% in controls and 80% in 800 and 900 Gy. The effect of gamma irradiation on survival was statistically not different ($P \geq 0.05$) from the controls (Appendix 6f).

The height of the plants varied depending on the dose of irradiation. It decreased as the dose of irradiation increased. The fastest growth (62.30cm) was observed when seeds were irradiated at 200 Gy followed by 100 Gy (55.20 cm) and 300 Gy (49.30 cm) in that order (Table 4.14). At these doses of irradiation, the heights of the plantlets were higher than the controls (48.25 cm). Thereafter, the growth (height) of the plantlets was retarded by the irradiation as increasing the dose gradually decreased the height of the plant. Statistical analysis using ANOVA showed that there was a significant difference ($p \leq 0.05$) between plants obtained from irradiation and controls (Appendix 6g).

The number of leaves produced by the plantlets under post-flask *ex vitro* conditions was also influenced by the irradiation. The effect of gamma irradiation on the morphology of the shoot and leaves is shown in Figure 4.20. The number of leaves produced followed the same trend as the growth of the plant as the dose of irradiation increased, the number of leaves decreased. The number of leaves produced by the irradiated plants ranged from 5.00 to 6.67 and these were less than the controls (7.00) indicating that the irradiation adversely affected leaf development. The number of leaves was high (6.67) when the seeds were irradiated at 300 Gy while the least (5.00) was produced by 500 and 900 Gy. Statistical analysis using ANOVA showed no significant differences between irradiation doses and the controls (Appendix 6b).

Gamma irradiation of the seeds also affected the number of suckers produced. The non-irradiated controls did not produce multiple shoots while the irradiated seeds produced a mean of 1.5 suckers per seed. Seeds irradiated at 500 Gy and 800 Gy produced the highest number of suckers (3.0), followed by 1000 Gy (2.40) (Table 4.14).

4.5.6 Effect of irradiation and days to maturity.

Plants obtained from irradiated seeds prior to sowing either had delayed flowering or enhanced early flowering. Gamma irradiation gradually delayed flowering until it peaked (140 days) at 500 Gy and thereafter it decreased days to flowering more than the controls. Although gamma irradiation reduced the days to flowering to 116 days at 600 Gy, the reduction was statistically not significant ($P \geq 0.05$) compared to the non-irradiated controls (137.5 days) (Table 4.14). The dose that significantly delayed flowering is 500 Gy where plants took 140 days to flower. Plants obtained from seeds irradiated at 600 Gy or higher reduced the number of days to flowering. With the exception of seeds irradiated at 600 Gy, all seeds irradiated took at least 125 days to flower.

Table 4.14: Effect of irradiation on plant height, number of leaves, multiple sucker development and number of days to flowering 117 days after sowing *ex vitro*.

Dose rate	Survived plantlet after weaning (%)	Plant Height (cm)	Number of leaves	Number of multiple suckers	Number of days to flowering
0Gy (control)	67±0.58[a]	48.25±0.35[abcd]	7.00±0.00[a]	1.00±0.00[a]	137.5±0.71[a]
100Gy	75±0.50[a]	55.20±4.95[ab]	5.67±0.58[a]	2.33±0.58[a]	125.3±13.65[a]
200Gy	100±0.00[a]	62.30±7.80[a]	5.75±0.50[a]	1.75±0.50[a]	138.2±1.26[a]
.300Gy	60±0.55[a]	49.30±2.69[abc]	6.67±3.79[a]	2.33±0.58[a]	139.3±1.16[a]
400Gy	60±0.55[a]	47.90±3.14[abcd]	5.67±1.16[a]	1.67±0.58[a]	139.3±1.16[a]
500Gy	50±0.58[a]	46.00±0.00[abcd]	5.00±0.00[a]	3.00±0.00[a]	140.0±0.00[a]
600Gy	60±0.55[a]	45.93±1.60[bcd]	5.67±1.53[a]	2.00±1.00[a]	116.0±17.16[a]
700Gy	60±0.55[a]	39.55±6.15[bcd]	5.25±1.71[a]	2.00±0.82[a]	125.0±8.64[a]
800Gy	80±0.45[a]	39.63±9.30[bcd]	5.50±1.00[a]	3.50±1.29[a]	126.2±8.69[a]
900Gy	80±0.50[a]	34.35±7.99[cd]	5.00±0.00[a]	1.50±0.71[a]	131.5±9.19[a]
1000Gy	100±0.00[a]	33.12±5.54[d]	5.20±0.84[a]	2.40±1.14[a]	129.0±6.52[a]

Means followed by the same letter are not significantly different at ($P \leq 0.05$) according to Tukey's pairwise comparisons

4.5.7 Effect of irradiation on the morphology of *C. indica*

Gamma irradiation created morphological variation on the leaves and height of the plant (Figure 4.20). The control plants were tall and developed broad leaves (Figure 4.20A) while the irradiated plants (800 Gy) were dwarfed with narrow leaf size as well as multiple shoots (Figure 4.20B). Seeds irradiated at 1000 Gy developed short but thick shoots (Figure 4.20C).

Figure 4.20: Effect of gamma irradiation on *C. indica* growth (A) Control (B) 800 Gy and (C) 900 Gy. Data was taken 117 after days after weaning. (Bar indicates 4mm).

CHAPTER FIVE

DISCUSSION

5.1 Survey on economic importance and mode of propagation of flowers in Ghana

The floriculture industry is rapidly emerging in Ghana offering job opportunities for both males and females mostly among the youth. To ascertain the economic importance of floriculture industry in Ghana, a survey was conducted in the Greater Accra and a small segment of Eastern Region. The survey also sought to identify challenges that confront the industry players. Out of the 120 respondents, more than 40% were between the ages of 36 – 50 indicating that young people are more engaged in the flower industry than the elderly with ages between 50 and 60 years. In several countries both in Africa and the developed countries, it has been estimated that the floriculture industry employs more young people. For example, it is estimated that in Kenya, the floriculture industry provides livelihood for over two (2) million people (Embassy of the Republic of Kenya, 2020).

Majority of the respondents were males (73.3%) with only (26.7%) females who are mostly helping their family members especially husbands in the industry. This is contrary to a report by FAO (2010), indicating that women in Tanzania are mostly employed as casual workers; planting, harvesting and grading in flower farms whiles men occupy a small number of managerial positions. All the respondents have had some form of formal education. However, half of the respondents have had only basic education and less than ten percent (10%) have been schooled to the tertiary level. A report by FAO (2010) suggests that education and training are fundamental determinants of employment outcomes in any labour market, especially in the flower industry where skilled labour is required in new technologies employed in the propagation and breeding of ornamental plants.

According to the survey, many plant species belonging to different families are cultivated for their aesthetic values in Ghana. However, in the present survey the most commonly propagated ornamental plant is the *Roystonea regia* (Arecaceae family). This observation may be attributed to its aesthetic appearance in landscaping, making it one of the most economically important plants in the world. In many historical cultures, palms are symbols for such ideas as victory, peace, and fertility. Today, palms remain a popular symbol for the tropics and vacation spots and have a lifespan up to 100 years depending on the species (Palm, 2008).

Majority of the respondent preferred flowers with multiple bright or mottled petal colours. The reason may be that naturally, these colours are aesthetically appealing and are employed as cut flowers which are used for decorations, funerals and other cultural activities or for landscaping to beautify the environment. According to Stratcomm Africa (2017), global celebrations such as New Year, Valentine's Day, Mother's Day and Christmas as well as individual occasions such as weddings, birthdays and funerals are all done using flowers to express sentiments towards each other.

The survey also revealed that the major challenge confronting the floriculturist is technical know-how on mode of propagation of ornamental plants. This lack of technical know-how may be attributed to the low level of education of the industry players. However, for the growth of the industry in Ghana, there is the need to develop new technologies for propagation of these ornamental plants. Thus, in this study, more than eighty percent (80%) of the respondents indicated that they were ready to accept and use modern methods of propagation and improved planting materials. Mishra *et al.*, (2015) have reported that modern biotechnological techniques such as micropropagation may be used for rapid multiplication of ornamental plants and has an added advantage of cleaning vegetatively propagated varieties off viruses. Such modern propagation methods can be used to address pest and diseases which the respondents indicated as a challenge in the horticultural industry.

5.2 Germination of *C. pulcherrima*

Floriculturists surveyed in Greater Accra and some part of Eastern region revealed that propagation is a major challenge in the industry. This may be attributed to several factors including the use of vegetative propagation for multiplication as most of the plants produce non-fertile seeds. However, in the present study, both yellow and mottled flower varieties of *C. pulcherrima* had more than 75% germination suggesting that industry players can use sexual propagation to multiply the plant to meet market demand. Although this study achieved higher percentage of germination, it needs to be improved. Several factors including dormancy and position of the seed in the pod are known to influence germination of leguminous seeds and *C. pulcherrima* is no exception. Ferreira *et al.*, (2019) observed that in the members of the Fabaceae family, the position of the seed in the pod has significant influence on germination and thus recommended that proximal/median positioned seeds should be used for propagation of the plant. In a study by Ferro *et al.*, (2019) on *C. pulcherrima,* they observed that freshly harvested seeds had 98% germination while those stored for 12 months had 80.5% germination due to possible secondary dormancy. In the present study, the seed used were not freshly harvested and this may have accounted for 75% germination compared to 98% reported by Ferro *et al.,* (2019).

Although seed dormancy is a problem in the members of the Fabaceae family, the high percentage of germination (75%) obtained in the present study does not seem to suggest that dormancy hindered germination. However, to achieve 100% germination, pre-treatment methods such as acid scarification to soften the hard seed coat prior to sowing should be investigated. Opoku *et al.*, (2018) have reported that pre-sowing treatments of seeds of *C. pulcherrima* using hot water, acid scarification and growth regulators effectively broke seed dormancy of leguminous species yielding percentage germination of 96, 86 and 60 percent respectively.

5.3 Gamma irradiation and germination in *C. pulcherrima*

In most ornamental plant species, gamma irradiation has been used to either break dormancy (FAO/IAEA, 2012) or create variation. The effect of irradiation on germination and growth generally depends on dose of irradiation, dose rate, exposure time and species of plant to be irradiated. Irradiation of *C. pulcherrima* seeds using Cobalt-60 gamma source significantly ($P \leq 0.05$) influenced germination. Irradiation of seeds at 100, 200 or, 300 Gy significantly increase germination over 80% compared to the non-irradiated controls suggesting that low doses of gamma irradiation stimulate germination in seeds. Datta (2009) reported that 300–500 Gy reduced germination of *Trigonella foenum-graecum* seeds while low doses of 100 – 200 Gy significantly increased frequency of germination. Low doses of gamma rays have stimulatory effects on seed germination and plant growth, according to Jan *et al.*, (2013), a phenomenon known as radiation hormesis. Low doses of ionising radiation, according to the principle of hormesis, are not only innocuous but also advantageous by boosting the immune system or repair mechanisms (Koch and Schlesinger 2005). Even though the theory applies to animal species it has been observed in several plant species including those used in this study. Jan *et al.,* (2013) explained that low doses of ionizing radiations have modulatory role in the metabolic and biochemical processes of seedlings thereby leading to enhanced growth. It has also been reported that the stimulating causes of gamma ray on germination may be attributed to the activation of RNA or protein synthesis, which occurred during the early stage of germination after seeds were irradiated (El-mahrouk *et al.*, 2013).

When the experiment was repeated using higher dose range (200-1000 Gy), germination significantly reduced when seeds were irradiated with 800-1000 Gy in both yellow and mottled flower varieties suggesting that higher doses had phytotoxic effect on growth. Also, at this same higher doses the germinated seedlings did not survive indicating the lethal effect of these higher doses. El-mahrouk *et al,*. (2013) observed that the inhibition of seed germination at high doses could be due to the damage to seed tissue, chromosomes and subsequent mitotic retardation with the severity of the damage depending on the dose of irradiation. According to Songsri *et al*., (2011), higher doses of gamma radiation reduced germination of physic nut seeds and number of plants that survived. In another study on *Cuminum cyminum,* Verma (2017) observed that germination and seedling survival improved at a lower dose (100 Gy) but declined at higher doses particularly at 500 Gy. Similarly in the present study, low doses of 200 Gy resulted in 100% seedling survival while higher doses of irradiation (600-1000 Gy) significantly reduced seedling survival, an observation similar to that of Verma (2017). Marcu *et al.,* (2013), have explained that growth inhibition induced by higher doses of irradiation may be attributed to cell cycle arrest during somatic cell division and/or to a variety of damages in the entire genome.

5.4 Gamma irradiation on morphometric traits and creation of genetic variability of *C. pulcherrima*

The effect of ionising radiation on both food and tree crops is well documented by several authors and have been used as basis for selection of several mutant varieties. Acute high external doses of ionising radiation have long been known to affect most aspects of shoot growth and development (Nishiguchi *et al.*, 2012; Sidler *et al.*, 2015), morphology (Celik *et al.*, 2014; Sever-Mutlu *et al.*, 2015), anatomy (De Micco *et al.*, 2014) and the development of bulbs (Mostafa *et al.*, 2015) resulting in the creation of genetic variability. There are also recent reports that acute irradiation at high doses, may have positive as well as negative effects on subsequent growth. Yue and Ruter (2020) reported that irradiation of *Panovia missionum* had some positive as well as negative effects on subsequent height, leaf area, flower diameter and stem diameter. In the present study, seeds of *C. pulcherrima* irradiated with gamma rays (200-1000 Gy) had significant effect on morphometric features of the surviving plants. At higher doses there were significant reductions in plant height, morphology of leaves and flowers, number of branches produced, days to flowering and number of flowers. Consequently, at 600 Gy a dwarfed plant with significantly reduced height (32 cm), reduced number of flowers and reduced pinnate leaf size with short fruit length was produced. Additionally, at 400 and 600 Gy some of the shoots had reduced spines or they were completely spineless as well as reduced petiole and pinnate leaf size. Furthermore, there were also reduced stamens compared to long ones in the controls (Figure 4.13). Such morphological variations have been observed in several plant species (Khah & Verma, 2015), suggesting possible genetic changes associated with gamma irradiation of seeds. Although these morphological variants may be attributed to genetic variations created in *C. pulcherrima* by irradiation of seeds at higher doses of 400 to 600

Gy, due to time constraints the M1 seeds could not be planted for subsequent M2 and M3 generation to ascertain or confirm these variations. In a study on *Curcuma longa*, Ilyas and Naz (2014) explained that irradiation of seeds may cause genetic variability that enable plant breeders to select new genotypes with improved characteristics. However, at the present M_1 generation it is difficult to classify them as putative mutants or mutant lines due to chimera formation within the population. Thus, they can only be classified as mutant lines at M_2 or M_3 generation but due to time constraints, this could not be done in the present studies. This study therefore was used to determine the optimal dose (LD_{50}) or sensitivity of the seeds to gamma rays as a precursor for mutation induction in *C. pulcherrima* species.

5.5 Lethal dose (LD_{50}) of *C. pulcherrima*

For mutation breeding programmes the determination of LD_{50} is a prerequisite because it limits the use of wide range of doses in large populations and also reduces labour involved in handling of large mutant populations. Thus, for future improvement of *C. pulcherrima,* the LD_{50} was determined using germination response 10 days after planting which was found to be 583.33 Gy and 645.39 Gy for yellow and mottled flowers variety respectively to serve as a guide or provide a baseline for choice of gamma dose to be used for irradiation. The LD_{50} determines the sensitivity of plant species or propagules to ionising radiations and may vary from species to species. It may be low indicating high sensitivity or high indicating low sensitivity. The differences in the LD_{50} between yellow and mottled flower variety suggests that yellow flower variety is more radiosensitive than the mottled flower variety. Datta (2009) has observed the varietal differences in radio-sensitivity in most of the species/varieties which indicate that a genotype dependent mechanism is involved in the damage or repair of radiation induced damage within the organism. According to Sparrow and Miksche (1961), there is a strong connection between a species' nuclear volume and its radio-sensitivity. They also stated that examining 23 species of herbaceous plants with a wide range of nuclear volumes indicated that as nuclear capacity increased, radio-sensitivity increased proportionally.

5.6 Propagation in *C. indica*

5.6.1 Scarification and germination

C. indica is also an ornamental plant propagated and marketed for its aesthetic value. However, the rate of multiplication is seriously constrained due to its seed coat induced dormancy making sexual propagation extremely difficult even though it has high frequency of seed production. Seed dormancy plays a critical role in seed storage and conservation. It allows seeds to be stored for a long period before sowing for the next season. According to Mensah and Ekeke (2016), the softening of hard seed coat is necessary to enhance imbibition of water and diffusion of oxygen which are needed to initiate germination process in the seed and eventual protrusion of the radical and subsequent germination. Thus, the current study was conducted to address sexual propagation challenges of *C. indica* as well as the use of micropropagation techniques for rapid multiplication of the plant. To overcome the seed coat dormancy limitation, seeds of *C. indica* scarified either at the micropylar end or any side of the seed resulted in germination. However, scarification on any side of the seeds was more effective as it resulted in high frequency of germination (70%) than the micropylar end. Scarification of the seeds allowed imbibition for water, which stimulated the enzymes involved in germination thereby leading to higher frequency of germination. The lower percentage of germination observed at the micropylar end of scratched seeds could be attributed to damage to the embryo. The embryo is the embryonic plant containing the radicle and plumule which subsequently develop into root and shoot of a plant and therefore damaging it through scarification destroys germination. These observations suggest that dormancy in *C. indica* seeds is seed coat-imposed which should be broken before germination commences. According to Gomes *et al*., (2016), mechanical scarification of *C. indica* seeds leads to optimum germination under wide range of temperature. Seed coat induced dormancy is caused by the presence of exotesta constituted by palisade Malpighian cell layers that provide mechanical strength but limit the absorption of water (María, 2012). The results obtained in this study corroborates that of Asif *et al*., (2020) who demonstrated that abrasion with sandpaper and side cutting of seeds were effective for breaking seed dormancy in *Prosopis juliflora* and *Dalbergia sissoo*. Mensah and Ekeke (2016), have observed that physical dormancy in the seeds of *Senna obtusifolia* is constrained by the hard impermeable coverings and was overcome by chemical scarification using sulphuric acid (H_2SO_4). Venugopal *et al* (2009) have reported that acid scarification and pre-treatment soaking of seeds enhanced germination.

5.6.2 Irradiation and scarification

Scarified *C. indica* seeds as described above resulted in high frequency of germination when cultured under *in vitro* conditions using Murashighe and Skoog(1962) basal salts without any hormonal supplements. In addition to moisture, oxygen, temperature and light, which are conditions necessary for seed germination, several other factors including the substrate, have been shown to influence seed germination. The higher percentage of germination achieved in this study could be attributed to the controlled *in vitro* environment with adequate supply of light in the growth room and availability of moisture in the semisolid Murashighe and Skoog (1962) basal medium. Even though, there were no phytohormones, water holding capacity and mineral composition might have played an important role in the germination of the scarified seeds. Soil with sufficient moisture content and important nutrients enhances the rate of germination as well as the further growth of a plant. Additionally, the medium was sterilised prior to culture ensuring that there were no microorganisms which could cause the seeds to rot. However, to improve frequency of germination for rapid propagation of the plant via sexual reproduction, the seeds were scarified and irradiated prior to *in vitro* culture and this resulted in 100% germination. Three factors may explain the higher frequency (100%) germination in this experiment. Firstly, an environment factor which includes ample supply of water, optimum temperature and well aerated medium of growth. Secondly, there was adequate supply of mineral nutrition to stimulate germination. And thirdly, the gamma irradiation stimulated germination in the seeds. As has been already reported in this thesis, low doses of irradiation have stimulatory effect on germination and growth (Jan *et al.*, 2013), a phenomenon known as hormesis. Although all irradiation dose treatments stimulated germination, subsequent plant development significantly affected the morphometric traits. Morphometric traits observed in the developing plantlets showed that higher doses of irradiation had adverse effect on plant height, number of leaves produced and multiple shoot induction except the survival of plantlets. As the dose of irradiation increased, the plant height, number of leaves as well multiple shoot induction correspondingly decreased.

5.6.3 Post-flask survival of plantlets

Successful post-flask acclimatization of plantlets regenerated under *in vitro* conditions makes large-scale micropropagation commercially viable. Post-flask survival rate of plantlets after weaning was high at higher doses of irradiation (700-1000 Gy) contrarily to the effect on morphometric traits. This could have been high cellular capacity for DNA repair, especially double stranded breaks and the development of reproductive structures *de* *novo* (which limits the multi-generation effect of deleterious mutations) consequently physiologically sensitive plantlets developed structures (roots and photosynthetic tissues) for survival against abiotic stress. Caplin and Willey (2018) observed that the individual plants' survival and their populations is based on a life strategy which is coping with stress using high cellular capacity for DNA repair, anti-oxidant pathways and the development of reproductive structures. Similar observation have been reported by Wafa *et al.,* (2016) that the gradual transition process from culture vessel to the greenhouse produced normal plant growth and morphology with plant survival rate as high as 75.0%.

The developing plantlets were influenced by irradiation dose; low doses of 200 Gy stimulated plantlet growth as expressed in increased plant height while high doses (700-1000 Gy) reduced plant height. Similarly, the number of leaves as well as suckers also decreased with increased irradiation dose. The stimulating effect of low doses of gamma irradiation could be attributed to increased enzymatic and cell division activities which resulted in increased morphological traits while the inhibitory effect could be due to chromosomal aberrations and mitotic inhibition (Hernández-Muñoz *et al.*, 2019). Several other authors including Oladosu *et al.,* (2014), Ariraman *et al.,* (2018) and Ke C, *et al.,* (2019) have explained that some plant morphological traits such as plant height, number of leaves, number of branches, and biomass were severely altered due to the inhibitory effects of high mutagen doses on planting materials. They further illustrated that mutagens such as gamma rays (acute and chronic) may cause either negative or positive genetic effects on plant growth and development depending on the nature and quantity of the dosage applied. In their study on diversity induction with gamma radiation on *Dendrobium odoardi orchid*, Fathin *et al.,* (2021) observed that gamma irradiation resulted in changes in morphological traits such as increased plant height and leaf width and also decreased the number of roots and root length as well as changes in leaf shape and colour. All these changes may be attributed to the effect of the gamma rays on genes constituting these traits.

At higher dose of irradiation some of the developing plants were dwarfed, an observation which has been made in *C. pulcherrima* in this study (Figure 4.8). There were also variations in floral and leaf morphology. Plantlets obtained from seeds irradiated at 800 and 1000 Gy developed curled leaf (Figure 4.20) suggesting the creation of genetic variability caused by the gamma rays. These trends are quite common in mutagenized populations due to drastic chromosomal aberrations in addition to genetic mutations (Taheri *et al.*, 2014). Such effects of gamma rays on leaf morphology have been reported by some authors. For example, Huang *et al.*, (2017) reported that different patterns of leaf variegation such as green to yellow sectors were obtained when *Monstera deliciosa* plants were irradiated. Anne and Lim (2020) reported variegated leaves and curled leaf morphology formation in *Hibiscus sp.* and *Rosa sp.* respectively as a result of high doses of gamma irradiation. Similar effects of gamma irradiation on leaves have been observed in this study. However, the curled leaf reported in this study needs further investigation as viral infection could also cause similar effect.

The study also revealed that the number of suckers was increased at 500 and 800 Gy compared to the controls. Sexual propagation of *C. indica* is difficult due to seed coat induced dormancy and therefore propagation is achieved via the use of rhizomes. Therefore the development of multiple suckers as a result of gamma irradiation will augur well for rapid multiplication of the plant for commercial exploitation. Both Barbosa *et al.*, (2005) and Venugopal *et al.*, (2009) have independently reported that propagation of *C. indica* can be achieved through the division of its rhizome which has buds for regeneration. Such division of the rhizome can be done throughout the year thereby increasing the rate of multiplication for commercial exploitation.

To meet the increasing demand for ornamental plants, there is the need to develop varieties with early flowering. Irradiation of seeds of *C. indica* at 600 Gy reduced days to flowering to 117 suggesting early maturity caused by irradiation compared to the controls. Nunoo *et. al.*, (2014) reported that wild tomato (*Solanum pimpinellifolium*) plants irradiated at 300 Gy were the first to attain 50% flowering at 40 days while the control was the last to flower (52 days). Similarly, Asare *et al.*, (2017) observed significant ($P \leq 0.05$) decreased in the number of days (92) to attain 50% flowering when seeds of *Abelmoschus esculentus* were irradiated at 400 Gy. In their study increasing the irradiation doses to 600Gy, 800Gy and 1000Gy increased the number of days to flowering. While the exact reason for early flowering in irradiated plants could not be ascertained in this study, Asare *et al.,* (2017) suggested it may be attributed to delayed germination caused by the mutagen in okra seeds. This is contrary to an observation by El-Khateeb *et al.*, (2017) which suggest that high dose (400 Gy) of gamma irradiation delay flowering and reduced the number of flowers produced in *Helichrysum bracteatum*. Anne and Lim, (2020) have also reported early flowering and spike emergence in *Polianthes tuberosa* at 20 Gy of gamma irradiation.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The flower industry is now emerging and it is a viable and lucrative industry employing both males and females with varied educational backgrounds. In spite of its lucrativeness, the industry has a number of challenges. These challenges range from lack of planting materials, low technical know-how and lack of application of modern propagation methods, land acquisition, lack of water for watering to pest and diseases attack. Both *Caesalpinia pulcherrima* and *Canna indica* are tropical ornamental plants with aesthetic and medicinal values but grow in the wild. Their exploitation for commercial gain is seriously constrained by poor germination as a result of seed dormancy and other limitations. This research therefore focused on developing methods of propagation as well as creation of variability with a view to enhancing its aesthetic values. The following conclusions can be drawn from investigations conducted.

Both males and females are engaged in the flower industry in some parts Greater Accra region and Nsawam in the Eastern Region, providing a means of livelihood for them.

Germination in both *C. pulcherimma* and *C. indica* is low due to seed coat induced dormancy. However, the dormancy effect in *C. indica* is significantly higher than *C. pulcherima*, thus simple scarification by scratching the side of the seed to enhance imbibition of water was enough to break dormancy in *C. indica* while *C. pulcherrima* does not need such treatments.. Additionally, rapid propagation via sexual multiplication can be achieved when scratched seeds are cultured *in vitro.*

This study revealed that low doses of gamma irradiation (100-300 Gy) enhanced germination in both seeds, a phenomenon known as hormesis while higher doses inhibited germination and other morphometric traits.

Thirdly, higher doses of irradiation (400 - 600 Gy) lead to the creation of variation expressed as dwarfed plant, reduced spines, increased number of branches as well as deciduousness in *C. pulcherrima.* However, further investigation is needed to confirm if this variation are genetically induced as a result of the gamma irradiation or epigenetic caused by environmental factors.

Furthermore, gamma irradiation also influenced days to flowering; low doses (200 - 400 Gy) of gamma irradiation induced early flowering, new flower colours, size and shape, in *C. pulcherrima* while high dosage delayed flowering.

Using data from germination, the lethal dose (LD_{50}) for *C. pulcherrima* was calculated to be 583.33 and 645.39 Gy for yellow and mottled flower variety respectively.

Also irradiation doses from 300 Gy to 800 Gy increased the number of shoots and roots of *C. indica* plantlets *in vitro*. The study also revealed that plantlets in *C. indica* seedlings which germinated from high dose irradiation had a higher post-flask survival rate.

Gamma irradiation also influenced days to flowering and morphological traits such as height and number of leaves in *C. indica*.

6.2 Recommendations

1. Since germination in *C. indica* after scarification is still low (70%), the experiment should be repeated using chemical pre-treatment such as sulphuric acid and other dormancy breaking compounds such as growth regulators.
2. To ensure that the variations observed at higher doses in both plants are genetically induced, further investigations are needed for confirmation by planting the M_1 seeds to generate M_2 plants. Seeds of plants irradiated at doses which caused the variation should be sown for confirmation of variants or putative mutants.
3. To achieve rapid multiplication in *C. indica*, further *in vitro* studies should be conducted to improve multiple shoot induction using different concentrations of growth hormones.

REFERENCE

Abou-Dahab, A. M., Heikal, A. M., Taha, L. S., Ahmed, M. M., Metwally, S. A., and Ali, A. R. (2017). *In vitro* mutagenesis induction in *Eustoma grandiflorum* plant using gamma radiation. *Journal of Environmental Science and Technology*, 10(4), 175-185.

Africa Business Insight (2010). Produce Cut flowers in Ghana. https//www.howwemadeitinafrica.com. Date accessed 6th July 2020.

Afshar A, Salehi Fallah Imani, A. S. Soorchi. and Maiver. S. Y. (2014). Effect of H_2SO_4 on Seed Germination and Viability of *Canna indica* L. Ornamental Plant. *International Journal of Advanced Biological and Biomedical Research*, *2*(1), 223–229.

Agrawal, P. K., Dadlani, M. (1995). Techniques in Seed Science and Technology. 2nd Ed. India: *South Asian Publishers Limited* 43(5), 321- 405.

Ahloowalia, B. S., Maluszynski, M., & Nichterlein, K. (2004). Global impact of mutation-derived varieties. *Kluwer Academic Publishers.*, *135*(14), 187–204.

Ahmad, N., Faisal, M., Anis, M., and Aref, I. M. (2010). *In vitro* callus induction and plant regeneration from leaf explants of Ruta graveolens L . *South African Journal of Botany*, *76*(3), 597–600.

Aida, R., Sasaki, K., & Ohtsubo, N. (2016). regeneration from leaf explants Production of chrysanthemum periclinal chimeras through shoot regeneration from leaf explants. *Plant Biotechnology*, 15(3), 1–6.

Al-snafi, A. E. (2015). Pharmacology & Toxicology of *canna indica* - an overview. *International Journal of Pharmacology & Toxicology*, 5(2), 71–75.

Altmann, M., Essen, M., & Essen, I. P. M. (2016). Developments and trends in the flower and plant market for 2015 / 2016. *EUROSTAT*, *7* (October 2015), 47–52.

Andrea K., and Rownak A., (2018). Physical and chemical mutagenesis. *Methods in Molecular Biology*. 16(5), 321-332.

Ariraman Paul, Arockia Naik, Sugali Nanu. Anandan, P and Arunachalam, Arun. (2020). Effect of boron application on growth, yield parameters, yield, quality, nutrient uptake and economics of sunflower. *International Journal of Chemical Studies*. 8(10), 75-85

Asare, A., Mensah F., Acheampong, S., Asare B. E., Armah J., (2017). Effects of Gamma Irradiation on Agromorphological Characteristics of Okra (Abelmoschus esculentus L. Moench.) *Advances in Agriculture* 17(3) 1-7.

Asif, M. J., Ali, A., Mazhar, M. Z., Tanvir, A., Zia, B., Anmbreen, I., Anjum, M. Z., and Mahr, M. S. (2020). Effect of different pre-treatments on seed germination of *Prosopis juliflora* and *Dalbergia sissoo*: A step towards mutation breeding. *Journal of Forest Science*, *66*(2), 80–87.

Barbosa TM, Serra CR, La Ragione RM, Woodward MJ, Henriques AO.(2005) Screening for bacillus isolates in the broiler gastrointestinal tract. *Applied Environmental Microbiology*. 71(2), 968-978.

Bado, S., Yamba, N. G. G., Sesay, J. V., Laimer, M., & Forster, B. P. (2017). Plant mutation breeding for the improvement of vegetatively propagated crops: Successes and challenges. *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, *12*(028), 1–21.

Beyaz, R., Kahramanogullari, C. T., Yildiz, C., Darcin, E. S., & Yildiz, M. (2016). The effect of gamma radiation on seed germination and seedling growth of Lathyrus chrysanthus Boiss. under in vitro conditions. *Journal of Environmental Radioactivity*, *162–163*(May 2016), 129–133.

Bruno, L. (2019). Trees and shrubs of Maldives. *In Journal of Chemical Information and Modeling* 53(6), 321-332

Caplin, N., & Willey, N. (2018). Ionizing Radiation , Higher Plants , and Radioprotection : From Acute High Doses to Chronic Low Doses. *Frontier in. Plant Science.*, *9*(6), 847.

Celik, O., Atak, C., and Suludere, Z. (2014). Response of soybean plants to gamma radiation: biochemical analyses and expression patterns of trichome development. *Plant Omics* 7, 382–391

Danso, K. E., O Safo-Katanka , Y Adu-Ampomah, V Oduro , H M Amoatey, D K Asare, E Ofori and Ayeh G Amenorpe, (2009). Induced Plant Mutations in the Genomics Era. Food and Agriculture Organization of the United Nations. (1st ed.; Q. Y. Shu, ed.). Rome: Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. pp545-559

Darakhshanda Neelam, T. T. (2014). Radiation Sensitivity of *Cajanus Cajan* to Gamma Radiations. *Journal of Food Processing & Technology*, *5*(12), 1–7.

Datta, S. K. (2009). Induced Plant Mutations in the Genomics Era. Food and Agriculture Organization of the United Nations. In Q. Y. Shu (Ed.), *induced plant mutations in the genenomics era* (1st ed., pp. 253–256). Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

De Filippis F, Pasolli E, Tett A, Tarallo S, Naccarati A, De Angelis M, Neviani E, Cocolin L, Gobbetti M, Segata N, Ercolini D. (2019). Distinct Genetic and Functional Traits of Human Intestinal Prevotella copri Strains Are Associated with Different Habitual Diets. Cell Host Microbe. 25(3)444-453.

De Micco, V., Paradiso, R., Aronne, G., De Pascale, S., Quarto, M., and Arena, C. (2014). Leaf anatomy and photochemical behaviour of *Solanum lycopersicum* L. plants from seeds irradiated with low-LET ionising radiation. *Scientific World Journal* 42(8)14-32.

Donkor, E., Victor, E. O., Samira, O., Jacqueline, S., Avame, H., Djamson, Y., Kwesi, E., & Serbeh, F. O. (2017). Assessing the financial viability of the floricultural industry in Ghana. *Applied Studies in Agribusiness and Commerce – APSTRACT*, *11*(1–2), 119–126.

El-Khateeb, M. A., Rawia A. Eid, Heba A. Mahfouze, Ashor, H. A. and Mabrouk, R. M. S. (2017). Induction of Mutation with Gamma Radiation in Helichrysum bracteatum L . and Identification of Mutants by Molecular Markers. *Middle East Journal of Agriculture Research*, *6*(2), 282–293.

El-mahrouk, Mohammed E. Minisi, F. A., & Nasr, M. E.-D. F. R. and M. N. (2013). Effects of Gamma Radiation on Germination , Growth Characteristics and Morphological Variations of Moluccella laevis L. *American-Eurasian Journal of Agric. & Environmental. Science.*, *13*(5), 696–704.

Embassy of the republic of Kenya in Japan, (2020) The Flower Industry in Kenya. http://www.kenyarep-jp.com/business/flower. Date assesed 15th June, 2021.

Essel, P., Glover, E. T., Yeboah, S., Adjei-Kyereme, Y., Nutifafa Doyi Yawo, I., Nyarku, M., Asumadu-Sakyi, G. S., Kudjoe Gbeddy, G., Agyiriba Agyiri, Y., Mawuli Ameho, E., & Atule Aberikae Background, E. (2016). Rainfall erosivity index for the Ghana Atomic Energy Commission site. *SpringerPlus 66*(2), 182–189 .

FAO/IAEA. (2018). *Manual on mutation breeding* (B. P. and Spencer-Lopes, M.M. Forster & L. P. Jankuloski (eds.); Third). Food and Agriculture Organization of the United Nations. pp 432-441.

FAO/IAEA. (2012). Plant mutation breeding and biotechnology. In H. N. Q.Y. Shu, B.P.Forster (Ed.), *Plant Mutation Breeding and Biotechnology* (1st ed.) pp. 169–209).

Food and Agriculture Organization of the United Nations / International Atomic Energy Agency (FAO/IAEA). (2017). on https://mvd.iaea. Org. Date assessed June 19, 2021.

Fathin, T. S., Hartati, S., & Yunus, A. (2021). Diversity induction with gamma ray irradiation on Dendrobium odoardi orchid. *IOP Conference Series: Earth and Environmental Science*, *637*(1),164-171.

Fern, K., (2019). Useful tropical plants database. https://www.feedipedia.org. Date assesed; 11th November, 2019.

Ferreira D. S., Patrick L, Monteiro C., R. M., & Pinheiro, R. R. (2019). Seed position and influences on *Caesalpinia pulcherrima* germination and reserve proteins. *Ornamental Holticulture*, *25*(2), 119–125.

Ferro, J. S., Rocha, D. F., Junior, J. L. A. M., Neto, J. C. A., Silva, J. V., & Pavão, J. M. S. J. (2019). Germination and Viability of Seeds of *Caesalpinia pulcherrima* Newly

Harvested and Stored. *Journal of Agricultural Science*, *11*(7), 73.

Fidler MM, Soerjomataram I, Bray F. (2017). A global view on cancer incidence and national levels of the Human Development Index. *Internationl journa of Cancer* 2016; 139: 2436– 46.

Frank, M. H., Chitwood, D. H., Frank, M. H., & Louis, S. (2018). Plant Chimeras : the good , the bad , and the ‘ Bizzaria .’ *Plant Science Center*, 6(314), 32–40.

Frimpong, S. (2019). How IAEA Support is Benefitting Ghana’s Floriculture Industry. https//www.gaecgh.org; Date assesed 4th March, 2020.

Gakpetor, P. M., & Nassar, N. M. A. N. (2017). Periclinal chimera technique : new plant breeding approach. *Genetics and Molecular Research*, 16(3), 1–16.

Ghana Meteorological Agency (2020), https://www.meteo.gov.gh. Date assesed; 18 July, 2020.

Gilman, E. F., & Watson, D. G. (2014). *Caesalpinia pulcherrima:* Dwarf Poinciana. *Agricultural Engineering*, *3*(11), 1–3.

Godofredo U. Stuart Jr., M. . (2016). Philippine medicinal plants. *StuartXchange*. pp26-32

Gomes, R. I. O., Filho, M., & Grossi, N. I. O. S. (2016). Sulfuric acid on breaking dormancy seeds and on emergence. *Ornamental Holticulture*, *22*(2), 221–227.

Hamideldin, N., and Hussien, O. S. (2014). Morphological, physiological and molecular changes in Solanum tuberosum L. in response to pre-sowing tuber irradiation by gamma rays. American Journal of Food Nutrition. 2, 1–6.

Hartmann, H. T.; Kester, D. E.; Davies, F. T.; Geneve, R. L.(2002). Plant propagation:

principles and practices. 7th ed. New Jersey: Prentice Hall.pp45-71.

Hasbullah, N. A., Taha, R. M., Saleh, A., & Mahmad, N. (2012). Irradiation effect on *in vitro* organogenesis, callus growth and plantlet development of *Gerbera jamesonii*. *Horticultura Brasileira*, 30(2), 252–257.

Hase *et al.*, (2010). Measurements and their uncertainties; a practical guide to modern error analysis. *OUP Oxford*. p321-341.

Hernández-Muñoz, S., Pedraza-Santos, M. E., López, P. A., Gómez-Sanabria, J. M., & Morales-García, J. L. (2019). Mutagenesis in the improvement of ornamental plants. *Revista Chapingo, Serie Horticultura*, *25*(3), 151–167.

Hua, J. feng. (2016). Taxonomy of caesalpinia. StuartXchange, 14(5), 1–15.

Huxley, A. (1992) The New RHS Dictionary of Gardening. MacMillan Press, London

Huylenbroeck, J. Van. (2018). *Ornamental Crops* (J. Van Huylenbroeck (ed.); 11th ed.). Springer International Publishing AG.pp411-432.

Ilyas, S. and S. Naz. (2014). Effect of gamma irradiation on morphological characteristics and isolation of curcuminoids and oleoresins of Curcuma longa L. Journal of Animal Plant Science., 24: 1396-1404.

Ingels, J. E. (2010). Ornamental Horticulture (S. M. & T. E. K. Cathy Haas (ed.); 4th editio), pp41-50.

International Atomic Energy Agency [IAEA] (1992). Effects of Ionizing Radiation on Plants and Animals at Levels Implied by Current Radiation Protection Standards. Technical Reports Series No. 332. Vienna: IAEA.

Jan, S., Parween, T., Hameed, R., & Siddiqi, T. O. (2013). Effects of presowing gamma irradiation on the photosynthetic pigments , sugar content and carbon gain of Cullen corylifolium (L .) Medik . *Chilean Journal of Agricultural Research*, *73*(4), 345–350.

Jensen, M.H. and Malter, A.J. (1995) Protected Agriculture: A Global Review. *World Bank Publications*, Washington DC.253(8) 241-270.

Johnson Elizabeth Ofosuah (2019). Kenya is the world's 4th-largest flower exporter and makes over $500m a year. https://face2faceafrica.com. Date assesed 15th March, 2020

Ke C, Guan W, Bu S, Li X, Deng Y, Wei Z, Wu W, Zheng Y. (2019) Determination of absorption dose in chemical mutagenesis in plants. *PLoS One*, 14(1).57-66.

Khah, M. A., & Verma, R. C. (2015). Assessment of the effects of gamma radiations on various morphological and agronomic traits of common wheat (*Triticum aestivum* L.) var. WH-147. *European Journal of Experimental Biology*, *5*(7), 6–11.

Khan Hina (2018). Top 10 flower producing countries in the world. World Blaze. https.//www.worldsblaze. Date assessed 20th March 2020.

Koch, J., and Schlesinger, T. (2002). Radiation hormesis: Beneficial effects of exposure to low levels of ionizing radiation A critical review. Proceedings of the 21 conference of the nuclear societies in Israel Final program and book of summaries, (p. 237).

Logah, F. Y., Obuobie, E., & Ofori, D. (2013). Analysis of Rainfall Variability in Ghana. *International Journal of Latest Research In Engineering and Computing (IJLREC)*, 1(1), 1–8.

Magee, R. V., Crowder, R., Winters, D. E., Beerbower, E., Bodhi, B., Schindler, S., Pfattheicher, S., Reinhard, M.-A., Haddock, G., Foad, C., Windsor-Shellard, B., Dummel, S., Adarves-Yorno, I., Furlotte, C., & Gorski, P. C. (2017). Assesing genetic diversity of cacao (*Theobroma cacao* L.) *Nativo chuncho in la convención, cusco-perú. Aba journal*, *102*(4), 24–25.

Mahajan N. S., Jadhav R. L., Pimpodkar N. V., Dias R. J., and Kodsu. P. P. (2008). Use of Canna indica Flower Extract As A Natural Indicator In Acid Base Titration. *Journal of Pharmacy Research*, 1(1), 84–87.

Maluszynski, M., Nichterlein, K., Zanten, L. V. A. N., Ahloowalia, S., Section, G., Atomic, I., & Agency, E. (2000). Officially released mutant varieties - the FAO/IAEA database. *International Atomic Energy Agency(IAEA).*, 12, 14–84.

María, de las M. C. (2012). Life Cycle in Natural Populations. In D. X. Zhang (Ed.), *Phenology and Climate Change* (1st ed., pp. 101–115). InTech.

Marcu, D., G. Damian, C. Cosma and V. Cristea. 2013a. Gamma radiation effects on seed germination, growth and pigment content, and ESR study of induced free radicals in maize (Zea mays). Journal of Biological Physiology., 39(4), 625-634.

McIntyre, E. & B. (2001). *Canna indica* – wild Canna , Indian Shot. *Botanical Society of SA - Garden Route Branch.*, 14 (2)104-112

Mensah, S., & Ekeke, C. (2016). Effects of Different Pretreatments and Seed Coat on Dormancy and Germination of Seeds of Senna obtusifolia (L.) H.S. Irwin & Barneby (Fabaceae). *International Journal of Biology*, *8*(2), 77.

Miri, S. M. (2018). Mutation Technique and Its Applications in the Breeding of

Ornamental Plants. *Research Gate*, *5*(10), 1–4. https://www.researchgate.net/publication/328601280_Mutation_technique_and_its_applications_in_the_breeding_of_ornamental_plants

Mishra, T., Goyal, A. K., & Sen, A. (2015). An overview on the in vitro regeneration of Canna. *International Journal of Fundamental & Applied Sciences*, 4(2), 39–49.

Mohammad R. S., Abdollah H., and Hussan. S. L. (2019). Mutation Induction in Chrysanthemum Cut Flowers Using. *Journal of Ornamental Plants*, 9(2), 143–151.

Mostafa, H. H. A., Wang, H. P., Shen, D., Qiu, Y., and Li, X. X. (2015). Sprout differentiation and mutation induction of garlic (Allium sativum L.) callus exposed to gamma radiation. *Plant Growth Regulation*. 75, 465–471.

Moteriya and Chanda (2017). Synthesis and characterization of silver nanoparticles using Caesalpinia pulcherrima flower extract and assessment of their in vitro antimicrobial, antioxidant, cytotoxic, and genotoxic activities. *Artificial Cell Nanomed Biotechnology*. 45(8);1556-1567.

Murashige, T. and Skoog, F. (1962) A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Plant Physiology*, 15, 473-497.

Nbangan (2019). Ghana's Beautiful Ornamental flora in a Nutshell. https//ghanacoffin.com. Date assesed 6th July 2020.

Nemali (2016) Indiana flower growers convene for a conference at Purdue https://www.purdue.edu/hla/sites/cea/indiana. Date assessed 14th April, 2020.

Nero, B. F., Kwapong, N. A., Jatta, R., & Fatunbi, O. (2018). Tree Species Diversity and

Socioeconomic Perspectives of the Urban (Food) Forest of Accra , Ghana. *Sustainability*, 10(17), 1–19.

New World Encyclopedia (2008). Psalm https://www.newworldencyclopedia.org. Date assesed; 4 March, 2021.

Nishiguchi, M., Nanjo, T., and Yoshida, K. (2012). The effects of gamma irradiation on growth and expression of genes encoding DNA repair-related proteins in Lombardy poplar (Populus nigra var. italica). *Journal Environmental Radioactivity*. 109, 19–28.

Nusrat Jahan, Suban. B. J. and Mustapha. A. (2014). *In vitro* regeneration from nodal explants in Caesalpinia pulcherrima L.- An anticancerous woody legume. *Agrotechnol*, 2(4), 98-181.

Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Rahim, H. A., Miah, G., & Usman, M. (2016). Principle and application of plant mutagenesis in crop improvement : a review. *Biotechnology & Biotechnological Equipment*, 30(1), 1–16.

Oliveira,R.A., Hamilton, R.S, Pauli, A., Davis, I., Nasmyth, K. (2010).Cohesin cleavage and Cdk inhibition trigger formation of daughter nuclei. *National cell Biology* 12(2); 185-192

Opoku, J. A., Amissah, J. N., Essilfie, M. E., Ashanti, M.-, & Norman, J. C. (2018). Seed Germination Percentage And Growth Performance Of *Caesalpinia pulcherrima* As Influenced By Different Pre-Sowing Treatment. *Journal of Forestry*, *5*(4), 1–14.

Pallavi, B., Nivas, S.K., D'Souza, L., Ganapathi, T.R., and Hegde, S., (2017). Gamma rays induced variations in seed germination, growth and phenotypic characteristics of

Zinnia elegans var. Dreaml and. *Advances in Horticultural Science*. 31(4): 267-273

Patil, S. D. and H. E. P. (2009). Impprovement of major ornamental crops through mutation breeding. *International Journal of Agricultural Sciences*, *5*(2), 628–632.

Pocket K. (2014). Biotechnology in ornamental plants. *International Service for the Acquisition of Agri-Biotech Applications* (ISAAA), 47, 12.

Puy Djdu; Labat JN; Rabevohitra R; Villiers JF; Bosser J; Moat J, (2002). The Leguminosae of Madagascar.Richmond, UK: *Royal Botanic Gardens*, pp737.

Raihana, Q. Z. Faridah, A. A. Julia, A. H. A. Abdel mageed and M. A. Kadir (2011), "*In vitro* culture of Curcuma mangga from rhizome bud," *Journal of Medicinal Plant Research*, 5 (28) 6418–6422.

Randall RP, 2012. A Global Compendium of weeds. Perth, Austrailla: Department of Agriculture and food western Australlia, pp1124.

Rodrigues, M. G. F., Mazzini, R. B., Pivetta, K. F. L., Alves, M. de C., & Desidério, J. A. (2012). Characterization of the genetic variability among *Caesalpinia pulcherrima* (L.) Sw. (Fabaceae) plants using RAPD molecular markers. *Acta Scientiarum - Agronomy*, 34(3), 259–263.

Saika Anne and Jin Hee Lim. (2020). Mutation Breeding Using Gamma Irradiation in the Development of Ornamental Plants: A Review. *Flower Research Journal*, *28*(3), 102–115.

Sarasan, V., Cripps, R., Ramsay, M. M., Atherton, C., McMichen , M., Prendergast,G., and Rowntree, J. K., (2006). Conservation in vitro of threateneted plants – progress in the past decade. *In vitro Cellular Development Biology* – Plant, 42, 206-214.

Selvam, R. P. N. T (2019). Trees and shrubs of Maldives. In *Journal of Chemical Information and Modeling* 53 (9), 205-220.

Sever-Mutlu, S., Djapo, H., Ozmen, S. F., Selim, C., and Tuncel, N. (2015). Gammaray

irradiation induces useful morphological variation in Bermuda grass. *National. Botany of Horticulture Agrobotany.* 43, 515–520.

Sidler, C., Li, D. P., Kovalchuk, O., and Kovalchuk, I. (2015). Developmentdependent expression of DNA repair genes and epigenetic regulators in Arabidopsis plants exposed to ionizing radiation. *Radiation. Resources.* 183, 219–232.

Songsri P., Suriham B, Sanitchon, J, S. S. and K. T. (2011). Effect of gamma radiation on germination and growth of jatropha *Journal of Biological Science*, 11(3), 268–274.

Sooch, B. S., Singh, R. S., & Puri, M. (2007). Optimization of medium and process parameters for the production of inulinase from a newly isolated Kluyveromyces marxianus YS-1. *Bioresource technology*, 98(13), 2518-2525.

Sparrow A. H. and Miksche J. P. (1961). Correlation of nuclear volume and DNA content with higher plant tolerance to chronic radiation. *Science* 134(3475), 321-321.

Stratcomm-Africa (2017). 5th anniversary edition of Ghana garden and flower show ends with six awards to individuals and organisations. https://www.stratcomm-africa.com. Date accessed 6th March 2020.

Sudha D. P., and Harshal E. P., (2009). Improvement of major ornamental crops through mutation breeding. *International Journal of Agricultural Sciences*, *5*(2), 628–632.

Tabbicca, A. K. (2018). An Investigation on the Entrepreneurial Opportunities in the Ornamental Plants Industry in Accra, Ghana. *Journal of Ornamental Horticulture*, 4(1), 43.

Taz and Zeiger (2017). Plant physiology and development. 6th edition Artmed, Porto Alegre, p858.

Tanaka (2004). "The utilization of edible Canna plants in southeastern Asia and southern China," *Economic Botany*,vol.58, no.1, pp. 112–114.

Taheri, S., Abdullah, T. L., Ahmad, Z., & Abdullah, N. A. P. (2014). Effect of acute gamma irradiation on Curcuma alismatifolia varieties and detection of DNA polymorphism through SSR marker. *BioMed Research International*, *2014*(631813), 1–18.

Telci, C., Yildiz, M., Pelit, S., Onol, B., Erkilic, E.G., Kendir, H., (2011). The effect of surface-disinfection process on dormancy-breaking, seed germination, and seedling growth of Lathyrus chrysanthus Boiss. under *in vitro* conditions. *Propagating Ornamental Plants* 11, (10)16.

Tinoi J, Rakariyatham N,Deming RL, 2006. Determination of Major Carotenoid Constituents in Petal Extracts of Eight Selected Flowering Plants in the North of Thailand,Chiang Mai *J. Science*., 33(2), pp.327 – 334.

Uffelen, Ruud, Groot, Nico (2005). Floriculture world-wide; production, trade and consumption patterns show market opportunities and challenges. http://purl.umn.edu/. Date assessed 30th April, 2020.39(4).

United Nations. (2008). Export Performance Following Trade Liberalization *: Some Patterns and Policy Perspectives*. Date accessed 6th March, 2021.

Venugopal, C. K., Yashaswini, S., Yashwantkumar, K. H., & Patil, V. S. (2009). Seed germination studies in Canna indica. *Journal of Ornamental Horticulture*, 12(1), 44–47.

Verchot, J., & Webb, A. (2017). Greenhouse Propagation of Ornamental Cannas Grown from Rhizomes or Seeds. *Oklahoma State University*, 2(3), 7333.

Viji, M. O., & Wilson, N. (2017). Studies on phytochemical , antimicrobial and cytotoxic properties of caesalpinia mimosoides leaf extracts. *VISTAS*, 6(1), 111–120.

Wafa, S. N., Mat Taha, R., Mohajer, S., Mahmad, N., & Ahmed, A. B. A. (2016). Organogenesis and Ultrastructural Features of in Vitro Grown Canna indica L. *BioMed Research International*, *20(1)*, 1–9.

Xie Emily, 2017. Effects of Radiation on Seed Survival and Germination coursework for PH241, Stanford University, Winter 2017. http://large.stanford.edu. Date assessed 6th June, 2021.

Yamaguchi, H. (2018). Mutation breeding of ornamental plants using ion beams. *Breeding Science*, *68*(1), 71–78.

Yildiz, M., Beyaz, R., Gursoy, M., Aycan, M., Koc, Y., & Kayan, M. (2017). Seed Dormancy. In *Advances in Seed Biology* 14(11)pp. 85–97).

Yue, Y., & Ruter, J. M. (2020). ^{60}Co Irradiation Influences Germination and Phenotype of Three Pavonia Species, *HortScience*, 55(12), 2037-2044.

Zanin Baldim, J. L., De Carvalho, B. A., Martineli, P. S., Dos Santos, M. H., Lago, J. H. G., Sartorelli, P., Viegas, C., & Soares, M. G. (2012). The genus Caesalpinia L. (Caesalpiniaceae): Phytochemical and pharmacological characteristics. *Molecules*, 17(7), 7887–7902.

Zofou, D., & Titanji, V. P. K. (2013). Antimalarial and Other Antiprotozoal Products from African Medicinal Plants. *Medicinal Plant Research in Africa*, 17(3), 1–38.

APPENDICES

Appendix 1: Survey of Flower growers and utilization of ornamental plants in Greater Accra Region

RESEARCH QUESTIONNAIRE

(For flower growers in Greater Accra and some parts of Eastern region)

1. Name of flower grower……………………………………………………..Area (suburb)…………………….
2. Sex; Male ☐ Female ☐
3. Age; 18 – 25 ☐ 26 – 35 ☐ 36- 50 ☐ Above 50 ☐
4. Level of education; Basic ☐ Secondary ☐ Tertiary ☐ None ☐
5. Why did you enter the floriculture industry?...
6. Which flower varieties normally sells faster…………………………………………………………………………
7. (a) Which flower colours do you prefer…………………………………………………………………………
   (b)Why………………………………………………………………………………..
8. (a)Which flower colour(s) do buyers prefer…………………………………………………………………………
   (b)Why………………………………………………………………………………..
9. Which of the flower colours of *Caesalpinia purcherrima* flower do buyers prefer?
   Red ☐ Yellow ☐ Mottled ☐

(a) Why………………………………………………………………………….

10. Which of the flower colours of *Canna indica* do buyers prefer? Yellow ☐ Red ☐

11. What is the unit price of *Caesalpinia purcherrima* flower?…………………………………………………………………………..

12. What is the unit price of Canna indica?

…………………………………………………………………………...

13. How much is the cheapest flower sold?

…………………………………………………………………………...

14. How much is the expensive flower sold?

…………………………………………………………………………..

15. How do you propagate *C. purcherrima*? Seeds ☐ Cuttings ☐ Other ☐

16. How do you propagate *C. indica*? Seeds ☐ Cuttings ☐ Other ☐

17. What are the challenges associated with propagation of Peacock flower?

………………………………………………………………………………….

18. What are the challenges associated with propagation of *Canna lily* ?

………………………………………………………………………………….

19. Would you be interested to buy improved seeds or cuttings? Yes ☐ No ☐

20. Would you be interested in participating in training on improved flower propagation? Yes ☐ No ☐

21. What are some of the challenges in the flower

industry?……………………………………………………………………………

22. How lucrative is the floriculture

business?…………………………………………………………………………..

Very lucrative ☐ Lucrative ☐ None ☐

Appendix 2:

Composition of the MS basal medium per litre.

Stock 1 (Macronutrient)

NH_4NO_3	33g
KNO_3	38g
$CaCl_2.2H_2O$	8.8g
$MgSO_4.7H_2O$	7.4g
KH_2PO_4	3.4g

Stock 2 (Micronutrient)

$MnSO_4.4H_2O$	4.46g
KI	0.16g
H_3BO_3	1.24g
$ZnSO_4.7H_2O$	1.72g
$Na_2MoO_4.2H_2O$	0.05g
$CuSO_4.5H_2O$	0.05g
$CoCl_2.6H_2O$	0.05g

Stock 3 10mls (Iron source):

$FeSO_4.7H_2O$	5.86g

Na2-EDTA	7.46
Stock 4 (Vitamins)	
Myo-inositol	10g
Nicotinic acid	0.05g
Pyridoxine HCl	0.05g
Thiamine HCl	0.01g
Glycine	0.2g

Appendix 3

Appendix 3a: ANOVA of germination test for *C. pulcherrima*

Source	DF	Adj SS.	Adj MS	F-Value	P- Value
Gamma Dose	1	0.0250	0.0250	0.14	0.714
Error	38	6.9500	0.1829		
Total	39	6.9750			

Appendix 3b: ANOVA of number of seeds of yellow flower *C. pulcherrima* germinated 5 days after planting

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	7	1.475	0.2107	0.88	0.526
Error	152	36.500	0.2401		
Total	159	37.975			

Appendix 3c: ANOVA of no. of seeds germinated for *C. pulcherrima* yellow flower – Day10

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	7	4.800	0.6857	2.97	0.006
Error	152	35.100	0.2309		
Total	159	39.900			

Appendix 3d: ANOVA of no. of seeds germinated for *C. pulcherrima* yellow flower – Day15

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	7	7.244	1.0348	5.34	0.000
Error	152	29.450	0.1937		
Total	159	36.694			

Appendix 3e: ANOVA of no. of seeds survived for *C. pulcherrima* yellow flower – Day 30

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma Dose	7	16.70	2.3857	15.84	0.000
Error	152	22.90	0.1507		
Total	159	39.60			

Appendix 3f: ANOVA of height at 28 days for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma Dose	7	552.8	78.976	8.75	0.000
Error	62	559.7	9.027		
Total	69	1112.5			

Appendix 3g: ANOVA of height at 42 days for C. pulcherrima yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma Dose	7	960.0	137.14	7.43	0.000
Error	62	1144.1	18.45		
Total	69	2104.1			

Appendix 3h: ANOVA of height at 56 days for C. pulcherrima yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma Dose	7	5726	818.1	4.10	0.001
Error	62	12368	199.5		
Total	69	18094			

Appendix 3i: ANOVA of number of leaves at 28 days for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma Dose	7	152.4	21.770	7.01	0.000
Error	62	192.5	3.105		
Total	69	344.9			

Appendix 3j: ANOVA of number of leaves at 42 days for C. pulcherrima yellow flower

Source	DF	Adj SS	Adj MS	F- Value	P- Value
Dose	7	39.99	5.713	4.25	0.001
Error	62	83.28	1.343		
Total	69	123.27			

Appendix 3k: ANOVA of number of leaves at 56 days for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma Dose	9	88.91	9.879	4.16	0.000
Error	60	142.36	2.373		
Total	69	231.27			

Appendix 3l: ANOVA of number of branches for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma Dose	7	12.11	1.7301	3.25	0.005
Error	62	32.97	0.5319		
Total	69	45.09			

Appendix 3m: ANOVA of Number of days to 50% flowering for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma Dose	7	1574	224.9	0.84	0.558
Error	62	16594	267.6		
Total	69	18168			

Appendix 3n: ANOVA of number of opened flowers for *C. pulcherrima* yellow flowers

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma Dose	7	10.51	1.502	0.24	0.975
Error	62	393.27	6.343		
Total	69	403.79			

Appendix 4a: ANOVA of no. of seeds germinated for *C. pulcherrima* yellow flower (200 Gy) – Day 5

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	5	3.342	0.6683	3.22	0.009
Error	114	23.650	0.2075		
Total	119	26.992			

Appendix 4b: ANOVA of no. of seeds germinated for *C. pulcherrima* yellow flower – Day 10

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	5	3.242	0.6483	2.76	0.021
Error	114	26.750	0.2346		
Total	119	29.992			

Appendix 4c: ANOVA of no. of seeds germinated for *C. pulcherrima* yellow flower – Day 15

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	5	2.567	0.5133	2.14	0.066
Error	114	27.400	0.2404		
Total	119	29.967			

Appendix 4d: ANOVA of no. of seeds survived for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	5	4.742	0.9483	7.81	0.000
Error	114	13.850	0.1215		
Total	119	18.592			

Appendix 4e: ANOVA of height at 28 days for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	3	394.8	131.61	6.90	0.001
Error	28	533.9	19.07		
Total	31	928.7			

Appendix 4f: ANOVA of height at 42 days for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	4	1182	295.40	6.13	0.001
Error	27	1302	48.21		
Total	31	2483			

Appendix 4g: ANOVA of height at 56 days for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	4	4224	1055.95	13.09	0.000
Error	27	2178	80.67		
Total	31	6402			

Appendix 4h: ANOVA of height at 50% flowering for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	4	3295	823.7	6.48	0.001
Error	27	3431	127.1		
Total	31	6726			

Appendix 4i: ANOVA of number of leaves at 28 days for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	4	180.4	45.10	4.48	0.007
Error	27	272.1	10.08		
Total	31	452.5			

Appendix 4j: ANOVA of number of leaves at 42 days for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	4	286.7	71.667	8.41	0.000
Error	27	230.2	8.526		
Total	31	516.9			

Appendix 4k: ANOVA of number of leaves at 56 days for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	4	330.0	82.50	5.76	0.002
Error	27	386.7	14.32		
Total	31	716.7			

Appendix 4l: ANOVA of number of branches for *C. pulcherrima* yellow 200gy

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	4	6.760	1.6901	3.59	0.018
Error	27	12.708	0.4707		
Total	31	19.469			

Appendix 4m: ANOVA of number of opened flowers for *C. pulcherrima* yellow flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	4	6.094	1.523	0.33	0.858
Error	27	125.875	4.662		
Total	31	131.969			

Appendix 4n: ANOVA of number of days to flowering for *C. pulcherrima* yellow 200gy

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	4	1597	399.3	2.21	0.095
Error	27	4885	180.9		
Total	31	6482			

Appendix 5a: ANOVA of no. of seeds germinated for *C. pulcherrima* mottle flower – Day 5

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	5	1.942	0.3883	2.48	0.036
Error	114	17.850	0.1566		
Total	119	19.792			

Appendix 5b: ANOVA of no. of seeds germinated for *C. pulcherrima* mottle flower – Day 10

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	5	5.600	1.1200	5.30	0.000
Error	114	24.100	0.2114		
Total	119	29.700			

Appendix 5c: ANOVA of no. of seeds germinated for *C. pulcherrima* mottle flower – Day 15

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	5	6.775	1.3550	6.67	0.000
Error	114	23.150	0.2031		
Total	119	29.925			

Appendix 5d: ANOVA of no. of seeds Survived for *C. pulcherrima* mottle flower – after Day 30

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	5	10.34	2.0683	15.46	0.000
Error	114	15.25	0.1338		
Total	119	25.59			

Appendix 5e: ANOVA of height at 28 days for *C. pulcherrima* mottle flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	3	2422	807.33	32.32	0.000
Error	44	1099	24.98		
Total	47	3521			

Appendix 5f: ANOVA of height at 42 days for *C. pulcherrima* mottle flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	3	11878	3959.5	29.61	0.000
Error	44	5884	133.7		
Total	47	17762			

Appendix 5g: ANOVA of height at 56 days for *C. pulcherrima* mottle flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	3	41508	13836.0	34.29	0.000
Error	44	17756	403.6		
Total	47	59264			

Appendix 5h: ANOVA of number of leaves at 28 days for *C. pulcherrima* mottle flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	4	638.3	159.57	13.50	0.000
Error	43	508.4	11.82		
Total	47	1146.7			

Appendix 5i: ANOVA of number of leaves at 42 days for *C. pulcherrima* mottle flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	3	2032.8	677.58	32.86	0.000
Error	44	907.2	20.62		
Total	47	2939.9			

Appendix 5j: ANOVA of number of leaves at 56 days for *C. pulcherrima* mottle flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	3	1915	638.41	26.03	0.000
Error	44	1079	24.53		
Total	47	2994			

Appendix 5k: ANOVA of number of branches for *C. pulcherrima* mottle flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	3	27.73	9.2431	20.59	0.000
Error	44	19.75	0.4489		
Total	47	47.48			

Appendix 5l: ANOVA of Height at flowering for *C. pulcherrima* mottle flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	3	55616	18538.5	50.69	0.000
Error	44	16090	365.7		
Total	47	71706			

Appendix 5m: ANOVA of number of days to flowering for *C. pulcherrima* mottle flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	3	3741	1247	0.82	0.492
Error	44	67209	1527		
Total	47	70950			

Appendix 5n: ANOVA of number of open flowers for *C. pulcherrima* mottle flower

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	3	80.92	26.972	6.49	0.001
Error	44	183.00	4.159		
Total	47	263.92			

Appendix 6a: ANOVA of *in vitro* germination of *C. indica*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	10	3.091	0.30909	4.03	0.000
Error	99	7.600	0.07677		
Total	109	10.691			

Appendix 6b: ANOVA of number of leaves of *in vitro C. indica*,

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	10	23.62	2.3618	2.74	0.005
Error	99	85.30	0.8616		
Total	109	108.92			

Appendix 6c: ANOVA of number of roots of *in vitro C. indica*,

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	10	1509	150.91	3.95	0.000
Error	99	3780	38.18		
Total	109	5289			

Appendix 6d: ANOVA of number of survived plantlets of *C. indica*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	10	1.233	0.1233	0.53	0.855
Error	38	8.767	0.2307		
Total	48	10.000			

Appendix 6e: ANOVA of number of days to flowering of *C. indica*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	10	1870	186.96	2.68	0.023
Error	24	1677	69.88		
Total	34	3547			

Appendix 6f: ANOVA of number of leaves during time of flowering of *C. indica*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	10	10.20	1.020	0.47	0.892
Error	24	51.97	2.165		
Total	34	62.17			

Appendix 6g: ANOVA of height during time of flowering of *C. indica*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	10	2821.7	282.17	8.15	0.000
Error	24	830.8	34.62		
Total	34	3652.5			

Appendix 6h: A of multiple suckers formed during time of flowering of *C. indica*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Gamma dose	10	14.15	1.4150	1.95	0.088
Error	24	17.45	0.7271		
Total	34	31.60			