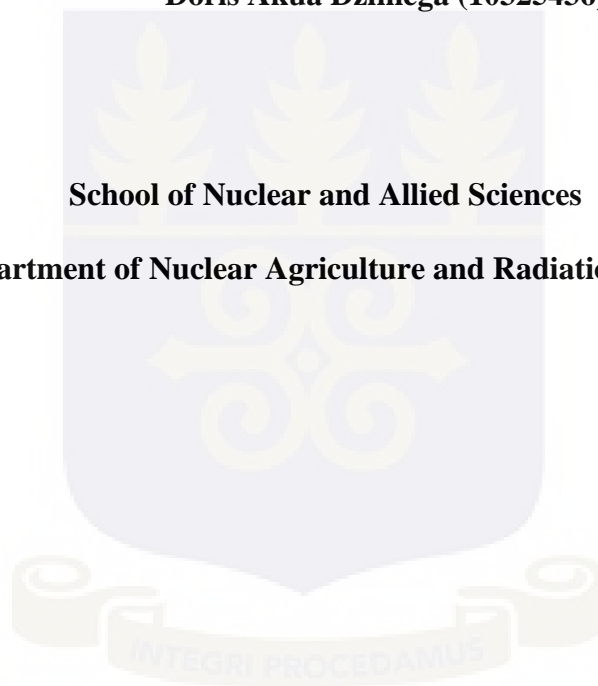


**EFFECT OF IRRADIATION AND COLCHICINE ON CALLUS AND SOMATIC
EMBRYO FORMATION IN CASSAVA (*Manihot esculenta* Crantz)**

Doris Akua Dzimega (10325436)

School of Nuclear and Allied Sciences

Department of Nuclear Agriculture and Radiation Processing



**This thesis is submitted to the University of Ghana, Legon in partial fulfillment
of the requirement for the award of M. Phil Nuclear Agriculture degree**

June, 2012

DECLARATION

This thesis is the result of research work undertaken by Doris Akua Dzimega in the School of Nuclear and Allied Sciences (Department of Nuclear Agriculture and Radiation Processing), University of Ghana, under the supervision of Prof. Kenneth Danso and Professor G.Y.P Klu.

No part of this work has been presented for another degree in this university or elsewhere and references of other people's work have been cited with full acknowledgement given.

Signed.....

Doris AkuaDzimega

(Candidate)

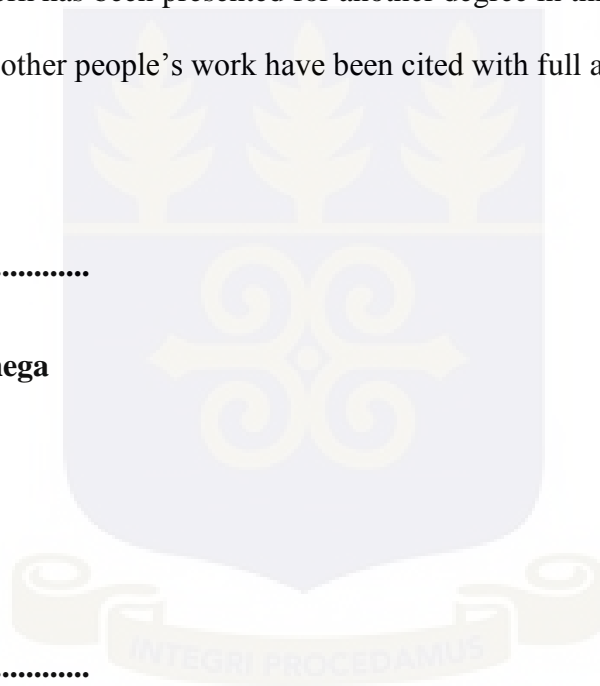
Signed.....

Prof. Kenneth Ellis Danso

(Supervisor)

Signed.....

Prof. G.Y.P Klu



(Supervisor)

DEDICATION

This work is dedicated to the Almighty God, the author and finisher of this thesis for how far He has brought me. To Him is all glory. To my mother Mrs. M.A Dzimega who has impacted so much to my life and without whose suffering I would not be what I am today. Thank you mom and God bless you, and to my dear husband and children for your prayers and support.



ACKNOWLEDGEMENTS

I wish to express my profound gratitude to the underlisted people for their valuable contributions.

- ❖ Prof. K.E Danso and Prof. G.Y.P Klu of the Ghana Atomic Energy Commission for supervision.
- ❖ Dr. Godwin Amenorpe for his contributions.
- ❖ My husband, my children, my family and very good friends from within and outside the departments for their prayers, and co-operation. They were a source of encouragement and laughter when the going got tough.
- ❖ Dr. Ebenezer Owusu and Dr. Sylvia Acheampong, all of Botany Department of the University of Ghana, Legon for their assistance.
- ❖ All the technicians at the Tissue Culture laboratory.

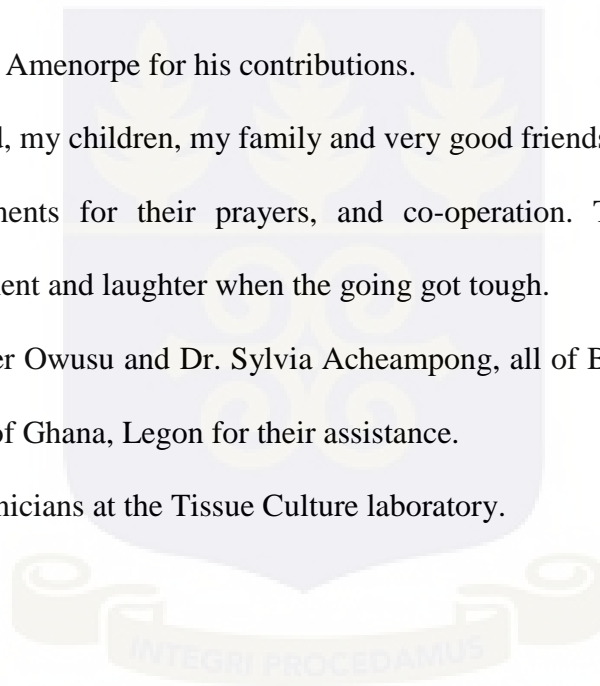


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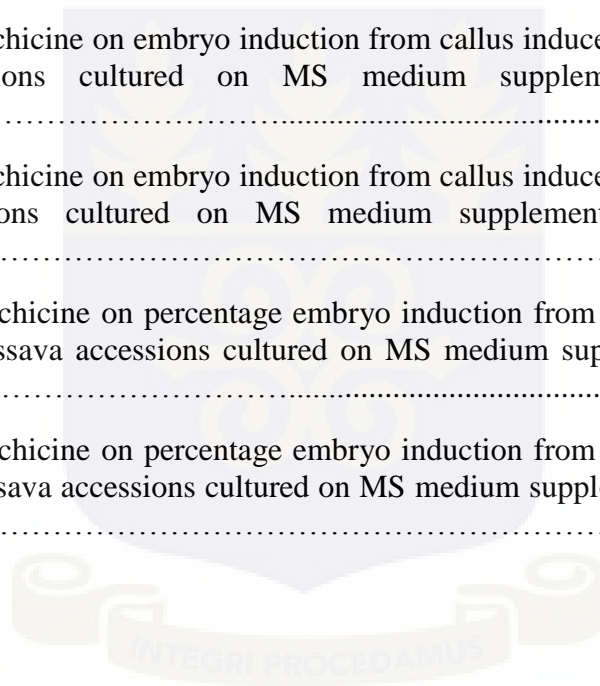
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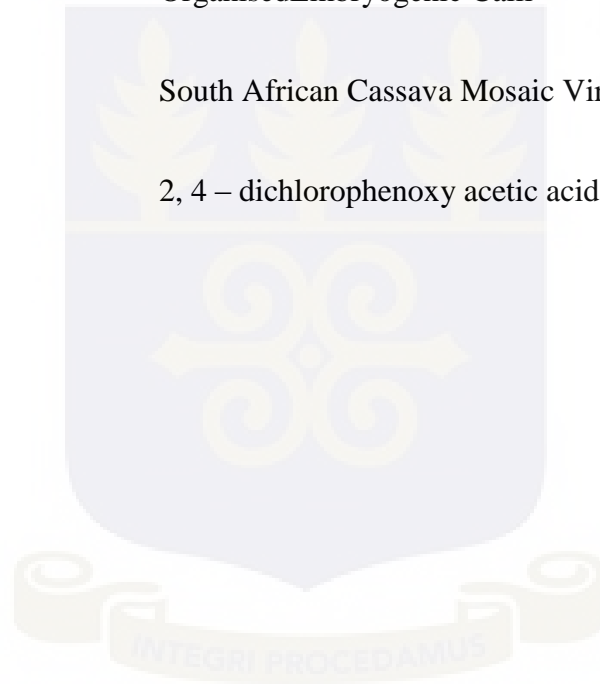
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LIST OF ABBREVIATION

ACMV	African Cassava Mosaic Virus
ANOVA	Analysis of variance
BAP	Benzylaminopurine
CBSD	Cassava Brown Streak Diseases
dES	diethyl sulphate
DMSO	Dimethyl sulfoxide
DNA	Dinucleotide acid
EACMV	East Africa Cassava Mosaic Virus
EMS	Ethyl methyl sulfonate
FAO	Food and Agricultural Organisation
FAOSTAT	Food and Agriculture Organisation Statistics
FEC	Friable embryogenic calli
Gy	Gray
HCl	Hydrochloric acid
IITA	International Institute of Tropical Agriculture

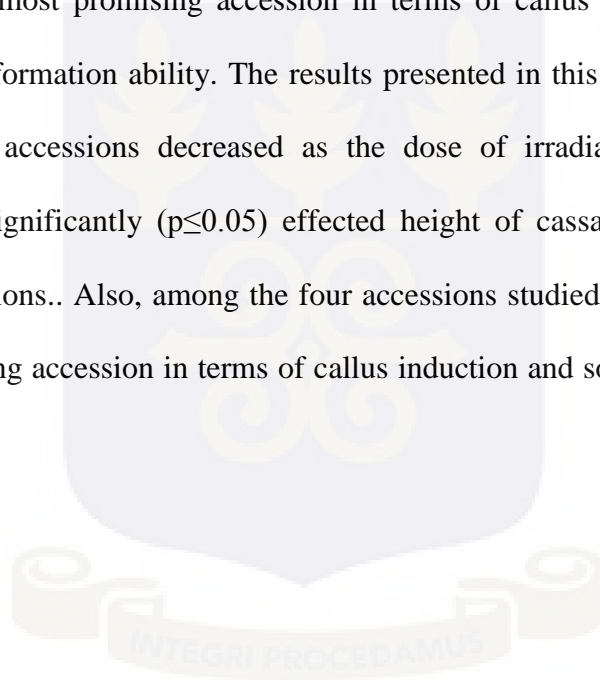
INDEL	Insertion and deletion
MS	Murashige and Skoog (1962) basal medium
NAA	Naphthalene Acetic Acid
NaOH	Sodium hydroxide
NARP	National Agriculture Research Programme
OEC	Organised Embryogenic Calli
SACMV	South African Cassava Mosaic Virus
2,4- D	2, 4 – dichlorophenoxy acetic acid



ABSTRACT

A study was conducted to assess the mutagenic effect of gamma radiation on sprouting and height in four local cassava accessions. The four cassava accessions were assessed for their callus induction and somatic embryo formation ability from leaf lobes from gamma irradiated stakes as well as colchicine treated leaf lobes on different concentrations of plant growth regulators, incorporated into Murashige and Skoog, (1962) (MS) basal medium. The cassava accessions were irradiated at 0, 32, 35, 45 and 50 Gy and planted in pots filled with loamy soil. The height of the shoots was measured with rule after sprouting. The leaf lobes were collected from the shoots and cultured on MS medium supplemented with 8 mg/l 2,4-D and 16 mg/l Picloram. Another set of leaf lobes were treated with 0.0, 0.05, 0.1, 0.2, 0.25 g/l colchicine for one hour and thereafter cultured on MS medium supplemented with 8 mg/l 2,4-D and 16 mg/l Picloram as described above. Callus induction from leaf lobes in 45 and 50 Gy were significantly ($p \leq 0.05$) affected by the irradiation. However, callus induced from leaf lobes in 32 and 35 Gy were not significantly ($p \leq 0.05$) affected by the irradiation. On the other hand, Callus induction from leaf lobes in 0.1-0.25 g/l colchicine were significantly ($p \leq 0.05$) affected by the mutagenic treatment whereas callus induced from leaf lobes in 0.05 g/l colchicine was not significantly ($p \leq 0.05$) affected. Callus induced on 8 mg/l 2,4-D and 16 mg/l picloram gave the best response in Ankrah and all control tested while Tomfa recorded the least. Colchicine at a concentration of 0.05 g/l and radiation dose of 32 Gy treatments gave the best response of callusing. Callus induction decreased with increasing colchicine concentration and gamma irradiation. Callus derived from irradiated and colchicine leaf

lobes appeared soft but friable and tiny, compact, respectively, predominately with creamy to brown colouration. Calli obtained were sub-cultured on embryo regeneration medium consisting of MS supplemented with 0.01mg/l NAA and 0.1 mg/l BAP. There was no plantlet regeneration. Instead, embryo formation and prolific root was observed in cases where there was no embryo. Similarly, somatic embryo formation was significantly ($p \leq 0.05$) different among the accessions. No response to callus formation was observed in 0.25 g/l colchicine treatment after 30 days. Among the four accessions evaluated, Ankrah was the most promising accession in terms of callus induction frequency and somatic embryo formation ability. The results presented in this thesis clearly show that, sprouting in all accessions decreased as the dose of irradiation increased. Gamma irradiation had significantly ($p \leq 0.05$) effected height of cassava plant but this varied among all accessions.. Also, among the four accessions studied Ankrah and Tuaka were the most promising accession in terms of callus induction and somatic embryo formation ability.



CHAPTER ONE

1.0 INTRODUCTION

Cassava is a dicotyledonous plant belonging to Euphorbiaceae family. The genus is reported to have about 200 species, with *Manihot esculenta* Crantz being the only commercially cultivated species (Alves, 2002). The species is believed to have originated in South America (Allem, 2002; Olsen and Schaal, 2001) and introduced in the old world tropics in the 16th century by Portuguese explorers when they established forts, trading stations and settlements on African coastal and nearby islands (Jones 1959).

Cassava is the second most important source of calories (providing more than 50% of the calories) after maize and a major staple food for more than 800 million people in the subtropics and tropics (FAO, 2006). The crop is widely adapted to a variety of soils and climates making it a suitable alternative to other grain staples in areas where population pressure and crop failure are a challenge (Al-Hassan, 1993; Nweke, 1996; Benesi, 2005). A unique advantage of cassava over other crops is its ability to be stored in the ground and harvested when needed, thus, making it a good food security (DeVries and Toenniessen, 2001).

The main nutritional component of cassava is carbohydrate, which is accumulated in the tuberous storage roots (Jaramillo *et al.*, 2005); a valuable source of energy for both humans and animals. It constitutes a greater part of the diets of most Africans south of the Sahara. More than two-thirds of the total production of cassava is used as food for humans, with small amounts

being used for animal feed and industrial use (Nwokoro *et al.*, 2002). There is a growing importance of cassava among Ghanaians because its contribution to the national economy and development is enormous (Manu-Aduening *et al.*, 2006).

In Ghana, cassava is produced predominantly in all the regions except the Upper East Region with per capita consumption of 152.9 kg/year. The fresh roots are used to prepare the most important traditional meal “fufu, or boiled into “Ampesi” and eaten with stew. They can as well be processed into gari (Amenorpe *et al.*, 2006; Baafi and Sarfo-Kantanka, 2008). Roots can also be used to prepare fermented starches as well as dried chips, or pellets for animal feed (Wheatley and Chuzel, 1993). Cassava is not only a staple food in this country, but also a source of income for farmers (Watananonta, 2006).

In recent times, cassava has gained attention as a potential industrial crop due to its high carbohydrate content and other qualities such as amylose and amylopectin ratio which makes it a good candidate crop for starch production (Parkes, 2001). Starch is one of the most important products from cassava and it forms an essential component of food by providing a large proportion of the daily caloric intake. Starch from cassava is used in the production of adhesives in the textile industries, filler and binding agents in the pharmaceutical industries (Manyong and Abass, 2007), as glue to improve strength and brightness in the paper industry and for powder production in the cosmetic industry (Tonukari, 2004).

In addition, cassava has the potential to become an important biofuel crop. It is used as a source of ethanol fuel and is gaining more attention in Mozambique, Ghana, Nigeria, Uganda and other parts of Africa (<http://www-mvd.iaea.org>). Rising oil prices coupled with the need to address concerns about emission from transportation fuels and the requirements of the Kyoto Protocol on carbon emission has led to the promulgation of a mandatory blending of biofuels (ethanol) with fossil fuels in Europe by 2020, which will require cassava chips as the alternative raw material feedstock (UNCTAD, 2009). According to FAO Food outlook (2009) cassava chips production will become a major emerging market opportunity for biofuels.

In spite of its economic importance, cassava has several negative characteristics such as low protein content of the tubers, high cyanogenic glucoside (Iglesias *et al.*, 2002) content in storage roots as well as poor storability of storage roots after harvest (Bokanga *et al.*, 1994). Furthermore, its cultivation is limited by high incidence of pests and diseases leading to very low yields on farmers' fields (Fargette and Thresh, 1994). The most prevalent diseases of cassava in Africa are African cassava mosaic virus (ACMV) (Hillocks and Thresh, 2002) which account for yield losses of about 90% and cassava brown streak disease (CBSD) (Thresh and Otim-Nape, 1994). These limitations lessen the potential use of the crop as an engine for growth and development. Breeding objectives have therefore aimed at increasing yield and starch content, reducing the propagation cycle, improving nutritional quality, cyanide content and development of ACMV tolerant varieties.

Conventional breeding efforts at addressing these problems are seriously limited by high

heterozygosity of the allopolyploid plants with low natural fertility, strong inbreeding depression and lack of resistance genes in sexually compatible germplasm (Kawano *et al.*, 1998, Fregene *et al.*, 2001, Nassar, 2001). Although traditional breeding programmes have led to the development of improved cultivars (Hershey and Jennings, 1992), the difficulty and time involved makes it laborious. Thus, the use of other methods are highly recommended to complement conventional genetic improvement programmes.

Genetic transformation is the most commonly used system to transfer foreign genes into plant cells. The technique was first reported in cassava in the second half of the 1990s (Li *et al.*, 1996; Raemakers *et al.*, 1996; Schöpke *et al.*, 1996; Zhang *et al.*, 2000b) and has been successful in some cassava cultivars. However, it requires a reliable regeneration system which is compatible with transformation methods allowing selection and regeneration of transgenic plants (Birch, 1997). Although, genetic transformation of cassava holds great promise for increasing productivity, especially in areas where conventional breeding lacks solution to problems facing the crop, it involves highly skilled labour and also expensive. Furthermore, the improvement of cassava using marker-free genetic transformation biotechnology can be significantly impaired by lack of a suitable *in vitro* regeneration procedure especially for the local cultivars (Aldemita and Hodges, 1996).

Somatic embryogenesis is the most efficient cassava regeneration method and it is mainly restricted to young leaves and shoots meristems (Puonti-Kaerlas, 1997; Raemakers *et al.*, 1997; Feitosa *et al.*, 2007). Thus far, it is the only reproducible morphogenic system in cassava which was first reported by Stamp and Henshaw (1982, 1987). Stamp and Henshaw (1982) induced

somatic embryos on a limited number of explants such as cotyledons or embryonic axes from zygotic embryos, whereas (Matthews *et al.*, 1993; Raemakers *et al.*, 1993a; Taylor *et al.*, 1996; Puonti-Kaerlas *et al.*, 1997; Li *et al.*, 1998) induced somatic embryos from immature leaf lobes. It is the process by which somatic cell differentiates into somatic embryos through characteristic embryological stages without fusion of gametes (Schumann *et al.*, 1995; Arnold *et al.*, 2002).

Somatic embryos are achieved by culturing leaf lobes, cotyledons, zygotic embryos on a medium supplemented with auxins such as picloram or 2,4-dichlorophenoxy acetic acid (2,4-D) or both (Sofiari, 1996). Such embryos are germinated further into plantlets through developmental steps that correspond to those of the zygotic embryos. Somatic embryogenesis has both advantages and disadvantages. Besides the use of somatic embryogenic calli for long term preservation via cryopreservation, somatic embryogenesis can be employed for the multiplication of diseased-free elite cassava clones and regeneration capacity of the propagules in a short time (Danso and Ford-Lloyd, 2004). However, this system is highly genotype dependent and for this reasons that it has not been adopted commercially in most of the crop species.

In the last few years successful long-term, highly regenerable embryogenic suspension cultures (Taylor *et al.*, 1996) and/or cyclic somatic embryogenic systems (Joseph *et al.*, 1999) were established for several varieties of cassava, making it possible to further create new varieties by genetic manipulations or induced mutations. Plant regeneration via somatic embryogenesis is widely applied in micropropagation, mutation breeding, cryopreservation and transformation (Bhojwani and Razadan 1996).

Tissue culture technique such as callogenesis is an essential step for any successful plant breeding (Guo and Zhang, 2005). *In vitro* plant regeneration can be exploited to raise transgenic plants using vector-mediated or direct gene delivery approaches or create variability by irradiation for cassava varieties. An important step in the somatic embryo technique is the development of an intermediate callus which could act as targets for inserting gene of interest either through microprojectile bombardment or co-culture with *Agrobacterium tumefaciens* to produce genetically transformed cassava. Joseph *et al.*, (2004), has reported that embryogenic calli could be used for induction of mutation by irradiation with X-rays or gamma rays for the production of valuable mutants.

The use of natural or induced mutations offers an alternative approach to crop improvement via physical and chemical mutagens. Mutations are heritable changes in the genetic material and are the ultimate source of all genetic variations between individuals (Ahloowalia *et al.*, 2004). Physical mutagens which are both ionizing and non-ionizing radiations as well as chemical mutagens are widely used to induce mutations in plants including cassava. The ionising radiations commonly used are X-rays and gamma rays. There are a number of chemical mutagens but for practical purpose of induction of mutation, only a few such as Ethyl methane sulphonate (EMS), diethyl sulphate (DES), ethylene imine (EI), and sodium azide are really useful. Other chemicals such as colchicine (though antimitotic) can as well be used for mutation induction.

Mutation breeding has been used to create useful variability in the DNA of several crops and it mostly results in insertions and deletions (INDEL) of part of the genome sequence (Lee *et al.*,

2003). Mutation induction breaks the nuclear DNA and cytoplasmic organelles which results in chlorophyll mutations, chromosomal or genomic mutations from which useful mutants are selected.

Mutagenic treatment may uncover recessive alleles by mutating or deleting corresponding dominant alleles. Mutation breeding as a tool for crop improvement becomes a good option for creating variability in vegetatively propagated crops (Sleper and Poehlman, 2006). It has been used extensively to improve several crops, without extensive hybridization and backcrossing (Maluszynski *et al.*, 2000; Ahloowalia *et al.*, 2004).

The induction and selection of mutants provide a simple, efficient, rapid and cheap method to alter the genetic make-up and obtain desired genotypes from otherwise well-adapted genotypes. Over 64% of the radiation induced mutant varieties was created via the use of gamma radiation (Ahloowalia *et al.*, 2004). In Ghana, mutation breeding resulted in the development of mutant cassava ‘Tek bankye’, with high dry matter content (40%) and good poundability and mutant cacao ‘Upper Amazon T85/799’ with resistance to cocoa ‘swollenshoot’ virus from Ghana (Danso *et al.*, 2008).

Besides hybridization and mutant induction, cassava improvement could also be achieved through the production of colchiploids as well as triploids. Polyploid breeding has unique advantages in cassava, because the economically useful product is a vegetative part. Generally, polyploids have enhanced resistance and enlarged plant organs. Thus, polyploid breeding in cassava could be used to improve the yield of the crop. Polyploid plants, for example,

tetraploids, can be produced by the chemical treatment (colchicine, oryzalin, etc.) of diploid plants. Moreover, other ploidy levels can be obtained by crossing different ploidy levels. The first triploid cassava variety “SreeHarsha” released in 1996 (Sreekumari *et al.*, 1999) was a cross between natural diploid and induced tetraploid plants. In this study, we propose the development of callus and somatic embryos via colchicine treatment.

In both seed and vegetatively propagated crops colchicine produces gene mutations instead of complete polyploidy in the plant (Datta, 1990) and has been used to induce morphological variants in pineapple (Mujib, 2005) and in Eucalyptus (Lin *et al.*, 2010).

The main objective of this research was to assess the effect of irradiation and colchicine on callus induction and germination in four local cassava accessions. The research had the following specific objectives;

- i. To determine the effect of radiation on plant height after sprouting.
- ii. To study the effect of doses of gamma radiation on callus induction and somatic embryo development in cassava.
- iii. To study the effect of different concentrations of colchicine on callus induction and somatic embryo development in cassava.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Origin and distribution of cassava

Cassava was introduced by Portuguese explorers when they established forts, trading stations and settlements on African coastal and nearby islands (Jones, 1959). The crop spread throughout Africa by various mechanisms of which the initial contact with the Portuguese-Brazilian culture appears to be the most remarkable. Cassava got to West Africa via the Bight of Benin and the Congo River at the end of the 16th century by European visitors to America (Sturtevant, 1969), and shortly after to the other parts of West and South-West Africa. The cultivation of cassava spread rapidly across Angola and Southern Zaire, but in West Africa only more slowly in the mid-19th century (Jones, 1959). The cultivation of cassava increased rapidly throughout tropical Africa during the 19th and early 20th centuries (Angladette, 1949). The first cultivation of cassava in Africa dates from 1558 (Carter *et al.*, 1992).

Central Brazil was suggested as one of the four centres of diversity of *Manihot* species. However, the exact area of origin of cassava as a crop plant is unknown, although several theories have been postulated. Recent nuclear DNA sequence studies by Kenneth (2002) suggested Mexico and Central America as centres of domestication. According to De Candolle (1886), cassava (*Manihot esculenta* Crantz) was first cultivated in north-eastern Brazil, based on the abundance of wild *Manihot* species.

Fregene *et al.*, (1994) reported that all species of the genus *Manihot* are native to the New World and exist in the western hemisphere between southern USA (33°N) and Argentina (33°S). Allem (1994) originally proposed that the modern cultivated cassava, *M. esculenta* subsp. *esculenta*, originated directly from the present wild subspecies *M. esculenta* subsp. *flabellifolia*. It has been reported that cassava was first cultivated in north-eastern Brazil.

In Ghana, cassava was grown by Portuguese around their trading ports, forts and castles and it was a principal food eaten by both Portuguese and slaves (Adams, 1957; Bentil, 2011). By the second half of the 18th century, the crop spread throughout the coastal plains and had become the main food of the people (Bentil, 2011).

2.2 Taxonomy and classification

The genus *Manihot* belongs to the Euphorbiaceae family and comprises over 200 species widely distributed throughout the tropics. About 98 species of *Manihot* are native to the New World, located in four regions in Brazil and Central America. *M. esculenta* is the only one of the 98 species widely cultivated for food production. Of the 98 species, 80 occur in Brazil, making the country an important genetic resource centre for the genus (Rogers and Appan, 1973; Onwueme 1978; Mkumbira 2002; Nassar and Marques, 2006). There are about five thousand known varieties of cassava adapted to different environmental conditions.

Although all the *Manihot* species examined so far are diploid with chromosome number $2n = 36$, research has shown that cassava may have originated by hybridization of two wild *Manihot*

species, (Olsen and Schaal, 1999) *M. esculenta* subsp. *flabellifolia*. Reports indicate that polyploid plants have been produced experimentally (O’Hair, 1990).

2.3 Economic importance of cassava

2.3.1 Food

Cassava is a vital staple food, which provides more than 50% of the calories consumed for more than 800 million people in sub-Saharan Africa (Osiru *et al.*, 1996). The dry root flesh contains up to 85% starch and very low protein. It is also rich in vitamins C (36 mg) and calcium (33 mg) with low fat (0.30 g) content. In Africa, the roots of cassava can be processed into various food forms which include gari, dried chips, flour and many other products (Nweke *et al.*, 1994). The fresh roots of cassava can also be peeled, boiled, fried or baked or fermented as dough (a processed cassava product) which is consumed in large quantities.

In Ghana, cassava can be used in various forms. The roots of sweet cassava varieties are eaten raw, roasted in an open fire, or boiled in water and used to prepare fufu. Cassava can also be dried and milled into flour to prepare a local meal “konkonte”. The fresh roots can also be grated or milled into cassava dough or processed into a granulated cassava product called gari.

The leaves are reported to have excellent nutritional value for humans and animals (Ceballos *et al.*, 2004). The tender shoots and leaves are eaten as vegetables in many parts of Africa and it provides protein (7 g protein per 100 g edible portion) with a high content of lysine, minerals and

vitamins (IITA 1990; Nweke *et al.*, 1994; Fregene *et al.*, 2000; Benesi, 2005). The seed is processed for oil and seed cake, used for formulating feed for livestock. The seed is also processed into a medicinal product to cure skin diseases (Popoola and Yangomodou, 2006).

2.3.2 Industrial uses

Cassava is an important commodity which can serve as a nucleus for many industrial applications mainly because of its starch. Starch is becoming multibillion dollar business worldwide and can be used to make products which maize, rice and wheat starch are currently being used for (Nduele *et al.*, 1993). Starch produced from cassava has a wide range of industrial applications. In the textiles industries, starch is used to increase the brightness and weight of the cloth and in the production of adhesives and glues for paper industry (Balagopalan, 2002). In the pharmaceutical industries starch is used for making gelatin capsules and as a thickener in syrups through the hydrolysis of glucose and dextrose.

Cassava starch is used as an additive in cement to improve the setting time and to improve the viscosity of drilling mud in oil wells. It is also used to seal the walls of bore holes and prevent fluid loss. Starch is also an important raw material for powder in the cosmetics industries. In the manufacture of detergent, starch is used to get better recovery and to improve the shelf life of detergents. (Nduele *et al.*, 1993) While in the rubber and foam industries, starch is employed for getting better foaming and colour.

2.3.3 Cassava as a biofuel crop

In recent times, interest in the usage of biofuels has increased due to rising global crude oil prices. Many countries are exploring a promising new market for cassava starch, as raw material for production of ethanol biofuel. Thus, besides its traditional role as a food crop, cassava is increasingly becoming a biofuel crop due to its high starch quality. As such ethanol used as a biofuel (Nguyen *et al.*, 2008).

In Ghana, the development and production of cassava bio-ethanol has the potential to create more jobs, increase exports, attract foreign investment and contribute to industrialization and modernization of Ghana's economy. The production of cassava as a bio-energy crop could minimise fossil fuel consumption, reduce environmental pollution and generate employment and income for the producers. In countries like China, cassava chips have been utilised in the production of ethanol (Getachew, 2009).

2.4 Cassava production

In Ghana, cassava is cultivated by more than 90 per cent of the farming population and it contributes about 22% of agricultural Gross Domestic Product (GDP). Cassava is earmarked as one of the alternatives to compete with maize for industrial processes in the tropics (Jaramillo *et al.*, 2005). In fact, it was declared as the crop of the decade by the African Union (AU) (Okai, 2001). Its high yield potential makes it a suitable option for other grain staples where population pressure and crop failure are a challenge (Al-Hassan, 1993; Nweke, 1996; Benesi, 2005). The exceptional ability of cassava roots to be stored in the ground and harvested when needed, makes

it a food security (DeVries and Toenniessen, 2001) and famine reserve crop (Nweke *et al.*, 2002).

Many reports on Ghana's economic growth and development have identified cassava as a single commodity that could generate desired economic growth and fight poverty (Dapaah, 1991; 1996; Al-Hassan and Diao, 2007; Nweke, 2004). There is a growing importance of cassava in Ghana (Dapaah, 1991; Al-Hassan, 1993; Manu- Aduening *et al.*, 2006); requiring therefore, that the demands of the growing cassava-based industry in Ghana be met in order to promote economic growth in Ghana and West Africa (Nweke, 2004; Al-Hassan and Diao, 2007; Dixon and Ssemakula, 2008). The annual cassava production in Ghana in 2006 was approximately 10 million tonnes representing 8% of total cassava production on the continent thus, rating Ghana as the third largest producer in Africa, after Nigeria and the Democratic Republic of Congo (FAO Food Outlook, 2009). Cassava ranks first in terms of area under cultivation and utilization in Ghana (NARP 1994). Production increased marginally to 11.3 metric tonnes (MT) in 2008 and by 2011, production has increased to 14.2 MT.

In Ghana, cassava is primarily produced for its roots which are a major and cheap source of carbohydrate in human diet, containing 20% amylose and 70% amylopectin, an important source of energy with a calorific value of 250 kcal/ha per day. About 90% of cassava produced is for human consumption, 10% for animal feed and industry. Cassava alone accounts for 34% of food crop consumption per annum (RMRDC, 2004; Bentil, 2011).

2.5 Diseases and pests of cassava

Insect pests and plant diseases can reduce cassava yield drastically, posing a threat to food security in the producing countries. More than 40 diseases caused by viruses, bacteria, fungi and phytomonas hinder cultivation of cassava. The cassava mealy-bug and cassava green mite are the two important pests of cassava in Africa. Additionally, most cassava-growing countries experience yield losses due to severe infection of the stakes with African Cassava Mosaic Virus (ACMV), East African cassava mosaic virus (EACMV), cassava anthracnose disease, cassava bacterial blight and South African cassava mosaic virus (SACMV) (Fargette and Thresh, 1994).

ACMV is the most devastating cassava disease which causes yield reduction up to 90% (Thresh and Otim-Nape, 1994). ACMV has been reported as occurring in all cassava growing countries in Africa and adjacent islands (Hillocks, 2002), while EACMV on the other hand is prevalent in Kenya, Tanzania, Malawi, Zimbabwe and other African countries including Ghana and causing significant yield losses (Thottapilly *et al.*, 2003). In Ghana, EACMV has been observed as a new viral infection with ACMV in some cassava cultivation (Fondong *et al.*, 1998; Offei *et al.*, 1999; Ogbe *et al.*, 1999).

2.6 Cassava improvement

2.6.1 Conventional hybridization

Cassava is conventionally propagated by stakes (Hallack, 2001; Alves, 2002). However, in some breeding programmes, seeds are used for propagation (Kizito *et al.*, 2005; Manu-Aduening *et al.*,

2005). But cassava propagation from true seed occurs under natural conditions and it is utilized in most breeding programmes (Iglesias *et al.*, 1994). However, propagation of cassava seeds result in segregation due to outcrossing in the crop (Osiru *et al.*, 1996). Besides, seedlings from seed propagation are smaller and less vigorous than plants from stem cuttings (Alves, 2002) and take a longer time to become established. Thus, cassava since its origin has been propagated vegetatively using stem cutting.

Cassava stem cuttings are bulky and highly perishable, drying up within a few days. Under favourable conditions, cassava stakes sprout forming adventitious roots within one week as such cannot be stored for a long time (FAO, 2000; Alves, 2002). However, propagation of cassava by stakes results in the production of true-to-type plants with high initial vigour. Propagation via stem cuttings could lead to systemic pests and disease build up which are transmitted from one season to the next thereby reducing yield (Thro *et al.*, 1998).

Conventional plant breeding is based on variation and selection of desired genotypes and is characterized by several years of crossing, selection and field evaluation before a desired genotype is released as an improved cultivar. Many vegetatively propagated crops including cassava have complex polyploidy levels, take several years to flower and fruit, are self-incompatible and/ or produce little or no seeds after crossing (Olsen and Schaal, 1999), hence, there is limited possibility to produce sufficiently large population to obtain desired recombinants in a short duration. Although, conventional breeding efforts are being made to overcome these problems, alternate breeding technique should be employed in cassava breeding.

2.6.2 Somatic embryogenesis

Tissue culture techniques such as genetic engineering, *in vitro* mutagenesis and somatic embryogenesis are important tools for varietal improvements of plants. The most widely used among these techniques for genetic transformation, mutant regeneration and somaclonal variation induction in many plant species is somatic embryogenesis. The technique has been documented in several cassava genotypes (Raemakers *et al.*, 1995a; Konan *et al.*, 1997; Atehnkeng *et al.*, 2006) and has been used to produce transgenic cassava in combination with gene transfer (Taylor *et al.*, 2001).

Somatic embryogenesis is the process of somatic cell differentiation into somatic embryos through characteristic embryological stages without fusion of gametes (Arnold *et al.*, 2002; Schumann *et al.*, 1995). Somatic embryogenesis can be initiated either directly on explant tissues, or indirectly from callus. For any successful plant breeding programme, a stable and active cell or tissue culture is required (Guo and Zhang, 2005). Somatic embryos or embryogenic calli produced can be used as target for transformation of the crop (Merkle and Dean, 2000). For cultivar generation and germplasm storage callus formation is an important material for establishing embryogenic culture. Embryogenic property can be maintained for prolonged period of time by repeated cycles of secondary embryogenesis (Raemakers, 1995), thereby making the technique useful for clonal multiplication.

Currently, the two most frequently used systems are shoot organogenesis from cotyledonous somatic embryos and embryogenic suspensions derived from friable embryogenic callus (FEC). All reported cases of cassava somatic embryogenesis involve the induction of organized

embryogenic structures from immature leaf lobes or cotyledons. The immature leaves have been reported to be most totipotent, capable of becoming de-differentiated tissues (Dhar and Joshi, 2005). These tissues are maintained on MS media with various concentrations of picloram and/or 2,4-D. Plant regeneration via somatic embryogenesis is widely applied in micropropagation, mutation breeding, cryopreservation and transformation (Bhojwani and Razadan 1996). Development of shoot primordia from germinating somatic embryos is induced directly on medium containing cytokinin. Cassava somatic embryogenesis involves optimization of embryo maturation and incorporation of a desiccation stage prior to induction of germination.

2.6.2.1 Induction of somatic embryos in cassava

Tissue culture technique such as somatic embryogenesis has been documented as the most efficient and reproducible cassava regeneration method to enhance the transformation of the crop (Raemakers *et al.*, 1995a; Konan *et al.*, 1997; Atehnkeng *et al.*, 2006). Studies on somatic embryogenesis of cassava were first reported by Stamp and Henshaw (1982). Several varieties of cassava plant have been produced via somatic embryogenesis in recent times (Danso and Ford-Lloyd, 2003). A number of reports documented a variety of improvement via cyclic embryogenesis (Raemakers *et al.*, 1993a). It is achieved by culturing leaf lobes, cotyledons, zygotic embryos cultured on a medium supplemented with auxins such as Picloram, 2,4-dichlorophenoxy acetic acid (2,4-D) or dicamba (Sofiari, 1996).

Somatic embryogenesis can be used to establish long-term embryogenic cultures by constant sub-culturing of such embryos, (Woodward and Puonti-Kaerlas 2001) via the development of cyclic secondary somatic embryogenic systems (Joseph *et al.*, 1999; Groll *et al.* 2001; Danso and

Ford-Lloyd 2002) which can be established on either liquid or solid medium. *In vitro* techniques such as somatic embryogenesis improves the effectiveness of mutation induction, by allowing the handling of large number of population (Jain and Maluszynski, 2004) and selection of mutants by means of rapid multiplication (Siddiqui *et al.*, 1994; Khatri *et al.*, 2002). The high frequency multiplication rate via somatic embryogenesis has been useful for mutation as well as gene transformation in crops including cassava (Munyikwa *et al.*, 1998; Li *et al.*, 1998; Konan *et al.*, 1994).

On the other hand, a combination of induced mutation and *in vitro* somatic embryo induction has been used to create desired variation and subsequent mutant production. Somatic embryos originate from single cells and mutagenesis of such cells may lead to the production of stable and solid mutants. This eliminates the need of repeated culture of tissues to separate mutated from non-mutated sectors (Ahloowalia, 1998). The induction and selection of mutants provide a simple, efficient, rapid and cheap method which alters the genetic make-up of desired genotypes obtained from otherwise well-adapted genotypes.

The induction of somatic embryos involves a two-step approach (Taylor and Henshaw, 1993; Konan *et al.*, 1994). In the first step, embryogenic calli are induced from leaf lobes of irradiated cassava stakes as well as leaf lobes treated with colchicine. These leaf lobes are subsequently cultured on MS medium supplemented with phytigel, 30g/l sucrose, 1 mg/l thiamine-HCl, 1.5 mg/l nicotinic acid, 1.5 mg/l pyridoxine-HCl, 2 mg/l glycine, 1ml of 2 μ M CuSO₄, 8mg/l, 2, 4-D or 16mg/l picloram. Organized embryogenic calli (OEC) are then transferred to a step two medium comprising of MS supplemented with 0.1mg/l benzylaminopurine (BAP) for

germination and maturation of somatic embryos. The primary somatic embryos can be used as source of explants to initiate a new cycle of embryogenesis. The process involves the fragmentation of matured primary embryos and culturing them on initiation medium (Raemakers *et al.*, 1993a). Several factors such as culture medium, growth condition, source of explant and genotype affect the development of embryos in somatic embryogenesis. These factors play key role to the successful embryo induction and subsequent plant regeneration.

2.6.3 Mutation breeding

Nuclear applications in food and agriculture have contributed greatly in enhancing agriculture production of seed and vegetatively propagated crops (Jain, 2005). Mutation breeding is an important tool in crop improvement of vegetatively propagated crops (Broertjes and Van Harten, 1998). It enhances genetic variability in plants, thereby allowing for selection of new cultivars. Mutation can either be spontaneous (natural) or induced (artificial). According to Li *et al.*, (1998) and Puonti-Kaerlas *et al.*, (1997), the genetic properties of cassava could be improved using mutagenesis. Usually, physical (gamma radiation) and chemical mutagens are used to alter the DNA structure. These mutagens cause random changes in the nuclear DNA or cytoplasmic organelles, resulting in gene, chromosomal or genomic mutations (Mensah *et al.*, 2005).

An important physical mutagen is gamma radiation. Gamma rays are electromagnetic radiations produced from radioisotopes with shorter wavelength. They have high penetrating power which leaves no traces of radioactivity in the irradiated material. The gamma rays cause breaks in the nuclear DNA and during the process of DNA repair mechanism, new mutations which are

heritable occur randomly. It has been used to develop 64% of the radiation induced mutant varieties (Ahloowalia *et al.*, 2004). Induced mutations have played important roles in cassava improvement programmes.

2.6.3.1 Mutation breeding in cassava

Mutations have been used over a period of time to create variability in many crops including cassava where natural genetic variation was limited and insufficient (Ahloowalia *et al.*, 2004; Maluszynski *et al.*, 2001). Over 200 to 300 mutant varieties have been released in China (Nagatomi, 1992) and Japan (Hitoshi, 2008). The use of induced mutation have resulted in the development of new and valuable alterations in plant characters and thus contributing to increased yield of specific crops. Many mutant varieties have been developed and among these varieties is cassava. Through induced mutation, cassava varieties with improved traits such as high yield, rind colour and putative mutants with altered starch qualities, suspected to be a novel free sugar have been developed (Amenorpe, 2010).

In Ghana, a mutant cassava cultivar “TekBankye” was released (IAEA-AFRA 2000). This mutant was developed by irradiating cassava stakes at 25 or 30 Gy using cobalt-60 source (Asare and Kantanka, 1997). It has very high dry matter content of 40% with good poundability and very popular among cassava growers. According to Ahiabu *et al.* (1997), radio-sensitivity tests conducted on the regenerated cassava plantlets identified 25, 30 and 35 Gy as suitable for *in vivo* and *in vitro* grown plants. However, 35Gy was identified as the optimum dose. Joseph *et al.*, (2004) found 50 Gy of γ -rays to be the optimal dose for inducing mutations in a cyclic somatic

embryogenic system in cassava PRC 60a under *in vitro* propagation. The estimated the effective radiation dose for the irradiated stakes was determined in previous research as 32 Gy (1 Gy = 1 J kg⁻¹= 100 rad) (Amenorpe *et al.*, 2004). Some varieties (Ankrah and Medzontem) with ACMV infection and poor yield characters were improved through irradiation of their stakes at 35 Gy LD50 dose from a gamma source (Amenorpe *et al.*, 2004).

2.6.3.2 Chemical mutagenesis

Chemical mutagens on the other hand, inhibit microtubule formation preventing the chromatin formation and inducing separation of the metaphase chromosomes. The mutagen breaks the nuclear DNA and new heritable mutations occur randomly during repair process. The use of chemical mutagens such as colchicine, sodium azide and dimethyl sulfoxide (DMSO) have been exploited in breeding programmes with great successes (Nilanthi *et al.*, 2009). Other mutagens such as ethyl methane sulfonate (EMS), sodium azide have been used to induce mutation in both seeds and vegetative propagated crops (Hossain, 2006).

Colchicine induces polyploidy in plant cells during cellular division by inhibiting chromosome segregation during meiosis (Doležel *et al.*, 1994). It has been used as both a polyploidising and mutagenic agent to produce polyploid plants by doubling of chromosome number. Polyploid plants generally have enlarged organs and exhibit disease resistance, delayed flowering, or lower fertility in some cases. Among artificially produced polyploids, triploids have a higher yield and higher starch potential (Sreekumari and Jos, 1996) and they are more vigorous than tetraploids (Nilanthi *et al.*, 2009).

These phenotypes that are considered to be favourable traits make them more desirable than the normal diploid parents. For this reason, this type of genetic manipulation is frequently used in breeding plants commercially. The mutagen induces genetic sterility in the crop without changes in vigour (Mensah *et al.*, 2005). The effects of colchicine on plant morphology, chlorophyll, sterility and yield have been reported by Castro *et al.* (2003) and Mensah (2005). Application of colchicine usually leads to increase in genome size and subsequent increased variation in the morphological characteristics. This observation is supported by Zel *et al.*, (1999).

A superior high yielding triploid hybrid mutant cassava (Sreekumari *et al.*, 1999) was produced from a cross between natural diploid and induced tetraploid plants. In cassava, colchicine can be applied to different parts of the plant including immature leaf lobes, meristems and shoot tips to induce polyploidy. However, the effectiveness of chemical mutagens depends on the concentration and the genotype (Singh *et al.*, 2000).

2.6.4 In vitro mutagenesis

A successful application of biotechnological methods for crop improvement requires a reliable *in vitro* plant regeneration system such as somatic embryogenesis (Raemakers *et al.*, 1993a). This technique requires less labour because embryos can grow into complete plants with a root or shoot axis, a vascular system and functional meristems are produced in a single step (Thorpe and Stasolla, 2001). *In vitro* mutagenesis involves the use of tissue culture along with mutation to induce phenotypic and genotypic variations in plants species to overcome the limitations of crop

improvement (Samad *et al.*, 2001). It is done by exposing propagules to mutagens (physical or chemical) and their subsequent culture of mutated explants on nutrient medium *in vitro*.

Different parts of the plant including shoot tips, leaf lobes, axillary buds can be used for *in vitro* mutation induction. *In vitro* induction and accelerated multiplication of polyploids was successfully done on shoot tips and nodal segments of cassava (Roy *et al.*, 2001; Nguyen *et al.*, 2003; Gu *et al.*, 2005; Viehmannova *et al.*, 2009). Pande and Khetmalas (2012) subjected leaf explants of Stevia to varying concentrations of Sodium azide and Colchicine (0 - 0.250%) solution for varying period of 12 and 24 hours at room temperature. Fu *et al.*, (1995) demonstrated maceration of leaf or shoot pieces in mutagenic solution of ethyl methane sulfonate (EMS) before tissue culture and obtained solid mutants.

In vitro techniques provide the mechanism to generate large populations for mutation induction, selection and rapid multiplication of the selected mutants (Siddiqui *et al.*, 1994). The success of any *in vitro* mutagenesis programme depends on the development of reproducible *in vitro* plant regeneration procedures, optimization of mutagenic treatments, and efficient screening of the mutagenized populations for desired variations (Van Harten, 1998; Jain, 2000, 2006, 2007).

According to Singh *et al.* (2000), explants can be irradiated and/or agitated in aqueous solutions of the chemical mutagen before culturing on the medium or the mutagen can be incorporated in the medium to induce mutation. The combined use of induced mutation techniques and *in vitro*

culture methods has a great potential in breeding programs (Lee *et al.*, 2003). In mutation breeding, determining the sensitivity of mutagen is the first step. *In vitro* mutagenesis has contributed to genetic improvement in several crop plants such as pineapple (Lepade *et al.*, 1995), banana (Rao *et al.*, 1995) and grape (Kuksova *et al.*, 1997).



CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Experimental site

The experiment was carried out in the Tissue Culture Laboratory of Biotechnology and Nuclear Agriculture Research Institute of Ghana Atomic Energy Commission (BNARI-GAEC).

3.1.1 Plant material

Four accessions of cassava were collected from BNARI farm and used for the study. The accessions were Ankrah, Dagati, Tumfa and Tuaka. Fresh stakes, 15cm long having 8 nodes each were planted in polythene bags in the heat chamber at 37⁰C for five weeks. The germinated shoot tips were excised and used as explants.

3.1.2 Culture media

The culture medium used consisted of Murashige and Skoog (1962) (MS) basal medium powder (Sigma Chemical Company, St. Louis, USA) prepared from MS stock solutions. The MS medium was supplemented with growth regulators according to the experimental requirements. All culture media were adjusted to pH 5.8 using 1M NaOH or 1M HCL and solidified with 3.5g/l phytigel and then sterilized in an autoclave at 121⁰C for 15minutes. Fifty millilitres (50ml) of the medium was dispensed into honey jars or Petri dishes (15ml). All media were kept at room temperature prior to usage.

3.1.3 Aseptic Manipulations

Aseptic manipulations were done under the laminar air flow hood (Nuaire Biological Safety Cabinet, UK). Forceps and scapels were sterilized in an oven (Gallenkamp Hotbox oven) at a temperature of 110°C for 2 hours. All inoculated Petri dishes were sealed with parafilm (Pechiney Plastic Packaging, USA) to prevent desiccation and contamination of cultures.

3.1.4 Incubation conditions

All cultures were kept in the growth room maintained at a temperature of 26± 1 °C under a 16/8-h (light/dark) photoperiod with light provided by white fluorescent tubes at an intensity of 2300 lux.

3.2 Sensitivity of cassava stakes to gamma irradiation

Cassava stakes of the four local accessions measuring 15cm were irradiated at 0 Gy, 32 Gy, 35 Gy, 45 Gy, and 50 Gy using 60Co source of gamma irradiation facility (GIF) prior to planting at the Radiation Technology Centre of the Ghana Atomic Energy Commission. The irradiated stakes were immediately planted in polybags filled with soil and sawdust mixture and kept in a heat chamber with a room temperature of 26°C at the Nuclear Agriculture Centre (NAC). The stakes were watered periodically after planting and allowed to sprout. Apical shoot tips obtained from germinated stakes were excised and used as explants. The heights of the shoots were measured with metre rule weekly for five weeks to assess the effect of irradiation dose on the height of the plant.

The experiment was set up as a completely randomized factorial design. The factors tested were (four accessions of cassava) x (gamma irradiation doses). Data were subjected to analysis of variance (ANOVA) and the means were separated, where appropriate, at the 5% significance level using the least significant difference (LSD). Data were analyzed using Genstat statistical package.

3.2.1 Induction of callus from leaf lobes of gamma irradiated cassava accessions

Apical shoot tips obtained from germinated gamma irradiated cassava stakes in the heat chamber were washed under running tap water for 30 min. These shoot tips were surface-sterilized by immersing in 70% alcohol with two drops of Tween 20 and vigorously agitated for 2 minutes under the laminar flow hood and thereafter rinsed with three changes of sterile distilled water. Leaf lobes about 3mm in length were excised from the sterilized shoot tips under a stereomicroscope (Lecia 2000, China) using a scalpel and pair of forceps and inoculated abaxially onto 25 ml callus induction medium in culture jars containing MS medium supplemented with 8 mg/l 2,4-D or 16 mg/l Picloram. The above treatment i.e 8 mg/l 2,4-D and 16 mg/l Picloram have been determined as the optimal concentration for callus induction under current laboratory condition previously by Danso and Ford-Lloyd, (2002). The cultures were subsequently incubated in total darkness at 21°C for 21 days and assessed for calli formation. Observations were made based on the size and colour of calli formed.

Each treatment was replicated three times with each replicate consisting of five leaf lobes.

3.2.2 Induction of callus from colchicine treated leaf lobes of cassava accessions

Another set of leaf lobes from sterilised shoot tips of the control were immersed in 10 ml of 0.00g/l, 0.05g/l, 0.10g/l, 0.20g/l and 0.25g/l colchicine for one hour in closed glass bottles. These bottles were placed on an orbital shaker at 6.5 rpm. Treated leaf lobes were thoroughly rinsed and then subsequently inoculated onto callus induction medium and incubated in total darkness at 21°C for 21 days and assessed for calli formation as described above. Observations were made based on the size and colour of calli formed.

The experiment was set up as a completely randomized factorial design. The factors tested were (four accessions of cassava) x (four concentrations of colchicine).

3.2.3 Somatic embryo formation from leaf lobe-derived callus of cassava

Primary calli obtained were sub-cultured on MS medium supplemented with 0.01 mg/l naphthaleneacetic acid (NAA) and 0.1 mg/l 6-benzylaminopurine (BAP) followed by the addition of 30 g/l sucrose and 100 mg/l myo-inositol. The pH was adjusted to 5.8 and 3.5 g/l phytigel added to the medium before autoclaving at 121°C for 15 minutes at 15 psi. The cultures were subsequently kept in a growth room at a temperature of 21°C under a 16/8-hr (light/dark) photoperiod with light provided by white fluorescent tubes (T 5 fluorescent fitting, UK) at an intensity of 3000 lux. The cultures were assessed for somatic embryo formation and the number of somatic embryos produced per lump was recorded after two weeks.

Each treatment was replicated three times with three calli clumps per each replicate.

3.3 Statistical Analysis

Statistical analysis was performed using Genstat software version 15. Analysis of variance (ANOVA) was done to separate the differences between means of treatment. The means were separated, where appropriate, at the 5% significance level using the least significant difference (LSD). Data were analyzed using Genstat statistical package.



CHAPTER FOUR

4.0 RESULTS

4.1 Radiation sensitivity in cassava accessions

4.1.1 Effect of gamma irradiation on plant height of cassava accessions

Plant height at the end of the 5th week was highest among the parent plants reaching a maximum of 24cm compared to plants irradiated at 32 Gy, 35 Gy, 45 Gy and 50 Gy. Among the four accessions, Tuaka had the highest height of 5.93cm at 45 Gy which was significantly ($p \leq 0.05$) higher than the other three accessions (Table 4.1). The gamma radiation had high significant ($P \leq 0.05$) effect on the height of plants and this was found to vary among the accessions and the interaction between accession and irradiation doses was also found to be statistically significant ($P \geq 0.05$).

Statistically ($p \geq 0.05$), the plant height after five weeks for the treated plants of the various doses was found to be variable among the four accessions. The four accessions showed significant ($p \leq 0.05$) differences as regards plant height.

Table 4.1: Effect of gamma irradiation on plant height of four Cassava accessions five weeks after sprouting.

Accessions	Irradiation dose (Gy)				
	Control	32	35	45	50
Ankrah	24.32cd	4.61ab	2.99a	5.43ab	4.50ab
Dagati	21.58cd	3.12a	3.31a	3.20a	4.69ab
Tumfa	16.32c	4.95ab	4.12a	3.12a	0.66a
Tuaka	19.38c	4.03a	5.89ab	5.93ab	3.25a

Values with same superscripts are not significantly different at $P \leq 0.05$

4.2 Effect of gamma irradiation on callus induction in cassava accessions

Callus formation/emergence was observed in all the leaf lobe explants of all four cassava accessions irradiated with gamma radiation when cultured on MS medium supplemented with 16 mg/l Picloram and 8mg/l 2,4-D between nine (9) and 21 days of culture. The earliest days to callus emergence was found to be nine days after culture at 32 Gy with leaf lobes cultured on Picloram (Table 4.2) and this occurred in Ankrah and Tuaka at 32 Gy with Tumfa and Tuaka having the longest day of emergence at 50 Gy (Table 4.3). Morphologically, calli formed were either compact with predominantly cream or yellow colour in the case of 2,4-D. In contrast, calli derived from leaf lobes cultured on Picloram were friable and soft with cream to brown colour (Figure 2 A and B). Days to callus emergence increased with increasing gamma irradiation dose in all accessions. However the increase was at different rates depending on the accession. A further increase in the level of dose from 45 to 50 Gy caused a decrease in callus formation. The gamma radiation had high significant ($P \leq 0.05$) effect on the days to calli emergence and this

was found to vary among the accessions but the interaction between accession and irradiation doses was however, not statistically significant ($P \geq 0.05$).

Table 4.2: Effect of gamma irradiation on callus induction from leaf lobes of four Cassava accessions cultured on MS medium supplemented with 16 mg/l Picloram.

Accessions	Irradiation dose (Gy)				
	Control	32	35	45	50
Ankrah	9.00 ^{ab}	9.00 ^{ab}	11.00 ^{ab}	10.00 ^{ab}	18.00 ^{de}
Dagati	9.00 ^{ab}	13.00 ^{abc}	15.00 ^{bcde}	17.00 ^{cde}	21.00 ^e
Tumfa	9.00 ^{ab}	9.00 ^{ab}	10.00 ^{ab}	16.00 ^{cde}	21.00 ^e
Tuaka	9.00 ^{ab}	9.00 ^{ab}	13.00 ^{abcd}	9.00 ^{ab}	20.00 ^e

Values with same superscripts are not significantly different at $P \leq 0.05$



Table 4.3: Effect gamma irradiation on callus induction from leaf lobes of four Cassava accessions cultured on MS medium supplemented with 8 mg/l 2,4-D.

Accessions	Irradiation dose (Gy)				
	Control	32	35	45	50
Ankrah	9.00 ^{ab}	9.00 ^{ab}	10.00 ^{abc}	11.00 ^{abc}	19.00 ^{efgh}
Dagati	9.00 ^{ab}	17.00 ^{def}	17.00 ^{cdef}	19.00 ^{efg}	21.00 ^{efgh}
Tumfa	10.00 ^{abcd}	10.00 ^{ab}	11.00 ^{ab}	17.00 ^{def}	21.00 ^{efgh}
Tuaka	9.00 ^{ab}	9.00 ^{ab}	11.00 ^{ab}	6.00 ^a	21.00 ^{efgh}

Values with same superscripts are not significantly different at $P \leq 0.05$.

4.2.1 Effect of gamma irradiation on percentage callus formation from leaf lobes of cassava accessions

Leaf lobes of gamma irradiated stakes of Ankrah at 32, 35 and 50 Gy cultured on MS medium supplemented with picloram did not develop callus except for 45 Gy. The same trend was observed in Tuaka for all the doses. However, in Tumfa and Dagati, only leaf lobes of 50 Gy irradiated stakes failed to form callus (Table 4.4). Unlike, leaf lobe explants cultured on Picloram, proliferation of callus continued in leaf lobe explants cultured on 2,4-D until the fourth week of culture, and the percentage callus induction was recorded. All the controls showed significant calli development. Leaf lobes explants obtained from cassava stakes irradiated at 50 Gy failed to produce callus. This was found in almost all the accessions irradiated with the above doses.

In addition, Tuaka failed to produce callus for all doses of the gamma radiation. All the accessions developed calli except for Ankrah and Tuaka which failed to induce calli at 35 Gy and 32 Gy respectively (Table 4.5). Statistical analysis using analysis of variance (ANOVA) showed highly significant ($P \leq 0.05$) differences in doses of gamma radiation in terms of calli formation. There was no statistical difference amongst all four accessions; likewise the interaction between the mutagenic treatments and the accessions was also not significant ($P \leq 0.05$). The effect of gamma irradiation on percentage callus formation varies greatly among the accessions ($p \geq 0.05$).

Table 4.4: Effect of gamma irradiation on percentage callus formation from leaf lobes of four Cassava accessions cultured on MS medium supplemented with 16 mg/l Picloram.

Accessions	Irradiation dose (Gy)				
	Control	32	35	45	50
Ankrah	79.00 ^g	0.00 ^a	0.00 ^a	9.33 ^{ab}	0.00 ^a
Dagati	50.00 ^f	29.33 ^{cde}	8.33 ^{ab}	8.33 ^{ab}	0.00 ^a
Tumfa	50.00 ^f	30.00 ^{cde}	3.33 ^a	3.33 ^a	0.00 ^a
Tuaka	79.00 ^g	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a

Values with same superscripts are not significantly different at $P \leq 0.05$

Table 4.5: Effect of gamma irradiation on percentage callus formation from leaf lobes of four cassava accessions cultured on MS medium supplemented with 8 mg/l 2,4-D.

Accessions	Gamma Irradiation				
	Control	32	35	45	50
Ankrah	70.00 ^{fg}	3.33 ^a	0.00 ^a	40.00 ^{def}	0.00 ^a
Dagati	34.00 ^{cde}	11.67 ^{ab}	21.67 ^{bcd}	8.33 ^{ab}	0.00 ^a
Tumfa	40.00 ^{def}	7.67 ^{ab}	3.33 ^a	3.33 ^a	0.00 ^a
Tuaka	59.33 ^{fg}	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a

Values with same superscripts are not significantly different at $P \leq 0.05$

4.3 Effect of colchicine on callus induction in cassava accessions

Leaf lobe explants of the four *Manihot esculenta* Crantz accessions; Ankrah, Tumfa, Tuaka and Dagati treated with varying concentrations of colchicine (0.05 – 0.25 g/l) and cultured on MS medium supplemented with 16 mg/l picloram induced calli except for 0.25 mg/l irrespective of the accession. However, all the four accessions used in this study produced calli on MS medium supplemented with 16mg/l Picloram, occurring after eight days of culture (Table 4.6). The days to callus emergence however, varied significantly ($P \leq 0.05$) from one accession to the other. The mean days to emergence of the Ankrah accession was the best followed by Tuaka, Tumfa and Dagati.

On the hand, leaf lobe explants cultured on callus induction medium supplemented with 8 mg/l 2, 4- D developed calli after a minimum of nine days of culture. The earliest days to callus emergence were recorded in Ankrah (controls and 0.1g/l). Leaf lobe explants of all the

accessions treated with 0.25g/l produced no calli after 30 days of culture (Table 4.7). Callus formation was found to be low with 2,4-D in the culture medium compared with Picloram. The colchicine treatment had significant ($P \leq 0.05$) effect on the days to emergence of the calli.

Generally, days to callus emergence increased with increasing colchicine treatment in all accessions. The earliest mean days to callus emergence, 8 days was observed in Ankrah leaf lobes treated with 0.05g/l colchicine (Table 4.4), this however, was not significantly different from the controls observed in all the accessions. Callus formation however, depended on the presence of the auxin in the culture medium. Colour of callus ranged from cream to yellow (Figure 3 A and B) with callus becoming tiny and or compact with intense colouration as concentration of colchicine in medium increased. Analysis of variance (ANOVA) shows that there are significant differences amongst the accessions with regards to days of callus emergence at $P \leq 0.05$.

Table 4.6: Effect of colchicine on callus induction from leaf lobes of four Cassava accessions cultured on MS medium supplemented with 16 mg/l Picloram.

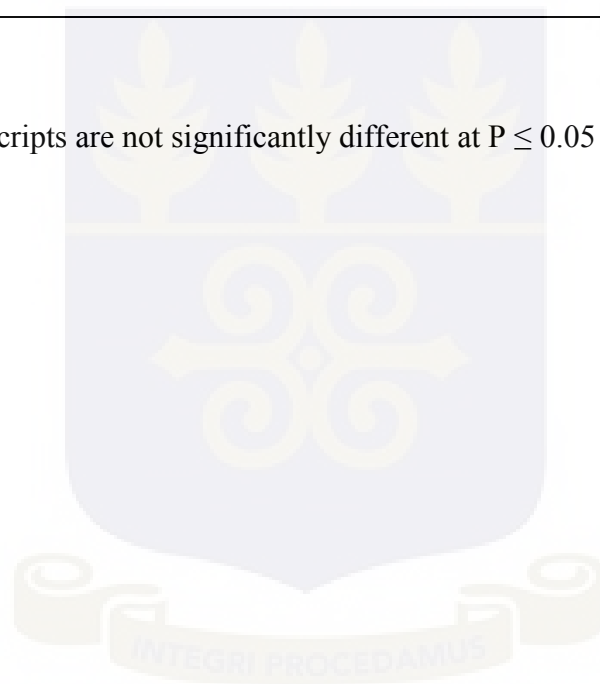
Accessions	Conc. of Colchicine (g/l)				
	Control	0.05	0.1	0.2	0.25
Ankrah	9.00 ^{ab}	8.00 ^a	20.00 ^{ef}	19.00 ^{def}	30.00 ^h
Dagati	9.00 ^{ab}	13.00 ^{abcd}	21.00 ^{efg}	23.00 ^{fg}	30.00 ^h
Tumfa	9.00 ^{ab}	11.00 ^{abc}	23.00 ^{fg}	26.00 ^{gh}	30.00 ^h
Tuaka	9.00 ^{ab}	9.67 ^{ab}	19.33 ^{cde}	19.33 ^{def}	30.00 ^h

Values with same superscripts are not significantly different at $P \leq 0.05$

Table 4.7: Effect of colchicine on callus induction from leaf lobes of four Cassava accessions cultured on MS medium supplemented with 8 mg/l 2,4-D.

Accessions	Conc. of Colchicine (g/l)				
	Control	0.05	0.1	0.2	0.25
Ankrah	9.00 ^{ab}	10.00 ^{abc}	26.00 ^{ghi}	22.00 ^f	30.00 ⁱ
Dagati	9.00 ^{ab}	14.00 ^{bcde}	25.00 ^{ghi}	27.00 ^{hi}	30.00 ⁱ
Tumfa	10.00 ^{abcd}	11.00 ^{abcd}	26.00 ^{ghi}	17.00 ^{cdef}	30.00 ⁱ
Tuaka	9.00 ^{ab}	11.00 ^{abcd}	26.00 ^{ghi}	20.00 ^{efgh}	30.00 ⁱ

Values with same superscripts are not significantly different at $P \leq 0.05$



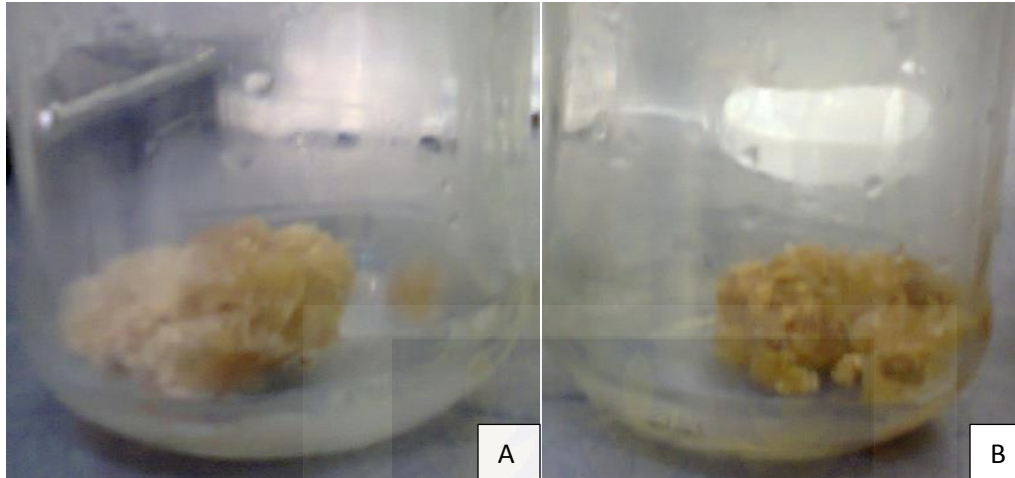
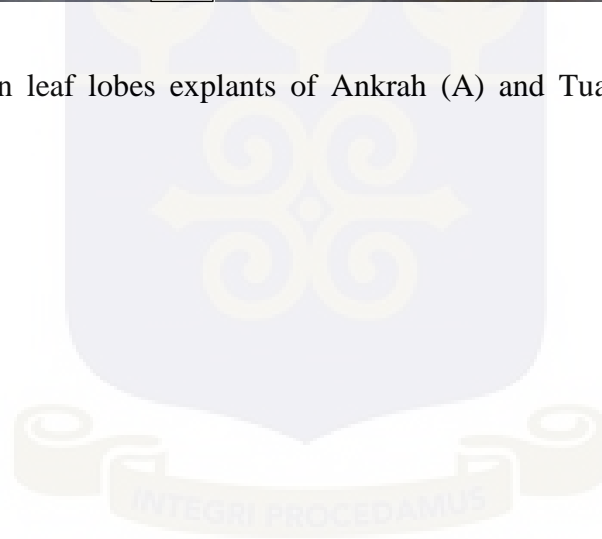


Fig.1: Calli formation in leaf lobes explants of Ankrah (A) and Tuaka (B) after 21 days of culture in the dark.



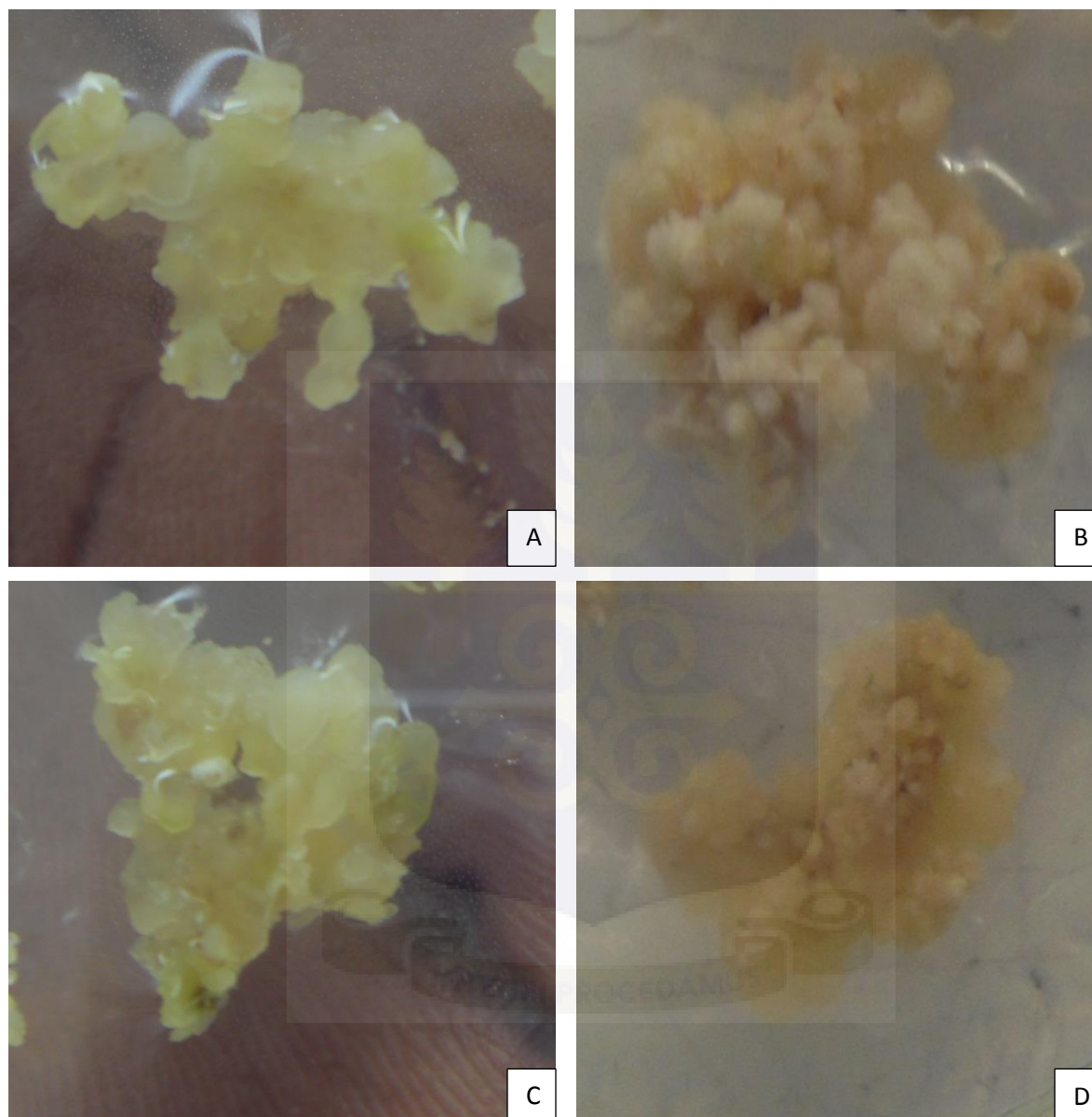


Fig.2: Calli formation in leaf lobe explants of *Manihot esculanta* (Crantz) accessions:

- (A) Dagati 0.05 g/l colchicine on 8 mg/l 2, 4 - D
- (B) Ankrah 0.05 g/l colchicine on 16 mg/l picloram
- (C) Tuaka 0.05 g/l colchicine on 8 mg/l 2, 4 - D
- (D) Tomfa irradiated at 32 Gy dose

4.3.1 Effect of colchicine on percentage callus formation from leaf lobes of cassava

accessions

Callus formation was observed on the adaxial surface of almost all leaf lobes after 7 days in culture. Globular somatic embryos developed on the upper surface of the leaf lobe cultures after 14 days. Development of calli from colchicine induced leaf lobes on picloram occurred after 21 days of culture. Morphologically, these calli were translucent and friable with predominantly, creamy to brown colouration. The highest percentage calli formation was observed in Ankrah (control) whilst Tuaka, Ankrah and Tomfa induced the lowest percentage of callus (Table 4.8). When the culture medium was supplemented with 8 mg/l 2, 4 – D, callus formation was found to increase in Ankrah and Tuaka. The mean differences between these accessions however, were highly significant ($P \leq 0.05$). Similarly, the mutagenic treatments had a highly significant effect on percentage callus formation with the controls producing more calli than those treated with colchicine.

In Ankrah, Dagati, and Tuaka accessions, the percentage callus formation was inversely proportional to the concentration of colchicine used in the treatment while there was no pattern in the relationship between irradiation dose and their corresponding callus formed (Table 4.9). Statistical analysis using analysis of variance (ANOVA) showed significant ($P \leq 0.05$) differences in concentration of colchicine and doses of gamma radiation in terms of calli formation. There was no statistical difference amongst all four accessions; likewise the interaction between the mutagenic treatments and the accessions was also not significant ($P \leq 0.05$).

Table 4.8: Effect of colchicine on percentage callus formation from leaf lobes of four Cassava accessions cultured on MS medium supplemented with 16 mg/l Picloram.

Accessions	Conc. of Colchicine (g/l)				
	Control	0.05	0.1	0.2	0.25
Ankrah	79.00 ^g	70.00 ^g	10.00 ^{abc}	3.33 ^a	0.00 ^a
Dagati	50.00 ^f	24.33 ^{bcde}	15.33 ^{bcd}	8.33 ^{ab}	3.33 ^a
Tumfa	50.00 ^f	66.67 ^g	33.33 ^{cde}	7.67 ^{ab}	0.00 ^a
Tuaka	79.00 ^g	70.00 ^g	11.67 ^{abc}	0.00 ^a	0.00 ^a

Values with same superscripts are not significantly different at $P \leq 0.05$

Table 4.9: Effect of colchicine on percentage callus formation from leaf lobes of four cassava accessions cultured on MS medium supplemented with 8 mg/l 2,4-D.

Accessions	Conc. Of Colchicine (g/l)				
	Control	0.05	0.1	0.2	0.25
Ankrah	70.00 ^{fg}	52.33 ^{ef}	8.33 ^{ab}	1.67 ^a	0.00 ^a
Dagati	34.00 ^{cde}	20.00 ^{bc}	13.33 ^{abc}	8.67 ^{ab}	3.33 ^a
Tumfa	40.00 ^{def}	43.33 ^{ef}	26.67 ^{bcd}	3.33 ^a	0.00 ^a
Tuaka	59.33 ^{fg}	45.00 ^{ef}	8.33 ^{ab}	0.00 ^a	0.00 ^a

Values with same superscripts are not significantly different at $P \leq 0.05$

4.4 Effect of gamma irradiation on embryo induction from leaf lobes of cassava accessions

For embryo formation, MS medium supplemented with BAP and NAA were used. Upon transfer to maturation medium, all the accessions were found to develop embryo between six to 14 days.

Tumfa and Tuaka had the highest number of shoots for control and 45 Gy respectively. However, at 50 Gy, no embryo was formed in all the accessions during the 30 days after transfer (Table 5.0). Embryo formation differed significantly among cultivars when cultured on MS supplemented with picloram. However, gamma irradiation caused significant influence on the number of shoots produced ($p \leq 0.05$). There were statistical differences in number of shoots produced at the various doses amongst all the accessions.

When callus tissues induced on MS medium supplemented with 2,4-D, were transferred to maturation media, some exhibited no response or became necrotic, but others developed either roots only (rhizogenic calli), or shoots (embryogenic calli). Upon transfer to maturation medium, Tumfa and Dagati calli induced on 8 mg/l 2,4-D resulted in the highest number of shoots for control and 45 Gy respectively. Shoot formation was observed as early as one week after calli were placed on maturation medium, especially from calli that had formed on medium supplemented with 8 mg/l 2,4-D (Table 5.1). The cultures were maintained for eight weeks to allow time for potential regenerative calli to respond. Observations made at the end of the culture period revealed that the percentages of embryogenic and rhizogenic calli were variable among genotypes (Fig.5 a-c). Most calli were found to be embryogenic.

Table 5.0: Effect of gamma irradiation on embryo induction from callus induced from leaf lobes of four Cassava accessions cultured on MS medium supplemented with 16 mg/l Picloram.

Accessions	Gamma Irradiation				
	Control	32	35	45	50
Ankrah	6.00 ^a	8.00 ^{ab}	12.00 ^{abcd}	17.00 ^{def}	30.00 ^h
Dagati	9.00 ^{ab}	7.00 ^a	11.00 ^{abcd}	18.00 ^{defg}	30.00 ^h
Tumfa	10.00 ^{abc}	7.00 ^a	9.00 ^{ab}	24.00 ^{fgh}	30.00 ^h
Tuaka	8.00 ^{ab}	8.00 ^a	13.00 ^{bcd}	25.00 ^{gh}	30.00 ^h

Values with same superscripts are not significantly different at $P \leq 0.05$

Table 5.1: Effect gamma irradiation on embryo induction from callus induced from leaf lobes of four cassava accessions cultured on MS medium supplemented with 8 mg/l 2,4-D

Accessions	Gamma Irradiation				
	Control	32	35	45	50
Ankrah	6.00 ^a	9.00 ^{abc}	13.00 ^{abcde}	17.00 ^{def}	30.00 ^g
Dagati	8.00 ^{ab}	8.00 ^{ab}	12.00 ^{abcde}	18.00 ^{def}	30.00 ^g
Tumfa	9.00 ^{abc}	9.00 ^{abc}	9.00 ^{abc}	16.00 ^{bcdef}	30.00 ^g
Tuaka	8.00 ^{ab}	8.00 ^{ab}	14.00 ^{bcdef}	16.00 ^{bcdef}	30.00 ^g

Values with same superscripts are not significantly different at $P \leq 0.05$

4.4.1 Effect of gamma radiation on percentage embryo induction from leaf lobes of cassava accessions

Percentage embryo formation declined linearly with increasing gamma irradiation dose in all accessions except in Dagati where the percentage embryo formation of 35% observed at 32Gy

unexpectedly increased to 42.3% at 35 Gy and then begun to decline with further increases in gamma irradiation dose (Table 5.2). No embryo formation occurred at 50 Gy in all the accessions. A significant increase in embryo formation in all the accession was associated with the NAA of the maturation medium and also due to the fact that most calli tissues were embryogenic. However, an increase in dose of 45 to 50 Gy failed to yield any significant difference in embryo formation.

Even though, all the accessions showed significant percentage of embryo in controls as well as 32 and 35 Gy, the increase in embryo formation was not statistically significant. Additionally, there was significant ($p \leq 0.05$) difference between percentages of embryos formed at all the doses amongst the accessions.

Table 5.3 displays results of the effect of gamma irradiation on percentage embryo formation of callus induced from leaf lobes of four cassava accessions cultured on MS medium supplemented with 8 mg/l 2,4-D. Upon transfer of callus tissues to maturation media, there was an increase in the number of shoots (embryos) resulting in Ankrah (control) and Dagati (35 Gy) recording the highest number of shoots. Generally, from these results shoot proliferation was optimum when callus was induced on MS supplemented with 2,4-D (8 mg/l). Some calli however, exhibited no response or became necrotic, therefore recorded no result at 50 Gy. The number of shoots produced was however significant ($p \leq 0.05$) but there were no statistical differences in number of shoots produced amongst the accessions.

Table 5.2: Effect of gamma irradiation on percentage embryo induction from callus induced from leaf lobes of four Cassava accessions cultured on MS medium supplemented with 16 mg/l Picloram.

Accessions	Irradiation dose (Gy)				
	Control	32	35	45	50
Ankrah	85.00 ⁱ	50.00 ^{gh}	26.30 ^{def}	10.00 ^{abc}	0.00 ^a
Dagati	51.70 ^{gh}	35.00 ^{ef}	42.30 ^{fg}	13.30 ^{abcd}	0.00 ^a
Tumfa	58.30 ^h	46.70 ^g	15.00 ^{abcd}	3.30 ^{ab}	0.00 ^a
Tuaka	80.00 ⁱ	50.00 ^{gh}	25.00 ^{cdef}	3.30 ^{ab}	0.00 ^a

Values with same superscripts are not significantly different at $P \leq 0.05$

Table 5.3: Effect of gamma irradiation on percentage embryo induction of callus induced from leaf lobes of four Cassava accessions cultured on MS medium supplemented with 8 mg/l 2,4-D.

Accessions	Irradiation dose (Gy)				
	Control	32	35	45	50
Ankrah	65.67 ^h	45.00 ^h	13.00 ^{abcd}	3.33 ^{ab}	0.00 ^a
Dagati	45.00 ^h	18.33 ^{bcde}	43.00 ^h	10.00 ^{abc}	0.00 ^a
Tumfa	50.00 ^h	20.00 ^{cdef}	7.67 ^{abc}	3.33 ^{ab}	0.00 ^a
Tuaka	55.67 ^h	43.67 ^h	13.33 ^{abc}	3.33 ^{ab}	0.00 ^a

Values with same superscripts are not significantly different at $P \leq 0.05$

4.5 Effect of colchicine on embryo induction from leaf lobes of cassava accessions

Despite the development of somatic embryos from all the cassava accessions cultured on MS medium supplemented with 16mg/l Picloram, the same could not be told of the mutagenic treatments as the calli from 0.2 mg/l to 0.25 mg/l showed little or no signs of developing

embryos at the end of the study. Generally, somatic embryos of the controls took shorter length of time to emerge compared to the colchicine treatment. Nonetheless, the days to embryo emergence declined with the increase in colchicine concentration. Consequently, the mutagenic treatments had a highly significant ($P \leq 0.05$) effect on the days to somatic embryo emergence. (Table 5.4). Although statistically there was no significant difference between the accessions,

Ankrah again comparatively performed better regarding days to embryo emergence compared to the other accessions. Embryo emergence began six days to one week after transfer onto regeneration medium in leaf lobe explants induced on 2,4-D. Apart from the control, the earliest embryo emergence (6 days) were all observed in the 0.05mg/l colchicine treatment. The days to embryo emergence among the various accessions (Figure 3) were significantly different from each another (Table 5.5). It was observed that shoot proliferation, was at best performance when callus was sub-cultured on MS supplemented with 0.1 mg/l BAP.

A number of shoots, as well as roots were produced at this concentration giving a significantly ($p \leq 0.05$) high number of shoots. However, some calli became necrotic immediately after transfer onto the step 2 medium whilst others were either embryogenic or rhizogenic. The mutagenic treatments had significant ($P \leq 0.05$) effect on the days to embryo emergence. However, the interaction between the accessions and the colchicine were not statistically significant ($P \leq 0.05$).

Table 5.4: Effect of colchicine on embryo induction from callus induced from leaf lobes of four Cassava accessions cultured on MS medium supplemented with 16 mg/l Picloram.

Accessions	Conc. Of Colchicine (g/l)				
	Control	0.05	0.1	0.2	0.25
Ankrah	6.00 ^a	11.00 ^{abc}	14.00 ^{bcd}	16.00 ^{cd}	23.00 ^{ef}
Dagati	9.00 ^{ab}	8.00 ^{ab}	13.00 ^{bcd}	16.00 ^{cd}	17.00 ^{def}
Tumfa	10.00 ^{abc}	9.00 ^{ab}	12.00 ^{abcd}	15.00 ^{cd}	17.00 ^{def}
Tuaka	8.00 ^{ab}	9.00 ^{ab}	12.00 ^{abcd}	14.00 ^{bcd}	16.00 ^{cd}

Values with same superscripts are not significantly different at $P \leq 0.05$

Table 5.5: Effect of colchicine on embryo induction from callus induced from leaf lobes of four cassava accessions cultured on MS medium supplemented with 8 mg/l 2,4-D

Accessions	Conc. Of Colchicine (g/l)				
	Control	0.05	0.1	0.2	0.25
Ankrah	6.00 ^a	12.00 ^{abcde}	15.00 ^{bcdef}	6.00 ^{bcdef}	22.00 ^f
Dagati	8.00 ^{ab}	8.00 ^{ab}	12.00 ^{abcde}	17.00 ^{def}	17.00 ^{def}
Tumfa	9.00 ^{abc}	9.00 ^{abc}	12.00 ^{abcd}	15.00 ^{bcdef}	17.00 ^{def}
Tuaka	8.00 ^{ab}	10.00 ^{abcd}	13.00 ^{abcde}	20.00 ^{ef}	17.00 ^{def}

Values with same superscripts are not significantly different at $P \leq 0.05$

4.5.1 Effect of colchicine on percentage embryo induction from leaf lobes of cassava accessions

In the step two or maturation medium, the percentage embryo formation varied from one mutagenic treatment to the other, ranging from 0 – 85%. Percentage embryo formation declined almost linearly with increasing colchicine concentration, thus embryo proliferation in the controls were significantly higher than in all mutagenic treatments. The accession type also had a significant effect on percentage embryo formation ($P \leq 0.05$). For instance, the highest (85%) percentage embryo formation in the controls was observed in Ankrah, whilst the least (51.70%) was observed in Dagati. Also, the interaction between the accessions and the mutagenic treatments were highly significant ($P \leq 0.05$).

Table 5.7 displays results of effect of colchicine on percentage embryo formation of callus induced from leaf lobes of four cassava accessions cultured on MS medium supplemented with 8 mg/l 2,4-D. Shoot proliferation was observed within one week after calli were placed on regeneration medium, especially from calli that had formed on 2,4-D (8 mg/l) medium. Most calli tissues were found to be embryogenic in all the accessions with only few being rhizogenic. However, the effect of the mutagenic treatments on percentage embryo formation was highly significant ($P \leq 0.05$). Embryos formed from calli induced at 0.25 g/l were very tiny and eventually became necrotic. Additionally, the colchicine concentration had an inverse relationship with percentage embryo formation.

Table 5.6: Effect of colchicine on percentage embryo induction from callus induced from leaf lobes of four Cassava accessions cultured on MS medium supplemented with 16 mg/l Picloram.

Accessions	Conc. of Colchicine (g/l)				
	Control	0.05	0.1	0.2	0.25
Ankrah	85.00 ⁱ	40.00 ^{fg}	33.30 ^{efg}	8.30 ^{abc}	7.00 ^{abc}
Dagati	51.70 ^{gh}	41.00 ^{fg}	41.00 ^{fg}	11.70 ^{abcd}	6.70 ^{ab}
Tumfa	58.30 ^h	45.00 ^{gh}	40.00 ^{fg}	10.30 ^{abcd}	8.30 ^{abc}
Tuaka	80.00 ⁱ	33.30 ^{efg}	36.70 ^{fg}	8.30 ^{abc}	18.00 ^{abcde}

Values with same superscripts are not significantly different at $P \leq 0.05$

Table 5.7: Effect of colchicine on percentage embryo induction of callus induced from leaf lobes of four Cassava accessions cultured on MS medium supplemented with 8 mg/l 2,4-D.

Accessions	Conc. of Colchicine (g/l)				
	Control	0.05	0.1	0.2	0.25
Ankrah	65.67 ^h	38.33 ^g	26.67 ^{def}	8.67 ^{abc}	3.33 ^{abc}
Dagati	45.00 ^h	34.33 ^{fg}	30.00 ^{efg}	8.33 ^{abc}	8.33 ^{abc}
Tumfa	50.00 ^h	26.67 ^{def}	30.00 ^{efg}	10.00 ^{abc}	8.33 ^{abc}
Tuaka	55.67 ^h	36.67 ^g	31.67 ^{efg}	5.00 ^{abc}	11.67 ^{abcd}

Values with same superscripts are not significantly different at $P \leq 0.05$

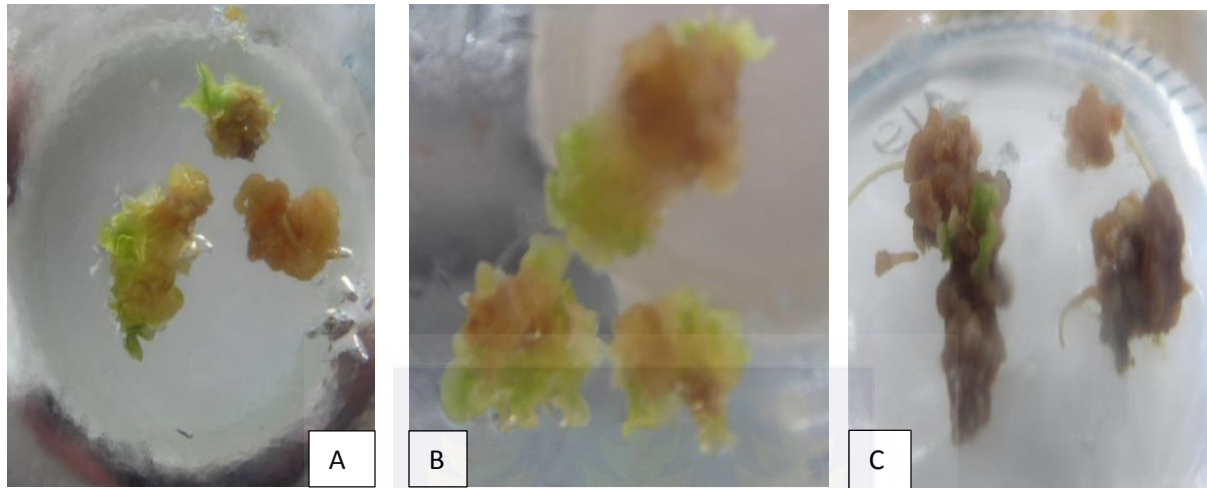
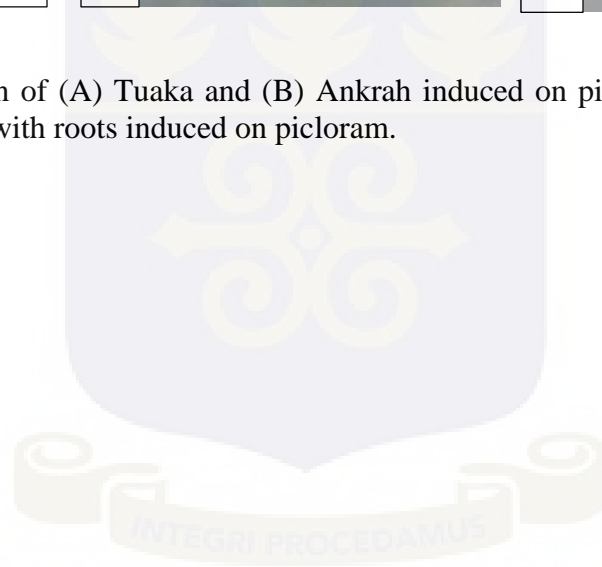


Fig.3: Embryo formation of (A) Tuaka and (B) Ankrah induced on picloram and (C) callus of Tuaka showing embryo with roots induced on picloram.



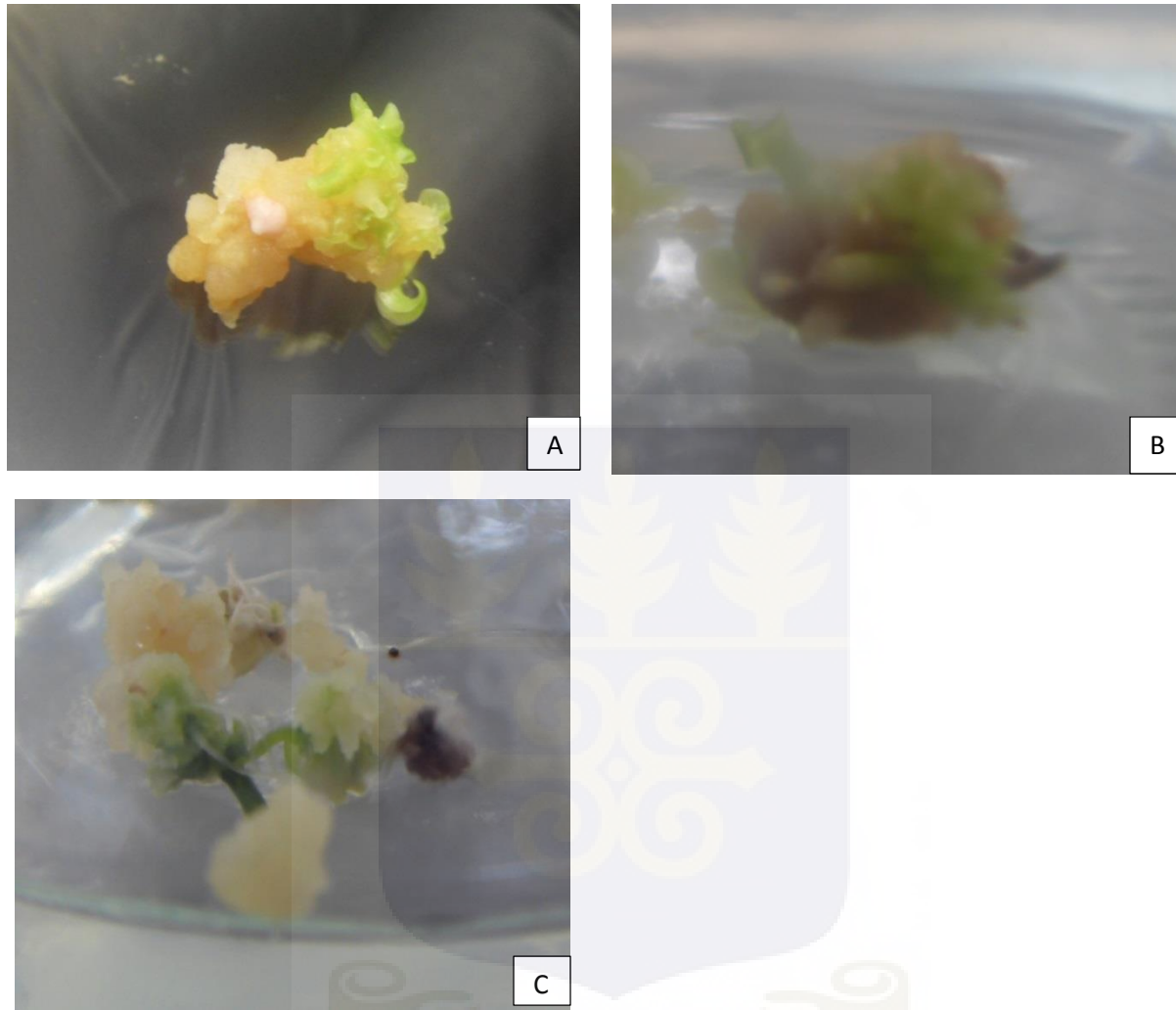


Fig. 4: Embryo formation in leaf lobe explants of (A) Ankra and (B) Tuaka irradiated at 32 Gy (C) Ankra treated with 0.05 g/l colchicine on 8 mg/l 2, 4 – D.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Radiation sensitivity on plant height as influenced by gamma radiation

The use of induced mutation creates new genetic variations within crop varieties. Induced mutations are ideal for augmenting natural variation in germplasm and as a supplement to hybridization. It also offers an excellent tool for identifying new genes (Micke *et al.*, 1990). In the present study, the irradiated cassava stakes reduced sprouting per cent with increase in dosage irrespective of genotypes used. Among the four accessions, Ankrah was found to be more sensitive to higher exposure whereas, lower dose had stimulatory effect. Survival percentage in the study also exhibited a decreasing trend with increasing levels of gamma rays. Similar observation on reduction in sprouting and survival per cent of treated material and radiation sensitivity among the accessions of a crop were reported by several workers in vegetatively propagated crops (Datta, 1991 and Hemalatha, 1998). Similar observation has also been recorded by Subramanian (2003) who reported that application of low doses of radiation (0.15 KGy) can arrest the sprouting of potatoes and onions. Radiation induces reduction in hydrolysis, resulting in lack of an energy source which may be the main cause for decreased survival rate and the reasons may be chromosomal and extra chromosomal damage to the cells. According to Kaicker (1992), reduction in sprouting may be due to the toxic effect of higher concentration of gamma rays whereas; the same at lower levels hastened the metabolic activity. The effect of gamma rays was more pronounced in increased doses in all four accessions and stimulatory effects at lower doses. Varieties differ in their sensitivity to radiation and this fact establishes that the genotypic control occurs within the species and it is the matter of sensitivity to radiation. (Pal, 1962).

5.2 Effect of gamma radiation and colchicine on callus induction in four cassava accessions

The mutagenic effect of different doses of gamma irradiations on callus induction and callus growth were examined. High dose of gamma irradiation showed necrosis of explants. The duration of callus induction in explants with high doses significantly increased as compared to control. It is evident that higher doses of gamma radiations mostly affect callus growth (Pande and Khetmalas (2012), hence, days to callus emergence increased. This is due to the disruption of the mitotic process essential for callus formation as a result of the destruction or possible damage of certain cellular processes or organelles at high dose of radiation. A similar trend was observed in a related study in *Citrus spp* by Altaf (2006).

On the other hand, the cassava accession showed significant difference in the days to their callus emergence. This was as a result of variations in the genetic make-up of the various accessions. This result is in agreement with Siddiqui and Javed (1982). The mutagenic effect of different concentrations of colchicine on callus induction and callus growth were examined, it was found out that the duration of callus induction significantly increased as compared to control. It was observed that at higher concentration i.e 0.25 g/l of colchicine mostly affects callus growth. In most of the treatments of mutagens ranging from 0.2 g/l to 0.25 g/l, there was no significant callus growth. Treatment duration therefore also affects callus generation and growth as observed in the experiment as there was no significant callus growth of explants treated with higher concentration above 0.2 g/l of colchicine. This study reveals that leaf explants treated with colchicine delays callus induction as concentration of the mutagens goes on increasing.

Comparatively, Colchicine being an anti-mitotic agent might have adversely restricted the mitotic process responsible for callus production, consequently the general delay in emergence of callus as observed in the colchicine treatments. Similarly, the non-emergence of callus in 0.25 g/l colchicine indicates that, at 0.25 g/l, concentration of colchicine is too high to allow for mitotic division to take place, hence the inability of the treatment to produce callus.

On the other hand, the days to emergence of calli on 2, 4 -D and Picloram supplemented medium were very similar. The lack of significant difference between the two auxin treatments could have been as a result of the use of only one concentration level, 8mg/l 2, 4 -D and 16mg/l picloram. However, the difference in days to callus emergence among the mutagenic treatments was highly significant ($P \leq 0.05$).

The study revealed that callus proliferation differs significantly among accessions and also depended upon the concentration of 2, 4-D and Picloram used. However, the accessions responded differently to the optimum concentration of 2,4-D and Picloram used. The use of 2,4-D require much lower concentration (8 mg/l) than picloram (16 mg/l) to induce more calli. The use of 8 mg/l 2,4-D for primary embryo induction in cassava is well documented (Danso and Ford-Lloyd, 2002; Ihemere, 2003). Khanna and Raina, (1998) reported that 2,4-D is the most suitable auxin for callus induction of rice in tissue culture.

5.3 Effect of gamma radiation and colchicine on percentage callus formation in four cassava accessions

Both physical and chemical mutagens are important in plant breeding as these create variability for crop improvement. All accessions provided good level of calli formation. However, differences were observed among the accessions, types of growth regulators, colchicine concentrations and doses of mutagen applied.

It was observed that callus induction occurred in all the explants of control. However in treated explants some of the treatment combinations failed to induce callus. There was significant effect of irradiation as the response of treated explants was low. Gahukar and Jambhule, (2000), also found similar type of decrease in callus obtained with increased dose of gamma rays in sugarcane. Higher doses of gamma irradiation caused considerable tissue damage, which perhaps in turn leads to reduction in the sizes of calli formed.

Apparently, callus fresh weight decreased with increasing colchicine treatment as explants that survived and formed calli showed low toxicity of colchicine since this antimetabolic is toxic to plants at high concentrations. Opposite results were reported by Viehmannova *et al.*, (2009), who studied yacon (*Smallanthus sonchifolius*) and observed a greater explants survival rate at higher concentrations of colchicine. Colchicine acts as a polyploidy inducer in plants *in vitro* but has limitations because it has a toxic effect at high concentrations, generating high mortality in the treated plants (Hamill *et al.*, 1992).

Additionally, leaf lobe explants (controls) of the four cassava accessions gave high percentages of callus formation when grown in medium containing 8mg/l 2, 4 -D or 16mg/l Picloram. Nonetheless, the percentage of callus formed in the 2, 4 -D treatment was comparatively higher

than in the Picloram treatment. In most tissue culture experiments, a high auxin/cytokinin ratio is used for starting embryogenic callus formation compared to a low ratio for the regeneration of plantlets (Ge *et al.*, 2006). The exact molecular function of plant growth regulators in tissue culture is unclear; however, it may probably be involved in the reprogramming of the expression of embryogenic genes (Ge *et al.*, 2006). In this study, the highest percentage of callus induction in all the varieties for leaf from colchicine treatment and gamma irradiated stakes were observed at 0.05g/l on 8mg/l 2,4-D and 16mg/l picloram. This suggests that the rate at which calli is induced depends on the concentration of the growth medium as well. 2,4-D was found to be more effective singly for the production of embryogenic calli. The results were in agreement with that of Rashid *et al.* (2001) but other researchers have found that 2,4-D in combination with kinetin was more effective in producing embryogenic callus (Ge *et al.*, 2006).

Cultures of explants also demonstrated rapid callus proliferation after subsequent sub culturing and transfer to shoot regeneration media supplemented with 0.1 mg/l BAP. This is in agreement with Zheng *et al.* (1996) where the use of ABA promoted the production of a larger number of somatic embryos per explant. Also, percentage callus production decreased with increasing irradiation doses. This is consistent with the observation made on sugar cane by Khan *et al.*, (2009).

High doses of radiation have been reported severally to have adverse effect on living tissue, thus, the decreased callus production observed in this study. Also, percentage callus production decreased with increasing colchicine concentration. Comparatively, colchicine treated explants produced more calli than those from gamma irradiated explants. Higher doses of gamma irradiation caused considerable tissue damage, which perhaps in turn led to a reduction in callus formation. In addition, this study proved that callus induction ability was greatly influenced by

the genotype used. This demonstrates that different varieties respond differently to callus formation. This confirms earlier reports by Gandonou *et al.* (2005) that callus induction is influenced by the genotype. The cultivars response to tissue culture was also dramatically different. Other reports indicate that some cultivars respond better than others and which seems to be genetically controlled (Li *et al.*, 2007; Ozawa *et al.*, 2003; Taguchi-Shiobara *et al.*, 1997). Different genotypes are reported to respond differently to callus *in vitro* under different 2,4-D concentrations (Elwafa and Ismail, 1999).

5.4 Effect of gamma radiation and colchicine on somatic embryo formation in four cassava accessions

Induction of mutation has been accepted as a useful tool in plant breeding programme and it has contributed significantly to the wealth of genetic resources. The capability of Gamma-rays in inducing desirable mutations in ornamental plants is well understood from a significant number of new varieties developed via direct mutation breeding. Higher doses of gamma irradiation caused considerable tissue damage, which perhaps in turn leads to reduction in callus fresh weight, hence, the days to somatic embryo formation. The gamma irradiation treatments unexpectedly increased the number of days to somatic embryo formation compared to the controls.

Nonetheless, the non-emergence of somatic embryo at 50Gy might have been due to the destruction of certain life processes in the explants, thus their inability to regenerate embryos.

Different mutagenic agents caused differential response of the treated calli to various tissue culture media. As such, the duration of exposure of leaf explants to colchicine influences callus

growth and regeneration to form somatic embryos. There were reductions in the germination of calli with increasing concentrations of due to the effects of mutagens on various explants. A similar observation was made in sesame seed by (Mensah *et al.*, 2005). At the end of the study, it was found out that tight calli with shoot organic potentiality failed to develop into somatic embryos but only loose calli without shoot organic potentiality which had lower duration of exposure to lower concentration of colchicine developed somatic embryos. The mutagenic treatments also significantly ($P \leq 0.05$) affected the days to somatic embryogenesis as was seen in the gamma irradiation and the colchicine treatments compared to the controls. This result was undoubtedly confirmed colchicine been an anti-mitotic agent, which rather suppressed the mitotic division and hence suppress somatic embryogenesis.

On the other hand, Somatic embryo formation is influenced by several factors such as explant, growth hormones and environmental factors. Of these factors, growth hormones are the most pronounced. In this study, MS basal medium supplemented with 8mg/l 2, 4-D led to early somatic embryo development as compared to 16 mg/l Picloram. Meanwhile, the days to somatic embryogenesis varied from one accession to the other in both Picloram and 2,4-D treatments. This, like the response of the varieties to days to callus emergence was also attributed the genotypic differences in the accessions.

5.5 Effect of gamma radiation and colchicine on percentage somatic embryo formation in four cassava accessions

Somatic embryogenesis is an important method in regeneration of cassava and cassava transformation. Somatic embryogenesis during callus induction is not unusual in cassava. The maximum number of somatic embryos was observed in the control in all four accessions. The mutagenic effect of different doses of gamma radiation on callus growth and regeneration of somatic embryos were studied. It was indicated that lower doses of gamma radiation influences callus induction and generation of somatic embryos. The somatic embryogenesis percent decreased with increasing doses of gamma radiations. Somatic embryo generation was significantly affected by higher doses of gamma radiation. The different irradiation doses showed significant impact on average number of somatic embryos produced, as the dose increased the embryogenesis potential decreased. Alain *et al.* (2002) made a similar observation in sugarcane that regeneration potential of calli into somatic embryos was inversely proportional to the mutagenic treatment.

Variations in the percentage of somatic embryos formed were recorded and it was observed that the percentages decreased with increasing concentrations of the colchicine used.

The frequencies of somatic embryo induction can be further increased and the method can be extended to many different cassava cultivars. The differential response of cassava cultivars to different auxin types and concentrations has been reported previously (Roca and Thro, 1993; Taylor *et al.*, 1993). The efficiency of somatic embryo induction is almost up to two-fold higher than the primary embryo induction in Ankrah (control), which confirms the observations that were reported earlier by Raemakers (1993). The high efficiency of somatic embryo production ensures the production of large numbers of explants for shoot regeneration.

This increasing evidence is also seen in the other tested cassava accessions. 2,4-D and NAA alone or in combination with Kinetin are essential for continuity of callus induction. However, a suitable combination of auxins and cytokinins is important for embryogenesis and organogenesis (Guohua, 1998). In some other species, induced somatic embryos might need a little cytokinin or other plant growth regulators in order to grow (Kumar *et al.*, 1989). Embryo maturation was achieved by cultivating clusters of developing somatic embryos in maturation medium, as described by Zhang *et al.* (2001). The highest percentage somatic embryos formation was observed in the controls, irrespective of the type of accession. Again, the mutagenic treatments had significant effect on the percentage somatic embryo formation. Also, the 16mg/l Picloram treatment produced high percentage of somatic embryos compared to 2,4-D in this study. This was as a result of the concentration of 2,4-D been too high to elicit optimal somatic embryo production from the accessions used in this study.

Firoozabady and Moy (2004) reported picloram as one of embryogenic potentials agent to increase the growth regulators in *Ananas comosus*. However, picloram regulated the embryogenic stages and produced maximum frequency of SEs and plant germination (Little *et al.*, 2000; Groll *et al.*, 2001). Somatic embryos maturation was stimulated by auxins combine with cytokinins in *Leptadenia reticulata* (Martin, 2004). It is been reported severally that 2,4-D at lower concentration induces production of more somatic embryos (Raemakers, 1993).

CHAPTER SIX

6.0 CONCLUSION

Cassava (*Manihot esculenta* Crantz) is a tropical crop is well adapted to a wide range of has received the research attention it requires so it could be improved in both yield and grain quality. With the many pressing needs in various parts of the world and the demand placed on agriculture to produce more food, breeding and improvement of traditional crops have become necessary in both developed and developing countries.

Callus induction and somatic embryo development are the two most important steps in plantlet regeneration in tissue culture. With the successful callus induction and somatic embryo development from young cassava leaf lobes via *in vitro* mutagenesis, this study sought to provide information necessary for *in vitro* manipulation of Cassava (*Manihot esculenta* Crantz) aimed at improving the yield and production of ACMV- free through application of physical and chemical mutagens. The following conclusions have been made:

- a) Amongst the four cassava accessions, Ankrah gave the best respond in terms of sprouting and height whilst Dagati gave the least response.
- b) Amongst the four Cassava accessions of used, Ankrah and Tuaka gave better response in terms of callus and embryo formation than Tomfa and Dagati in both control and colchicine treated accessions.
- c) Colchicine concentration of 0.05 g/l and 32Gy of gamma irradiation gave better results in terms of callus formation.
- d) Callus and somatic embryo formation was observed in all control treatment.
- e) Somatic embryo formation was genotype dependent.

- f) Concentration of colchicine treatment delayed callus formation in all the four accessions.
- g) Callus formation decreased with increased gamma irradiation.
- h) Maximum callus formation was obtained on MS medium supplemented with concentration of 16 mg/l and 8 mg/l 2,4-D for leaf lobe explants.
- i) Almost all the accessions induced callus and somatic embryos within eight and six days respectively in control treatment but the treated accessions induced callus between 6 and 19 days and embryos between 8 and 15 days depending on the accession involved.
- j) Gamma radiation had significant effect on sprouting as well as the height of all four accessions.

6.1 RECOMMENDATIONS

Based on the findings of this study, the following recommendations would be of significant importance.

1. In future research, Tomfa and Dagati should be considered since callus formation and *in vitro* plant regeneration is genotype-dependent.
2. Further research should be carried out on plantlet regeneration using these accessions via mutagenesis.
3. Further manipulations should be made to 0.05g/l colchicine as well as 32 and 35 Gy treatment to induce callus and regenerate of plantlets

REFERENCES

- Adams, C. D. (1957). Activities of Danish Botanists in Guinea 1738–1850. Transactions of the Historical Society of Ghana III. Part 1.
- Ahiabu, R. K., Lokko, Y., Danso, K. and Klu, G. Y. P. (1997). Mutagenesis for ACMV resistance in Ghanaian cassava cultivar 'Bosom nsia'. IAEA-TECDOC-951:9-18
- Ahloowalia, B.S. (1998). Radiation techniques in crop and plant breeding. Multiplying the benefits, IAEA Bulletin, pp 37-39.
- Ahloowalia, B.S., Maluszynski, M and Nichterlein, K. (2004). Global impact of mutation derived varieties. *Euphytica*, **135** (2): 187–204.
- Alain, R., Ralinian, A.B.M.M. and Gupta, S. (2002). *In vitro* plant regeneration from leaf sheath cultures sugarcane via organogenesis. *Plant Cell Biotech. and Mol. Biol.*, 3(314):131-136.
- Aldemita, R. R. and Hodges, T. K. (1996). *Agrobacterium tumefaciens*-mediated transformation of *japonica* and *indica* rice varieties. *Planta*, **199**:612-617
- Al-Hassan, R.M. (1993). Cassava in the Economy of Ghana in product Development for Root and Tuber Crops. Volume III – Africa and Latin America. Breeding projects work to stabilize productivity without increasing pressures on limited natural resources. *BioSciences* 43:441-452.
- Al-Hassan, R.M. and Diao, Xinshen. (2007). Regional disparities in Ghana: Policy options and public investment implications IFPRI discussion papers 693, International Food Policy Research Institute (IFPRI).
- Allem, A.C., (1994). Origin of *Manihot esculenta* Crantz (Euphorbiaceae) 41: 133-150.
- Allem, A.C., (2002). The origins and taxonomy of cassava. In: R.J. Hillocks, J.M. Thresh, and A.C. Bellotti (Eds.), *Cassava: Biology, Production and Utilization*. CABI Publishing, pp. 1- 16.
- Altaf, N. (2006). Embryogenesis in undeveloped ovules of citrus cultivars in response to gamma radiation. *Pak. J. Bot.*, 38(3): 589-595.

- Alves, A. A. C. (2002). Cassava Botany and physiology. In: Hillocks, R. J., Thresh, J. M. and Bellotti, A.C. (Eds.). Cassava: Biology, Production and utilization. CABI Publishing, Oxon, New York. pp 67-89.
- Amenorpe, G., Amoatey, H.M. and Asare, D. (2004). Breeding for drought tolerance/avoidance and mealiness in cassava using gamma radiation. GAEC/BNARI 2004 Annual Report, pp. 22-23.
- Amenorpe, G., Carson, G. and Tetteh, J.P. (2006). Ethnobotanical characterization of cassava (*Manihot esculenta* Crantz) in Western Region of Ghana. *Ghana J. of Agricultural Science*, 39 (2):123-130.
- Amenorpe, G. (2010). Mutation breeding for *in planta* modification of amylose starch in cassava (*Manihot esculenta* Crantz).
- Angladette, M.A. (1949). La situation et l'avenir de la production du manioc dans les territoires d'outre-mer de l'Union Française. In: *Proc. Cong. Du Manioc et des Plantes Esculentes Tropicales des Territoires de L'Union Française*, Inst. Colon. Marseille, pp: 63-68. (cited from Lancaster *et al.*, 1982)
- Arnold, V.S., Sabala, I., Bozhkov, P., Dyachok, J. and Filonova, L. (2002). Developmental pathways of somatic embryogenesis, 69: 233-249.
- Asare, E. and Safo-Kantanka, O. (1997). Improvement of cassava cooking quality through mutation breeding. In: Ahloowalia BS (ed.). Improvement of basic food crops in Africa through plant breeding, including the use of induced mutations. International Atomic Energy Agency, Vienna, IAEA-TECDOC-951. pp. 19– 24.
- Atehnkeng, J., Adetimirin, V.O. and Ng, S.Y.C. (2006). Exploring the African Cassava (*Manihot esculenta* Crantz) germplasm for somatic embryogenic competence, 5 (14): 1324-1329
- Baafi, E. and Sarfo-Kantanka, K. (2008). Agronomic and Processing Attributes of some cassava (*Manihot esculenta* Crantz) genotypes affected by location and age at harvest in Ghana, 3(3): 211-218.
- Balagopalan, C. (2002). Cassava utilization in food, feed and industry. In: Hillocks, R. J., Thresh, J.M., and Bellotti, A.C (Eds.). Cassava: Biology, Production and Utilization. CABI Publishing, Oxon, New York.

- Benesi, I.R.M. (2005). Characterisation of Malawian cassava germplasm for diversity, starch extraction and its native and modified properties. PhD Thesis, Department of Plant Sciences (Plant Breeding), University of the Free State, South Africa.
- Bentil, B. (2011). Assessment of three different drying technologies (Sun, Solar And Bin) used for the production of cassava (*Manihot esculenta* Crantz) chips in Ghana. PP 1-4
- Bhojwani, S.S. and Razdan, M.K. (1996). Clonal Propagation, pp 483-536. In: Bhojwani, S.S. and Razdan, M.K.(eds). Plant Tissue Culture: Theory and Practice. Rev. ed. Elsevier, Amsterdam.
- Birch, R.G (1997). Plant transformation: problems and strategies for practical application, 48: 297-326
- Bokanga, M., Ekanayake, I.J., Dixon, A.G.O and Porto, M.C.M. (1994). Genotype-environment interactions for cyanogenic potential in cassava. *Acta Horticulturae*, **375**: 131-139
- Broertjes, C., van Harten, A.M. (1998). Shoot apices: Organization and post-irradiation behavior. *In*: Broertjes, C., van Harten, A. M. (Eds.). Developments in crop science 12: Applied mutation breeding for vegetatively propagated crops. Elsevier: 29-44.
- Carter, S.E, Fresco, L.O., Jones, P.G., Fairbain, J.N. (1992). An atlas of cassava in Africa: historical, agro ecological and demographical aspects of distribution, CIAT, Cali, Colombia.
- Castro, C.M., Oliveira, A.C. and Calvaho, F.I.F. (2003). Changes in allele frequencies in colchicine treated Ryegrass population assessed with APD marker, 9 (2):107- 112.
- Ceballos, H., Iglesias, C.A., Pérez, J.C. and Dixon, A.G.O. (2004) Cassava breeding: opportunities and challenges, 56: 503-516.
- Danso, K.E. and Ford-Lloyd, B.V (2002). Induction of high-frequency somatic embryos in cassava for cryopreservation, 21: 226-232
- Danso, K.E. and Ford-Lloyd, B.V. (2003). Encapsulation of nodal cuttings and shoot tips for storage and exchange of cassava germplasm, 21:718-725.

- Danso, K.E. and Ford-Lloyd, B.V. (2004). Cryopreservation of embryogenic calli of cassava using sucrose cryoprotection and air desiccation, 22: 623-631.
- Danso, K.E., Safo-Katanka, O., Adu-Ampomah, Y., Oduro, V., Amoatey, H.M., Asare, O.K., Ofori Ayeh, E., Amenorpe, G., Amiteye, S. and Y. Lokko. (2008). Application of induced mutation techniques in Ghana: Impact, challenges and the future. In: Book of Abstracts, FAO/IAEA international symposium on induced mutations in plants, 12-15 Aug., 2008, Vienna, Austria, p.108.
- Dapaah, S.K. (1991.) Contributions of root and tuber crops to socio-economic changes in the developing world: the case of Africa, with special emphasis on Ghana. In: Ofori F, Hahn SK (eds.) Tropical root crops in a developing economy. Proceedings of the ninth symposium of the international society for tropical root crops, 20-26 October, Accra, Ghana, pp. 21-24.
- Dapaah, S.K. (1996). The way forward for accelerated agricultural growth and development. A paper presented to the Government of Ghana on behalf of the Ministry of Food and Agriculture.
- Datta, S.K. (1990). Colchicine induced mutations in seed and vegetatively propagated plants. J. Indian Bot. Soc. **69**, 261-266.
- Datta, S.K. (1991). Evaluation of recurrent irradiation on vegetatively propagated ornamental: Chrysanthemum, **20** (2), 81-86.
- De Candolle, A. (1886). Origin of Cultivated Plants, 2nd ed. Reprinted, Hafner Publishing Company, New York, pp 468 (cited from Veltkamp, 1986)
- DeVries, J. and Toenniessen, G. (2001) Cassava. CABI Publishing Oxon, UK.
- Dhar, U. and Joshi, M. (2005). Efficient plant regeneration protocol through callus for *Saussurea obvallata* (DC.) Edgew. (Asteraceae): effect of explant type, age and plant growth regulators, 24: 195-200.
- Dixon, A.G.O. and Ssemakula, G. (2008). Prospects for cassava breeding in Sub-Saharan Africa in the next decade, 6: 256-262.

- Doležel, J., Lucretti, S. and Schubert, I. (1994). Plant chromosome analysis and sorting by flow cytometry, 13(3): 275 – 309.
- Elwafa, A.A.A. and Ismail, A.E.A. (1999). Callus induction and plant regeneration from cultures of immature embryos of spring wheat. *Assiut J. Agric. Sci.*, 30: 13–23.
- Fargette, D. and Thresh, J.M. (1994). The Ecology of African Cassava Mosaic Geminivirus. In: Bakeman, J.P., Williamson, B. (Eds). *Ecology of Plant Pathogens*, CABI.
- Feitosa, T., Bastos, J.L.P., Ponte, F.A., Juca, T.L and Paiva Campos, F.A. (2007). Somatic embryogenesis in cassava genotypes from the northeast of Brazil. *Brazilian Archives of Biology and Technology*. Volume 50 (2): 112-116
- Firoozabady, E and Moy, Y. (2004). Regeneration of pineapple plants via somatic embryogenesis and organogenesis. *In Vitro Cell. Dev. Biol.*, **40**, 67-74.
- Fondong, V.N., Pita, J.S., Rey, C., Beachy, R.N and Fauquet, C.M. (1998). First report of the presence of East African cassava mosaic virus in Cameroon, 82, 287-297.
- Food and Agriculture Organization (2000). Review of cassava in Africa with country case studies on Nigeria and Ghana-Cassava Development in Ghana.
- Food and Agriculture Organization (2006). Production yearbook. FAO, Rome.
- Food and Agriculture Organization (2009). Food Outlook : Corporate document repository, December, 2009.
- Fregene, M.A., Vargas, J., Ikea, J., Angel, F and Tohme, J. (1994). Variability of chloroplast DNA and molecular ribosomal DNA in cassava (*Manihot esculenta* Crantz) and its wild relatives. *Theoretical and Applied Genetics*, **89**: 719-727.
- Fregene, M., Bernal, A., Duque, M., Dixon, A.G.O and Tohme, J. (2000). AFLP analysis of African cassava (*Manihot esculenta* Crantz) germplasm resistant to the cassava mosaic disease (CMD). 100: 678-685.

- Fregene, M., Okogbenin, E., Mba, C., Angel, F., Suarez, M.S., Gutierrez, J., Chavarriaga, P., Roca, W.M., Bonierbale, M and Tohme, J. (2001). Genome mapping in cassava improvement: challenges, improvements and opportunities. 120: 159-165.
- Fu, R., Anderson, R. L., Cheng, Z. M. and Liu, Z. R. (1995). Studies on induced mutation of fruit trees *in vitro*. *Acta Horticulturae*, **403**: 111-117.
- Gahukar, S. J and Jambhale, N. D. (2000). Callus induction and regeneration in *Saccharum* cultivar as influenced by mutagen treatment. *J. Maharashtra Agric. Univ.*, **25**: 2, 219-220.
- Gandanou, C., Errabii, T., Abrini, J., Idaomar, M., Chibi, F. and Senhaji, N. (2005). Effect of genotype on callus induction and plant regeneration from leaf explants of sugarcane (*Saccharum sp.*). *African Journal of Biotechnology*.4 (11), 1250-1255.
- Ge, X.J., Chu, .ZH., Lin, Y.J. and Wang, S.P. (2006) .A tissue culture system for different germplasm of indicarice. 25: 392-402.
- Getachew, E., (2009). Genetics and Breeding of Cassava (*Manihot esculenta* Crantz). A term paper Submitted to the course instructor Dr. Sentayehu Alamirew, for the partial fulfillment of the course Advanced Genetics and Breeding of Horticultural crops. November, 2009 Jimma, Ethiopia.
- Groll, J., Mycock, D.J., Gray, V.M and Lamenski, S. (2001). Secondary somatic embryogenesis of *Cassava* on picloram supplemented media. *Plant Cell Tiss. Org. Cult.*, **65**, 201-210.
- Gu, X.F., Yang, A.F., Meng, H., and Zhang, J.R. (2005). In vitro induction of tetraploid plants from diploid *Zizyphus jujube* Mill. cv. Zhanhua. *Plant Cell Reports* 24: 671–676.
- Guo, Y. and Zhang, Z. (2005). Establishment and plant regeneration of somatic embryogenic cell suspension cultures of the *Zingiber officinale* Rosc. 107: 90-96.
- Guohua, M. (1998). Effects of Cytokinins and Auxins on Cassava Shoot Organogenesis and Somatic Embryogenesis from Somatic Embryo Explant. *Plant Cell Tiss. Org. Cult.*, **54**: 1-7.

Hallack, H. (2001) Cassava. Scientific Name: *Manihot esculenta* Family: Euphorbiaceae, Spurge
Duration: Short-lived shrubby perennial. www.wam.edu/-mathewsc/cassava.html

Hamill, S.D., Smith, M.K. and Dodd, W.A. (1992). *In vitro* induction of banana autotetraploid by colchicines treatment of micropropagated diploids. 40(6): 887-896.

Hemalatha, K., (1998). Induction of mutation in carnation (*Dianthus caryophyllus L.*) through gamma rays and ethyl methane sulphonate. *Ph.D. Thesis*, University of Agricultural Sciences, Bangalore, p. 165.

Hershey, C.H, and Jennings, D.L, (1992). Progress in breeding cassava for adaptation to stress. 62: 283 - 831.

Hillocks, R.J. (2002). Cassava in Africa. In: Hillocks R.J., Thresh J.M., and Bellotti A.C. (Eds.) *Cassava Biology, Production and Utilization*. CABI Publishing, Oxon, New York.

Hillocks, R.J. and Thresh, J.M. (2002). *Cassava: Biology, Production and Utilization*. CABI Publishing, pp. 261–280.

Hitoshi, N. (2008). Induced mutations in plant breeding and biological researches in Japan. 2008. In: *Book of Abstracts, FAO/IAEA International Symposium on Induced Mutations in Plants*, 12-15 Aug., 2008, Vienna, Austria, p.5.

Hossain, Z. (2006). Isolation of a NaCl-tolerant mutant of *Chrysanthemum morifolium* by gamma radiation: *in vitro* mutagenesis and selection by salt stress. *Functional Plant Biology* **33**, 91–101.

<http://www-mvd.iaea.org>. Accessed on 5th May 2012.

IAEA/AFRA (2000). Project “Development of Improved Crop Varieties” (RAF/5/042) International Atomic Energy Agency, Vienna, Austria.

IITA (1990). *Cassava in Tropical Africa. A reference manual*. Edited and designed by Chayce Publications Services, United Kingdom. Printed and bound in the United Kingdom by Balding Mansell International, Wisbech.

- Iglesias, C.A., Hershey, C., Calle, F and Bolanos, A. (1994). Propagating cassava (*Manihot esculenta* Crantz) by sexual seed. 30: 283-290
- Iglesias, C.A., Sanchez, T and Yeoh, H.H. (2002). Cyanogens and linamarase activities in storage roots of cassava plants from breeding program. *Journal of Food Composition and Analysis*, 15: 379-387
- Ihemere, U.E. (2003). Somatic embryogenesis and transformation of cassava for enhanced starch production. Ohio State University, p.59-60.
- Jain, S.M. (2000). Mechanisms of spontaneous and induced mutations in plants. Proc. 11th International Congress of Radiation Research. Dublin, Ireland 18–23, July 1999, p.255–258
- Jain, S.M. and Maluszynski, M. (2004). Induced mutations and biotechnology in improving crops. In: A. Mujib, M.J. Cho, S. Predieri and S. Banerjee (eds.), *In Vitro Application in Crop Improvement*, Science Publishers, London. p.170–202.
- Jain, S.M. (2005). Major mutation assisted plant breeding programs supported by FAO/IAEA. *Plant Cell Tiss. Org. Cult.* 82, 113–123.
- Jain, S.M. (2006). Major mutation-assisted plant breeding programs supported by FAO/IAEA. 82: 113-123.
- Jain, S.M. (2007). Recent advances in plant tissue culture and mutagenesis. *Acta Hort.* 736:205–211.
- Jaramillo, G., Morante, N., Pérez, J.C., Calle, F., Ceballos, H., Aria, B and Bellotti, A.C. (2005). Diallel Analysis in cassava adapted to the mid altitude valleys environment. 45:1058-1063.
- Jones, W.O. (1959). *Manioc in Africa*. Stanford University Press, Stanford, CA
- Joseph, T., Yeoh, H.H and Loh, C.S. (1999). Cyanogenesis in somatic embryos and plantlets of cassava (*Manihot esculenta* Crantz). *J Sci Food Agric* 79:1071–1074
- Joseph, R., Yeoh, H. and Loh, C. (2004). Induced mutations in cassava using somatic

- embryos and the identification of mutant plants with altered starch yield and composition. *PlantCell Rep.*, **23**:91–98.
- Kaicker, U. S., (1992). Rose breeding in India and cytology of induced mutation of H. T. Cv. 'Folkfore', *Acta Horticulturae*, **320** : 105 – 112.
- Kawano, K., Narintaraporn, K., Narintaraporn, P., Sarakarn, S., Limsila, A., Limsila, J., Suparhan, D., Sarawat, V and Watananonta, W. (1998). Yield improvement in a multistage breeding program for cassava. *38*: 325-332
- Kenneth, M. O. (2002). Population history of *Manihot esculenta* (Euphorbiaceae) inferred from nuclear DNA sequences. *11*: 901-911.
- Khan, I. A., Dahot, M. U., Seema, N., Yasmin, S., Bibi, S., Raza, S. and Khatri, A. (2009). Genetic variability in sugarcane plantlets developed through *in vitro* mutagenesis. *Pak. J Bot.*, **41(1)**: 153-166.
- Khanna, H. K. and Raina, S. K., (1998). Genotype x culture media interaction effects on regeneration response of three indica rice cultivars. *Plant Cell Tissue Organ Cult.*,**52**:145–153.
- Khatri, A., Khan, I.A., Javed, M.A., Siddiqui, M.A., Khan, M.K.R., Khanzada, M.H., Dahar, N.A. and Khan, R. (2002). Studies on callusing and regeneration potential of indigenous and exotic sugarcane clones. *Asian J. Plant Sci.*, 1(1): 41-43.
- Kizito, E.B., Bua, A., Fregene, M., Egwang, T., Gullberg, U. and Westerbergh, A. (2005). The effect of cassava mosaic disease on the genetic diversity of cassava in Uganda. *146*: 45-54
- Konan, N.K., Sangwan, R.S., and Sangwan-Norreel, B.S. (1994). Efficient *in vitro* shoot-regeneration systems in cassava (*Manihot esculenta* Crantz). *113*:227-236.
- Konan, N.K., Schöpke, C., Carcamo, R., Beachy, R.N. and Fauquet, C. (1997). An efficient mass propagation system for cassava (*Manihot esculenta* Crantz) based on nodal explants and axillary bud-derived meristems. *Plant Cell Rep* *16*:444-449
- Kuksova, V.B., Piven, N.M and Gleba, Y.Y. (1997). Somaclonal variation and *In vitro* induced mutagenesis in grapevine. *Plant Cell Tiss and Org Cult.*, *49*: 179-186.

- Kumar, A., Ghosh, P., Lee, Y.M., Hill, M.A and Preiss, J. (1989). Biosynthesis of bacterial glycogen: determination of the amino acid changes that alter the regulatory properties of a mutant *E. coli* ADP-glucose synthase. *J BiolChem* 264: 10464-10471.
- Lepade, A.G., Velug, A.M.S and Santos, I.S. (1995). Genetic improvement of the queen variety pineapple through induced mutation and *In vitro* culture techniques. FAO/IAEA In: *International Symposium, Vienna, 19-23 June 1995*.
- Lee, Y. S., Oh S. H., Lee, J.W., Kim, J.H., Kim, D. S. and Byun, M.W. (2003). Effects of gamma irradiation on physicochemical and textural properties of starches. *Food SciBiotechnol*, 12:508–12.
- Li, H.Q., Sautter, C., Potrykus, I. and Puonti-Kaerlas, J. (1996). Genetic transformation of cassava (*Manihot esculenta* Crantz. 14: 736 - 740.
- Li, H.Q, Guo, J.Y., Huang, Y.W., Liang, C.Y., Liu, H.X., Potrykus, I. and Puonti-Kaerlas, J. (1998). Regeneration of cassava plants via shoot organogenesis. 17:410-414
- Li, L., Duan, S., Kong, J., Li, S., Li, Y. and Zhu, Y. (2007). A single genetic locus in chromosome 1 controls conditional browning during the induction of calli from mature seeds of *Oryza sativa* ssp. Indica. 89: 237-245.
- Lin, H. Jinn M., Liang, L. Y., Pei, W. J., Lui, X. Z. and Zhang, H. Y. (2010). Production of polyploids from cultured shoot tips of *Eucalyptus globules* Labill by treatment with colchicines. 9(15): 2252-2255.
- Little, E.L., Magbana, Z.V and Parrott, W.A. (2000). A protocol for repetitive somatic embryogenesis from mature peanut epicotyl. *Plant Cell Rep.*, **19**, 351-357.
- Maluszynski, M. K., Nichterlein, K., van Zanten, L and Ahloowalia, B.S. (2000). Officially released mutant varieties-the FAO/IAEA Database. *Mut. Breed.*, **12**: 1–84.
- Maluszynski, M., Szarejko, I., Barriga, P. and A. Balcerzyk. (2001). Heterosis in crop mutant crosses and production of high yielding lines using double haploid systems. *Euphytica*, **120**: 387–398.
- Manu-Aduening, J., Lamboll, R., Dankyi, A and Gibson, R. (2005). Cassava diversity in Ghanaian farming systems. 144: 331-340.

- Manu-Aduening, J.A., Lamboll, R.I., Ampong Mensah, G., Lamptey, J.N., Moses, E., Dankyi, A.A and Gibson, R.W (2006). Development of superior cassava cultivars in Ghana by farmers and scientists: The process adopted outcomes and contributions and changed roles of different stakeholders. 150: 47-61
- Martin, K.P. (2004). Benzyl adenine induced somatic embryogenesis and plant regeneration of *Leptadeniareticulate*. *Biol. Plant.*, **48**, 285-288.
- Matthews, H., Schöpke, C., Carcamo, R., Chavarriaga, P., Fauquet, C and Beachy, R.N, (1993). Improvement of somatic embryogenesis and plant recovery in cassava. 12:328-333.
- Manyong, V.M. and Abass, A. (2007). Cassava; The king crop. Volume 1, Issue. [www. iita.org](http://www.iita.org). Visited 06/04/2008.
- Mensah, J.K., Akomeah, P.A. and Ekpekurede, E.O. (2005). Gamma irradiation induced variation of yield parameters in cowpea (*Vigna unguiculata* (L.) Walp.) *Global J. Pure.* 11(3): 327-330.
- Merkle, S.A and Dean, J.F.D. (2000). Forest tree biotechnology. *CURRENT OPINION BIOTECH.* 11: 298-302.
- Micke, A., Donini, B. and Maluszynski, M. (1990). Induced mutations for crop improvement. *Mutation Breeding Review.* 20001-2039. pp. 59-61.
- Mkumbira, J. (2002). Cassava development for small scale farmers: approaches to breeding in Malawi. Doctoral thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden. *Acta Universitatis Agriculturae Sueciae. Agraria* 365. Tryck: SLU Service/Repro, Uppsala 2002.
- Mujib, A. (2005). Colchicine induced morphological variants in pineapple. 15(2): 127-133
- Munyikwa, T.R.I., Raemakers, C.J.J.M., Schreuder, M., Kok, R., Rozeboom, M., Jacobsen, E. and Visser, R.G.F. (1998). Pinpointing towards improved transformation and regeneration of cassava. *Plant Sci.*, 135: 87-101.
- Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. 15: 73-97

Nagatomi, S. (1992). Selection of self compactible mutants in tea plant under long-term chronic Irradiation. Technical news, p. 40.

NARP (National Agricultural Research Programme) (1994). Annual Report. CSIR. Ghana.

Nassar, N.M.A. (2001). The nature of apomixes in cassava (*Manihot esculenta Crantz*). 134: 185-187

Nassar, N. M. A. and Marques, A.O. (2006). Cassava leaves as a source of protein. *J. of Food, Agriculture and Environment*, **4 (1)**: 187-188.

Nduele, M., Ludwig, A. and Van Ooteghem, M. (1993). The use of cassava starch in the formulation of gelatin capsules. *Journal de Pharmacie de Belgique*, vol. 48, no. 5, p. 325-334.

Nguyen, T.L.T., Shabbir, H., Gheewala and Bonnet, S. (2008). Life cycle cost analysis of fuel ethanol produced from cassava in Thailand. 13: 564-573.

Nilanthi, D. (2009). Influence of Gene Dose on In Vitro Culture Responses of Purple Coneflower (*Echinacea purpurea* L.). *Bioinformatics and Biomedical Engineering*, pp1-6

Nweke, F.I., Dixon, A.G.O., Asiedu, R and Folayan, S.A. (1994). Attributes of cassava varieties desired by farmers in sub-Saharan Africa. In: Akoroda MO (ed.) *Root Crops for Food Security in Africa*. Proceedings of the 5th triennial symposium of the International Society for Tropical Root Crops-Africa Branch (ISTRC-AB), 22-28 November 1992, Kampala, Uganda, ISTRC-AB/CTA, Wageningen/IITA, Ibadan, Nigeria, pp. 65-92.

Nweke, F.I. (1996). Cassava: A cash crop in Africa. Collaborative study of cassava in Africa Working Paper No. 14, International Institute of Tropical Agriculture, Ibadan, Nigeria.

Nweke, F. I., Spencer, D.S.C. and Lynam, J.K. (2002). *The cassava transformation: Africa's best kept secret*. Michigan State University Press, Lansing, Michigan, USA.

Nweke, F.I. (2004). *New Challenges in the Cassava Transformation in Nigeria and Ghana*. Discussion Paper No. 118, Environment and Production Technology Division, International Food Policy Research Institute, Washington, DC, USA.

- Nwokoro, S.O., Orheruata, A.M and Ordiah, P.I. (2002.) Replacement of maize with cassava sievates in cockerel starter diets: effect on performance and carcass characteristics. *Tropical Animal Health and Production*, vol. 34, no. 2, p. 163-167.
- Offei, S.K., Owuna-Kwakye, and Thottappily, G. (1999). First report of East African cassava mosaic begomovirus in Ghana. 83: 877.
- Ogbe, F.O., Atiri, G.I., Robinson, D., Winter, S., Dixon, A.G.O., Quin, F.M and Thottappily, G. (1999). First report of East African cassava mosaic begomovirus in Nigeria. 83: 398.
- O’Hair, S.K. (1990). Tropical root and tuber crops. *Horticultural Reviews* 12, 157-166.
- Okai, E. (2001). Genetic Diversity in some local cassava cultivars in Ghana. M.Phil. Thesis, Crop Science Department, Faculty of Agriculture, University of Ghana Legion, Ghana.
- Olsen, K.M., and Schaal, B.A. (1999). Evidence on the origin of cassava: Phylogeography of *Manihot esculenta*. *Proceedings of National Academy Science*, 96: 5586-5591.
- Olsen, K.M., and Schaal, B.A. (2001). Microsatellite variation in cassava and its wild relatives: Further evidence for a southern Amazonian origin of domestication. 88(1): 131–142.
- Onwueme, I.C. (1978). *The Tropical Tuber Crops: Yam, Cassava, Sweet potato, Cocoyam*. John Wiley and Sons Ltd. New York
- Osiru, D.S., Porto, M.C.M and Ekanayake, I.J. (1996). Morphology of cassava plant. IITA, Research Guide. Ibadan, Nigeria.
- Ozawa, K., Kawahigashi, H., Kayano, T and Ohkawa, Y. (2003). Enhancement of regeneration of rice (*Oryza sativa* L.) calli by integration of the gene involved in regeneration ability of the callus. 165: 395-402.
- Pal, B. P. (1962). A note on rose breeding. *Indian Journal of Horticulture*, 26 : 34 – 41.
- Pande, S and Khetmalas, M. (2012). Biological effect of sodium azide and colchicine on seed germination and callus induction in *Stevia rebaudiana*. Dr D.Y Patil Biotechnology and Bioinformatics Institute, Maharashtra, India, pp 93-97.
- Parkes, E.Y. (2001). Assessment of genetic diversity, combining ability, stability and farmer preference of cassava germplasm in Ghana. Pp 1-4, 9-12

- Popoola, T.O.S. and Yangomodou, O.D. (2006). Extraction, properties and utilization potential of cassava seed oil. 5(1): 38-41.
- Puonti-Kaerlas, J., Frey, P., and Potrykus, I. (1997). Development of meristem gene transfer techniques for cassava. African Journal Root Tuber Crops, 2:175-180.
- Raemakers, C.J.J.M, Amati, M., Staritsky, G., Jacobsen, E. and Visser, R.G.F. (1993a). Cyclic somatic embryogenesis and plant regeneration in cassava. 71:289-294.
- Raemakers, C.J.J.M., Schavemaker, C.M., Jacobsen, E., and Visser, R.G.F. (1993b). Improvement of cyclic somatic embryogenesis of cassava (*Manihot esculenta* Crantz). 12: 226-229.
- Raemakers, C.J.J.M, (1995a). NAA-induced somatic embryogenesis in cassava. In: cassava Biotechnology Network (Eds), A paper presented at Proceeding, 2nd International Scientific Meeting. CIAT working document. Bogor, Indonesia. pp 150: 355-363.
- Raemakers, C.J.J.M, Sofiari, E., Taylor, N., Henshaw, G., Jacobsen, E. and Visser, R.G.F. (1996). Production of transgenic cassava (*Manihot esculenta* Crantz) plants by particle bombardment using luciferase activity as selection marker. 2:339-349.
- Raemakers, C.J.J.M. and Jacobsen, E. (1997). Micropropagation of *Manihot esculenta* Crantz. In: Biotechnology in agriculture and forestry. High tech and micropropagation. Springer, Berlin Heidelberg New York, ISBN: 978-3-540-61606-1.
- Rao, P. S., Ganapathi, T.R, Bapat, V.A and Suprasanna, P. (1995). *In vitro* propagation and mutation induction in banana. IAEA-SM-340: 191-192.
- Rashid, H., Bokhari, S.Y.A and Quraishi, A. (2001). Callus induction, regeneration and hygromycin selection of rice (Super Basmati) OnLine 1: 1145-1146.
- Raw Materials Research and Development Council [RMRDC] (2004). Cassava Report on Survey of Agro Raw Materials in Nigeria-Raw Materials Research and Development Council (Maiden Edition), Abuja, Pp: 1-2, 18-19.

- Roca, W.M. and Thro, A.M. (eds) (1993). Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network. Working document 123. CIAT, Cali.
- Rogers, D. J., and Appan, G.J. (1973). *Manihot* and *Manihotoides* (Euphorbiaceae), a computer assisted study, 272-278.
- Roy, A.T., Leggett, G and Koutoulis, A. (2001). *In vitro* tetraploids induction and generation of tetraploids from mixoploids in hop (*Humulus lupulus L*) 20: 489-495.
- Samad, M.A., Begum, S and Majid, M.A. (2001). Somaclonal variation and irradiation in sugarcane calli for selection against red rot, water logged conditions and delayed or nonflowering characters. *IAEA-TECDOC-1227*: 45-50.
- Schöpke, C.; Taylor, N.; Cárcamo, R.; Konan, N. D. K.; Marmey, P.; Henshaw, G. G.; Beachy, R. N. and Fauquet, C. (1996). Regeneration of transgenic cassava plants (*Manihot esculenta* Crantz) from microbombarded embryogenic suspension cultures. 14, 731-735.
- Schumann, G., Ryschika U., Schulze, J., and Klocke, E. (1995). Anatomy of somatic embryogenesis. In: Bajaj, Y.P.S. (Ed.) Somatic embryogenesis and synthetic seed. *Biotechnology in Agriculture and Forestry*, 30: 71-86.
- Siddiqui, S.H. and Javed, M. (1982). Mutation breeding in sugarcane (*Saccharum* sp. Hybrid) by gamma irradiation of cuttings and tissue culture. In: *Induced mutations in vegetatively propagated plants II. Proc. IAEA, Vienna*, pp. 155-166.
- Siddiqui, S.H., Khatri, A., Javed, M.A., Khan, I.A and Nizamani, G.S. (1994). *In vitro* culture: A source of genetic variability and an aid to sugarcane improvement. *Pak. J. Agric. Res.*, 15 (1):127-133.
- Singh, K. R., Singh, B., Raghava, S. P. S. and Kalia, C. S. (2000). Induced flower colour mutation in carnation through *in vitro* application of chemical mutagen. *Indian Journal of Genetics and Plant Breeding*, 60:535-539.
- Sleper, D.A. and Poehlman, J.M. (2006). *Breeding Field Crops*, 5th Edition, Blackwell Publishing, Iowa.
- Sofiari, E. (1996). Regeneration and transformation of cassava. PhD Thesis, Agricultural University Wageningen, Netherlands, pp 1-119.

- Sreekumari, M.T and Jos, J.S., (1996). Scope of triploidy in cassava improvement, in: Trop Tuber Crops: Problems, Prospects and Future Strategies, Oxford and IBHPublishing Co., New Delhi, pp. 95–99.
- Stamp, J.A and Henshaw, G.G. (1982). Somatic embryogenesis in cassava. *Zeitschrift fur Pflanzen-physiologie*,105: 97-102 (cited from Raemakers *et al.*, 1993a)
- Stamp, J.A and Henshaw, G.G. (1987). Somatic embryogenesis from clonal leaf tissues of cassava. *Annals of Botany*, 59: 445-450
- Sturtevant, W.C. (1969). History and ethanography of some West Indian starches. In: The Domestication and Exploitation of plants and Animals, Ucko, P.J and Dimbleby, G.W. (Eds.), Duckworth, London, pp: 177-199.
- Subramanian, T.S. (2003). Radiation for food preservation. India's National Magazine from the Publishers of the Hindus., 20 (17)1-4.
- Taguchi-Shiobara, F., Lin, S.Y., Tanno, K., Komatsuda, T., Yano, M., Sasaki, T. and Oka, S. (1997). Mapping quantitative trait loci associated with regeneration ability of seed callus in rice, *Oryza sativa* L. 95: 828-833.
- Taylor, N.J. and Henshaw, G.G. (1993). The induction of somatic embryogenesis in fifteen African and one South American cassava cultivars. In: W.M. Roca and A.M. Thro (Eds),Proceedings of First International Scientific Meeting of the Cassava Biotechnology Network. Cartagena, Colombia, pp. 134-140.
- Taylor, N. J., Edwards, M., Kiernan, R. J., Davey, C. D. M., Blakesley, D. and Henshaw, G. G. (1996). Development of friable embryogenic callus and embryogenic suspension culture systems in cassava (*Manihot esculenta* Crantz). *Nature Biotech.*,14, 726-730.
- Taylor, N.J., Masona, M.V., Carcamo, R., Ho, T., Schöpke, C. and Fauquet, C. (2001). Production of embryogenic tissues and regeneration of transgenic plants in cassava. *Euphytica* 120: 25-34.
- Thorpe, T.A and Stasolla, C. (2001). Somatic embryogenesis. In: Bhojwani S.S. and Soh W.Y. (eds) Current trends in the embryology of angiosperms. Kluwer Academic Publishers, Dordrecht, pp 279-336

- Thottappily, G., Thresh, J. M., Calvet, L. A. and Winter, S. (2003). Cassava In: Virus and virus-like diseases of major crops in developing countries. G. Loebenstein and G. Thottappily (Eds). Kluwer Academic Publishers, Netherlands. pp 101-163.
- Thresh, J. M., and Otim-Nape, G. W. (1994). Strategies for controlling African cassava mosaic gemmini virus. *Advances in Disease Vector Research*, 10: 215 – 236.
- Thro, A.M., Roca, W., Iglesias, C., Henry, G. and Ng, S.Y.C. (1998). Contributions of *in vitro* biology to cassava improvement. *African Crop Science Journal* Volume. 6 (3): 305-315
- Tonukari, N.J. (2004). Cassava and the future of starch. 7 (1): 219-228.
- UNCTAD (2009). The Biofuels Market: Current Situation and Alternative Scenarios. United Nations Conference on Trade and Development. New York. Pp 118.
- Van Harten, A.M. (1998). Mutation Breeding: Theory and Practical applications, Cambridge Univ. Press, Cambridge.
- Viehmánová, I., Cusimamani, E.F., Bechyne, M., Vyvadilová, M and Greplova, M. (2009). *In vitro* induction of polyploidy in yacon (*Smallanthus sonchifolius*). 97: 21-25.
- Watananonta, W. (2006). Present Situation and its Future Potential of Cassava Production and Utilization in Thailand. FEALAC Interregional Workshop on Clean Fuels and Vehicle Technologies: the Role of Science and Innovation, Bangkok, Thailand. <http://as.doa.go.th/fieldcrops/res/0949-3.pdf>
- Wheatley, C.C. and Chuzel, G. (1993). Cassava: the nature of the tuber and use as a raw material. In: R. Macrae, R.K.Robinson and M.J. Sadler (Eds.), *Encyclopaedia of Food Science, Food Technology and Nutrition*. Academic Press, San Diego, CA, pp. 734–743.
- Woodward, B. and Puonti-Kaerlas, J. (2001). Somatic embryogenesis from floral tissue of cassava (*Manihot esculenta* Crantz). 120: 1-6
- Zel, J., Malkarm, D and Manj, U. (1999). The efficient regeneration of the potato (*Solanum tuberosum* L.) cultivar Igor *in vitro*. 39: 277-282.

Zhang, P., Legris, G., Coulin, P and Puonti-Kaerlas, J. (2000b). Production of stably transformed cassava plants via particle bombardment. *Plant Cell Rep.* 19: 939 – 945.

Zhang, P., Phansiri, S., Puonti-Kaerlas, J. (2001). Improvement of cassava shoot organogenesis by the use of silver nitrate *in vitro*. *Plant Cell Tiss Org Cult* 67:47-54

Zheng, Q., Dessai, A. P. and Prakash, C.S. (1996). Rapid and repetitive plant regeneration in sweet potato via somatic embryogenesis. In: *Plant Cell Rep.* 15, 381-385.



7.0 APPENDICES**Appendix 7.1**

Composition of Murashige and Skoog (1962) basal medium (MS)

Constituents	Concentration mg/l
Macro elements	
NH_4NO_3	1650.00
KNO_3	1900.00
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	440.00
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	370.00
KH_2PO_4	170.00
Micro elements	
KI	0.83
H_3BO_3	6.20
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	22.30
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	8.60

$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.25
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.025
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.025
Iron source	
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	27.80
$\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$	37.30
Vitamins	
Myoinositol	100.00
Nicotinic acid	0.50
Pyridoxine-HCl	0.50
Thiamine-HCl	0.10
Glycine	2.00



Appendix 7.2

ANOVA of plant height of cassava accessions after five weeks of sprouting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	3	346.56	115.52	3.06	0.028
Irradiation dose	4	17311.98	4328.00	114.68	<.001
Accession.Irrad.dose	12	818.15	68.18	1.81	0.045
Residual	380	14341.65	37.74		
Total	399	32818.34			

Appendix 7.3ANOVA of days to callus emergence of leaf lobes of *Manihot esculanta* Crantz cultured on MS medium supplemented with 8mg/l 2, 4 - D

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	8	5139.52	642.44	33.07	<.001
Treatment	3	273.37	91.12	4.69	0.005
Conc.Treatment	24	501.30	20.89	1.08	0.392
Residual	72	1398.67	19.43		
Total	107	7312.85			

Appendix 7.4

ANOVA of days to callus emergence of leaf lobes of *Manihot esculanta* Crantz cultured on MS medium supplemented with 16mg/l Picloram

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	8	4774.17	596.77	44.54	<.001
Treatment	3	191.66	63.89	4.77	0.004
Conc.Treatment	24	260.43	10.85	0.81	0.713
Residual	72	964.67	13.40		
Total	107	6190.92			

Appendix 7.5

ANOVA of days to embryo emergence of leaf lobes of *Manihot esculanta* Crantz cultured on MS medium supplemented with 8mg/l 2, 4 - D

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	8	4374.17	546.77	27.93	<.001
Treatment	3	41.52	13.84	0.71	0.551
Conc.Treatment	24	162.65	6.78	0.35	0.998
Residual	72	1409.33	19.57		
Total	107	5987.67			

Appendix 7.6

ANOVA of days to embryo emergence of leaf lobes of *Manihot esculanta* Crantz cultured on MS medium supplemented with 16mg/l Picloram

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	8	4909.74	613.72	42.87	<.001
Treatment	3	10.92	3.64	0.25	0.858
Conc.Treatment	24	312.33	13.01	0.91	0.589
Residual	72	1030.67	14.31		
Total	107	6263.66			

Appendix 7.7

ANOVA of percentage callus formation of leaf lobes of *Manihot esculanta* Crantz cultured on MS medium supplemented with 8mg/l 2, 4 - D

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	8	31822.74	3977.84	55.89	<.001
Treatment	3	797.11	265.70	3.73	0.015
Conc.Treatment	24	8526.89	355.29	4.99	<.001
Residual	72	5124.67	71.18		
Total	107	46271.41			

Appendix 7.8

ANOVA of percentage callus formation of leaf lobes of *Manihot esculanta* Crantz cultured on MS medium supplemented with 16mg/l Picloram

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	8	59159.33	7394.92	89.64	<.001
Treatment	3	395.59	131.86	1.60	0.197
Conc.Treatment	24	10768.74	448.70	5.44	<.001
Residual	72	5940.00	82.50		
Total	107	76263.67			

Appendix 7.9

ANOVA of percentage embryo formation of leaf lobes of *Manihot esculanta* Crantz cultured on MS medium supplemented with 8mg/l 2, 4 - D

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	8	29915.30	3739.41	42.35	<.001
Treatment	3	508.69	169.56	1.92	0.134
Conc.Treatment	24	4948.56	206.19	2.33	0.003
Residual	72	6358.00	88.31		
Total	107	41730.55			

Appendix 8.0

ANOVA of percentage embryo formation of leaf lobes of *Manihot esculanta* Crantz cultured on MS medium supplemented with 16mg/l Picloram

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	8	48721.9	6090.2	53.47	<.001
Treatment	3	214.4	71.5	0.63	0.600
Conc.Treatment	24	4594.7	191.4	1.68	0.048
Residual	72	8201.3	113.9		
Total	107	61732.3			

