IN-VITRO PROPAGATION OF SELECTED CASSAVA (*Manihot esculenta* CRANTZ) CULTIVARS USING MULTIPLE SHOOT INDUCTION AND SOMATIC EMBRYOGENESIS.

Kenneth Ellis Danso B.Sc (Hons). Dip. Ed.

A dissertation presented to the University of Ghana
in partial fulfilment of the requirements for the award
of degree of Master of Philosophy

November, 1997.

DECLARATION

I, the undersigned candidate do hereby declare that work reported in this thesis is my original work and also wish to state that the work has not be presented to any other university for the award of any degree by me.

Signed.....

Kenneth Ellis Danso
(CANDIDATE)

Signed.....

Dr. Elizabeth Acheampong
(SUPERVISOR)

DEDICATION

*This work is dedicated to the **GLORY of GOD** and to Charity, Cobby and Kofi whose prayer support enabled me to complete this work.*

ACKNOWLEDGEMENT

I sincerely do acknowledge the dedicated supervision of this work by Dr. Elizabeth Acheampong of Botany Department, University of Ghana. Her helpful suggestions and guidance saw me through this work.

My sincere thanks also go to Dr. H.M. Amoatey, the Head of Department of Plant and Soil Sciences, Biotechnology and Nuclear Agriculture Research Institute (BNARI), Ghana Atomic Energy Commission, Kwabenya for his helpful suggestions and guidance.

I also wish to thank Prof. R. G. F. Viser and Dr. C. J. J. M. Raemakers of Plant Breeding Department, Wageningen Agricultural University, The Netherlands for their helpful suggestions on work done on somatic embryo germination.

The helpful suggestions offered by Prof. Oteng Yeboah, Dr. Enu Kwesi, Prof. G.T. Odamtten and all the lecturers of the Department of Botany, University of Ghana is duly acknowledged.

My gratitude also goes to all the staff of the Tissue Culture Unit of the Botany Department of the University of Ghana, Legon my staff colleagues at the Department of Plant and Soil Sciences of BNARI/GAEC, and Mr. Kojo Gyampiah Montford and Dr. Josephine Nketsia-Tabiri for their assistance on the use of the computer for graphical representation and statistical analysis.

I finally wish to thank the Government of Ghana and the International Atomic Energy Agency for their financial support during the period of research.

May the **Lord God Almighty** bless them all.

LIST OF ABBREVIATIONS

ABA.....Abscisic acid

ACMV.....African Cassava Mosaic Virus

AgDP.....Agriculture gross domestic product

BA.....Benzylaminopurine

CIAT.....Centro International de Agriculture Tropical

Dicamba.....3,6-dichloro-2-methoxybenzoic acid

ESCaPP.....Ecologically Sustainable Cassava Plant Protection

2,4-D.....2,4-dichlorophenoxy acetic acid

DGIS.....Directorate General for International Corporation

FAO.....Food and Agricultural Organisation of the United Nations

GA_3..... Gibberellic acid

NAA.....α-Naphthalene acetic acid

MS.....Murashige and Skoog basal medium plus Gamborg B5 vitamins

Picloram.....4-amino-3,5,6-trichloropicolinic acid

RAPD.....Random Amplified Polymorphic DNA

RFLP.....Restriction Fragment Length Polymorphism

TDZ (Thidiazuron).....N-phenyl-N(123)-thiadiazolyl urea.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES.

ABSTRACT

The study showed that farmers cultivate different cassva cultivars based on popularity, duration to maturity and tolerance to African Cassava Mosaic Virus (ACMV) disease. All the five selected local were propagated *in vitro* using meristem explants on Murashige and Skoog (1962) basal salts and Gamborg B5 vitamins modified with benzylaminopurine (BA) (0.0 - 0.15 mg/l). There was profuse callus formation in all the cultivars. The optimal BA concentration for shoot proliferation in this medium was 0.10 mg/l BA. With reduced NAA and (GA_3) concentrations (0.02 and 0.04 mg/l respectively) in the MS medium 0.05 mg/l was optimum and resulted in 100% and 46% shoot regeneration in Bosomnsia ans Santom respectively compared to 37% and 0% in the previous treatment.

In M. Col 22 multiple shoots were produced from apical meristems as well as single nodal cutting explants. In the local cultivars multiple shoots were produced from nodal cuttings alone. The number of shoots produced by nodal cuttings in M. Col 22 were comparatively higher than the local cultivars at all the BA levels in the medium. In all, the number of shoots produced was dependent on the BA concentration in the medium.

Leaf lobe explants of both greenhouse and *in vitro* plantlets developed embryogenic calli on MS amended with 0.0-16 mg/l 2,4-D on a step one-induction medium. However, calli formation was depended on the type of explants. Calli formation from young leaf lobe and apical meristem explants was significantly higher than stipule explants. On transfer to a Step two BA amended maturation medium, embryogenic calli derived from *in vitro* plantlets only formed matured somatic embryos.

Embryo formation was depended on the concentration of the auxin in the induction medium and the cassava cultivar. Somatic embryo formation was higher on a medium with 16 mg/l 2,4-D. Santom produced the highest percentage of embryos (25%) among all the cultivars tested. Embryogenic calli which did not form somatic embryos formed foliose structures and/ or roots which also depended on the concentration of 2,4-D in the induction medium.

NAA induced somatic embryos required desiccation to stimulate normal germination in all the four cultivars studied. The best desiccation procedure was the petri dish method. The desiccated embryos required a medium supplemented with BA and kinetin for germination . Incubation of cultures in darkness increased frequency of germination and also reduced germination period to 14 days when compared to cultures incubated in light under growth room conditions. The development of the seedling was dependent on the concentration of BA as well as the dark/light photoperiod of the incubation period. Cultures on medium with 0.1 mg/l BA and incubated in the dark produced normal single shoots whereas most cultures on 1 mg/l in the dark produced multiple shoots.

Germination of somatic embryos was dependent on the level of moisture loss and the BA concentration in the medium. At optimal moisture loss (40%) higher frequency of germination was achieved on a medium with lower concentration of BA (0.1 mg/l). At lower moisture loss 1 mg/l BA was needed for higher germination in Gading and Adira 4.

Wrapping of cultures with aluminium foil to achieve desiccation in complete darkness did not enhance embryo germination. Low light intensity (64 lux) resulted in optimum germination. Light regimes had significant effect on seedling morphology but not on frequency of germination

2,4-D-induced embryos showed a different response from NAA-induced embryos. In both NAA- and 2,4-D - induced somatic embryos desiccation followed by incubation in the dark stimulated root formation than shoot.

At a concentration of 1 mg/l, ABA shoot formation only in somatic embryos of Adira 4; while at 40 mg/l, it had an inhibitory effect on the germination of these embryos.

Prewashing of somatic embryos with water for 24 hours enhanced whole plant germination on MS medium modified with BA.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 ORIGIN AND CULTIVATION OF CASSAVA

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub which belongs to the family Euphorbiaceae. The genus Manihot consists of at least 98 species (Roggers and Appan, 1973). The centres of diversity are Central Brazil, Northeastern Brazil, Southwestern Mexico and Eastern Bolivia (Naser, 1987). Cassava was introduced into West Africa from South America in the 16th century and into East Africa in the 18th century (Byrne, 1984). The crop is now widely cultivated in Africa between latitudes 30 degrees north and south of the equator. It has been reported that the crop is grown in 39 African countries. Although the crop is grown in every country in sub-Saharan Africa, cultivation is concentrated in the humid tropics.

A number of agricultural advantages have made the cassava crop of utmost importance for food security in Africa. First, productivity of cassava in terms of calories per unit land area per unit time is significantly higher than that of other staple food crops. The crop has high biological efficiency as food producer possibly due to its structural engineering considerations: the edible part of the tuberous roots lies beneath the ground and does not have to be supported by the plant in the form of heavy stems and branches (Byrne, 1984; Cock,1985). Also the crop is productive even on poor soils and under adverse climatic conditions where other crops fail. Typically, cassava is the only crop able to grow on eroded or marginal lands including those abandoned as a result of failed attempts to grow other crops. Growth of cassava requires low input of man hours compared to other crops. Furthermore, the roots can be left in the ground without harvesting for up to two or three years until needed (DGIS, 1991). Thus, the certainty of obtaining some yield even under most adverse conditions and the

possibility of maintaining continuity of supply throughout the year make this root crop a basic component of the farming system in many areas of Africa south of the Sahara (Nweke *et al*, 1994). It has further reported that famine rarely occurs in areas where cassava is widely grown, since it provides a staple base to the food production system (DGIS, 1991). This indicates that cassava has a potential for eliminating food crises and famine.

1.2 UTILISATION OF CASSAVA

Despite its low protein content (1.0%; Purseglove, 1968) cassava is mainly utilised for human consumption in Africa . It provides a source of carbohydrate for 450-500 million people (Cock, 1982; FAO, 1993) ranking ninth on the world scale. In the developing countries it ranks fourth on the list of major food crops after rice, wheat and maize (FAO, 1989). The tuberous root can be used as an industrial source of starch. Cassava starch is used in the food industry in many preparations , including glucose production, confectionery and baking products (IITA, 1990). In the livestock industry also, cassava is used in feed rations or as animal feed concentrate. It is estimated that about 65 percent of world production is used for human consumption, 19 percent for cattle feed, 6 percent for industrial starch and 10 percent are lost (Cock, 1985).

Cassava is the most widely grown crop in rural areas of Ghana. It is cultivated either as a monocrop or intercrop. Al-Hassan (1990) reported that cassava production in Ghana grew from 2.225 million tonnes in 1985 to 3.3 million tonnes in 1988, an average growth rate of 14 percent. The per capita consumption is high (148 kg/yr) compared to that of plantain at 83 kg/yr (Annor-Frempong, 1994). According to the Ministry of Agriculture (MOA, 1990), the contribution of cassava to the Agriculture Gross Domestic Product (AgDP) is 22 and is the

highest for any crop grown in the country. This high cultivation rate is attributed to the fact that cassava is cheap to produce, affordable and is utilised in many diets across the country. However, there are constraints to full utilisation of the crop; the tuberous root deteriorates after harvest, and has poor quality and quantity of proteins (Byrne, 1984). Cassava is also known to be cyanophoric, containing cyanogenic glucosides, limanarin and lotaustralin which liberate toxic cyanogenic compounds when the tissues are crushed (Roca, 1984; Obigbesan, 1994). Intake of improperly processed cassava leads to neurological disorder (Cock, 1985) and can also contribute to the aetiology of goitre and parpareis which are endemic in several African countries (Annon, 1990). Bokanga *et al.*, (1990) have however reported that submerged fermentation of cassava and the efficient processing procedures (grinding, grating, and pounding) are effective in removing limanarin.

1.3 YIELD OF CASSAVA

Cassava has high potential yield of 90 tonnes per hectare per year (Cock, 1985). Yields of more than 80 tonnes per hectare per year have been obtained under experimental conditions (Hershey, 1993) and yields of 50 to 60 tonnes have been obtained by farmers cultivating cassava as first crop on newly cleared lands. However the average cassava yield (8.8 t/ha) in the world is low and it is even lower (6.4 t/ha) in Africa (Cock, 1985).

In Ghana estimates on cassava production show that the average yield for the period between 1987-1990 is 7.8 t/ha (MOA, 1991). Ezumah (1988) reported that farmers who use improved technologies have the potential for achieving over 30 t/ha. The current lower yield in Ghana is mainly due to low adoption of improved technologies in the cultivation of cassava including lack of improved genotypes or varieties thereby forcing farmers to rely on susceptible genotypes for planting which are easily affected by diseases and pests.

1.4 CASSAVA DISEASES

The most notable diseases which affect cassava are African Cassava Mosaic Virus (ACMV) disease, Cassava Bacterial Blight disease (CBB) and Cercospora leaf spot. CBB is the most widespread bacterial disease of cassava. The causal organism is *Xanthomonas campestris pathovar manihotis*. In Africa it was first reported in Madagascar in 1946 (IITA, 1990) and now occurs in all cassava-growing areas. The symptoms of the disease include water soaked leaf spot, stem die-back and vascular necrosis. Severe attack results in rapid defoliation of the plant. Estimates of yield losses vary from 20 to 100% depending on the cultivar (IITA, 1990.

Cassava brown leaf spot caused by *Cercosporidium henningsi* is the most important cercospora leaf spot disease. Symptoms which are brownish are restricted to upper and lower leaf surfaces of older leaves. It has been estimated that the disease can caused about 20% yield loss in cassava (Theberge, 1985).

The African Cassava Mosaic Virus (ACMV) disease is the most widespread disease of cassava in Tropical Africa. It is transmitted by the whitefly vector *Bemisia tabaci* (Gennadius) which is prevalent in many parts of Africa (Annon, 1990). The etiology of ACMV has been widely documented in West Africa (Hahn *et al*., 1989; Fauquette and Fargette, 1990) and also in East Africa (Thresh *et al*., 1994).

Symptoms of ACMV disease include a characteristic light green, yellow or white patches, irregularly distributed. The chlorotic areas may be only small flecks or spots or they may cover the entire leaf surface. The mottling is sometimes accompanied by leaf deformation and a general stunting of the plant.

Estimates of yield reduction per plant from ACMV infection generally range from 24% in highly resistant genotypes to 75% in highly susceptible varieties (Terry and Hahn, 1980; Fargette *et al.*, 1985). Bocks and Woods (1983) have estimated that in highly susceptible clones yield losses of up to 95 % can occur. In East Africa , Seif (1982) showed that the mean yield loss of several Kenyan varieties was 57.7 % and disease incidence and yield were highly correlated.

The use of cassava stakes infected with the disease and pests enhance their rapid spread. In Ghana there is lack of good, healthy planting materials. Hence the prevalence of the disease. The control of ACMV disease can be achieved either through sanitation or the use of resistant/tolerant varieties obtained through breeding.

1.5 GERMPLASM COLLECTION AND SELECTION

Cassava and its wild relatives show an enormous genetic variation and there is a large number of cultivars. Africa abounds in wild varieties of cassava. African farmers cultivate over 2000 varieties of the crop (Iweke, *et al.*, 1994) and in Ghana farmers cultivate about 78 varieties (Annor-Frempong, 1994). The extensive and complete collection of local germplasm of cassava in Africa is important because native varieties have usually been selected over long periods for adaptability and palatability.

These varieties differ in their morphology, time-to-maturity and the type of food for which they are used. Besides these local clones carry genes for adaptation to local conditions especially tolerance to drought, diseases and pests and therefore serve as base material for cassava breeding.

Selection of desired genotypes is a critical step in plant breeding (Ahloowalia, 1995). Germplasm collections made from local farmers so as to select for cultivars with desirable traits is highly recommended. This would be used as base material for crop improvement and breeding using advance biotechnological techniques. For maintenance of these selected genotypes an efficient regeneration system is a necessary prerequisite.

1.6 PROPAGATION AND REGENERATION OF CASSAVA

Two methods of propagating can be distinguished. Cassava may be propagated by stem cutting which is the traditional method and by the tissue culture (*in-vitro*) method.

1.6.1 TRADITIONAL METHOD OF PROPAGATION

True seeds of cassava have hardly been used by local farmers for propagation of the crop due to its high level of heterogeneity which is a result of genetic segregation. They rely on cuttings prepared from matured stakes. This traditional method of propagation is slow and also has an inherent problem of disease transfer from one vegetative generation to the next because very often the cuttings carry pests and diseases (example, ACMV). The method also poses an enormous limitation during maintenance and international transfer of germplasm.

1.6.2 *IN-VITRO* REGENERATION

1.6.2.1 Meristem Culture

In-vitro culture of cassava is almost always accomplished by vegetative propagation of stem and bud cuttings. For production of disease-free planting materials, either meristem culture or a combination of meristem culture and thermotherapy are employed. These *in-vitro*

techniques result in non-adventitious shoot regeneration where existing meristems are allowed to regenerate into plants.

The first *in-vitro* procedure for cassava regeneration using meristem culture was described by Kartha *et al.* (1974) and a year later by Liu (1975). The procedure employed the culture of meristematic dome from shoot tips on a medium supplemented with naphthalene acetic acid (NAA), benzylaminopurine (BA), gibberelic acid (GA_3) and 20 g/l sucrose.

The meristem culture was later applied to the production of disease-free plants (Kartha and Gamborg, 1975). In cases where viruses are difficult to eliminate by meristem culture alone, a combination of thermotherapy and meristem culture has proved beneficial. Kaiser and Teemba (1979) combined meristem culture with thermotherapy to eradicate cassava mosaic diseases (CMD) and another "virus-like" pathogen from several East African cassava varieties successfully. Kartha and Gamborg (1975) further reported that after heat treatment, relatively larger meristem tips (0.8 mm) could be cultured. It is believed that the higher temperature either restricts multiplication of the virus or enhances vegetative growth, resulting in the production of virus-free shoots.

1.6.2.2 Multiple shoot induction

Healthy planting materials can also be produced in large numbers by induction of multiple shoots from either single nodes or apical meristems. Smith *et al.* (1986) induced multiple shoots from shoot tips and single buds on a medium supplemented with 0.5 mg/l NAA and 0-0.5 mg/l BAP. On this medium, between 4.2 and 18 nodes were produced within three weeks. Roca (1984) has reported that the number of apical nodes produced depends on the concentration of BA in the medium.

However, these techniques require several tissue culture steps (mechanical isolation of cuttings, rooting of cuttings and hardening of plants) which makes the procedure labour intensive.

1.6.3 ORGANOGENESIS

Organogenesis refers to the process in which a unipolar structure is formed from multicellular origin (Reinert *et al.*, 1977; Thorpe, 1990; Terzi and Loschiavo, 1990). The unipolar structure is either a shoot or a root and is physically connected to the tissue of origin. Plant regeneration through organogenesis can occur either directly or indirectly. Direct organogenesis involves the emergence of adventitious organs directly from the explant without callus phase. In contrasts, adventitious organs are produced through a callus phase in indirect organogenesis.

The first successful attempts of organogenesis in cassava were reported by Tilquin (1979) and Shahin and Shephard (1980). However, they were not repeatable by others.

A high concentration of auxin and low concentration of cytokinin promotes callusing. Tisserat *et al* (1979) reported that callus production on an agar medium could also be induced by the inclusion of 2,4-D alone. The cellular composition of the callus is heterogeneous (Evans *et al.*, 1981). Meristematically active cells in the organogenic callus are the precursors of root and shoot organs.

1.6.4 SOMATIC EMBRYOGENESIS

1.6.4.1 Origin and uses

Somatic embryogenesis is the development of embryos from somatic cells through a series of developmental stages similar to zygotic embryogenesis. The embryo produced is a bipolar structure with no vascular connection to the parental tissue (Tulucke, 1987).

Production of somatic embryos may occur either directly or indirectly (Sharp *et al.*, 1980). Direct embryogenesis involves the production of embryos without an intervening callus phase. During indirect embryogenesis, callus formation precedes embryo development (Carman, 1990; Sharp *et al.*, 1980; Wann , 1988).The somatic cell which gives rise to the embryo is called embryogenic cell. Even though every living cell is totipotent only a limited number of cells from explants give rise to structures that exhibit embryogenesis. Embryo induction from cells is dependent on the culture environment, such as hormonal balance, sugars, amino acids, salt concentration and the growth room condition (Vasil and Vasil, 1981; Franklin and Dixon, 1994). The methods for achieving appropriate conditions for embryo induction are still the main concern in regeneration research. In different plant species somatic embryogenesis is achieved on media with different inductive growth regulator(s). In monocots, embryogenesis is exclusively induced by an auxin supplemented medium whereas in the dicotyledonous species growth regulator-free, cytokinin and cytokinin plus auxin supplemented media are used (Raemakers, 1993). Synthetic auxins such as Picloram, Dicamba or 2,4-D are frequently used in the dicots.

Somatic embryogenesis has some advantages over other regeneration methods. Plants produced by somatic embryogenesis have taproots while in shoot regeneration adventitious roots are produced (Raemakers et al., 1995b). Also in somatic embryogenesis it is possible

to produce a bipolar structure bearing both root and shoot apices in one step. The system also enhances large scale clonal propagation compared to vegetative propagation. More recently somatic embryogenesis has been interfaced with gene transformation methods to obtain transgenic cassava (Schopke *et al.*, 1996).

1.6.4.2 Primary embryogenesis in cassava.

Somatic embryogenesis was defined by Haccius (1978) as a non sexual developmental process which produces a bipolar embryo which has its own vascular tissue. In cassava somatic embryogenesis was first reported by Stamp and Henshaw (1982). Since then, several groups of workers have described it as an efficient regeneration system in the crop (Szabados *et al.*, 1987; Mathews *et al.*, 1993; Raemakers *et al.*, 1993a). In the original method, embryos were induced on cotyledons, embryo axis or zygotic embryos using a two-step procedure . Induction of embryos on young leaf lobes have also been achieved (Stamp and Henshaw, 1987a; Szabados *et al.*, 1987; Raemakers *et al.*, 1993abc). In step one, embryos were induced on MS medium supplemented with 20 g/l sucrose and 16 mg/l 2,4-D. In step two, embryos were transferred to MS medium supplemented with 20 g/l sucrose, 0.1 mg/l 2,4-D and 0.1 mg/l BA for further maturation of embryos. The efficiency of primary embryogenesis was however very low. Mathews *et al.*, (1993) improved embryogenic frequency by transferring 2,4-D-induced globular embryos attached to the explant to a hormone-free medium supplemented with 0.5% charcoal after 15 days of induction. This procedure increased the number of embryos from 0.4 to 3.4 per initial leaf lobe.

In another method, Raemakers *et al.*, (1993b) treated donor plants with liquid 2,4-D two days before leaf lobes were isolated for culture for embryo production. In this method 9.4 mature embryos were produced per leaf explant of the genotype M. Col 22 compared with 3.5 mature

Although exogenous application of auxin has a positive effect on embryo induction, continuous exposure of somatic embryos to auxin has detrimental effect on the development of the apical meristem (Halperin and Witherel, 1965) which result in poor plant regeneration. Parrot *et al.*, (1991) reported that continued exposure of somatic embryos to auxin lock them into a given developmental phase and establish cultures undergoing repetitive embryogenesis. In soybean, it has been shown that one week exposure of explant tissue to auxin was necessary for embryo production and that increasingly longer exposure resulted in decreased conversion frequency (Parrot *et al.*, 1991).

Desiccation is also another characteristic of zygotic embryogenesis and can also play a role in germination of somatic embryos. It influences developmental processes and triggers the germination of embryos (Rosenberg and Rhine, 1986). Desiccation causes changes in tugor pressure, membrane permeability and levels of endogenous absissic acid (ABA) which in turn induce expression of specific genes involved in plant development (Oishi and Bewley, 1990). Gray (1987) has reported that in *Vitis longii,* 20% of somatic embryos that were desiccated germinated as opposed to 5% in non-desiccated embryos. Similarly, in spruce 50% of the desiccated embryos germinated whereas in the non-desiccated embryos only 5% germinated (Roberts *et al.*, 1990).

1.6.4.6 Somatic embryo germination in cassava.

Two main methods have been described for shoot conversion in cassava. In the first method Stamp and Henshaw (1987a) regenerated cassava plants from mature 2,4-D-induced embryos by culturing them in a basal medium supplemented with 0.1 mg/l BA plus 0.01 mg/l 2,4-D. Raemakers *et al.*, (1993b) improved shoot conversion by increasing the BA concentration to 1.0 mg/l in the basal medium. Depending on the genotype, 10% (M. Col

1505) to 70% (M. Col 22) of the embryos developed into plants. However, the process of embryo germination was long and plants were initially malformed and lacked roots. Adventitious roots were formed after transfer of plants to growth regulator-free medium. Mathews *et al.*, (1993) further improved embryo germination by culturing desiccated mature embryos on a hormone-free medium. With this method, 85% of M.Col 1505 embryos induced on 2,4-D medium germinated into plants.

1.7 OBJECTIVES

The current significant changes of cassava utilisation in the world, due to international market development and domestic demand will put pressure on the cassava breeder to supply the desired cultivars in a short time. To achieve this goal, breeding programmes in cassava should emphasize on the use of novel biotechnological techniques for genetic modification of the crop. An efficient regeneration system is therefore a necessary prerequisite for production of transgenic cassava. The most reliable regeneration system for cassava, thus far, has been through somatic embryogenesis and cyclic embryogenesis (Stamp and Henshaw, 1987a,b; Szabados *et al.*, 1987; Raemakers, 1993). The multiplication of virus-free planting materials would be greatly increased, if an efficient regeneration system such as somatic embryogenesis is developed for cassava in the producing countries. Although somatic embryos and plantlets formation have been obtained from Latin-American clones (Stamp and Henshaw, 1982, 1987a,b; Szabados *et al.*, 1987), this is yet not possible with African clones (Ng, 1992). Secondly, *in-vitro* methods used to produce healthy planting materials of cassava have not been employed in the local genotypes cultivated by local farmers.

The objective of this study was to select for cassava cultivars with desired agronomic traits from local farmers and to regenerate the selected cultivars *in vitro* using meristem culture,

multiple shoot induction and somatic embryogenesis. Other specific objectives include i. studies on embryogenic competence of selected local cassava cultivars ii. comparison of multiple shoot induction in Latin-American cultivar M. Col 22 and local cultivars and iii. high frequency germination of mature somatic embryos of African cultivars and Latin-American cultivars.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 EXPERIMENTAL LAYOUT.

Work described in this thesis is divided into two phases. The first phase involved germplasm collection of cassava (*Manihot esculenta* Crantz) from local farmers in Dodowa, Kasoa, Kwabenya and North Taifa. Selected cultivars were then propagated *in-vitro* using meristem culture, multiple shoot induction and somatic embryogenesis. These set of experiments were conducted at the Botany Department of the University of Ghana. Experiments in the second phase were conducted at the Plant Breeding Department, Wageningen Agricultural University, The Netherlands.

The second phase involved studies on germination of mature somatic embryos using selected cultivars from Nigeria (TMS 90853), Zimbabwe (Line 11), Indonesia (Adira 4 and Gading) and CIAT, Columbia (M. Col 22).

2.2 GERMPLASM COLLECTION AND PROPAGATION

Twenty-five farmers from Dodowa, Kwabenya, Kasoa and North Taifa were interviewed using a structured questionnaire for the collection of qualitative information on cassava cultivars planted, duration to maturity, incidence of diseases, utilisation of tubers and yield of cultivars (Appendix I). Ten-centimetre long cuttings were prepared from stakes of each cultivar and were planted in black polythene bags (15 cm diameter) filled with topsoil. The pots were kept in the greenhouse at the Botany Department, University of Ghana. The temperature in the greenhouse was 28-35°C in normal daylight. The pots were watered every other day with

tapwater. The cuttings sprouted within seven days of planting. After 28, days apical shoot tips were harvested and used for *in-vitro* culture.

The severity of ACMV on the local cultivars was scored using ESCaPP Survey Software, IITA Benin Station, (Appendix ii) in order to determine their susceptibility to ACMV. Six month-old cassava plants on the farm were used for the score test. Shoot tips of 25 plants of each cultivar were selected at random and scored for ACMV by examination of the shoot tip.

2.3 SELECTION OF CULTIVARS.

Based on popularity, duration to maturity (early bulking), yield and susceptibility to ACMV, five local cultivars namely, Ankrah , Bosomnsia, Biafra , Santom and Afisiafi were selected for this studies on multiple shoot induction and somatic embryogenesis. The cultivars Ankrah and Santom were collected from farmers at Dodowa whilst Bosomnsia and Biafra were collected from Kwabenya. Afisiafi is an introduction from the International Institute of Tropical Agriculture (IITA).

For studies on somatic embryo germination, the cassava cultivars: Line 11(provided by University of Zimbabwe), TMS 90853 (provided by IITA), Adira 4 and Gading (provided by Central Research Institute for food Crops, Indonesia) and M. Col 22 (provided by CIAT, Columbia) were used.

2.4 SOURCE OF EXPLANTS

Four-week old shoot tips harvested from greenhouse grown cuttings were used as source explants for *in vitro* studies (Phase 1). Meristematic tissues (0.2 mm) were dissected from the apical shoot tips for culture. Mature embryos of Line 11, Adira 4, TMS 90853, and Gading used for studies on embryo germination (Phase 2) were provided by the Plant Breeding Department, Wageningen Agricultural University, The Netherlands.

2.5 MEDIA PREPARATION AND STERILISATION

Culture media were prepared from Murashige and Skoog basal salts (1962) supplemented with Gamborg B5 vitamins (Gamborg *et al,* 1968) formulated by Sigma Chemical Company, USA (MS). The composition of the MS medium is shown in Table 1. The MS medium was supplemented with sucrose and growth regulators as required. For solid media, MS was solidified with 7 g/l agar (oxoid). The pH of the media was adjusted to 5.7 prior to autoclaving. Media were dispensed into sterilised testtubes (25 mm in diameter). Each testtube contained 10 ml of the culture medium. Petri dishes contained 5 ml of the media.

Culture media, testtubes, distilled water, forceps and scapel blades were sterilised by autoclaving for 15 minutes at 121°C. Petri dishes were sterilised in an oven (Gallenkamp Oven, 300 Plus Series) at a temperature of 160°C for 24 hours. Growth regulators were filter-sterilised using 0.22 μm millipore filter sterilizer and were added to autoclaved MS under the laminar air flowhood.

2.6 STERILISATION OF EXPLANTS.

Shoot tips were trimmed of older leaves and washed thoroughly in running tap water. They were then transferred to sterilised GA-7 flasks under the laminar air flow hood (Dalton, Ogawa Seiki Co. Ltd). Explants were washed again with two changes of sterilised distilled water, immersed in 70% alcohol for 1 minute and rinsed with three changes of sterilised distilled water.

2.7 CULTURE PROCEDURES AND INCUBATION.

In-vitro procedures involving sterilisation of plant tissues, growth regulators, dissection of meristems and leaf lobes, inoculation and subculture were done under the laminar air

flowhood. All cultures were incubated in a growth room at a temperature of 27±1°C and 12 hour photoperiod and light intensity of 3,500 lux. The light was provided by fluorescent tubes.

Table 1. Composition of Murashige and Skoog (1962) basal salts and Gamborg B5 vitamins (MS).

Macro salts	**mg/l**	**mM**
$CaCl_2.2H_2O$	332.02	2.99
KH_2PO_4	170.00	1.25
KNO_3	1900.00	18.79
$MgSO_4.7H_2O$	370.00	1.50
NH_4NO_3	1650.00	20.61
Micro Salts		
$CoCl_2.6H_2O$	0.025	0.11
$CuSO_4.5H_2O$	0.025	0.10
H_3BO_3	6.20	0.10
KI	0.83	5.00
$MnSO_4.H_2O$	16.90	0.10
$Na_2MoO_4.2H_2O$	0.25	1.03
$ZnSO_4.7H_2O$	8.60	29.91
FeNaEDTA	36.70	0.10
B5 Vitamins		
myo-inositol	100.00	0.56
nicotinic acid	1.00	8.12
pyridoxine HCl	1.00	4.86
thiamine HCl	10.00	29.65

2.8 EXPERIMENTAL PROCEDURES

2.8.1 Plant Regeneration

Three methods of plant regeneration were used. These are meristem culture, multiple shoot induction and somatic embryogenesis.

2.8.2 Plant regeneration from apical meristem of cassava.

The effect of different concentrations of BA on shoot induction from apical meristem was studied in Bosomnsia, Ankrah, Santom, Biafra , Bodwease bosomnsia and Afisiafi using two media formulations, Medium A and Medium B as indicated below. Four-week old shoots were harvested from greenhouse grown cuttings. Explants were derived from four week old shoot sterilised as described in section 2.6. The meristem (0.2-0.4 mm) was dissected under a stereomicroscope and were cultured on 10 ml of Medium A or Medium B.

Medium A

Murashige and Skoog (1962) basal salts and vitamins (full strength)

Naphthalene acetic acid (NAA)	0.1 mg/l
Gibberelic acid (GA_3)	0.1 mg/l
Sucrose	30 g/l
Agar	7 g/l

Medium B

Murashige and Skoog (1962) basal salts and vitamins (full strength)

Naphthalene acetic acid (NAA)	0.02 mg/l
Gibberelic acid (GA_3)	0.04 mg/l
Sucrose	30 g/l
Agar	7 g/l

Both Medium A and B were supplemented with the following BA concentrations, 0.0 (control), 0.05, 0.10, 0.15 or 1.0 mg/l BA. Medium B was formulated to reduce the callusing effect of NAA in the medium. Media were adjusted to pH 5.7 before autoclaving (see 2.4). Cultures were incubated in a growth room at 27 ± 1°C. The cultures were observed daily for organ formation.

Shoots were defined as organs with distinct nodes, internodes and at least one primary leaf. Shoots were transferred to a hormone-free medium after six weeks of culture for further growth. Plantlets obtained thereafter were subcultured onto hormone-free medium every four weeks using nodal cuttings.

The leaves of thirty six plantlets of each cultivar were examined *in-vitro* for ACMV symptoms after 28 days of culture on a hormone-free medium.

2.8.3 Induction of Multiple shoots from apical meristems.

Induction of multiple shoots from apical meristems were compared in Latin-American cultivar M. Col 22 and the Ghanaian cultivar Biafra. Apical meristems were cultured on MS medium supplemented with 20 g/l sucrose, 0.1 mg/l NAA, 0.1 mg/l GA_3 and 2.0, 1.0, 0.1 or 0.0 (control) mg/l BA. For each treatment, 10 ml of this medium were dispensed into a test tube which formed an experimental unit. One apical meristem was inoculated in a test tube. Each experimental treatment was replicated six times and arranged in a completely randomised design. The number of shoots and buds developed at four weeks was recorded.

2.8.4 Induction of multiple shoots from single bud cuttings.

To enhance the multiplication rate of plantlets multiple shoots were induced in Ankrah, Bosomnsia, Biafra and M. Col 22. Single nodal explants were prepared from four weeks

old *in-vitro* plantlets and cultured on 10 ml of medium. Each experimental unit was replicated four times and arranged in a completely randomised design. The number of shoots which sprouted per node as well as the number of buds per shoot were recorded.

2.8.5 Induction of somatic embryos from young leaf lobes of green house grown and *in vitro* plants.

Primary embryos were produced using a two-step procedure described by Stamp and Henshaw (1982) and Raemakers *et al.* (1993a,b). Four weeks old shoot tips from greenhouse-grown cassava and two weeks old *in vitro* plantlets were used. Shoot tips were sterilised as described in section 2.6. Young leaf lobes (0.5-2 mm) were dissected from the shoot tips using a stereomicroscope in the laminar air flowhood. The leaf lobe explants (from greenhouse grown cassava and *in vitro* plantlets) were cultured on MS medium supplemented with adenine sulphate(80 mg/l), sucrose (20 mg/l), agar (7 g/l) and 2,4-D at a concentration of 16, 8,4 or 0 mg/l (control). The explants were cultured in petri dishes with their adaxial surfaces in contact with the medium and were incubated in the dark (Step one).

After 24 days of culture, the number of explants which formed embryogenic calli were recorded and transferred onto MS medium supplemented with 0.1 mg/l BA (Step two) and incubated in the light. Each experimental treatment was replicated five times with 5 explants per treatment in a completely randomised design. The number of somatic embryos, foliose structures, roots and shoots produced were recorded.

In another experiment, young leaf lobes, apical meristems and stipules were dissected from four weeks old greenhouse grown cassava and cultured on MS medium supplemented with 16 mg/l 2,4-D and incubated as described above.

2.8.6 Desiccation of somatic embryos.

Mature NAA-induced embryos were subjected to various desiccation treatments for a period of seven days using the following procedures:

i. **Partial desiccation using petri dish.** Mature embryos of Adira 4 (8g) induced in liquid MS medium were transferred to sterile Whatmann 3 mm filter paper for one hour to absorb water from the embryos under the laminar air flowhood. Four grammes (4g) of these embryos were transferred to sterile petri dishes, weighed, sealed with parafilm and kept in the growth room for seven days. The petri dishes were changed every other day to remove condensed moisture. The percentage moisture loss through desiccation was calculated from the ratio between the weight of the embryos after and before desiccation, multiplied by 100. Desiccated embryos were cultured on solid MS medium supplemented with 1.0 mg/l BA. Cultures were incubated in the light. The number of embryos which germinated was recorded.

ii. **Desiccation using glycerol.** Four grams of mature embryos of Adira 4 were transferred to petri dishes and sealed with parafilm. The petri dishes were then placed in desiccators containing 200 ml of glycerol to control the internal humidity for seven days. The desiccated embryos were reweighed to determine the moisture loss before culture on solid MS medium with 1 mg/l BA.

iii. Desiccation by using sugar and other solutions. Four grams of matured embryos were transferred to solid or liquid MS medium supplemented with 0-60 g/l sucrose or liquid MS supplemented with 0-10 g/l mannitol. The control was water supplemented with 0-60 mg/l sucrose. After seven days half of each treatment was cultured on MS medium supplemented with 1.0 mg/l BA while the other half was desiccated using petri dish method for further seven days before they were cultured for germination.

2.8.7 Effect of ABA pre-treatment on embryo germination.

Four grams of mature embryos of Adira 4 were transferred to 300 ml culture jars containing MS medium supplemented with 0, 1 or 40 mg/l abscissic acid. The jars were incubated on a gyrator at 120 rpm in the growth room. After seven days half of the embryos of each treatment were cultured on solid MS medium supplemented with 1.0 mg/l BA for germination while the other half was desiccated for further seven days before cultured.

2.8.8 The effect of cytokinins and auxins on embryo germination.

The effects of three cytokinins Thidiazuron (TDZ), Benzylaminopurine (BA) and Kinetin as well as two auxins (NAA and IBA) on germination of somatic embryos were studied. Mature NAA liquid-induced somatic embryos of Adira 4 were desiccated using the petri dish method (Section 2.8.6) before culture on solid MS medium supplemented with 20 g/l sucrose as control or solid MS medium supplemented with various concentrations of cytokinins and auxins. Cultures were all incubated in the light.

2.8.9 Relation between moisture loss and BA on embryo germination

The relationship between the degree of moisture loss and BA on embryo germination was studied using mature embryos of Adira 4 and Gading. Four grams of mature embryos were

transferred to sterile petri dishes and sealed with parafilm. The petri dishes were then placed in 2L jars filled with 50 ml of various concentrations of sodium chloride solution. After seven days the amount of moisture loss was determined and the embryos which lost 20, 40 or 75 percent moisture were cultured for germination on MS medium supplemented with 0.1 mg/l BA.

2.8.10 Effect of prewashing of embryos on germination.

Four grams of mature embryos of Gading were transferred to 300 ml culture jars each containing 200 ml of sterile distilled water. The jars were incubated on a gyrator at 120 rpm. After 1, 5, 10, or 14 days half the embryos were cultured for germination while the other half were petri dish desiccated for a further one week before they were cultured for germination.

2.8.11 Influence of light/dark regimes on germination of desiccated embryos.

Mature NAA- or 2,4-D-induced embryos of TMS 90853, Line 11, Adira 4 and Gading were desiccated using the petri dish method. The moisture loss was determined as previously described for seven days. Desiccated embryos were cultured for germination on MS amended with 0.1, 1.0, or 4.0 mg/l BA including control (0 mg/l). After seven days one-half of the cultures were incubated in a dark cardbox while the remaining half were incubated under growth room light conditions of 3,500 lux. Cultures were evaluated after 14 or 28 days .

2.8.12 Effect of different light intensities on embryo germination.

Four grams of NAA induced embryos of Adira 4 were transferred to sterile petri dishes and desiccated using the petri dish method. After seven days the desiccated embryos were cultured on MS medium supplemented with various concentrations of BA. The cultured embryos were incubated in different boxes covered with transparent polyethylene sheets to

control the light intensities. The light intensities used were 0 6.4, 64, 640, or 6400 lux. The light intensity in each box was determined using a light meter.

2.8.13 Influence of different light regimes on embryo germination.

Four grams of matured embryos of Adira 4 were transferred to sterile petri dishes and incubated in white, red, blue, far red or dark cabinets.

2.8.14 Effect of desiccation in complete darkness on embryo germination.

Four grams of matured embryos of Adira 4 were transferred to sterile petri dishes, wrapped in aluminium foil to prevent entry of light or placed in the light (L) for 7 days. At the end of the 7 days, half of the embryos wrapped with aluminium foil were cultured for germination in the dark while the other half was incubated in light. Embryos desiccated in light were also cultured and incubated in the light.

2.8.15 Evaluation

Embryos cultured in the dark/light experiment were evaluated for germination after 14 and 28 days and all other experiments after 28 days. The embryos were evaluated for presence or absence of organs, the morphology of the root whether thin or thick, taproots or adventitious. Also the morphology of the shoot was determined, whether thin or thick, single or multiple. An embryo is said to be germinated when it had both shoot and root. A shoot was present when an internode has appeared with at least one primary leaf.

2.8.16 STATISTICAL ANALYSIS

Statistical analysis, where applicable, was performed using Statistical Graphics Corporation Software.

CHAPTER THREE

3.0 RESULTS

3.1 GERMPLASM COLLECTION.

Germplasm collections of cassava were made from local farmers in Dodowa, Kwabenya and North Taifa in the Greater Accra Region in order to identify cultivars which have desired agronomic traits using a structured questionnaire (Appendix 1). These collections will serve as basis for improvement of the crop.

The survey indicates that farmers distinguish cassava genotypes by local names which are often descriptive of the physical characteristics of the plant. The name may also describe the original source of the genotype such as the place from which it was introduced for the first time. Twelve (12) descriptive names of cassava were recorded including two IITA introduced cultivars (Table 2). The name of the cultivar may vary from village to village. For example Bosomnsia is associated with the village where the farmers collected it from. Hence Bodwease bosomnsia and Kuntunse bosomnsia refer to the same variety collected from Bodwease and Kuntunse, respectively.

Almost all the farmers cultivate Bosomnsia either as single cultivar or with other cultivars on the same farm. Sixty (60%) of the farmers interviewed cultivate Ankrah and Biafra. The remaining cultivars are cultivated on a minor scale.

The cultivars collected show different morphological features. For instance, the colour of the petiole is either red or green. A petiole is considered red if it has any red spot on the petiole,

otherwise green. All the cultivars collected had red petiole except Afisiafi which had green petioles.

The leaves of cassava are either described as broad or narrow. All the cultivars collected had broad leaves. However, the leaves of Biafra, Bankye tumtum and Ankrah were much broader than the other cultivars.

For the cultivars collected duration to maturity varied from 6-18 months (Table 2). For instance farmers indicated that Bosomnsia matures at six months after planting compared to 18 months in Saakwanyah, Bankye tumtum and Ankrah.

Farmers interviewed also indicated that the yield obtained in Bosomnsia is lower compared to Bodwease bosomnsia, Ankrah and Biafra.

3.2 Severity of ACMV disease on cassava cultivars

Symptoms of two main types of cassava diseases were identified on the leaves of cultivars collected. These are Brown leaf spot (Plate 1) and ACMV disease (Plate 2). Severity of ACMV symptoms was scored for five of the most frequently cultivated cultivars. Results are as shown in Table 2.

Symptoms of ACMV disease observed on the farms are light green or chlorotic patterns on leaves, wrinkled or distorted leaf surface (Plate 2) and reduction of leaf size. Symptoms of ACMV disease were observed in all the cultivars in every village. The severity of the symptoms however varied from cultivar to cultivar. Among the cultivars scored ACMV was found to be most conspicuous and severe in Bosomnsia compared to the other cultivars Table 2. Severity was least in Bodwease bosomnsia and Biafra. In Bosomnsia the symptoms were observed on both older and younger leaves whereas in Biafra and Bodwease

bosomnsia, symptoms were observed only on younger leaves. For Ankrah and Biafra the symptoms are not conspicuous at some locations. Farmers indicated that symptoms of ACMV appear four (4) weeks after shoots have sprouted in Bosomnsia and Ankrah compared to six (6) weeks after sprouting in Bodwease bosomnsia and Biafra.

Field observations also showed that brown leaf spot affected only older leaves of cultivars studied in this work. In addition cultivars with conspicuous symptoms of brown leaf spot express very low symptoms of ACMV disease.

Table 2. Cassava landraces collected from farmers during the survey.

Cultivars	maturity period (months)	Yield	ACMV Score	Remarks
Ankrah	18	high	2	Has good fufu making qualities
Bosomnsia	6	medium	4	used for making fufu, ampesi
Bodwease bosomnsia	9	high	1	used for making fufu, gari, ampesi
Biafra	12	high	2	used for making gari, agblema
Santom	9	medium	2	has sweet taste, good for ampesi
Afisiafi[1]	12	high	2	good for making gari, agblema
Bankye broni	12	medium		used for making fufu, gari
Bankye tumtum	18	high		used for making gari, agblema
Katawire	18	medium		used for making gari, agblema
Koasekohwe	9	medium		used for making fufu, ampesi
Saakwanyah	18	medium		used for making gari
Kuntunse bosomnsia	9	high		used for making gari, agblema
Gblomaduade[1]	-	high		used for making gari, agblema

Note 1: IITA introduced cultivar.

Plate 1. Biafra cultivar showing symptoms of brown leaf spot.

Plate 2. Bosomnsia showing symptoms of ACMV disease

3.3 Effect of Medium A on apical meristem of cassava cultivars.

In this experiment, apical meristems were cultured on MS medium amended with 0.1 mg/l NAA, 0.1 mg/l BA and 0.1 mg/l GA_3 (Medium A) . Theresults are as indicated in Table 3.

Explanted meristems became chlorophyllous within seven (7) days and after 14 days formed creamy callus. Explants cultured on a hormone-free medium did not form callus. The size of the callus was dependent on the concentration of BA in the medium. In all the cultivars callus formation was high in MS medium supplemented with high BA but decreased at the controls. Callus formation was however low in Santom explants.

In Bankye tumtum there was no shoot development at any of the BA levels. Only shoot-like structures developed when explants were cultured on 0.10 mg/l BA. These shoot-like structures had no distinct nodes or internodes and could also not develop into functional shoots when transferred to hormone-free medium.

In general, shoot development by Biafra and Bodwease bosomnsia was comparatively higher than Ankrah, Santom and Bosomnsia. The optimal treatment for shoot development was 0.15 mg/l BA for Ankrah and Bodwease bosomnsia whereas for Santom and Biafra the optimal treatment was 0.10 mg/l. The highest percentage number of shoots (75%) occurred on Biafra followed by Bodwease bosomnsia (74%), Santom (50%) and Ankrah (33%). Most explants which did not form shoots developed into shoot-like structures. These shoot-like structures did not developed into functional shoots when transferred onto hormone-free medium. Shoots were transferred to a hormone-free medium after 28 days of culture for further *in vitro* studies.

Table 3. Effect of Medium A on apical meristems of cassava cultivars at four weeks.

Cuitivar	BA (mg/l)	No. of explants [a]	Callus	Roots (%)	Shoots (%)	Shoot-like structures (%)
Ankrah	0.00	8	-	0	0 (0)	0 (0)
	0.05	8	++	0	2 (25)	0 (0)
	0.10	10	+++	0	0 (0)	3 (30)
	0.15	15	+++	0	5 (33)	3 (20)
Bosomnsia	0.00	8	-	0	0 (0)	0 (0)
	0.05	8	+++	0	3 (37)	1 (13)
	0.10	10	+++	0	2 (20)	3 (30)
	0.15	11	+++	0	3 (27)	3 (27)
Santom	0.00	8	-	0	0 (0)	0 (0)
	0.05	8	+	0	0 (0)	0 (0)
	0.10	10	++	0	5 (50)	1 (10)
	0.15*	-	-	-	-	-
Biafra	0.00	12	-	0	0 (0)	0 (0)
	0.05	12	++	0	7 (58)	2 (17)
	0.10	12	+++	0	9 (75)	3 25)
	0.15	11	+++	0	8 (73)	2 18)
Bodwease bosomnsia	0.00	9	-	0	0 (0)	0 (0)
	0.05	7	+++	0	5 (71)	2 (28)
	0.10	7	+++	0	4 (57)	0 (0)
	0.15	19	+++	0	14 (74)	4 (21)
Bankye tumtum	0.00	8	-	0	0 (0)	0 (0)
	0.05	12	++	0	0 (0)	0 (0)
	0.10	11	+++	0	0 (0)	2 (18)
	0.15	10	+++	0	0 (0)	0 (0)

Note: - no callus + low callus ++ moderate +++ high callus

a: Number recorded after contaminated cultures had been discarded.

b: Figures in parenthesis indicate percentages.

3.4 Effect of Medium B on apical meristem of cassava cultivars.

Following the experiment on Medium A, it was thought that excessive callus formation caused by high concentration of NAA and BA may have hinder shoot and root development. The hypothesis was tested by culturing apical meristems on MS supplemented with reduced concentrations of NAA (0.02 mg/l) and GA_3 (0.04 mg/l), while varying the concentration of BA. This constituted medium B.

The explants became chlorophyllous after seven days followed by callus formation and shoot regeneration within fourteen days. However, callus formation was moderate compared to Medium A and in Santom and Afisiafi most of the meristem explants did not form callus. The results are presented in Table 4.

In general, shoot regeneration was enhanced in Medium B compared to Medium A.
In all the cultivars, explants on a hormone-free medium became chlorophyllous but did not develop into shoots or roots.

For Bosomnsia, Santom and Afisiafi the optimal concentration of BA for shoot formation was 0.05 mg/l. At this optimal concentration, all the explants of Bosomnsia formed shoots compared to only 27% in Medium A. Similarly, in Afisiafi and Santom 90 and 46%, respectively, formed shoots. For Ankrah and Biafra, the optimal concentration was 0.15 mg/l BA and shoot formation was 90% and 85% respectively. In Medium A, only 33% of Ankrah meristems cultured on the same BA treatment were able to form shoots.

Multiple shoots were observed in Ankrah and Santom explants cultured on 0.15 mg/l BA compared to single shoots in Medium A. In Ankrah 26% of the cultures developed multiple shoots with a range of 2-4 shoots per meristem explant. In Santom, only one meristem explant developed three shoots.

Fifty five percent (55%) of Ankrah cultures on 0.15 mg/l BA developed thick roots after 4 weeks. In Bosomnsia and Santom, 38 and 6% respectively developed thick roots.
Cultures were transferred to a hormone-free medium and root development occurred within seven (7) days in all the cultivars.

Symptoms of ACMV disease were observed on 25% of the plantlets of Santom (Plate 3) suggesting that meristem culture alone could not eradicate the virus. This observation was not found on the remaining cultivars. The ACMV symptoms were not observed in the other cultivars.

Table 4. Effect of Medium B on apical meristems of cassava cultivars at 4 weeks.

Cultivar	BA mg/l	No. of Explants	Callus	Roots[a] (%)	Shoots (%)
Ankrah	0.00	12	-	0 (0)	0 (0)
	0.05	14	+	0 (0)	8 (57)
	0.15	20	++	11 (55)	17 (85)
	1.00	15	+++	0 (0)	12 (80)
Bosomnsia	0.00	14	-	0 (0)	0 (0)
	0.05	10	++	0 (0)	10 (100)
	0.15	13	++	5 (38)	11 (85)
	1.00	10	+++	0 (0)	5 (50)
Santom	0.00	12	-	0 (0)	0 (0)
	0.05	13	+	0 (0)	6 (46)
	0.15	18	+	1 (6)	7 (39)
	1.00	11	+	0 (0)	2 (18)
Biafra	0.00	12	-	0 (0)	0 (0)
	0.05	12	++	0 (0)	7 (58)
	0.15	10	+++	0 (0)	9 (90)
	1.00	12	+++	0 (0)	9 (75)
Afisiafi	0.00	12	-	0 (0)	0 (0)
	0.05	10	+	0 (0)	9 (90)
	0.15	10	+	0 (0)	7 (70)
	1.00	11	++	0 (0)	6 (5)

Note : - no callus, + low callus formation, ++ moderate callus, +++ high callus formation

a: Number recorded after contaminated cultures had been discarded.

b: figures in bracket indicate percentages

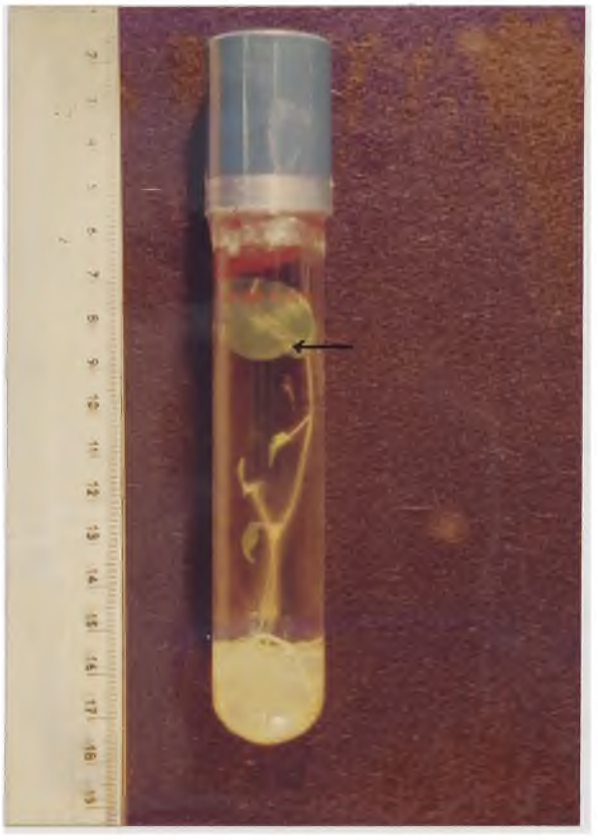

Plate 3. In vitro plantlet of Santom showing symptoms of ACMV disease on leaf (arrowed)

3.5 Effect of BA on induction of multiple shoots from apical meristem.

Apical meristems of M. Col. 22 and Biafra were induced to produce multiple shoots on an MS medium supplemented with 0.1 mg/l GA_3, 0.1 mg/l NAA and varying concentrations of BA (0.0-2.0 mg/l). The objective of this experiment was to compare multiple shoot induction in a Latin-American cultivar (M. Col. 22) and a Ghanaian cultivar (Biafra). Table 5 shows the number of shoots or buds produced by the two cultivars. The two cultivars were cultured at different times.

In M. Col. 22, there was shoot induction on a hormone-free medium (control) as well as the treated media whereas in Biafra shoot induction occurred only on hormone-amended media. The number of apical shoots or buds produced by the two cultivars differed significantly ($P \leq 0.05$) Appendix 3a, b. Also BA had a positive effect on the induction of multiple shoots from apical meristem of M. Col. 22 but not Biafra.

The number of apical shoots produced in M. Col. 22 correlated highly ($r = 0.9$) with the concentration of BA in the medium. Statistical analysis of individual data showed significant difference in the number of shoots produced between the controls and the BA treated media at $P \leq 0.05$ in both cultivars (Table 5, Appendix 4a). The highest number of shoots (7.0) was produced on a medium supplemented with 2.0 mg/l BA.

The highest number of buds (19) in M. COl. 22 was produced on a medium supplemented with 2.0 mg/l BA but this was not significantly different from the controls (Table 5, Appendix 4b).

In Biafra, single shoots were produced irrespective of BA concentration in the medium. Also the number of buds produced in the BA-amended media did not differ significantly at P≤0.05 (Appendix 5, Table 5).

Table 5. Effect of BA on induction of multiple shoots and buds from apical meristems of M. Col. 22 and Biafra.

BA mg/l	Mean number of apical shoots and buds			
	M. Col 22		Biafra	
	Apical shoots	Buds	Apical shoots	Buds
0.0	1.0 a	9 a	0.0 a	0.0 a
0.1	3.0 ab	15 a	1.0 b	10.0 b
1.0	4.8 ab	16 a	1.0 b	9.0 b
2.0	7.0 c	19 a	1.0 b	12.0 b

Note: Numbers in a column followed by different letters are significantly different (P≤0.05). M. Col 22 and Biafra were cultured at different times.

3.6 Effect of BA on induction of multiple shoots from single bud cuttings.

Single nodal explants were cultured on MS medium supplemented with 0.0-2.0 mg/l BA. The mean number of shoots and buds produced by the nodal cuttings are presented in Tables 6 and 7 respectively. The results indicate that BA induced multiple shoots from buds in all the cultivars tested (Plate 4) but the number of shoots produced was dependent on the

concentration of BA in the medium and the cultivar. Analysis of Variance (ANOVA) showed highly significant differences ($P \leq 0.05$) between the number of shoots produced by the Ghanaian cultivars (Ankrah, Bosomnsia and Biafra) and the Latin American cultivar (M. Col. 22) and also between the BA treatments (Appendix 6a).

In all the cultivars except Biafra, the optimum concentration of BA for highest number of shoot production was 0.1 mg/l BA. At this concentration, M. Col 22 produced 6.4 which was the highest whilst the local cultivar Ankrah produced 3.1 which was the lowest. Thus the later showed higher potential for multiple shoot production. The number of shoots produced was significantly different from the controls.

Statistical analysis did not show significant differences between the number of buds produced between the cultivars (Appendix 6b). Bud production in M. Col 22 was lowest on the control medium. However, Ankrah showed the highest bud formation on the same medium. On the whole, bud production on BA media was higher in M. Col 22 than in the African cultivars.

Plate 4. Single nodal cutting of Biafra showing multiple shoots. Note five shoots from one explant.

Table 6. Effect of BA on induction of multiple shoots from nodal cuttings of cassava cultivars.

BA mg/l	Mean number of shoots produced by cultivars[a]			
	M. Col 22	Ankrah	Bosomnsia	Biafra
0.00	1.0 a	1.0 a	1.0 a	1.0 a
0.02	-	1.5 ab	2.1 ab	1.6 a
0.10	6.4 c	3.1 c	3.8 c	2.0 a
1.00	3.9 b	2.8 bc	3.3 ab	4.2 b
2.00	5.0 bc	*	*	*

Note a: * no shoots were produced at the time records were taken.
b: Numbers in a column followed by different letters are significantly different (P≤0.05).

Table 7. Mean number of buds produced by cassava cultivars at 28 days of culture.

BA mg/l	Mean number of buds produced by cultivars[a]			
	M. Col. 22	Ankrah	Bosomnsia	Biafra
0.00	4.9 a	9.1 a	5.1 a	7.8 a
0.02	-	6.3 a	7.9 ab	5.7 a
0.10	16.0 b	7.1 a	11.4 c	5.9 a
1.00	13.0 b	6.0 a	7.8 ab	8.8 a
2.00	13.0 b	*	*	*

Note a: * no shoots were produced due to excessive callus formation.
b: Numbers in a column followed by different letters are significantly different (P≤0.05).

3.7 Effect of 2,4-D on induction of somatic embryos from greenhouse- grown cassava.

Young leaf lobes of greenhouse-grown four cassava cultivars were cultured on MS medium supplemented with 2,4-D in the dark (induction medium, step one). After 21 days, the cultures were transferred to MS medium amended with 0.1 mg/l BA (maturation medium, step two) to study their embryogenic competence.

Within five days of culture on the induction medium (step 1) the explants developed abundant callus. However, on a hormone-free medium (control), only swellings were observed at the cut surfaces of the leaf lobes explants. Examination of the calli seven days later showed that they contained embryogenic tissue indicated by numerous globular structures. The calli were compact and creamy . When the data for percentage number of leaf lobes that callused were analysed, significant differences were detected between the cultivars and also between the 2,4-D treatments (Appendix 7, Table 8). Callus formation was significantly higher in Santom than from the remaining cultivars. Callus formation was low in Ankrah but not significantly different from Biafra and Bosomnsia.

The data for calli formation were also analysed separately for Ankrah, Bosomnsia and Biafra (Appendix 8a,b and c) and in each cultivar, there were significant differences between the controls and 2,4-D concentrations used (Table 9). For Ankrah, calli formation on explants cultured on 4 and 8 mg/l 2,4-D differed significantly. At 16 mg/l 2,4-D, all explants of the tested cultivars developed calli and in Santom all leaf explants formed calli independent of 2,4-D concentration in the induction (step one) medium.

Table 8. Effect of 2,4-D on callus formation ofleaf lobes cultures of different cassava cultivars from the greenhouse.

Cultivar	Percentage calli formation ± SE
Ankrah	81.50 ± 5.95 a
Biafra	90.89 ± 4.18 ab
Bosomnsia	91.25 ± 2.95 ab
Santom	100.00 ± 0.00 c

Note a: Figures are means of four replicates.
b: Numbers in a column followed by different letters are significantly different (P≤0.05).

Table 9. Effect of 2,4-D on callus formation of leaf lobe cultures of greenhouse grown cassava.

2,4-D concentration (mg/l	Percentage calli formation			
	Ankrah	Bosomnsia	Biafra	Santom
0	0.0 a	0.0 a	0.0 a	0.0 a
4	55.0 ± 2.04 b	92.0 ± 4.83 b	87.6 ± 4.29 b	100.0 ± 0.00 b
8	89.5 ± 4.11 c	81.8 ± 10.57 b	85.0 ± 5.93 b	100.0 ± 0.00 b
16	100.0 ± 0.00 c	100.0± 0.00 b	100.0± 0.00 b	100.0 ± 0.00 b

Note a: Figures are means of four replicates.
b: Numbers in a column followed by different letters are significantly different (P≤0.05).

Within 14 days of culture on the maturation medium, 36% and 4% of Santom and AFI calli respectively developed adventitious roots (Plate 5). The roots were initially white but became chlorophyllous after 28 days.

Within 21 days of culture on the maturation medium embryogenic tissues were overgrown by calli and consequently none could grow into a mature somatic embryo. Foliose structures however frequently developed on some calli indicating that leaf lobes used as explants were morphologically competent. After 28 days calli became brownish and senesced.

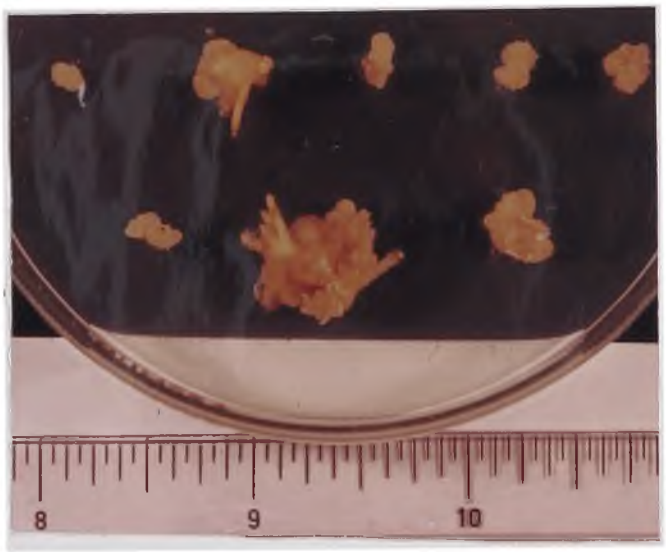

Plate 5. Leaf lobe cultures of Santom showing calli with roots (arrowed).

3.8 Induction of somatic embryos from leaf lobes, apical meristems and stipules from greenhouse plants.

In this experiment, young leaf lobes, apical meristems and stipules of greenhouse-grown cassava were cultured on 16 mg/l 2,4-D in order to identify other sources of explants for primary embryo production in cassava. Responses of explants following a 21-day incubation inthe dark on 16 mg/l 2,4-D are summarised in Table 10.

Within 15 days of culture on the induction medium, prolific callus formation occurred with globular structures appearing on apical meristem explants and on the adaxial surfaces of the leaf lobe explants of all the cultivars tested. Calli developed by apical meristem explants were friable, brownish and large in size compared to compact, creamy and small size calli developed by leaf lobe explants. Stipule explants developed swellings at the cut surfaces and only Santom and Afisiafi explants could form compact and creamy calli which were embryogenic in nature. Calli were however smaller in size compared to those obtained from explants of leaf lobes and apical meristems.

Statistical analysis showed that callus formation differed significantly ($P \leq 0.05$) between explants (Appendix 9). All explants from apical meristems developed calli independent of the cultivar (Table 10, Appendix 10a and b). In Bosomnsia and Santom, the differences between leaf lobe and meristem explants were significant whereas for Ankrah, Biafra and Afisiafi they were not. Stipule explants of Santom developed the highest percentage calli (74%) but this was not found to be significantly different from the leaf lobe explants of the same cultivar.

When embryogenic calli were transferred to the maturation medium (step two) growth of calli continued without embryo maturation. Only one callus out of the 12 cultures developed

cotyledonary stage embryo from apical meristem of Bosomnsia after 14 days of culture on the maturation medium.

There was no organ development in any of the cultures except in Santom, where one leaf lobe explant developed thick adventitious roots.

Table 10. Effect of 16 mg/l 2,4-D on callus formation from various explants after 21 days culture in the dark.

Explants	Percentage calli formation				
	Ankrah	Bosomnsia	Biafra	Santom	Afisiafi
Stipules	0 ± 0.0 a	52 ±3.7 a	0 ± 0.0 a	74 ± 8.1 a	10 ±10.0 a
Leaf lobes	94 ± 4.0 b	72 ±13.9 ab	100 ± 0.0 b	94 ± 4.0 ab	100 ± 0.0 b
Meristem	100 ± 0.0 b	100 ± 0.0 c	100 ± 0.0 b	100 ± 0.0 c	100 ± 0.0 b

Note: Letters in a column followed by different letters are significantly different ($P \leq 0.05$)
Figures are means of 5 replicates.

3.9 Induction of somatic embryos from leaf lobes of in vitro cassava plantlets.

Leaf lobes of all cultivars formed white creamy callus with globular structures at the adaxial surfaces within 15 days of culture on induction medium. Statistical analysis showed significant differences ($P \leq 0.05$) between the cultivars and 2,4-D concentrations (Appendix 11). However, analysis of individual data did not show significant differences between 2,4-D concentrations (Table 11). Leaf lobe explants cultured on 8 mg/l 2,4-D formed the highest percentage callus in all the tested cultivars but these were not significantly different from

explants cultured on 4 or 16 mg/l 2,4-D (Table 11). On a hormone-free medium (control), leaf lobes formed swollen structures which did not result in callus formation. Results were therefore not included in the statistical analysis shown in Appendix 11 and Table 11.

For maturation of embryos, it was essential that embryogenic calli with globular embryos were transferred to MS supplemented with 0.1 mg/l BA and incubated in the light. After 21 days of transfer to the maturation medium globular embryos of Santom, Bosomnsia and Biafra cultured on 8 or 16 mg/l 2,4-D matured into embryos. During maturation, globular embryos passed through cotyledonary and torpedo stages before they matured (Plate 6,7). Figure 1 shows percentage calli with matured somatic embryos after 21 days of incubation in light. Most of the globular embryos were overgrown by callus. In some cases callus developed foliose structures (Plate 8). The number of embryos formed were generally low. Also, most of the embryos isolated were malformed (Plate 8) except in Santom where all the embryos formed were normal (Plate 6).

Table 11. Effect of 2,4-D on callus production from leaf lobes explants in five cassava cultivars.

2,4-D (mg/l)[1]	Ankrah	Bosomnsia	Biafra	Santom	Afisiafi
4	90.75 ± 9.2 a	85.07 ± 5.6a	76.77 ± 10.1a	100.00 ± 0.0a	65.17 ± 3.7a
8	97.00 ± 3.0 a	100.00 ± 0.0a	92.75 ± 7.2 a	100.00 ± 0.0a	92.20 ± 4.8a
16	93. 75 ± 6.2 a	97.00 ± 3.0a	91.65 ± 8.3 a	100.00 ± 0.0a	89.40 ± 3.7a

Note 1: No callus formation at control treatments. Figures are means of four replicates. Figures in a column followed by the same letter are not significant.

The highest number of matured embryos was isolated from Santom (25%) when cultured on 16 mg/l 2,4-D (Figure 1). On this same treatment, only 9% embryogenic calli of Biafra matured into somatic embryos.

Bosomnsia, Biafra and Santom produced 18.6, 6.3 and 14.2% matured embryos respectively on a medium supplemented with 8 mg/l 2,4-D (Figure 1). Ankrah could form mature embryos. Mostly, single embryos were obtained with one embryo per callus.

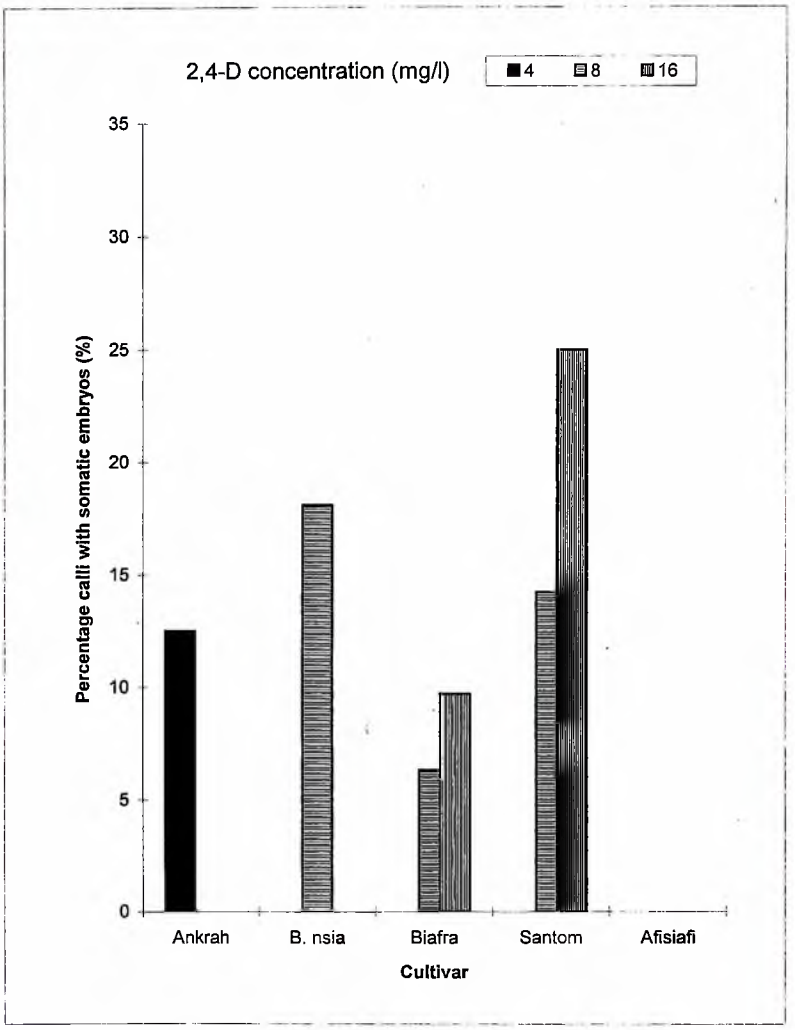

Figure 1. Percentage of callus which formed somatic embryos after 21 days of culture in maturation medium in light. B. nsia = Bosomnsia.

Root formation was also promoted within 14 days of transfer to the maturation medium but was dependent on the cultivar and the concentration of 2,4-D on the induction medium. Figure 2 shows root formation from calli 21 days after transfer to maturation medium. Generally, root induction decreased with increased concentration of 2,4-D in the induction medium. With the exception of Bosomnsia, 4 mg/l 2,4-D enhanced root development in all the cultivars. Ankrah developed the highest percentage of roots (63.5%) followed by Afisiafi (27.6%) and Bosomnsia (28.2%). In Bosomnsia, the highest percentage of roots was induced on 8 mg/l 2,4-D. Morphologically, the roots were thin and adventitious in nature (Plate 9) with two to four roots per callus.

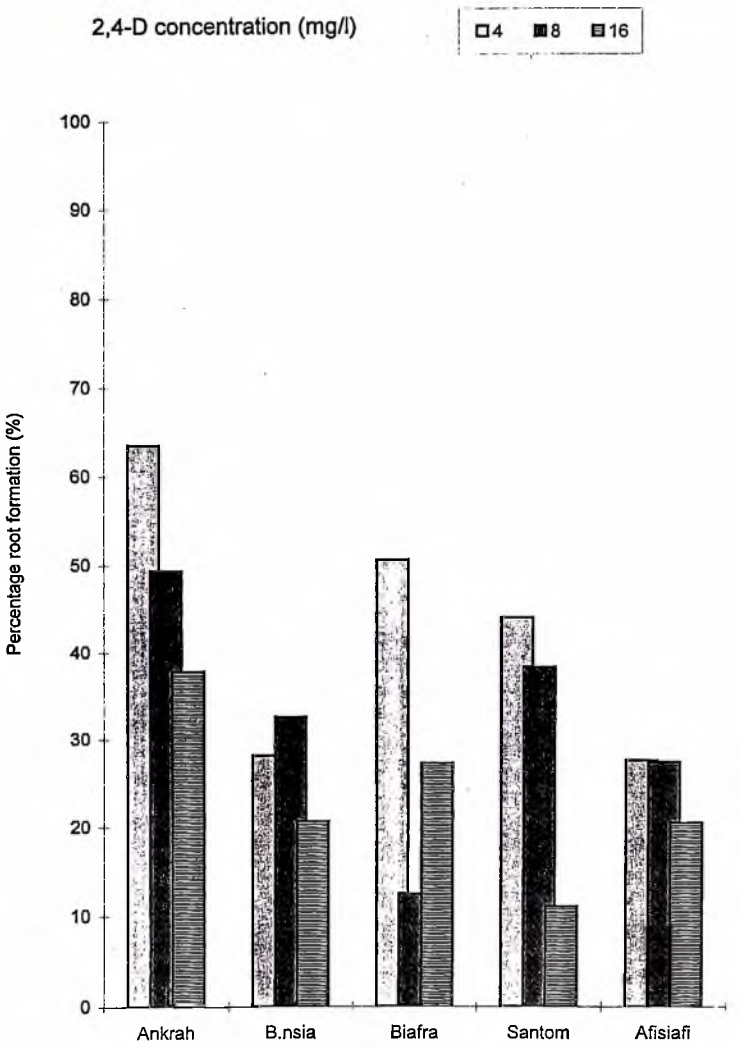

Figure 2. Percentage of callus which formed roots after 21 days on maturation medium.

Plate 6. Callus of Santom with matured somatic embryo (arrowed).

Plate 7. Callus of Santom with matured somatic embryo (a). Note foliose structures (b) at the base of embryo.

Plate 8. Callus of Bosomnsia with malformed somatic embryos (a) and foliose structures (b

3.9.1 Embryo germination

The use of somatic embryogenesis as an efficient system of propagation will be fully realised if somatic embryos regenerate into plants. Germination of mature somatic embryos were studied using Adira 4, Gading, Line 11 and TMS 90853 .

3.9.2 The effect of different desiccation methods on embryo germination.

Different methods of desiccation were used to stimulate germination of NAA-induced somatic embryos of Adira 4. As initial experiments have indicated that sugar solutions above 20 g/l have adverse effect on germination of somatic embryos, optimal concentrations only were recorded in Table 12. After 21days in culture non-desiccated embryos did not germinate into complete plants shoots and roots. Only 17% and 3% of the cultured embryos developed into either roots only or shoots only respectively.

Embryos desiccated using the petri dish method lost 40% of their fresh weight as moisture while those desiccated using glycerol lost 55%. In these two treatments, 88% of cultured embryos germinated into complete plants with roots and shoots. Only 4% of the embryos developed roots only.

In the desiccation method using sugar solutions and mannitol, the moisture loss could not be determined exactly. At lower concentrations of sugars, the embryos continue to grow, indicated by an increase in fresh weight. When higher concentrations of sugars were used embryo growth ceased and the cotyledons became yellowish. The highest percentages of shoot formation (with or without roots) were found in the treatments without or with relatively lower sugar concentrations (10 or 20g/l). In these optimal treatments, shoot formation was

higher than in non-desiccated embryos (control) but lower than petri dish desiccated embryos. The highest shoot formation (68%) among the optimal treatments occurred on liquid MS medium with 20 g/l sucrose with further one week desiccation. Most of the shoots formed were malformed and did not possess roots.

Table 12. Effect of different desiccation methods on somatic embryo germination. (Optimal concentrations only indicated).

Method of desiccation	No.of SEs cultures	Roots only[1] (%)	Shoots only (%)	Germination (%)
without desiccation (control)	30	5 (17)	1 (3)	0 (0)
petri dish	17	0 (0)	2 (12)	15 (88)
desiccator + glycerol	25	1 (4)	1 (4)	22 (88)
Solid MS + sucrose (20 mg/l)	78	2 (3)	27 (35)	5 (6)
liquidMS + sucrose (20mg/l) - desiccation	19	0 (0)	11 (55)	0 (0)
liquidMS + sucrose (20mg/l)+ desiccation	19	0 (0)	13 (68)	3 (16)
liquidMS - desiccation	15	0 (0)	6 (40)	1 (7)
liquid MS + desiccation	19	1 (5)	12 (63)	1 (5)
water + sucrose (30 mg/l) - desiccation	20	0 (0)	5 (25)	1 (5)
water + desiccation	19	1 (5)	1 (5)	17 (89)

Note 1: Figures in bracket indicate percentages. Ses = somatic embryos. Embryos were cultured for 28 days on MS supplemented with 1 mg/l BA and incubated in the light for germination.

3.9.3 Effect of ABA pre-treatment on embryo germination.

The effect of abscissic acid (ABA) on the germination of NAA-induced somatic embryos of Adira 4 was studied. Mature NAA-induced somatic embryos transferred to different concentrations of ABA (0-40 mg/l) were either cultured directly or desiccated before culturing on MS medium with 0.0-4.0 mg/l BA. After 7 days the cotyledons of the embryo grew very large with deep green coloration. With increased concentration of ABA (40 mg/l) there was no growth and the embryos turned yellowish. The results are as indicated in Table 13a.

Embryos pre-treated with ABA but without desiccation did not stimulate germination. Only 13% of the embryos pre-treated with 1 mg/l ABA germinated into shoots (without roots) when cultured on 0.1 mg/l BA.

Embryos which had been pre-treated with ABA and desiccated showed higher organ development than non-desiccated pre-treated embryos. With desiccation, germination and organ (root) development generally were higher in the control than in the ABA treated cultures (Table 13b). However, shoot development was comparatively higher when embryos were pre-treated with 1 mg/l ABA (Table 13b)

High ABA concentration of 40 mg/l inhibited both shoot or root formation. Only 7% showed shoot development on 0.5 and 1.0 mg/l BA compared with 13 and 28% in the control.

Table 13a. Effect of ABA pre-treatment (without desiccation) on embryo germination.

ABA mg/l	BA mg/l	No. of SEs cultured.	Roots only(%)	Shoots only (%)	Germination (%)
0.0	0.0	16	0	1 (6)	0
	0.1	15	0	2 (13)	0
	0.5	23	0	5 (22)	0
	1.0.	13	0	0 (0)	0
	4.0	22	0	6 (27)	0
1.0	0.0	15	0	0 (0)	0
	0.1	16	0	2 (13)	0
	0.5	-	-	-	-
	1.0.	16	0	0 (0)	0
	4.0	15	0	1 (7)	0
40	0.0	15	0	0 (0)	0
	0.1	15	0	0 (0)	0
	0.5	16	0	0 (0)	0
	1.0.	15	0	0 (0)	0
	4.0	15	0	0 (0)	0

Note: Figures in parenthesis indicate percentages.

Embryos were pre-treated with different concentrations of ABA for seven days and cultured for germination on 0-4 mg/l BA.

Ses = somatic embryos.

Table 13b. Effect of ABA pre-treatment (with desiccation) on embryo germination.

ABA mg/l	BA mg/l	No. of SEs cultured	Roots only (%)	Shoots only (%)	Germination (%)
0.0	0.0	15	3 (20)	0 (0)	3 (20)
	0.1	16	5 (31)	3 (19)	5 (31)
	0.5	15	5 (33)	2 (13)	6 (40)
	1.0	18	2 (11)	5 (28)	9 (50)
	4.0	15	4 (27)	1 (7)	5 (33)
1.0	0.0	15	0 (0)	0 (0)	0 (0)
	0.1	16	0 (0)	3 (19)	0 (0)
	0.5	15	7 (7)	10 (67)	1 (7)
	1.0	15	1 (7)	2 (13)	3 (20)
	4.0	15	0 (0)	7 (47)	0 (0)
40	0.0	15	0 (0)	0 (0)	0 (0)
	0.1	15	0 (0)	0 (0)	0 (0)
	0.5	15	0 (0)	1 (7)	0 (0)
	1.0	15	0 (0)	1 (7)	0 (0)
	4.0	15	0 (0)	0 (0)	0 (0)

Note: Figures in bracket indicate percentages
Embryos were pre-treated with ABA for seven days and desiccated for a further one week before culture for germination.
Ses = Somatic embryos.

3.9.4 Influence of auxins and cytokinins on embryo germination

Table 14 shows the effect of auxins and cytokinins on somatic embryo germination. The embryos used in this experiment lost 20% moisture from their fresh weight. None of the embryos cultured on hormone-free medium could form shoots and only 5% formed roots.

The auxins IBA and NAA stimulated root formation with NAA being more effective. More than 55% of the embryos formed roots at all the NAA levels tested. At 4.0 mg/l NAA 82% of the embryos formed roots. Thirteen (13%) and 18% of the embryos developed into whole plants when cultured on 0.1 and 4.0 mg/l NAA. The roots were numerous and thin.

All the cytokinins tested stimulated higher shoot formation compared to the auxins. The highest percentage of shoot development was observed on the BA supplemented media. The optimal BA concentration was 1.0 mg/l. At this concentration, 67% of the cultured embryos developed shoots without roots and 10% germinated into plants with roots. The morphology of the roots and shoots of plants was dependent on the concentration of BA in the medium. Embryos cultured on 1.0 or 4.0 mg/l BA formed thick roots. The shoots stout (had short internodes) with many branched (Plate 10).

With kinetin, germination increased with higher concentrations. On a medium supplemented with 4 mg/l kinetin, 36% of the embryos germinated and 18% formed only shoots. Morphologically, the roots were all thin and normal while the shoots had more than one apical meristem each (Plate 9).

Thiadizuron (TDZ) gave the lowest percentage germination; at all the concentrations tested not more than 12% germinated into complete plants.

Table 14. Influence of different growth regulators on shoot and root formation of Adira 4 somatic embryos.

Growth regulator	concentration (mg/l)	No of SEs cultured	Roots only (%)	Shoots only (%)	Germination (%)
Control	0.0	20	1 (5)	0 (0)	0 (0)
Thiadiazuron (TDZ)	0.1	25	2 (8)	2 (8)	3 (12)
	1.0	24	1 (4)	1 (4)	2 (8)
	4.0	23	4 (17)	3 (13)	1 (4)
Kinetin	0.5	24	1 (4)	0 (0)	2 (8)
	1.0	20	0 (0)	1 (5)	1 (5)
	4.0	22	0 (0)	4 (18)	8 (36)
Benzyladenine (BA)	0.5	15	0 (0)	6 (40)	2 (13)
	1.0	10	1 (10)	14 (67)	3 (10)
	4.0	22	0 (0)	7 (32)	2 (13)
Napthalene acetic acid(NAA)	0.1	15	9 (60)	0 (0)	2 (13)
	1.0	20	11 (55)	0 (0)	0 (0)
	4.0	11	7 (64)	0 (0)	2 (18)
Indole butyric acid (IBA)	1.0	20	3 (15)	0 (0)	0 (0)
	5.0	10	3 (30)	0 (0)	0 (0)
	10.0	15	0 (0)	0 (0)	0 (0)

Note 1: Figures in bracket indicate percentages.
SEs = Somatic embryos.

Plate 9. Germinated somatic embryos of Adira 4 on MS medium with 4 mg/l kinetin. Note the slender stems and thin roots.

Plate 10. Germinated somatic embryos of Adira 4 on MS medium with 1 mg/l BA. Note thick roots and multiple shoots (x 2.5).

3.9.5 Relation between moisture loss and BA on embryo germination.

Figure 3 shows the relationship between moisture loss and BA on embryo germination in Gading (A,B,C) and Adira 4 (D,E,F). In both cultivars, embryo germination was dependent on moisture loss and the concentration of BA in the cultured medium (Figure 3). Embryos with a moisture loss of 20% did not germinate into complete plants when cultured on a hormone-free medium (Figure 3A,D). Three percent of the Gading somatic embryos developed only roots while 11% developed only shoots (Figure 3A). For Adira 4 somatic embryos, 17% developed roots (Figure 3D). Somatic embryos of Gading with a moisture loss of 20% gave the highest percentage (31%) shoot development on a medium with 1 mg/l BA (Figure 3A). For Adira 4, the highest was on a medium with 4 mg/l BA (Figure 3D).

In both cultivars, embryos with a moisture loss of 40% gave a higher percentage of complete plant germination (Figure 3B,E). In Gading, formation of roots was comparably higher at all the BA treatments than when embryos lost 20% moisture. The highest germination of somatic embryos 72% and 82% in both Gading and Adira 4 respectively occurred on 0.1 mg/l BA (Figure 3B,E).

In Gading, shoot development at 75% moisture loss was comparatively higher on all BA treated media than when embryos had lost 20% moisture. In Adira 4, complete plant germination from embryos with moisture loss of 75% was not comparatively better than when embryos had lost 20% of their moisture (Figure 3F and 3D).

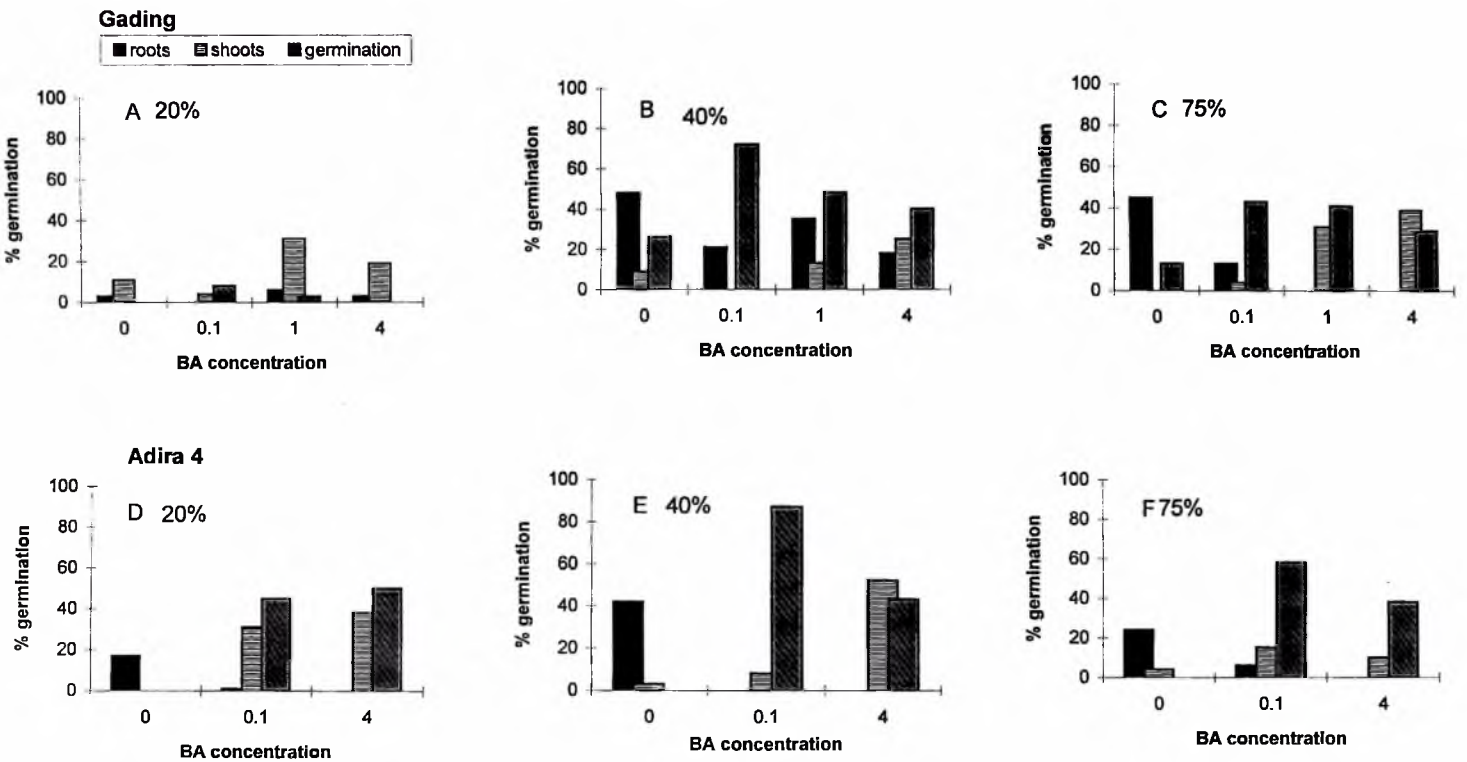

Figure 3. Effect of moisture loss and different BA concentrations on germination of somatic embryos of Gading (A,B,C) and Adira 4 (D,E,F)

3.9.6 Effect of prewashing of somatic embryos on germination.

In the earlier experiments, somatic embryo germination into complete plants was below 90%. This experiment was conducted with the aim of improving the frequency of somatic embryo germination. Embryos of Gading were therefore transferred to sterile distilled water for periods of 1, 5, 10 or 14 days and then cultured directly or desiccated for a further one week before culture. The results obtained are presented in (Table 15a).

Embryos washed for one or five days without further desiccation did not enhance germination into whole plants nor development of roots only. Shoot formation only was stimulated at day 10 and 14. The highest shoot formation (29%) occurred at 14 days of washing on a medium supplemented with 1.0 mg/l BA.

Root and shoot formation were comparatively higher when washed embryos were desiccated. On hormone-free media, root formation increased with period of washing. The highest percentage (67%) of root development occurred in the treatment that involved 10 days of washing. Root formation on media supplemented with various concentrations of BA, in contrast, decreased with the days of washing.

Germination of embryos into whole plants did not follow any obvious trend but was higher when embryos were washed for 1 day (Table 15b). At one or five days of washing, the optimal BA concentration needed for whole plant development was 0.1 mg/l BA whereas at 10 and 14 days, it was 1.0 mg/l. The highest percentage germination (47%) occurred at day 1 on a medium supplemented with 0.1 mg/l BA and the lowest at day 14.

Table 15a. Effect of prewashing and BA concentrations on germination of Gading somatic embryos (without desiccation).

Period of Washing (days)	BA (mg/l)	No. of SEs cultured	Roots only (%)	Shoots only (%)	Germination (%)
1	0.0	20	0 (0)	0 (0)	0 (0)
	0.1	26	0 (0)	3 (12)	1 (4)
	1.0	26	0 (0)	2 (8)	0 (0)
	4.0	30	0 (0)	2 (7)	0 (0)
5	0.0	20	0 (0)	0 (0)	0 (0)
	0.1	19	0 (0)	0 (0)	0 (0)
	10	20	1 (5)	1 (5)	0 (0)
	4.0	21	0 (0)	0 (0)	0 (0)
10	0.0	31	0 (0)	1 (3)	0 (0)
	0.1	28	0 (0)	2 (7)	6 (21)
	1.0	31	1 (3)	6 (19)	0 (0)
	4.0	28	0 (0)	2 (7)	1 (4)
14	0.0	22	0 (0)	0 (0)	0 (0)
	0.1	26	2 (8)	1 (5)	2 (8)
	1.0	24	0 (0)	7 (29)	1 (4)
	4.0	24	0 (0)	3 (13)	1 (4)

Note: Figures in brackets indicate percentages.
SEs = somatic embryos.

Table 15b. Effect of prewashing and BA concentrations on germination of desiccated somatic embryos of Gading.

Period of Washing (days)	BA (mg/l)	No. of SEs cultured	Roots only(%)	Shoots only (%)	Germination (%)
1	0.0	36	0 (0)	0 (0)	0 (0)
	0.1	36	11 (31)	1 (3)	17 (47)
	1.0	48	14 (29)	9 (19)	18 (38)
	4.0	28	4 (14)	5 (18)	12 (43)
5	0.0	27	6 (22)	0 (0)	0 (0)
	0.1	27	8 (30)	0 (0)	4 (15)
	1.0	36	11 (31)	7 (19)	2 (6)
	4.0	28	0 (0)	3 (10)	0 (0)
10	0.0	30	20 (67)	3 (10)	0 (0)
	0.1	31	5 (16)	6 (19)	6 (19)
	1.0	23	4 (17)	3 (13)	9 (39)
	4.0	39	4 (10)	15 (38)	4 (10)
14	0.0	31	20 (65)	6 (19)	0 (0)
	0.1	26	5 (19)	2 (6)	2 (8)
	1.0	24	2 (8)	8 (33)	1 (4)
	4.0	32	0 (0)	14 (44)	1 (4)

Note: Figures in bracket indicate percentages
SEs = somatic embryos

3.9.7 Influence of light/dark regimes on plantlet development from somatic embryos.

Desiccated NAA-induced embryos of TMS 90853 (Figure 4A), Gading (Figure 4B) and Line 11 (Figure 4C) germinated at higher frequencies than 2,4-D-induced embryos of the same cultivars (Figure 4 A,B and C). This was observed independent of the light/dark conditions and the concentration of BA used in the germination medium. For Adira 4 (Figure 4D) only NAA-induced embryos cultured on 0 and 0.1 mg/l BA germinated at a higher frequency than 2,4-D-induced embryos. On a medium with 1 and 4 mg/l BA, there were no differences (Figure 4D).

In all four genotypes, NAA-induced embryos cultured in the dark showed the highest percentage germination with roots in a medium supplemented with 0 or 0.1 mg/l BA and this was higher than in the light. Under dark conditions, root development occurred within 4 days followed by the elongation and the curvature of the hypocotyl and finally the opening of the hypocotylar hook after 10 days. The stems of the shoots were slender with long internodes. Embryos cultured on control or 0.1 mg/l BA formed single shoots mostly (Plate 11) whereas those cultured on 1 mg/l BA formed mostly two or more shoots (Plate 12). The shoots were etiolated but de-etiolation occurred seven days after transfer to the light.

Germination of 2,4-D-induced embryos was low in TMS 90853 (Figure 4A), Gading (Figure 4B) and Line 11 (Figure 4C) when cultured under both light and dark conditions. In Adira 4, 40% to 50% of the embryos cultured in the light and on a medium with 1 or 4 mg/l BA germinated. This was higher than comparable treatments in the dark.

Desiccation of 2,4-D-induced embryos had a clear effect on root development in all genotypes. The type of roots appeared four to seven days after culture and they were

dependent on the light/dark conditions; predominantly adventitious in light and taproots in the dark. Also there was comparatively higher root formation in 2,4-D-induced than NAA-induced somatic embryos incubated in the dark than light in all cultivars (Figure 5). This observation was more profound in Line 11 embryos.

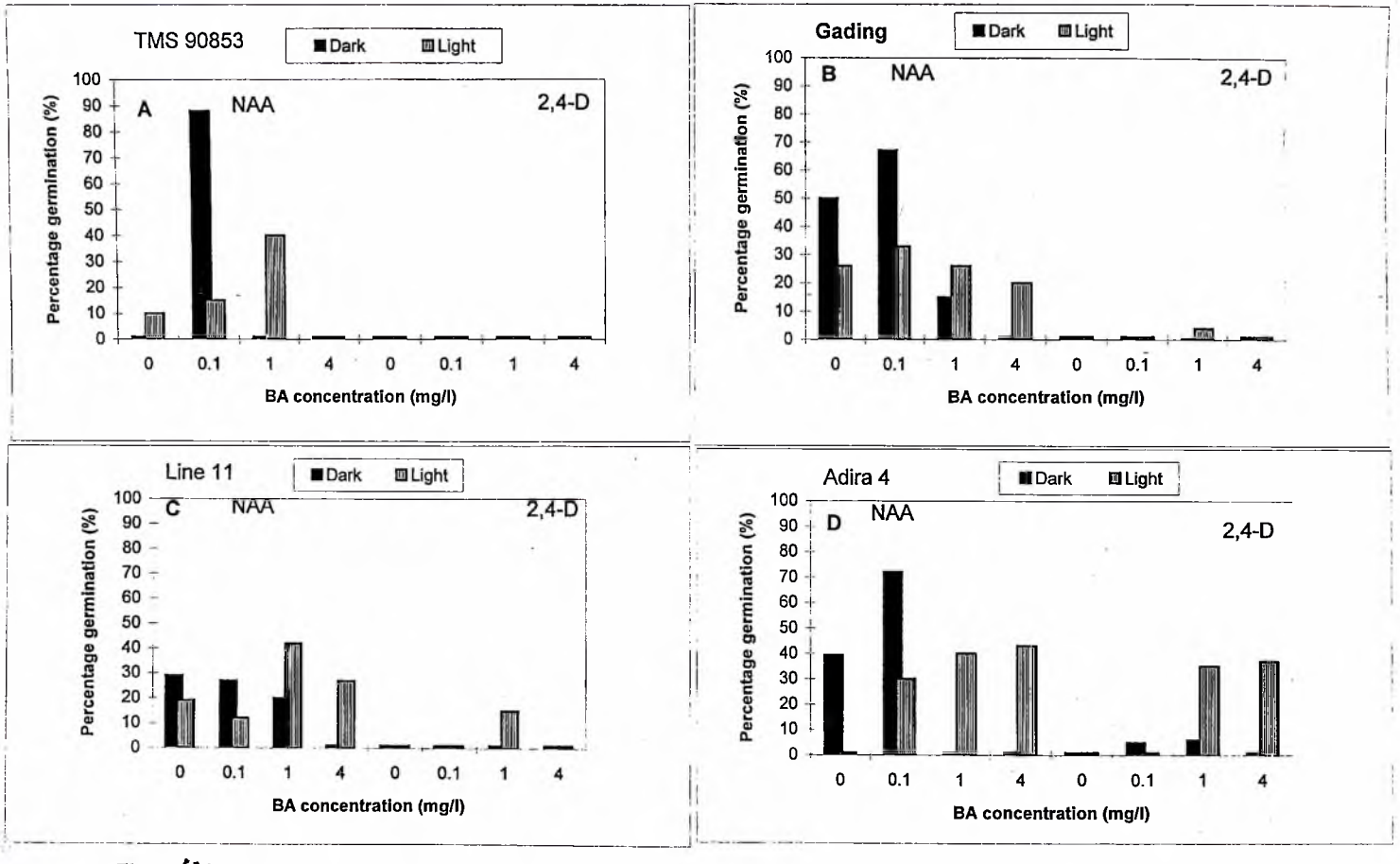

Figure 5: Influence of light/dark conditions on germination of NAA-and 2,4-D-induced somatic embryos of TMS 90853, Gading, Line 11 and Adira 4.

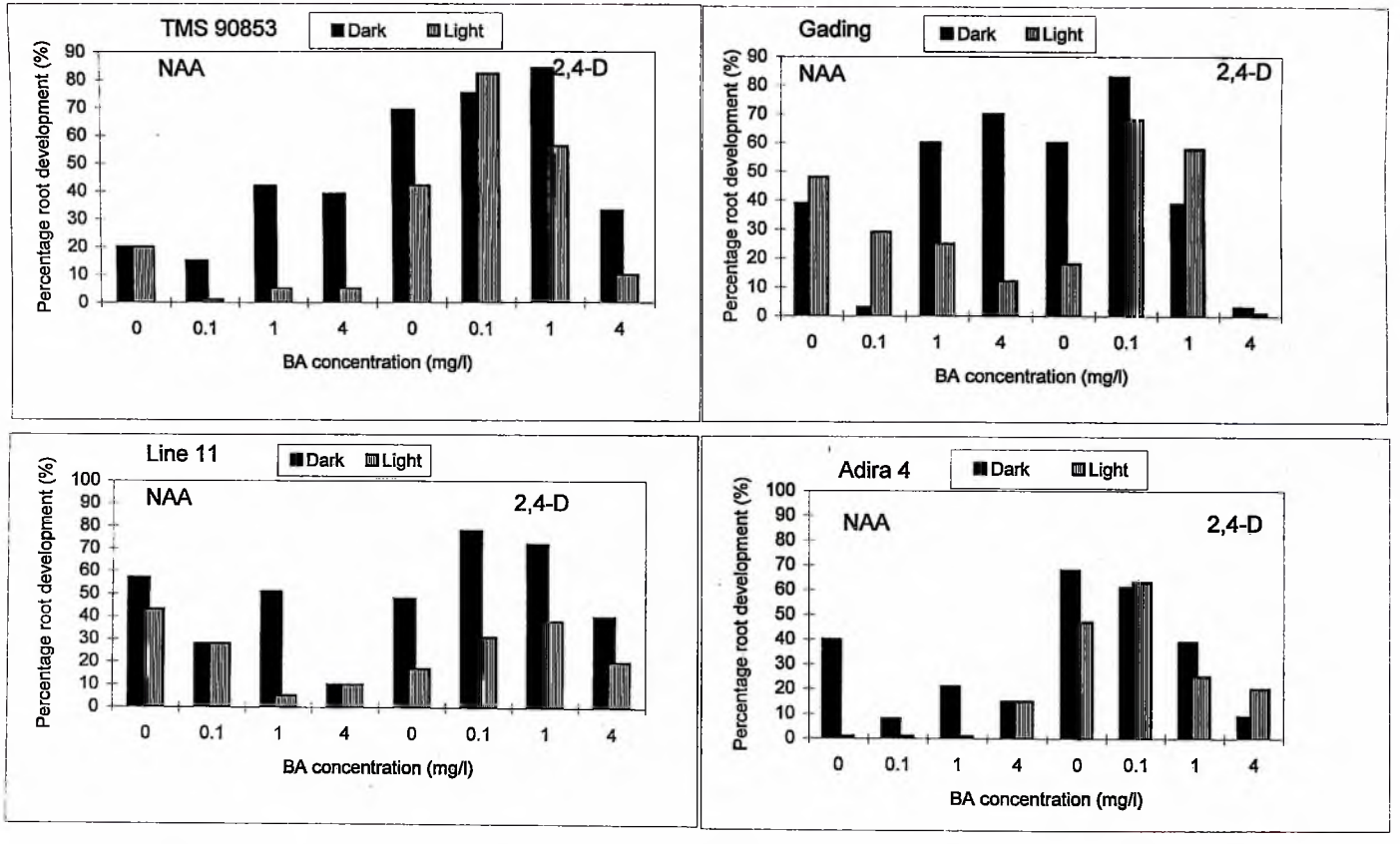

Figure 5. Influence of light/dark conditions on root formation of NAA- and 2,4-D - induced somatic embryos of TMS 90853, Gading, Line 11 and Adira 4

Plate 11. Germinated somatic embryos of Adira 4 cultured on 0.1mg/l BA in the dark. Note single shoots emerging from embryos (× 1.5).

Plate 12. Germinated somatic embryos of Adira 4 cultured on 0.1mg/l BA in the dark. Note multiple shoots emerging from embryos (×1).

3.9.8 Influence of different light intensities on embryo germination.

Since germination of some seeds are sensitive to light intensities, it was suggested that germination of somatic embryos might also be influenced by light intensities. To test this assumption, NAA-induced embryos of Adira 4 were desiccated for 7 days and cultured on media supplemented with 0-4 mg/l BA. The cultures were incubated at different light intensities as indicated in Figure 6.

The optimum light intensity for whole plant germination was 64 lux and complete plant formation was higher at all the BA levels investigated. At 64 lux, the optimal BA concentration for germination was 1.0 mg/l and percentage germination was 73%. Morphologically, the shoots were all etiolated. However, they became green 7 days after transfer to growth room light conditions.

The formation of shoot only was generally low at all the light intensities compared to either the formation of root only or the formation of root with shoot.

When compared to the control (0 lux), root formation at all the light intensities was high when the embryos were cultured on a hormone-free medium. At 4 mg/l BA, no root only development was observed at all the light intensities except 64 lux. The highest percentage (65%) of root only formation occurred at a light intensity of 6410 lux when embryos were cultured on a hormone- free medium.

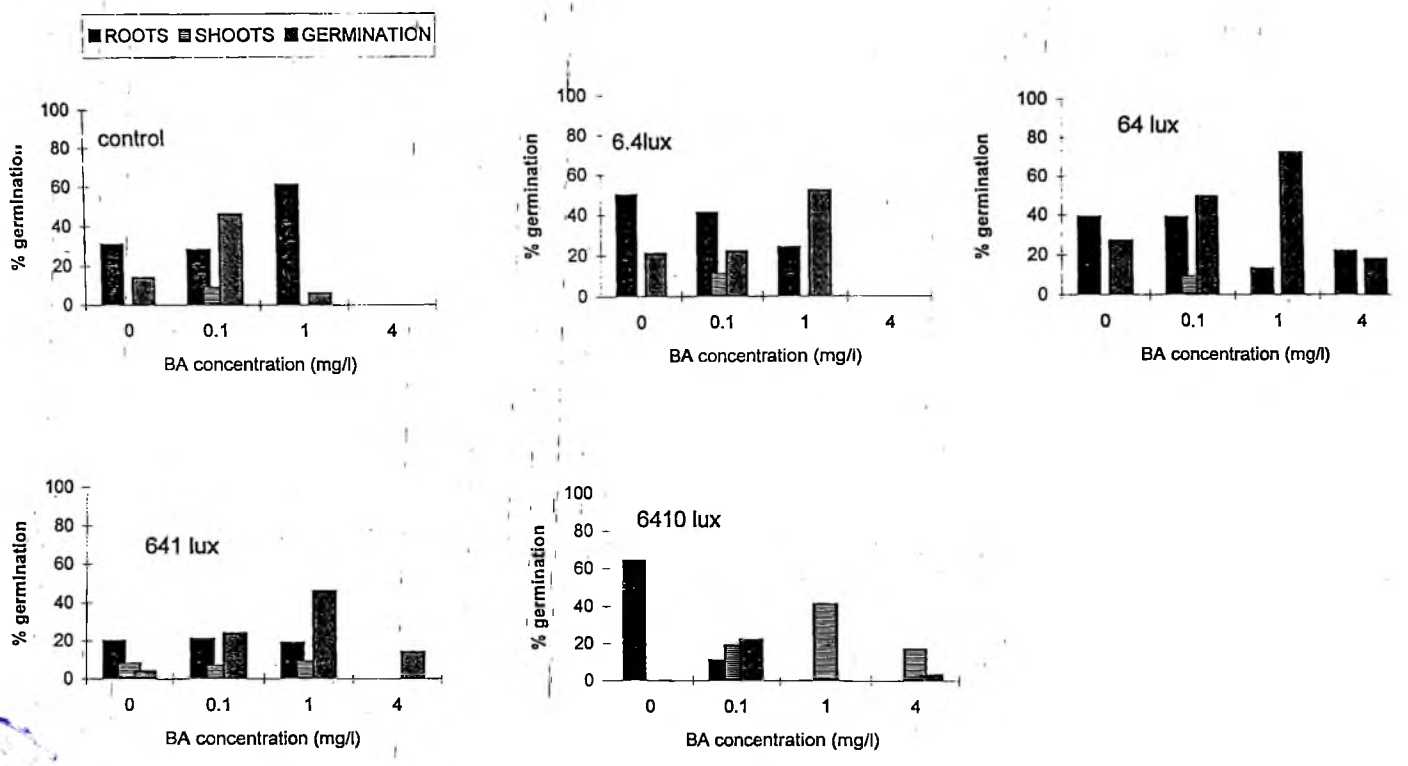

Figure 6. Effect of different lightintensities on root, shoot and plant germination in Adira 4 somatic embryos.

3.9.9 Effect of different light regimes on somatic embryo germination.

Four grams of matured somatic embryos of Adira 4 were transferred to sterile petri dishes and incubated in white, red, blue, far red and dark cabinets for 7 days and the results obtained are as shown in Figure 7.

In white and blue light there was no germination of embryos into roots, shoots nor complete plants on a hormone free - medium. Only seven (7) percent of the embryos incubated under blue light developed roots. In the hormone-supplemented media, germination of embryos into complete plants was below 20% under both light regimes. The optimal BA concentration for germination was 1 mg/l under both light regimes.

Cultures incubated under red, far red and dark cabinets showed higher germination compared to blue and white light. The highest percentage germination (40%) of embryos into complete plants was observed under dark conditions on 1.0 mg/l BA medium. Under far red light conditions, however, the highest germination (37%) was observed when embryos were cultured on 0.1 mg/l BA amended medium. The development of shoots only was higher in far red and dark regimes than red light.

Morphologically, embryos incubated under far red and dark regimes produced plantlets which were normal, single and etiolated.

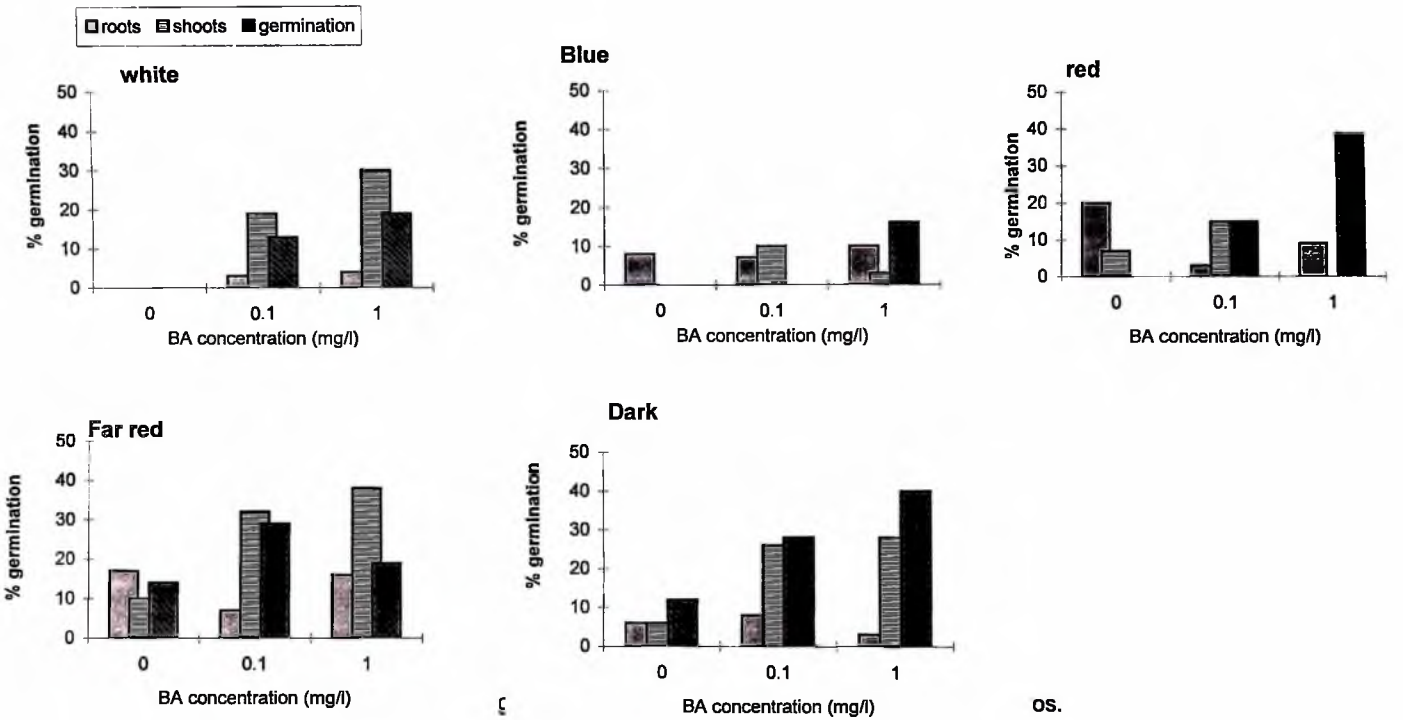

Figure 7. Effect of different light regimes on root, shoot and plant germination of Adira 4 somatic embryos.

3.9.10 Germination of embryos desiccated in complete darkness or light.

Mature NAA-induced somatic embryos of Adira 4 desiccated in complete darkness (CD) for 7 days were cultured for germination and incubated in complete darkness and in the light. The results are presented in the Table 16.

Desiccation of embryos in the light (L) followed by culture in complete darkness (CD) enhanced formation of roots as well as whole plant germination. Whole plant germination was high (46%) on a medium with 0.1 mg/l BA whereas the highest roots only formation (61%) occurred on a medium with 1.0 mg/l BA. There was no shoot only formation except on a medium with 0.1 mg/l BA.

When embryos were desiccated in complete darkness (CD) followed by culture in the light (L) whole plant germination decreased (Table 16). Only 13% of the embryos germinated on a medium with 1.0 mg/l BA.

Desiccation of embryos in complete darkness followed by culture in complete darkness had a negative effect on shoots only formation. In this treatment the highest number of shoots only was 5% and was obtained when embryos were cultured on a hormone-free medium. When embryos were cultured on BA-supplemented medium only 3% of the embryos formed shoot on a 1 mg/l BA-amended medium. Also, the highest whole plant germination was 20% and this occurred on 0.1 mg/l BA amended medium. On a medium with 4.0 mg/l BA there was neither root nor shoot development. The highest percentage (28%) of root only formation occurred when embryos were cultured on a medium amended with 1.0 mg/l BA.

Table 16. Effect of desiccation in complete darkness and light on germination of Adder 4 somatic embryos.

BA (mg/l)	Desiccation treatment	Incubation treatment	No.of SEs	Roots only (%)	Shoots only (%)	Germination (%)
0.0	CD	CD	20	3 (15)	1 (5)	0 (0)
0.1	CD	CD	30	5 (17)	0 (0)	6 (20)
1.0	CD	CD	29	8 (28)	1 (3)	3 (10)
4.0	CD	CD	31	2 (6)	0 (0)	0 (0)
0.0	CD	L	28	0 (0)	1 (4)	0 (0)
0.1	CD	L	27	4 (15)	3 (11)	0 (0)
1.0	CD	L	32	6 (19)	3 (9)	4 (13)
4.0	CD	L	32	1 (3)	0 (0)	0 (0)
0.0	L	CD	35	11 (33)	0 (0)	5 (14)
0.1	L	CD	35	10 (28)	3 (9)	16 (46)
1.0	L	CD	36	22 (61)	0 (0)	2 (6)
4.0	L	CD	31	0 (0)	0 (0)	0 (0)

Note: Figures in parenthesis indicate percentages.
CD = complete darkness; L = light

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1 Germplasm collection.

The germplasm survey indicated that farmers in the Dodowa, Kwabenya, Kasoa and North Taifa cultivate cassava cultivars principally based on early bulking, yield and the use to which they are put. Consequently, a wide range of cassava with tolerance to ACMV are cultivated. In this study, over 12 descriptive names of cassava cultivars with different morphological traits were identified. This result agrees with findings by Annor-Frimpong (1994) who reported that farmers in the Central and Western regions of Ghana cultivate different cultivars. Iweke *et al.*, (1994) collected over 1200 descriptive names of cassava from East and West African farmers in their Collaborative Study of Cassava in Africa (COSCA) project suggesting that farmers in various locations of Africa cultivate more than one cassava cultivar. They however pointed out that such descriptive names cannot be used for botanical classification because the names vary from village to village. In this study, it was observed that the cultivar Bosomnsia had different names associated with the villages of origin. A more detailed study using molecular markers such as Restriction Fragment Length Polymorphism (RFLP) or Random Amplified Polymorphic DNA (RAPD) may be used to ascertain their relationships and botanical classification.

The results obtained in this study show that ACMV and brown leaf spot disease were the most common disease of the cultivars collected. The results further show that ACMV disease affects all the cultivars popularly cultivated by farmers in Ghana. Hahn *et al.* (1989) has reported that ACMV is the most widespread and economically important disease in West

Africa and can cause up to 95% yield loss in most susceptible cultivars. It has also been reported that the disease is caused by three related geminiviruses which occur in West Africa, East and Southern Africa and India (Hahn *et al.*, 1989). Its spread may be due to lack of resistant cultivars. Intensive breeding programmes should be initiated by research institutions and the universities to produce resistant cultivars for farmers as ACMV has serious effect on yield. Secondly, a wider survey covering all the cassava growing regions should be conducted in order to identify landraces which are tolerant to the disease and can serve as bases for the improvement of the crop.

4.2 Meristem culture

In this study, Medium B with 0.5 or 0.15 mg/l BA and a lower concentration NAA (0.02 mg/l) gave much higher shoot formation except in Santom than Medium A with the higher concentration of NAA. (0.1 mg/l). There was 100% shoot formation in Bosomnsia when Medium B was used but most of them had no roots. The results obtained in Medium B was in agreement with that of Adejara and Coutts (1981) who obtained 100% shoot formation in all their tested cassava cultivars cultured on 0.02-0.2 mg/l BA in combination with 0.02 mg/l NAA. Work done at CIAT with over 300 cultivars has shown that shoots do not form roots on a medium amended with 0.01-0.1 mg/l BA and 0.02-0.04 mg/l NAA and 0.04 mg/l GA_3. (Raemakers *et al.,* 1997). A second step is therefore necessary for rooting of the shoots thus making the procedure more labour intensive. This is similar to observation in this study whereby on Medium B high percentage of the shoot formed had no roots.

The high concentration of NAA in Medium A caused high callus formation. According to Raemakers *et al.*, (1997) NAA at concentrations higher than 0.02 mg/l decreased shoot

formation but increased callus formation. Excessive callus formation during shoot formation can cause somaclonal variation. Therefore, this medium cannot be used for clonal multiplication. However, for breeding purposes somaclonal variation may result in the production of desirable mutants.

Visual observation of the plantlets produced from the meristem culture showed conspicuous symptoms of ACMV on some leaves of Santom cultures despite the fact that cultures were derived from meristem explants. Meristem culture technology has been used to free vegetatively propagated crops from viruses (Hartmann *et al.,* 1990). Kartha (1981) reported that potato, sweet potato, yam and cassava have been freed of viral pathogens by meristem culture. This is in contrast with the observations made in Santom in this study. However, the observation made on Santom is similar to the report of Mellor and Stace-Smith (1977) that potato leaf roll virus (PLRV) and virus Y (PVY) could not be eliminated by meristem culture alone. Virus elimination by meristem culture depends on the size of the meristem cultured and host -virus combinations (concentration of virus) (Kartha, 1981). High concentration of the virus in the stake of Santom could possibly explain its presence in the *in vitro* plantlets. It has been suggested that in cases where viruses are difficult to eliminate by meristem culture alone, a combination of thermotherapy and meristem culture has proved beneficial. Kartha and Gamborg (1975) reported that mosaic disease of cassava and seed-borne mosaic virus in pea were eliminated in 60% and 90% frequency respectively by culturing meristems not exceeding 0.4 mm in length whereas 100% mosaic-disease-free plants were regenerated from cassava meristems grown at 35°C for 30 days prior to meristem excision. Kaiser and Teemba (1979) also reported that donor explants of F 29 cassava cultivars treated to high temperatures (35-40 °C) freed them from brown streak virus in East Africa. In Canada,

meristem culture was combined with heat therapy to collect a virus-free germplasm of potato (Wright, 1988).

Enzyme-linked immunosorbent assay (ELISA) could be an effective method to detect the presence or absence of the ACMV virus in the remaining cultivars.

4.3 Multiple shoot induction

Both *in vivo* and *in vitro* cassava exhibit strong apical dominance. Breaking of this apical dominance results in the production of multiple shoots. In M.Col 22 multiple shoots were produced from apical meristems as well as nodal cuttings and the number of shoots produced depended on the BA concentration in the culture medium. In Ankrah, Biafra and Bosomnsia multiple shoots were produced from single nodal cuttings alone. The observation in this study is similar to the work of Roca (1984) and Smith *et al.*, (1986). Roca (1984) produced multiple shoots from nodal cuttings of cassava on a medium amended with only 1.0 mg/l BA. According to his report about 20 shoots and nodal cuttings were harvested every three weeks from the rosette multiple shoot culture but the number decreased with time. For rooting the harvested nodes were cultured in a liquid medium supplemented with 0.05 mg/l BA, 0.04 mg/l GA_3 and 0.02 mg/l NAA Smith *et al.*, (1982) also produced multiple shoots from single bud cuttings and between 2-6 apical shoots and 4-19 buds were produced depending on the cultivar. The results of this study and the above observations shows that multiple shoot production is dependent on BA concentration in the medium as well as the cassava cultivar.

4.4 Primary somatic embryogenesis

Leaf lobe explants from from greenhouse and *in vitro* plantlets produced embryogenic calli on 2,4-D-amended step one-induction medium. However, only embryogenic calli from *in vitro* plantlets produced matured somatic embryos on step two maturation medium. Embryogenic tissues obtained from *in vitro* leaf lobes matured into somatic embryos on a second-step maturation medium while those from greenhouse grown explants did not, indicating that the source of donor explant plays a great role in embryo production.

The failure of the greenhouse derived embryogenic calli to produce somatic embryos may have been caused by the rigorous sterilisation procedure applied to greenhouse grown explants. According to Raemakers *et al.,* (1993b), rigorous sterilisation procedures applied to donor explants had damaging effects on the tissues and subsequent somatic embryo production. It is also possible that the growth conditions of the donor plants were unfavourable for subsequent somatic embryogenesis.

There was excessive callus production when embryogenic calli derived from leaf lobes of both greenhouse grown cassava and *in vitro* cassava plantlets were transferred to the second step medium which contained BA. According to Raemakers *et al.* (1997) excessive callusing tend to have a negative effect on further development of embryogenic tissue. It was explained that during callusing the embryogenic tissue itself can proliferate into callus. Also, many of the embryogenic tissue may become arrested by the proliferating callus. This means that only a fraction of the globular embryos in the embryogenic calli would develop into torpedo - shaped embryos.

Matured embryos isolated from the explants were few in number. In most cultures, only one embryo per callus was obtained. Such low frequency of embryo production has been reported by many researchers. Finer (1995) has also observed that somatic embryo formation from cotyledon explants in soybean and maize occurred at very low frequency. In cassava, Raemakers (1993) reported that embryo production in eight Nigerian genotypes was very low and varied from 0.1 to 1.1 embryo per explant. Excessive callusing in the maturation medium (step two) could possibly explain the low frequency of embryo production in this study.

Results of this study seem to suggest that embryo production was dependent on the cassava cultivar and 2,4-D concentration in the step one medium. Of five local cultivars tested, four formed somatic embryos. Afisiafi did not produce somatic embryos on any of the media tested. Somatic embryogenic potential has been reported to vary from species to species and between varieties. Szabados *et al.* (1987), found wide variation among 15 cassava cultivars tested for embryogenic capacity using immature leaf lobes as explants. It was observed that *M. cercopiaefolia* and *M. aesculofolia* did not form somatic embryos whereas M. Ven and M. Col. 1505 formed between 60-70% somatic embryos when cultured on the same medium. Mroginsky and Scocchi (1993) have also reported that only two of eleven Argentinean genotypes formed somatic embryos.

Results obtained in this study show that frequency of embryo formation was dependent on the concentration of auxin in the induction medium as well as the cultivar (Figure 1). In Biafra and Santom somatic embryo production was higher on a medium with 16 mg/l 2,4-D than 8 mg/l. The observation made in this study is similar to that of Raemakers *et al.*, (1993). It was reported in their study that on a higher concentration of 2,4-D (8 mg/l), 49% of leaf lobe

explants of M. Col 22 formed embryos compared to 15 % on a lower concentration of 2,4-D (4 mg/l).

This study showed that the type of explant used for embryo induction influenced calli formation and subsequent somatic embryogenesis. A comparison of leaf lobe, apical meristem and stipule explant showed that apical meristem formed the highest embryogenic calli on all the auxin concentrations tested in the induction medium.

Foliose structures were observed on calli derived from both *in vitro* and greenhouse grown leaf lobe explants indicating that they are morphogenetically competent for somatic embryo production. This observation is similar to that of Raemakers *et al.*, (1993 a) who obtained foliose structures from embryogenic calli derived from leaf lobe explants of M. Col 22. They explained that the development of the foliose structures were favoured by low 2,4-D concentrations and short duration of explants on the induction medium. According to Stamp and Henshaw (1987a) foliose structures are caused by partial expression of morphogenetic competence.

Root development occurred when calli derived from *in vitro* explants were transferred to the step two medium. However, root development was high on calli derived from low 2,4-D (4 mg/l) medium (Figure 1). Results obtained in this study is similar with observation made by Krikoran *et al.* (1987) who found out that formation of somatic embryos and adventitious organs ensues when the auxin is removed. It has further been reported that although high concentrations of 2,4-D are considered herbicidal, the auxin is able to cause cell division Krikoran *et al.* (1987).

4.5 Germination of somatic embryos.

The result of this study showed that, of the various desiccation methods used, the highest somatic embryo germination was obtained in the petri dish method , desiccator plus glycerol method and then water plus desiccation method. Because of the simplicity of the petri dish method, it was considered the best and was therefore used in all the subsequent experiments.

Results of this study show that both NAA- and 2,4-D-induced somatic embryos require desiccation for normal germination (section 3.9.2). This observation is similar to the observation made by Finer (1995) that desiccation broke embryo dormancy and greatly enhanced germination of soybean somatic embryos. Kermode (1990) suggested that *in vivo* desiccation terminates the developmental process of embryo formation and activates a switch from maturation to germination. It has also been reported that desiccation causes changes in tugor pressure, membrane permeability and levels of endogenous ABA which in turn induces the expression of specific genes involved in plant development (Parrot *et al.*, 1991). However, it was argued that the somatic embryos had to be developmentally mature before the desiccation treatment could be effective. Mathews *et al.*, (1993) have also reported that desiccation improves germination of matured somatic embryos of cassava cultivar M. Col 1505.

The result obtained in this study show that BA and Kinetin were necessary to stimulate somatic embryo germination. Among the cytokinins tested, BA enhanced shoot formation in somatic embryos than TDZ and Kinetin. It was also observed in this study that BA stimulated both taproot and shoot formation. Of kinetin-supplemented medium, highest percentage of

shoot formation and full germination occurred at 4 mg/l which was the highest level employed. This suggest that its effects should be investigated at higher concentrations.

No shoot only formation occurred in the auxin amended media. Instead there was high root formation in the NAA and IBA media (Table 14). These cultures, however, did not form shoots on subsequent transfer to hormone-free media.

The finding in this study is in agreement with the report of Ammirato (1983) that cytokinins are known to organise the shoot apex during early maturation of somatic embryos. It has also been shown that cytokinins are needed to break dormancy in poorly germinating somatic embryos (Gray and Puhit, 1991). The high root formation in the auxin media observed in this study agrees with the report of Krikoran *et al.* (1987) that high auxin levels leads to root formation during organogenesis.

Observation made in this study shows that the BA concentration needed for somatic embryo germination was dependent on moisture loss (Figure 3). Of the moisture loss levels used (20, 40 and 75%), 40% was found to be the best for embryo germination in both Gading and Adira 4. At this optimal moisture loss, 0.1 mg/l BA gave the highest percentage of germination. Although germination occurred at higher BA concentrations, the developing plantlets had thick stems with multiple shoots.

Germination of NAA-induced somatic embryos was found to be faster in the dark than in the light. In the dark, full plant germination occurred within 14 days whereas in the light germination occurred within 28 days. Also, cultures incubated in the dark enhanced higher

root development than in the light (Figure 5). Furthermore, this study showed that BA concentration needed in the dark for germination was lower than in the light. In the dark, 0.1 mg/l BA gave the highest percentage germination but in light 1 mg/l was needed. Morphologically, developing seedlings from somatic embryos cultured on 0.1 mg/l in the dark had single shoots whereas embryos on 1 mg/l BA in dark had multiple shoots (Plate 11 and 12).

For 2,4-D-induced somatic embryos, dark condition stimulated higher germination in only one cultivar (Figure 4). The results obtained in this study are in contrast with the work of Mathew *et. al.* (1993) and Raemakers *et al.* (1993b,c) who found that embryos formed in liquid media amended with 8 mg/l 2,4-D did not posses root meristem. On culturing these embryos without desiccation on BA medium, the embryos formed shoots but there was no root formation. The result of this study indicate that the desiccation process may have triggered the development of the root meristem and subsequent outgrowth of the roots. The contrasting results between this study and that of Mathews *et al* (1993) and Raemakers (1993 b,c) could be explained by the way the embryos were induced. The induction of embryos using 2,4-D in this study differed from that of Mathews *et al.*, (1993). In the later study, 2,4-D exposure time was shorter, the concentration was lower and for maturation the embryos were transferred to charcoal-amended medium. Secondly, in this study, the embryos were obtained in liquid medium whereas in Mathews method the embryos were induced from solid medium. Aithough no direct evidence is available, the culture system in this study may have favoured higher germination of NAA - induced somatic embryos than 2,4-D induced embryos. Their culture system on the other hand, may have activated the root meristem of 2,4-D-induced embryos and subsequent whole plant germination. Furthermore, the charcoal incorporated in their culture medium may have removed the inhibitory effect of 2,4-D on apical meristem of

somatic embryos. According to Parrot *et al.*, (1991) charcoal removes excess auxins which are detrimental to apical meristem organisation in somatic embryos.

In this study, the embryo germination process lasted 4 weeks. Thus, the seedlings were produced within four weeks of a new cycle. The shorter *in vitro* culture period is advantageous. Especially as it may lead to few changes in the phenotypic characteristics of the resultant plants. In the procedure described by Mathews *et al.*, (1993), embryo germination process lasted 10 weeks.

For direct delivery of somatic embryos to the field through synthetic seed development, taproot formation will be desirable as it will enhance quick seedling establishment. But for the farmer, single taproot formation will be disadvantageous because the yield of cassava tuber will be lower since it is dependent on the number of roots formed. To use synthetic seed therefore will require further research to produce somatic embryos which are morphologically similar to their zygotic counterparts and also to determine optimal desiccation and dark period required for germination.

When a range of various light intensities of 0, 6.4, 64, 641 and 6410 lux were used, 64 lux was found to enhance embryo germination. Different light intensities have been reported to influence adventitious root and shoot formation in stem cuttings (George and Sherington, 1984).

Different light regimes were found to affect somatic embryo germination. In this study red light was found to enhance germination of somatic embryos whereas blue light was inhibitory. This observation is similar to the observation of Henrington and Mcpherson (1993) who reported

that shoot proliferation of *Prunus insita* was high when cuttings were exposed to continuous red light. According to Norton *et al.,* (1988) blue light inhibited shoot proliferation of *Spiraea nipponica.* It has been explained that red light appears to have either the same effect as cytokinin or it reinforces cytokinin in the medium. Leshem *et al.* (1988) have reported that light regime may be used to reduce high concentration of exogenous growth regulator such as BA *in vitro* which causes vitrification and bushiness in *ex vitro* developed plants.

Desiccation of somatic embryos in light followed by incubation in darkness enhanced germination of somatic embryos (Table 16). This results may seem to suggest that somatic embryo germination may not be under phytochrome control. Further research is, however, needed to verify this inference.

Abscissic acid has been shown to improve desiccation tolerance in seeds. It is also known to mobilise storage proteins which are needed for germination in zygotic seeds (Parrot *et al.*, 1991). It has also been shown that in somatic embryogenesis ABA prevents precocious germination (Krikorian *et al.,* 1987) Etienne *et al.*, (1993), showed that ABA improves germination of somatic embryos In the rubber plant (*Heavea brasiliensis).* However, in this study ABA was found to decrease somatic embryo germination. The reason for this contrasting result might be due to the high concentration of ABA applied to the mature embryos. Secondly, the methods used for *Heavea brasiliensis* are different from what have been described in this thesis. Etiene *et al.*, (1993) incorporated ABA in the maturation medium. In this report, already matured somatic embryos were transferred to ABA-amended medium for a further one week before culture for germination. This procedure might have had detrimental effect on organisation of shoot and root apices resulting in poor germination. Their culture system mimics what happens during zygotic seed development and may have

prepared the somatic embryos for desiccation as ABA induces desiccation tolerance and brings about normal desiccation (Krikorian *et al.*, 1987).

Washing of embryos for one day followed by desiccation was found to stimulate germination of Gading somatic embryo. Also root development of somatic embryos on a hormone-free media increased with the period of washing. The observation made in this study could be explained by the fact that the washing of the somatic embryos with water removed excess auxins absorbed by the embryos in the induction medium which were detrimental to the development of the apical meristem. It has been reported that in soybean longer exposure of somatic embryos to auxin in the induction medium decreased the frequency of germination of somatic embryos (Parrot *et al.* 1991).

The ever-growing demand for cassava products on the international market has resulted in a trend towards more efficient methods of propagation. Induction of multiple shoots and somatic embryogenesis has the potential to speed up propagation of elite cassava and provide significant economic advantage to the cassava industry.

SUMMARY

Farmers in Ghana cultivate cassava cultivarsbased on the following criteria: early bulking, yield and potential use. ACMV and brown leaf spot disease are the common diseases of the cultivars collected. ACMV disease affects all the cassava cultivars popularly cultivated by farmers in Ghana.

High concentration of NAA in a medium caused high callus formation.

Meristem culture alone does not free cassava cultivars example, Santom from viruses

Multiple shoot production is dependent on BA concentration in the medium as well as the cultivar of cassava.

Apical meristem formed the highest embryogenic calli on all auxin concentrations tested in the induction medium when compared to leaf lobe and stipule as explants.

NAA- and 2,4-D induced embryos require desiccation for normal germination. BA concentration needed in the dark for germination of somatic embryos was lower than in the light (0.1 mg/l BA in the dark compared to 1.0 mg/l in the light).

The study has also indicated that for field synthetic seed development, further research will be needed to produce somatic embryos which are morphologically similar to their zygotic counterparts and also to determine optimum desiccation and dark period required for germination.

REFERENCES

Ahloowalia, B.S. (1995). *In vitro* selection of mutants. 14th IAEA/FAO International Training Course on Advances in Plant Mutation Techniques. 18th April-26th May, 1995. Vienna, Austria. pp 1-3.

Adejara, G.O. and Coutts, H.A. (1981). Eradication of cassava mosaic disease from Nigerian cassava clones by meristem culture. Plant Cell Tissue and Organ Culture. 1: 25-32.

Al-Hassan, R.M. (1990). Cassava in the economy of Ghana. In: Iweke, I. F., Lynam, J. and Coffi, P. Status of data in major producing countries in Africa: Cameroun, Cote d'Ivoire, Ghana, Tanzania, Uganda and Zaire

Ammirato, P.V (1983). Embryogenesis. In: Evans D.A., Sharp, W.R., Ammirato P.V. and Yamada, Y. (eds) Handbook of Plant Cell Culture Vol 1 pp 82-123. Macmillan, New York.

Annor-Frempong, C. (1994). A survey of cassava cultivation practices in Ghana. In: Ofori, F. and Hahn S.K. (eds) Tropical Root Crops in a developing economy. Proceedings of the ninth symposium of the international society for tropical root crops, 20-26th October, 1991. Accra, Ghana. pp

Baker, C.M. and Wetzetein H.E. (1992). Somatic embryogenesis and plant regeneration from leaflets of peanuts (*Arachis hypogea*). Plant Cell Report. 11:122-125.

Bai, K.B.V. (1987). Recent advances in cassava genetics and cytogenetics. In: Hershey, C.H. (eds) Cassava breeding: a multidisplinary review, CIAT Columbia, pp 35-49.

Bewley, D.D. and Black, M. (1985). Seed development and maturation. In: Seed physiology of development and germination. Plenum Press. New York. pp 22-88.

Bocks, K.K. and Woods, R.D. (1983). The aetiology of African Cassava Mosaic Virus disease. Plant disease 67:994-995.

Bokanga, M , O' Hair SK, Narayanan, K.R. and Steinkrans K.H. (1990). Cyanide detoxification and nutritional changes during cassava (*Manihot esculenta* Crantz) fermentation. In: Howler, R.H. (ed) Proceedings of 8th Sympossium of the International Society for Tropical Root Crops, 30th October-15th November, 1988, Bangokok, Thailand. pp 385-392

Byrne, D. (1984). Breeding Cassava. In Janick J (ed) Plant Breeding Reviews, 2: 73-113.

Carman, J.G. (1990). Embryogenic cells in plant tissues: occurrence and behaviour. In Vitro Cellular Developmental Biology 26:746-753.

Cock, J.H. (1982). Cassava: a basic energy source in the tropics. Science 218: 755-762.

Cock, J.H. (1985). Cassava: New Potential for a neglected crop. Wetview Press, Boulderland, London. p 191.

Crouch, M.L. (1982). Non-zygotic embryos of *Brassica napus* L. contain embryo specific proteins. Planta 156: 520-524.

DGIS (1991). Cassava and Biotechnology. Proceedings of workshop held in Amsterdam, 21-23 March, 1990. The Hague:Directorate General for International Co-operation. p 4.

Durham, R.E and Parrot W.A. (1992). Repetitive somatic embryogenesis from peanuts cultivars in liquid medium. Plant Cell reports 11: 122-125.

Eskes, A.B, Varga, A, Staritsky, G. and Bruinsma, J. (1984). Callus growth and rooting of cassava stem segments cultured *in vitro*. Acta Botanica Neerlandica. 23:315-320.

Etienne, H., Montoro, P., Michaux-Ferriere, N. and Carron, M. P. (1993). Effects of desiccation, medium osmolarity and abscissic acid on the maturation of *Heavea brasiliensis* somatic embryos. Journal of Experimental Botany, 44 (267):1613-1619.

Evans, D.A., Sharp, W.R. and Flick, C.E. (1981). Growth and behaviour of cell cultures: embryogenesis and organogenesis. In Thorpe, T.A. (ed) Plant Tissue Culture: Methods and Applications in Agriculture. Proceedings of UNESCO Symposium, Saulo Paulo. Academic Press, New York, pp 45-113.

Ezumah, H.C. (1988). Cassava-based cropping system research collaboration. Cassava based cropping system research. Vol. 1. IITA, Ibadan.

FAO, (1989). Food and Agricultural Organisation of the United Nations. Production yearbook, 1988. Rome.

FAO (1993). Food and Agriculture Organisation of United Nations. Production yearbook 1992, Rome, pp 281.

Fargette, D., Fauquet, C. and Thouvenel, J.C. (1985). Field studies on the spread of African mosaic virus. Annals of Applied Biology: 106: 285-295.

Fauquet, C. and Fargette, D. (1990). African Cassava Mosaic Virus: Etiology, epidemiology and control. Plant Disease 74 (6): 404-441.

Finer, J.J. (1995). Direct somatic embryogenesis. In: Gamborg, O.L. and Phillips, G.C (eds.) Plant Cell, Tissue and Organ Culture. Fundamental methods. Springer, Berlin pp 90-99.

Franche, C., Bogusz, D., Schokpe, C., Faquet, C. and Beechy, R.N. (1991). Transient gene expression in cassava using high velocity microprojectiles. Plant Molecular Biology 17:493-498.

Franklin, C.I. and Dixon, R.A. (1994). Initiation and maintenance of callus and cell suspension cultures. In: Dixon, R.A and Gonzales, R.A. (eds). Plant Cell Culture. A practical approach. Second Edition. IRL Press.,Oxford University Press. pp 1-22.

Gamborg, O.L., Miller, R.A. and Ojima, K. (1968). Nutrient requirements of suspension culture of soybean root cells. Experimental Cell Res. 50: 151-158.

George, E.F. and Sherrington, P.D. (1984). Plant propagation by tissue culture. Handbook and directory of commercial laboratories. Exegetics Ltd. England. pp 141-158.

Gray, D.J. (1987). Quiescence in monocotyldonous and dicotyledonous somatic embryos induced by dehydration. Hortscience 22: 810-814.

Gray, D.J. and Puhit, A (1991). Somatic embryogenesis and development of synthetic seed technology. Critical Reviews in Plant Sciences 10: 33-61

Haccius, B. (1978). Question of unicellular origin of non-zygotic embryos in callus cultures. Phytomorphology. 28:74-81

Hahn, S.K., John, C., Isoba, G., and Ikotun, T. (1989). Resistant breeding of root and tuber crops at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Crop Protection 8: 147-168.

Halperin, W. and Witherel, D.F. (1965). Ontogeny of adventitious embryos in wild carrot. Science 147: 756-8.

Hartmann, H.T., Kester, T.T. and Davies, T.T. (1990) Plant propagation, 5th edition, Prentice Hall, Englewood Cliffs, N.J.

Herrington, E .and McPherson, J.C. (1993). Light quality growth promotion of *Spiraea nipponica*: the influence of low photon fluence rate and transfer time to a higher fluence rate. Plant Cell, Tissue and Organ Culture 32: 161-167.

Hershey, C.H. (1993). Cassava (*Manihot esculenta* Crantz) In: Kalloo, G. and Bergh, B.O. (eds). Genetic improvement of vegetable crops. Pergamon Press. Oxford-New york pp 669-691.

IITA, (1990) Cassava in Tropical Africa : a reference manual IITA, 1990. pp 60-89.

Iweke, F.I. Poison, R. and Strauss, J. (1994). Cassava production trends in Africa. In: Ofori, F. and Hahn, S.K. (eds). Tropical Root Crops in a developing economy. Proceedings of the ninth symposium of the international society for tropical root crops, 20-26th October, 1991. Accra, Ghana.

Kaiser, W.J. and Teemba, L.R. (1979) Use of Tissue Culture and Thermotherapy to free East African cultivars of African Cassava Mosaic and Brown streak disease. Plant Science Reporter 63: 780-784.

Kartha, K.K. (1981). Tissue Culture techniques for virus elimination and germplasm preservation. In:Rachie, K.O. and Lyman, J.M. (eds), Genetic engineering for crop improvement. Working papers, Rockefeller Foundation, pp 123-134.

Kartha, K.K., Gamborg, O.L. Constabel, F. and Shylnk ,J.P. (1974). Regeneration of cassava plants from apical meristems. Plant Science Lett. 2: 107-113.

Kartha, K.K. and Gamborg O.L. (1975). Elimination of Cassava Mosaic Virus disease by meristems. Plant Science Lett. 107-113.

Kermode, A.R. (1990). Regulatory mechanism involved in the transition from seed development to germination. CRC Crit. Rev. Plant Sci. 9: 155-195.

Komatsuda, T. and Ohyma, K. (1988). Genotypes of high competence for somatic embryogenesis and plant regeneration in Soybean max. Theoretical and Applied Genetics 75: 695-700.

Krikorian, A.D., Kelly, K. and Smith, D. L. (1987). Hormones in plant tissue culture and micropropagation. In: Peter, J. D. (Ed). Plant Hormones and their role in plant growth and development. Martinus Nijhoff Publishers, Dordrect, pp 593-613.

Leshem, B., Shaley, D.P and Izhar, S. (1988). Cytokinin as an inducer of vitrification in melon. Ann. Bot. 61: 255-260.

Liu, M.C. (1975). The *in-vitro* induction and regeneration of cassava plant from apical meristem. Taiwan Sugar 22: 171-177.

Mathews, H, Schokpe, C, Carcamo, R, Chavarriaga, P, Fauquet, C and Beachy, R.N. (1993). Improvement of somatic embryogenesis and Plant regeneration in cassava. Plant Cell Report. 12: 328-333.

Mellor, F.C., and Stace-Smith, R. (1977). Virus-free potatoes by tissue culture. In J. Reinert and Y.P.S. Bajaj (eds.), Applied and fundamental aspects of plant cell, tissue and organ culture. Springer-Verlag, Berlin pp. 616-635.

Merkle, S.A. and Wiecko, A.T. (1990). Somatic embryogenesis in three Magnolia species. Journal of American Horticultural Science. 115: 858-860.

MOA (1990). Ministry of Agriculture. Ghana medium term agricultural development programme: an agenda for sustained agricultural growth and development (1991-2000) MOA, Accra.

Mroginsky, L.E. and Scocchi, a. (1993). Somatic embryogenesis of Argentine cassava varieties. In: Roca, W.M. and Thro, A.M. (eds.). Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network. Carte-agena, Columbia, 25-28 August, 1992. Cali, Columbia: Centro International de Agricultura Tropical, 1993.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco cultures. Physiologum Plantarum. 15: 473-494.

Nasser, N.M.A. (1978). Conservation of genetic resources of cassava (*Manihot esculenta*): determination of wild species localisation with emphasis on possible origin. Econ. Bot. 311-320.

Ng, S.Y.C. (1992). Tissue Culture of root and tuber crops at IITA. In: Thottapilly, G., Monti L.M, Mohan Raj D.R. and More A.W. (eds), Biotechnology: Enhancing research on Tropical Crops in Africa. pp 135-141.

Norton, C.R., Norton, M.E., Herrington, E. and Philips, D. (1988). Light quality and light pipes in the micropropagation of woody ornamental plants. In: von Hetig W-U and Gruber, G. (eds) International sympossium on propagation of ornamental plants. Acta Hort. 26: 413-416.

Obigbessan, G.O. (1994). Issues of cassava cyanide: Nigeria's experience. In: Ofori, F. And Hahn, S.K. (eds) Tropical Root Crops in a developing economy. Proceedings of the ninth sympossium of the international society for tropical root crops, 20-26th October, 1991. Accra, Ghana. pp 233-236.

Oishi, M.Y. and Bewley, J.D. (1990). Distinction between the responses of developing maize kernels to flouridone and desiccation in relation to germinability, a- amylase activity and abscissic acid content. Plant Physiol. 94: 592-598.

Parrot, W.A., Merkle, S.A. and Williams, E.G. (1991). Somatic embryogenesis: Potential for use in propagation and gene transfer systems. In: Murray, D.R. (ed) Advanced methods in plant breeding and biotechnology. CAB Int. Wallingford. pp 158-200.

Purseglove, J.W. (1968). Tropical Crops: Dicotyledons. Longman Group Ltd. pp 172-185.

Raemakers, C.J.J.M, Bessember, J., Staristy, G., Jacobsen, E. and Visser, R.G.F. (1993a). Induction, germination and shoot development of somatic embryos in cassava. Plant Cell, Tissue and Organ Culture 33:151-156.

Raemakers, C.J.J.M, Amati, M., J., Staristy, G., Jacobsen, E. and Visser, R.G.F. (1993b). Cyclic embryogenesis in cassava. Annals of Botany 71:289-294.

Raemakers, C.J.J.M, Schamakers, C.M., Jacobsen, E. and Visser, R.G.F. (1993c). Improvement of Cyclic embryogenesis of cassava *(Manihot esculenta* Crantz). Plant Cell Reports 12:2269-229.

Raemakers, C.J.J.M, Jacobsen, E. and Visser, R.G.F. (1995). Secondary somatic embryogenesis and application in plant breeding. Euphytica. 81:93-107

Raemakers, C.J.J.M, Sofiari, E., Kanju, E, Jacobsen, E. and Visser, R.G.F. and van Lammeren A.M.M. (1995b). NAA induced somatic embryogenesis in cassava. In: Roca W.M., and Thro, A.M. (eds) Proceedings of the second international Scientific meeting of the Cassava Biotechnology Network, Bogor, Indonesia.

Raemakers, C.J.J.M, Sofiari, E., Kanju, E, Jacobsen, E. and Visser, R.G.F. and van Lammeren A.M.M. (1996). Production of transgenic cassava (*Manihot esculenta* Crantz) plants by particle bombardment using luciferase activity as selection marker. Molecular Breeding, Kluwer Academic Publishers, Belgium. pp 1-11.

Raemakers, C.J.J.M., Visser, R. G. F. and Jacobsen, E. (1997). Micropropagation of cassava. (*Manihot esculenta* Crantz). In Y.P.S. Bajaj (ed). Biotechnology in Agriculture and forestry, High-tech and micropropagation, Springer-Verlag Berlin. 39:77-102.

Raemakers C.J.J.M. (1993). Primary and cyclic embryogenesis in cassava (*Manihot esculrenta* Crantz). Ph.D Thesis, Agricultural University, Wageningen, The Netherlands.

Reinert J, Bajaj, Y.P.S and Zbell, B. (1977). Aspects of organogenesis and embryogenesis. In: Street E.D. (ed) Plant tissue and cell culture. University of Columbia Press, Berkley, pp 389-429.

Roberts, D.R., Lazarroff, W.R. and Webster, F.B. (1990). Interaction between maturation and relative humidity treatments and their effects on germination of Stika spruce somatic embryos. Journal of Plant Physiology 138: 1-6.

Roca, W.M. (1984). Cassava. In: Sharp, W.R., Evans W.A., Ammirato, P.V. and Yamada (eds) Handbook of plant cell culture, Vol 2, Crop species. Macmillan Publishing, New York pp 269-301.

Roggers, D.J. and Appan, S.G. (1973). Manihot and Manihotoides (Euphorbiaceae). Flora Neotropica Monograph 13. Hafner press, New York.

Rosenberg, L.A and Rhine, R.W. (1986). Moisture loss, a prerequisite for seedling growth in seeds of soybean (Glycine max L. Merr). Journal of Experimental Botany 37: 1663-1674.

Schopke, C., Taylor, N., Carcamo, R., Konan, N.K., Marmey, P., Henshaw, G.G., Beachy, R.N., Fauquet, C. (1996). Regeneration of transgenic cassava *plants (Manihot esculenta* Crantz) from microbombarded embryogenic suspension cultures. Nature Biotechnology 14: 731-735.

Seif, A.A. (1982). Effect of Cassava Mosaic Virus on yield of cassava. Plant Diseases. 66:661-662.

Seitz, Kris M.H. and Bingham E.T. (1988) Interactions of highly regenerative genotypes of alfafa (*Medicago sativa)* and tissue culture protocols. *In-vitro* cell and developmental Biology pp. 84

Shahin, E.A. and Shephard, J.F. (1980). Cassava mesophyll protoplasm: isolation, proliferation and shoot formation. Plant Sci. Lett 17:459-465.

Sharp W.R., Sohndahl M.R., Evans, A.E., Caldas L.A. and Maraffa, S.B. (1980). The physiology of *in vitro* asexual embryogenesis. Horticultural Reviews 2: 268-310.

Slack, S.A. and Tufford, L. A. (1995). Meristem culture for disease elimination. In: Gamborg, G.L. and Philips G.C. (eds). Plant cell, tissue and organ culture. Fundamental methods, Springer-Verlag Berlin. pp 11-128.

Smith, M.K., Biggs, B. J. And Scott, K.J. (1986). *In vitro* propagation of cassava (*Manihot esculenta* Crantz). Plant Cell, Tissue and Organ Culture 6:221-229.

Stamp, J.A and Henshaw, G.G. (1982). Somatic embryogenesis in cassava. Z. Pflanzenphysiol 105: 183-187.

Stamp, J.A. and Henshaw G.G. (1987a). Somatic embryogenesis from clonal leaf tissue of cassava. Annals of Bot. 59: 445-450.

Stamp, J.A. and Henshaw G.G. (1987b). Secondary somatic embryogenesis and plant regeneration in cassava. Plant Cell Tissue and Organ culture10: 227-233.

Szabados, L., Hoyos, R. And Roca, W. (1987). *In vitro* somatic embryogenesis and plant regeneration of cassava. Plant Cell Reports. 6:248-251.

Terry, E.R. and Hahn, S.K. (1980). The effect of cassava mosaic disease on growth and yield of a local and improved variety of cassava. Tropical Science 34: 26-421

Terzi, M. and Loschiavo, F. (1990) Somatic embryogenesis. In Bhojawani, S.S. (ed) Development in Crop Science, Plant Tissue Culture: Applications and limitations pp 1-33.

Theberge, R.L. (1985). Common African pests and diseases of cassava, yam, sweet potato and cocoyam. IITA Handbook. pp 14.

Thomas, E.T. and Wenzel, G. (1975) Embryogenesis from microspores of *Brssica napus*: Zeitscrif fur Pflanzenzuchtung 74:77-81.

Thorpe, T.A. (1990). The current status of plant tissue culture. In Bhojawani, S.S. (ed) Development in Crop Science, Plant Tissue Culture: Applications and limitations pp 1-33.

Thresh, J.M., Fargette, D. and Otim-Nape, G.W. (1994). Effect of African Cassava Mosaic geminivirus on yield of cassava. Tropical Science 34:26-42.

Tiliquin, J.P. (1979). Plant regeneration from stem callus of cassava. Canadian Journal of Botany 57: 1761-1763.

Tisserat, B., Esan, E.B., and Murashige, T., (1979). Somatic embryogenesis in Angiosperms. Horticultural Review 1:1-77.

Tulucke, W. (1987). Somatic embryogenesis in woody perennials. In: Bonga, J.M. and Durzan, D.J. (eds) Cell and tissue culture in forestry, Vol 2, specific principles and methods: growth and development, Martinus Nyhoff Publishers, Dordrecht, Boston, Lancaster, pp 61-91.

Umbeck, P., Johnson, G. Barton, K. and Swain, W. (1987). Genetically transformed cotton (*Gossypium hirstum*) plants. Bio/technology 5: 263-266.

Vasil, V., and Vasil, I. K. (1981). Somatic embryogenesis and plant regeneration from suspension cultures of Pearl Millet (*Pennisetum americanum*). Annals of Botany 47: 669-678.

Wann, S.R. (1988). Somatic embryogenesis in woody species. Horticultural Review 10: 153-189.

William , E.G. and Maheswaran, G. (1986). Somatic embryogenesis: factors influencing coordinated behaviour of cells as an embryogenic group. Annals of Botany 57: 443-462.

Wright, N.S (1988). Assembly, quality control and use of potato cultivar collection rendered virus-free by heat therapy and tissue culture. Am Potato Journal, 65:181-198.

APPENDIX

Appendix 1

GERMPLASM COLLECTION QUESTIONAIRE

1. Name of farmer (optional).....
2. Location of farm.....
3. Local names of cassava cultivated.....
4. Type of farm I. Peasant/subsistence ..... ii. Government.....
5. Source of variety/cultivar .....
6. Region of origin.....
7. Country of origin.....
8. Morphological features
   - i. colour of stem.....
   - ii. colour of petiole.....
   - iii. number of leaflets.....
   - iv. leaf arrangement.....
   - v. leaf shape.....
   - vi. branching habits a. no branching b. branching
   - vii. Does your cultivar flower a. yes b. No
   - viii. Colour of petal.....
9. Diseases
   - i. Which diseases affect your cultivar?.....
   - ii. Where are the symptoms noticed?.....
   - iii. Describe the symptoms briefly.....

     .....

iv. At what stages are the symptoms first noticed?

a. 1 month after shooting

b. 2-4 months after shooting

c. 4-6 months after shooting

d. 6-10 months after shooting

10. The variety/cultivar is good for making a. fufu b. ampesi. c. gari d. agbelema

e. konkonte f. others.....

11. How long does your cultivar take to mature?

a. 6 months b. 9 months c. 12 months d. 18 months

e. 24 months

12. The yield per hectare is a. high b. medium c. low.

Appendix 2

ACMV INCIDENCE AND SEVERITY SCORING SHEET

(C ESCaPP Survey Software, IITA, Benin Station.

Village.....

Name/I.D no. of..... Crop age.....

Name/I.D. no. of researcher:..... Cultivars.....

Date:..... Field size.....

Plant sample	No. of shoot tips per ACMV symptom score 1-5					Total no. of shoot tips	Average score per tip	ACMV score / whole plant
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
Total								
Mean								
S.D.								

Appendix 3a

ANOVA for the effect of cultivar and BA on induction of multiple shoots from apical meristem of M. Col 22 and Biafra.

Source of variation	SS	d.f.	MS	F-ratio	Sig.level (P≤0.05)
Main effects	210.18750	9	23.35417	5.451	0.0001
Cultivars	123.52083	(1)	123.52083	28.833	0.0000
Replicates	7.60417	(5)	1.52083	0.355	0.8758
BA concentration	79.06250	(3)	26.35417	6.152	0.0016
Residual	162.79167	38	4.2839912		
Total (Corr)	372.97917	47			

Appendix 3b

ANOVA for the effect of cultivar and BA on induction of multiple shoots from single bud cuttings of M. Col 22 and Biafra.

Source of variation	SS	d.f.	MS	F-ratio	Sig.Level (P≤0.05)
Main effects	1464.1875	9	162.68750	7.429	0.0000
Cultivars	595.0208	(1)	595.02083	27.172	0.0000
Replicates	80.4375	(5)	16.08750	0.735	0.6021
BA concentration	788.7292	(3)	262.90972	12.006	0.0000
Residual	832.12500	38	21.898026		
Total (Corr.)	2296.3125	47			

Appendix 4a

ANOVA for the effect of BA on induction of multiple shoots from apical meristem M. Col 22 .

Source of variation	SS	d.f.	MS	F-ratio	Sig. level (P≤0.05)
Main effects	133.3333	8	16.666667	2.240	0.0848
Replicates	15.20833	(5)	3.041667	0.409	0.8353
BA concentration	118.12500	(3)	39.375000	5.291	0.0109
Residual	111.62500	15	7.4416667		
Total (Corr.)	244.95833	23			

Appendix 4b

ANOVA for the effect of BA on the number of buds produced by apical meristem of M. Col 22.

Source of variation	SS	d.f.	MS	F-ratio	Sig. level (P≤0.05)
Main effects	154.83333	8	68.229167	1.904	0.1344
Replicates	251.83333	(5)	50.366667	1.406	0.2780
BA concentration	294.00000	(3)	98.000000	2.735	0.0803
Residual	537.50000	15	35.833333		
Total (Corr.)	1083.3333	23			

Appendix 5

ANOVA for the effect of BA on the number of buds produced by apical meristem of Biafra .

Source of variation	SS	d.f.	MS	F-ratio	Sig. level (P≤0.05)
Main effects	542.33333	8	67.79167	13.446	1.0000
Replicates	25.20833	(5)	5.04167	1.000	1.0000
BA concentration	517.12500	(3)	172.37500	34.190	1.0000
Residual	75.625000	15	5.0416667		
Total (Corr.)	617.95833	23			

Appendix 6a

ANOVA for the effect of cultivar and BA on induction of shoots from single bud cuttings of M. Col 22, Ankrah, B. nsia and Biafra.

Source of variation	SS	d.f.	MS	F-ratio	Sig. Level (P≤0.05)
Main effects	116.16473	9	12.907192	9.995	0.0000
Replicates	5.28512	(3)	1.761706	1.364	0.2635
BA concentration	71.03637	(3)	23.678789	18.337	0.0000
Cultivar	39.84324	(3)	13.281081	10.285	0.0000
Residual	69.84324	54	1.2913238		
Total (Corr.)	185.89621	63			

Appendix 6b

ANOVA for the effect of cultivar and BA on induction of buds from single bud cuttings of M. Col 22, Ankrah, Bosomnsia and Biafra.

Source of variation	SS	d.f.	MS	F-ratio	Sig. Level (P≤0.05)
Main effects	322.61975	9	35.846639	3.886	0.0008
Cultivar	233.67598	(3)	77.891993	8.444	0.0001
Replicate	21.90617	(3)	7.302056	0.792	0.5039
BA concentration	67.03760	(3)	22.345868	2.422	0.0758
Residual	498.11595	54	9.2243694		
Total (Corr.)	185.89621	63			

Appendix 7

ANOVA for the effect of cultivar and 2,4-D concentration on calli formation from leaf lobes of greenhouse grown cassava.

Source of variation	Sum of squares	d.f.	MS	F-ratio	Sig. level (P≤0.05)
Main effects	4600.0608	8	575.0076	4.001	.0015
Cultivars	2055.5056	(3)	685.1685	4.767	.0063
Replicate	328.9225	(3)	109.6408	0.763	.5218
2,4-D conc.	2215.6329	(2)	1107.8165	7.708	.0015
Residual	5605.1440	39	143.72164		
Total (Corr.)	10205.205	47			

Appendix 8a

ANOVA for the effect of 2,4-D concentration on calli formation of greenhouse grown cassava (Ankrah) cultivar.

Source of variation	SS	d.f.	MS	F-ratio	Sig. level (P≤0.09)
Main effects	24474.000	6	4079.0000	262.691	0.0000
Replicates	113.250	(3)	37.7500	2.431	0.1321
2,4- D conc.	24360.750	(3)	8120.2500	522.950	0.0000
Residual	139.75000	9			
			15.527778		
Total (Corr)	24613.750	15			

Appendix 8b

ANOVA for the effect of 2,4-D concentration on calli formation of greenhouse grown cassava (Bosomnsia) cultivar.

Source of variation	SS	d.f.	MS	F-ratio	Sig. level (P≤0.05)
Main effects	25778.219	6	4296.3698	25.658	0.0000
Replicates	115.672	(3)	38.5573	0.230	0.8730
2,4- D conc.	25662.547	(3)	8554.1823	51.086	0.0000
Residual	1507.0156	9	167.44618		
Total (Corr)	27285.234	15			

Appendix 8c

ANOVA for the effect of 2,4-D concentration on calli formation of greenhouse grown cassava (Biafra) cultivar.

Source of variation	SS	d.f.	MS	F-ratio	Sig. level (P≤0.05)
Main effects	25604.434	6	4267.4056	114.510	0.0000
Replicates	308.467	(3)	102.8223	2.759	0.1039
2,4- D conc.	25295.967	(3)	8431.9890	226.260	0.0000
Residual	335.40062	9	37.266736		
Total (Corr)	25939.834	15			

Appendix 9

ANOVA for the effect of explant and cultivar on calli formation leaf lobes, apical meristem and stipules.

Source of variation	SS	d.f.	MS	F-ratio	Sig. level (P≤0.05
Main effects	86080.000	10	8608.000	20.169	0.0000
Cultivar	5821.333	(4)	1455.333	3.410	0.0137
Explant	79690.667	(2)	39845.333	93.360	0.0000
Replicate	568.000	(4)	142.000	0.333	0.8550
Residual	27314.667	64	426.79167		
Total (Corr)	113394.67	74			

Appendix 10a.

ANOVA for the effect of 2,4-D on calli formation from leaf lobe, apical meristem and stipules of Santom.

Source of variation	SS	d.f.	MS	F-ratio	Sig. level (P≤0.05)
Main effects	2746.6667	6	457.77778	4.905	0.0216
Explant	1853.3333	(2)	926.66667	9.929	0.0068
Replicate	893.3333	(4)	223.33333	2.393	0.1366
Residual	746.66667	8	93.333333		
Total (Corr)	3493.3333	14			

Appendix 10b.

ANOVA for the effect of 2,4-D on calli formation from leaf lobe, apical meristem and stipules of Bosomnsia

Source of variation	SS	d.f.	MS	F-ratio	Sig. level (P≤0.05)
Main effects	7120.0000	6	1186.6667	3.327	0.0600
Explant	5813.3333	(2)	2906.66667	8.150	0.0117
Replicate	1306.6667	(4)	326.6667	0.916	0.4994
Residual	2853.3333	8	356.6667		
Total (Corr)	9973.3333	14			

Appendix 11.

ANOVA for the effect of cultivar and 2,4-D concentration on calli formation *in-vitro* explants.

Source of variation	SS	d.f.	MS	F-ratio	Sig. level (P≤0.05)
Main effects	4804.6558	9	533.85065	3.347	0.0028
Cultivar	2274.4157	(4)	568.60392	3.565	0.0123
Replicate	621.4978	(3)	207.16594	1.299	0.2851
2,4-D concentration	1908.74234	(2)	954.37117	5.984	0.0047
Residual	7973.9940	50	159.47988		
Total (Corr)	12778.650	59			