IN VITRO CONSERVATION OF SWEET POTATO (*IPOMEA BATATAS*, (L.) LAM USING SLOW GROWTH MEDIA

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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They provided me with great love and understanding during the course of the study.

and nburu for being a kenyan. african.

TABLE OF CONTENTS.

List of Tables and Figures and Plates

List of plates

List of Figures

List of Appendices

List of Abbreviations referred to in the text.

ACIAR	- Australian Centre for International Agricultural Research
ABA	- Absiscic Acid
ALFP	- Amplified Length polymorphism
AVRDC	- Asian Vegetable and Development Centre
BAP	- Benzyl amino purine
CCC	- Cycocel
CIP	- International Potato Centre
CRD	- Complete Randomized Design
DNA	- Deoxy-ribonucleic Acid
DMSO	- Dimethyl Sulphoxide
HCL	- Hydrochloric acid
IAA	- Indole-3-Acetic acid
MDH	- Malate Dehydrogenase
MH	- Maleic Hydrazide
MS	- Murashige and Skoog
NAA	- Naphthalene acetic acid
RAPDs	- Random Amplified Polymorphic DNAs
RFLP	- Restriction Length Fragment Polymorphism
SOD	- Superoxide Dismutase
SSR	- Small Sequence Repeats

ABSTRACT

An *in vitro* protocol was developed for short-term conservation of sweet potato by modification of medium osmotic potential through incorporation of growth retardants namely abscisic acid (ABA), mannitol, sorbitol and glyphosate (commercial Round up). Single node cuttings from virus tested sweet potato genotypes were obtained from the Plant Quarantine Station, Kenya. These materials were multiplied *in vitro* on MS basal medium supplemented with 30g/l sucrose, 0.1g/l inositol, 0.1 g/l calcium pantothenate, 0.1 g/l l-arginine, 0.02 g/l putrescine- HCL, 0.1 g/l calcium nitrate and 0.4 mg/l thiamine-HCl. The media was gelled with 2.5 mg/l phytagel. This multiplication medium was used to multiply plants for conservation studies.

Single node cutting were cultured on slow growth medium as follows; In one experiment five sweet potato genotypes: NC-1582, IRA-048, CPT 560, Lohafinjo and KSP-20 were cultured designated as SW1to SW7. The medium: SW2, SW3, SW4, SW5, SW6 and SW7 contained 4% mannitol, 6% mannitol, 2% sorbitol and 2% mannitol combined and ABA at 0.5, 1.0, and 2.0 mg/l), respectively while the control (SW1) contained no growth retardant. Single node cuttings of sweet potato genotypes NC-1582 (440094), IRA-048 (440143), CPT560, Lohafinjo (440391) and KSP-20 were inoculated on the medium. A total of three hundred and fifty cultures were made (5 genotypes x 7 treatments x 10 replications).

In second experiment two genotypes namely: Nyaluolo (local) and IITA-TIS-3290 (440068) and three medium treatments namely: SG1, SG2 and SG3 were utilised. SG1 did not contain any glyphosate (control), whereas SG2 contained 5 mg/l glyphosate, and SG3 contained 10mg/l glyphosate. All the tissue culture media were autoclaved at a temperature of 121°C and a pressure of 15 pounds per square inch

(p.s.i.) for 15 minutes. The plants were assessed on height on a monthly basis up to five months. The number of roots, leaves and nodes, % leaf abscission and percentage survival were scored at 2, 5 and 8 months respectively.

Analysis of variance (ANOVA) for height indicated significant differences for genotypes, growth retardants and their interactions. However four treatments (4 % mannitol, 6% mannitol, 1.0 mg/l ABA and 2.0 mg/l ABA) were the best slow growth recipes. However treatments of mannitol significantly ($p = 0.05$) reduced % survival compared to the control, and the best ABA treatments. Percentage survival was 65% (control), 51.49 % (4% mannitol), 47.73 % (6 % mannitol), 74.8% (1.0 mg/l of ABA) and 80.5 for (2.0 mg/l of ABA). Mannitol also significantly ($p = 0.05$) reduced internode lengths, leaf sizes and root means of five sweet potato genotypes.

The treatment combining sorbitol and mannitol (2% of each on w/w basis) was unable to significantly retard growth of sweet potato genotypes, IRA-048 and NC-1582. Plants significantly ($p = 0.05$) outgrew the control after one month and four months for the two genotypes, respectively. However the treatment was significantly superior to the control in conservation of three genotypes namely: CPT- 560, KSP-20 and Lohafinjo, throughout the study period.

Overall, ABA at concentration of 1-2 mgl^{-1} was the best retardant for the five genotypes studied due to conservation of genotypic integrity, high percent survival, minimal effect on photosynthetic surface and effective plant height retardation.

In the second experiment glyphosate was studied for *in vitro* conservation at 0 mg/l, 5 mg/l and 10 mg/l to conserve two sweet potato genotypes: Nyaluolo and IITA-TIS-3290. Glyphosate treatments significantly ($p = 0.05$) retarded plant growth throughout the 5 months of *in-vitro* culture. The two levels did not significantly ($p = 0.05$) differ from one another. In addition, the two treatments completely inhibited root formation in both genotypes. Mean survival was also significantly ($p = 0.05$) reduced by glyphosate.

CHAPTER 1

INTRODUCTION

Sweet potato (*Ipomea batatas (L.*) conservation by slow growth medium

Origin, Botany and Classification

The sweet potato probably originated in or near north-western South America. The crop belongs to the Convovulaceae family, the tribe *Ipomeae*, genus *Ipomea*, sub-genus Q*uamoclit*, section, *Batatas* and species *Ipomea batatas* (L) Lam. The species is classified in the genus *Ipomea* on the basis of the stigma shape and the surface of the pollen grains.

Within the Section *batatas* there are 13 wild species considered related to sweet potato. These include; *I. tricharpa, I. cyanchifolia, I. lacunosa, I. x leucantha, I. littralis, I. ramosissima, I. tabascana, I. tenissima, I. tilicea, I. trifida, I. triloba and I. umbraticola* (Huaman, 1992).

The number of chromosomes in the sweet potato plant is 2n = 6x = 90. This indicates that the plant is hexaploid with a basic chromosome number, x = 15. Some of the wild relatives are tetraploids, while others are diploids. The ploidy levels of some species is still unknown (Huaman, 1987).

A large number of sweet potato cultivars exists with many of them arising through systematic breeding efforts, whereas others have arisen through natural hybridization and mutations. On the basis of texture after cooking sweet potato cultivars generally fall into three groups:

- Those with firm dry, mealy flesh after cooking;
- Those with soft, moist, gelatinous flesh after cooking and
- Those with very coarse tubers suitable only for animal feed or for industrial uses (Onwueme, 1978; Onwueme and Charles, 1994).

Genetics and Cytology

Most sweet potato cultivars are self-incompatible. However, a few are self-compatible and genes for this characteristic can conveniently be incorporated into other cultivars (Onwueme, 1978). Cross compatibility also exists between some sweet potato cultivars. Some cultivars produce shrunken and defective pollen. Sweet potato also benefits from hybrid vigour following hybridisation of inbred lines (Onwueme, 1978).

Environment for sweet potato

The crop grows best at temperatures above 24°C. Severe growth retardation occurs when the temperature falls bellow 10°C. The crop takes between 4 and 6 months to mature. The sweet potato does best in regions with 75-100 cm of rainfall /annum with about 50 cm falling during the growing season. The crop grows best on sandy loam soils and poorly on clays. A soil pH of 5.6 –6.6 is preferred for sweet potato. It is sensitive to alkaline or saline soils (Onwueme, 1978; Onwueme and Charles, 1994).

Economic importance and utilisation of sweet potato

a) Approximate nutritional composition.

The fresh sweet potato tuber contains: 50-81% moisture, 8-29% starch, 0.95-2.4% protein, 1.8-6.4% ether extract, 0.5-2.5% reducing sugars, 0.5 -7.5% non-starch carbohydrates and 0.88-1.38% mineral matter. The fresh tuber contains the following vitamins (on mg/100 gm fresh weight basis): 1-12 mg carotene, 0.1mg thiamine, 0.06 mg riboflavin, 0.90 mg nicotinic acid and 29-40 mg ascorbic acid (Onwueme, 1978).

b) Sweet potato for human consumption

In most parts of the tropics, it is utilised without processing. The fresh tuber is boiled, baked roasted or fried and then consumed directly. Processed forms include chips and refined flour. The chips are produced by slicing tubers into thin slices and dried in the sun. Sometimes the fried chips are packaged and eaten as snacks. Also, spray-drying the peeled potatoes produces refined flour. The flour may be mixed with wheat for baking bread (Onwueme, 1978; Woolfe, 1992).

c) Sweet potato as livestock feed.

Tubers and foliage can be fed to livestock. The dried product can be fed whole or ground to cattle, pigs, sheep or poultry (Yen, 1982).

d) Sweet potato for industrial uses

Sweet potato can supply starch for industrial uses. However, production costs are high. The starch is used for textile manufacture and production of syrup and alcohol (Onueme, 1978).

e) Leaves as vegetables and livestock feed

Leaves and tender shoots of sweet potato are used as vegetable foods in many parts of the tropics. Leaves contain, on dry matter basis, about 8% starch, 27% real protein and 10% ash. It also contains about 15% carotene per 100gm dry matter. The leaf is much richer than the tuber in proteins, minerals and vitamins. The leaves are usually either boiled or incorporated into soups and stews. They may also be fed to livestock either in form of silage or fresh foliage (Onwueme, 1978; Yen, 1982).

Production and consumption trends

The sweet potato crop is presently produced in 111 countries of the world of which 101 are classified as developing nations (Woolfe, 1992). Approximately 90% of the World production comes from Asia, 5% from Africa and the remaining 5% from the rest of the world (Woolfe, 1992). The world's leading producer is China. Annually it produces nearly 100 million tonnes. Uganda, Indonesia and Vietnam are second to China with a total annual output of 2 million metric tonnes. Although the global sweet potato production has increased in the recent past, it has reduced in Japan, United States and other industrialised nations. However, the production in Africa has risen steadily since the 1960's (Woolfe, 1992).

Regional patterns of production and consumption in Africa

Sweet potato production in Africa has increased over the last 25 years while it has reduced or stagnated in other regions (Ewell and Mutuura, 1991). Within sub-Saharan Africa sweet potato is the third most important root and tuber crop, next to Cassava (*Manihot esculenta*) and Yam (*Dioscorea spp*). Nearly 90% of the total output comes from eastern and southern Africa. Uganda is by far the largest producer in Africa with a total output of 1.7 million tonnes, and the fourth in the world. Rwanda and Burundi come next in total production but being much smaller countries they lead in per-capita production. Other notable producers are Kenya, Madagascar and Tanzania (Ewell and Mutuura, 1991).

In Kenya over 75% of the national production is found in the western region, in the lake Victoria basin (Mutuura *et al.*; 1992). The area under sweet potato cultivation in Kenya is approximately 30,000 Ha (Horton, 1988). Production in Kenya has risen steadily since the early sixties, reaching a record output of approximately 530,000 metric tonnes in the early

eighties (Horton, 1988). Nevertheless, recent surveys show that the production is constrained by several factors including: weevil damage, drought, infection of plants by virus diseases, destruction by moles, lack of planting materials and loss of genetic resources (Wambugu, 1991, Mutuura *et al.*, 1992).

The crop is typically grown by small-scale growers. However in many areas it is an important cash crop, besides being used for livestock feed or for industrial input such as starch. Both leaves and tubers are eaten. The roots, tender leaves and shoots provide high energy, vitamins, proteins and a substantial amount of minerals (Woolfe, 1992).

Status of sweet potato genetic resources

Sweet potato is typically propagated vegetatively due to the heterozygous nature of the seeds. In the recent past there has been attempts to introduce new sources of genetic diversity due to narrow genetic base of germplasm in most breeding programmes. However, in spite of this concern has been raised on rapid loss of plant genetic resources as a result of rising population pressure and changing farming systems.

Many factors such as change in land use, the introduction of modern agricultural techniques, use of fertilisers, pesticides and fungicides has made traditional cultivars obsolete due to their replacement with improved cultivars (Rao and Riley, 1994). Sweet potato land races and farmer varieties have special attributes such as taste, disease and pest resistance and hence the need to conserve them as seed or as vegetative materials in the field. The great wealth of genetic diversity existing in genepools holds vast potential for the current and future uses for the benefit of humankind. The available genepool could be exploited either through conventional means or use of biotechnology. Conservation can be divided into two; *in-situ* and *ex-situ*. *Ex-situ* conservation maintains germplasm outside its original habitats, in

facilities that have specifically created such as seed, field, *in vitro* banks or botanical gardens, pollen and DNA libraries. On the other hand *in-situ* conservation relies on natural ecosystems and natural habitats (Perino, 1992; Rao and Riley, 1994).

Clonal maintenance in the field is expensive and risky due to attack of crop by pests, infectious diseases and unfavourable climatic conditions. At the moment limited effort to conserve the crop both at the centres of diversity and areas of introduction. For example, a major centre in the South Western U.S.A. maintained only 50-60 lines of sweet potatoes (Austin, 1987). Therefore there is an urgent need to conserve sweet potato both in centres of origin and countries recipient of the collected germplasm (Austin, 1987).

Because of the problems associated in field maintenance, other methods such as *in vitro* conservation are required. *In vitro* methods have several advantages;

a) Tissue culture possess the potential for very rapid multiplication of high value plants (Dodds and Roberts, 1985).

b) Tissue culture systems are aseptic and can be easily kept free from fungi, bacteria, viruses and insect parasites. Tissue culture is used routinely to obtain virus free plants (Waithaka, 1992). In addition, propagation and storage of plants saves on space as opposed to field maintenance (Jarret and Florkowski, 1990). Also, utilisation of specialised *in vitro* techniques, such as pollen and anther culture produces haploid plants which may be used in breeding programmes (Henshaw, 1975).

c) Plant tissue culture conservation techniques are useful in plant breeding programmes as a means of rescuing and subsequently culturing zygotic embryos from incompatible crosses, which normally result in embryo abscission (Henshaw, 1975).

d) *In vitro* based techniques are not as expensive as field collections (Jarret and Florkowski, 1990). Problems of genetic erosion in stocks which can be serious under field conditions are completely avoided (Henshaw, 1975).

Limitations of *in vitro* based conservation techniques for conservation.

A number of problems are encountered in establishing efficient *in vitro* based conservation technologies (Henshaw, 1975). These include:

- With certain species difficulties can be encountered in establishing suitable cultures.
- Difficulties in regenerating plants from certain cultures of certain species arise due to not knowing the precise nutritional requirements necessary to release the morphogenic potential of these species.

In case of sweet potato, many attempts to establish an efficient slow growth medium have failed due to strong genotypic response to modified culture medium, low survival percentage under restrictive growth conditions, or formation of callus and vitrification during storage. Despite, the shortcomings of *in vitro* conservation, it provides an opportunity to rescue germplasm currently under farmer or breeders care. These materials have lately reduced due to limited research funding and damage of the same by pests and diseases. Unfavourable weather conditions have also imparted negatively on conservation.

The addition of osmotica or growth retardants has proved efficient for reducing growth rates of different plant species (Rao and Riley, 1994; Kuo, 1991). A study was undertaken at Plant Quarantine Station with an aim of optimising medium for conservation of seven elite sweet potato genotypes. The main objectives of the study were:

1) To investigate the effects of different levels of mannitol, sorbitol and ABA on plant height, number of roots, leaves, internodes, percentage abscission, survival and effect on the phenotype of various sweet potato cultivars.

2) To investigate the effect of different levels of glyphosate supplied on the nutrient culture medium on height, roots and survival of sweet potato genotypes for five months *in vitro*.

CHAPTER 2

LITERATURE REVIEW

Sweet potatoes *(Ipomea batatas* (L.) Lam

The sweet potato (*Ipomea batata l)*, Lam was one of the first root crops to be introduced into Europe after Columbus landed in Caribbean Islands (Sauer, 1969). Today the crop remains one of the three most important root crops in the world, following potato (*Solanum tuberosum)*, and cassava (*Manihot esculenta*) (Austin, 1987). The crop is mainly grown for its underground roots. It contains approximately 20 percent starch, and 5 percent simple sugars. The sweet potato tubers contain (20-30 mg/100 g) vitamin C, 0. 8-1. 0 mg/1000 kcal of vitamin B_2 (thiamine), a substantial amount of pro-vitamin A. Deep yellow varieties have a higher pro-vitamin A content compared to white fleshed genotypes (Wang, 1982). In addition to vitamins, sweet potato roots contain substantial minerals, with potassium being most predominant at level 200-300 mg/100 g. Roots also contain 0.1 - 0.2 percent fat, 4-6 percent protein, besides a high content of most amino acids. Although sweet potato contains most amino acids, the leucine content is very low (Wang, 1982). Other parts of the crop, especially the foliar portions of the crop contain proteins, minerals and vitamins. Leaves contain 2. 7 - 3. 4 g protein per 100 g of fresh leaves, substantial amounts of pro-vitamin A, vitamin B_6 (Pyridoxine), C, E, pantothenic acid, biotin, niacin, thiamine, and a wide range of trace elements (Kays, 1992).

Use of sweet potato in food processing

Due to high starch and vitamin content tubers are now commonly used for making flour, starch, and beverages (Truong, 1991). In livestock industry, dried or raw chips are used in constituting poultry, pig and cattle feed. In poultry nutrition, sweet potato

chips can replace maize by as much as 30 percent in broiler feeds. Likewise in pig feeding dried chips can replace maize by as much as 25 percent (Yen, 1982). Cattle fed on fresh sweet potato vines produce more milk, without ill effect on health, or milk quality (Yen, 1982).

Genetic diversity of genus *Ipomea*

The genus *Ipomoea* contains twelve species, two named hybrids and one un-named hybrid. The species are; *I. batatas, I. cordatotriloba, I. cyanichifolia, I. grandifolia, I. gracilis, I. lacunosa, I. littoralis, I. cordata, I. peruviana, I. ramosissima, I. tenuissima, I. trifida, I. tiliacea and I. triloba*. One of these *I. corditotriloba* has three named hybrids viz: *I.* x *grandifolia* and *I.* x *leucantha*. The third variety is not yet named, however, sweet potato is supposedly one of its parents and the third parent is yet to be determined (Austin, 1987). The origin of *I. cordatotriloba and I. lacunosa*; is North America whereas *I. tenusissima, I. triliacea and I. triloba* are from Caribbean. Most of the other related species are found in Central and South American regions. These include *I. cyanochitolia, I.* x *grandifolia, I. peruviana, I. ramosissima* and *I. Trifida. I. littoralis* is an Asian species, mostly abundant in the Pacific and Indian Ocean regions (Austin, 1987). In spite of excellent cytological studies made over the past two and half decades (Jones, 1980), chromosome counts exist for only five species and two hybrids and hence seven species and one proposed hybrid remain unexamined (Austin 1976, 1977 and 1978). Available data suggest that within the genus *Ipomea* three ploidy levels exist; diploid, tetraploid and hexaploid. Cultivated sweet potato (*Ipomea batatas*) is hexaploid (2n=90) though it may have tetraploid races. *Ipomea triliacea* is tetraploid

while *I. cordato, I. triloba, I. lacunosa* are diploid. *I. trifida* may have both diploid and tetraploid races (Austin, 1987).

Breeding programs for sweet potatoes have existed in user countries for decades yet the original material and the current genome of these breeding programs are incredibly limited (Austin, 1978). For example, a major centre in south-western U. S. A. until recently maintained between 50 and 60 lines of sweet potato. This is in spite of availability of data suggesting a continuous introduction of 'wild' genes to sweet potato in its centre of origin. Central and South America has the greatest diversity of *I. trifida*. *I. trilotea* and *I. tiliacea* are mostly found in the Caribbean islands and the surrounding regions. Guatemala, Colombia, Ecuador and Peru constitute an area of greatest sweet potato genetic diversity (Austin 1987).

Germplasm conservation

Genetic diversity provides key solution to future food security and thus should be conserved (Christensen, 1987). The adopted conservation strategies need to consider four key issues; security offered by technology, ease at which the conserved germplasm can be accessed, applicability of the technology and the cost aspect of the technology (Jarret and Florwoski, 1990). Technology is considered efficient if it allows exchange of germplasm (Scowcroft, 1984). Germplasm is conserved either as an *in situ* or ex *situ* collection.

***In situ* conservation.**

In situ conservation involves maintenance of plants within their natural habitats (Hoyt, 1988; Perrino, 1992). It may involve, conservation of large tracts of land to

protect both plants and animals in their area of cultivation (Perrino, 1992). The major advantage of *in situ* conservation include:

(a) Allows germplasm to co-evolve with diseases, drought and pests. More genetic diversity is possible under this system than *in situ* system.

(b) Since large amounts of population genes and their complexes are conserved *in-situ* it minimises random genetic drift and inbreeding and gene sampling effects associated with small sample size characteristic of *ex-situ* conservation.

Despite the advantages offered by the *in-situ* conservation, increased human population has put significant pressure land thus making it difficult to rely on this system solely. In addition, the conserved plants are exposed to adverse weather, and pest and disease attack. Because of the limitations of *in situ* germplasm conservation, adoption of other strategies is necessary (Perrino, 1992).

Ex situ conservation

Ex situ conservation of germplasm involves collection, and conservation of seeds, plants, plant parts, tissues or cells in artificial habitat or in habitat different from original one (Hoyt, 1988; Perrino, 1992). It entails conservation in seed banks, field collections, botanical gardens, in *vitro* conservation, and gene libraries (Perrino, 1992). Seed regeneration is also part of conservation. Regeneration and re-invigoration reduce the possibility of germplasm loss through ageing (Hoyt, 1988). A breeder's collection is an excellent example. Such a collection should contain 100-1500 accessions (Huaman, 1997). The method has a disadvantage in that minimal international exchange of material is possible due to disease build up in such collections (Jarret and Florkowski, 1990). For this reason focus is now shifting to the

use of other techniques including; pathogen tested *in vitro* cultures and seed conservation (Nelson and Mantell, 1988).

Ex-situ seed conservation

Seeds are by far the most convenient parts of the plant for storage (Hoyt, 1988). Seeds of most plants withstand drying and low temperature storage without losing viability. With few exceptions, every seed has different genetic constitution. This ensures storage of a wide range of genetic variability in small samples (Perrino, 1992; Withers 1993). Seed conservation in sweet potato is now a feasible option. This follows demonstration that virtually without exception even non-flowering genotypes can, with certain treatments be induced to flower and produce seed. Flower induction protocols include: photoperiodic treatment, temperature regimes and grafting on appropriate rootstocks (Huaman, 1997). Only a few number of sweet potatoes may fail to respond to flowering pre-treatment. Despite tremendous progress in seed production technology, sweet potato seeds do not breed true-to-type. Therefore, genetic stability is ascertained when germplasm is conserved as vines in field, or by *in-vitro* cultures of meristems, and shoot tips (Jones *et al.*, 1986, Jarret and Gawel, 1991). There is also a problem of compatibility between pollen and female parents for seed formation. The advantage of *in vitro* include: mass cloning of a single species or cultivar, as well as storage under "slow growth" condition and cryopreservation (Perrino, 1992). This *in vitro* based strategy, is based on the premise that all of the genetic information in a plant is present in every vegetative cell (Hoyt, 1988).

Slow growth technology.

Slow growth technology delays reculture of conserved germplasm due to reduced growth rate. *In vitro* conservation of sweet potato is advantageous over seed banks since sweet potato seeds do not breed true-to-type (Jarret and Florlowski, 1990). In addition some sweet potato varieties are shy flowering which limits production of seeds (Onwueme and Charles, 1994; Ng and Ng, 1991). *In vitro* conservation of sweet potato involves conservation of pathogen-tested materials derived from cultured meristems and maintained through reculture of auxiliary buds (Jarret and Florkowski, 1990). Virus diseases are routinely eliminated before culture using meristem excision and thermotherapy (Frisson and Ng, 1981; Bentham and Mason, 1992; Wambugu, 1991; Dodds *et al.*, 1992; Waithaka, 1992). Conservation of *in vitro* auxiliary buds or shoot tip cultures ensures maintenance of true- to- type germplasm. Despite the advantages offered by *in vitro* techniques, the technology is yet to be applied in a wide-scale proportion (Kuo, 1991). This is attributed to lack of specific conservation protocols for different sweet potato genotypes (Jarret and Gawel, 1991). Extent of controlled growth retardation achieved together with level of maintenance of trueness-to-type *in vitro* is the yardstick of the effectiveness of the technique (Jarret and Gawel, 1991).

Growth retardation is achieved in several ways namely: use of sub-optimal culture temperature (Withers, 1992; 1993; 1985), use of sugar alcohols including, sucrose, mannitol and sorbitol (Mix 1985; Withers 1985; Jarret and Gawel 1991; Dodds *et al.*, 1991; Kuo, 1991 and Ng and Ng 1991), and use of growth regulators such as abscisic acid (ABA) (Jarret and Gawel, 1991; Withers, 1990; Kuo, 1991).

Effects of mannitol on *in-vitro* conserved germplasm

Mannitol is a non-metabolisable sugar derived from manose. It controls rate of growth by raising osmotic potential of the culture medium (Jingyu *et al.*, 1989). This property has been exploited in conservation of many crops including: cassava, sweet potato, Irish potatoes, coleus and yams (Ng and Ng 1991; Roca *et al.*, 1982, Desamero, 1990, Jarret and Gawel 1991, AVRDC 1992). For example, Jarret and Gawel (1991) reported reduction of both fresh weight and shoot height of sweet potato conserved *in vitro* in Murashige and Skoog (MS) (1962) medium supplemented with mannitol. The extent to which growth was retarded dependent on the concentration of mannitol used. Mannitol when used at 18.2 g/l or more induced distortions in stems and leaves (Jarret and Gawel, 1991). In a similar study, Bessembinder *et al.*, (1993) reported storage of a clone of *colocasia esculenta* at 24/28°C, 12 hrs photoperiod, with mannitol levels: 0, 15, 30, 45 and 60g/l. However with 45 –60 g/l many cultures had abnormal growth. No cultures conserved in medium containing 45-60g/l mannitol survived at a lower temperature of 9°C. In another study, Desamero (1990) used mannitol levels ranging from 30 - 40 g l^{-1} to conserve sweet potato genotypes "Regal" and "Jewel" for eight to 12 months. Mean survival for these cultivars ranged from 62.3% to 97.2%. In the same study, cultures maintained in medium containing 45 - 60 g l^{-1} mannitol recorded low percent survival and had distorted growth habits (Desamero, 1990).

In yam conservation MS basal medium supplemented with 30 g/l mannitol and 50 g/l^{1} sucrose delayed need for reculture in *Discorea alata* for 12-15 months (Ng and Ng 1991).

Effect of sorbitol on *in-vitro* conversation

Sorbitol like mannitol is a widely used osmoticum. It acts by increasing osmotic potential of medium thus reducing water uptake by plants (Desamero, 1990). In a study where different levels of sorbitol: 0, 5.46, 18.2, 36.4 and 54.6 g l^{-1} were used to conserve sweet potato, use of 5.46 g l^{-1} and 18.2 gl^{-1} resulted in increased plantlet height and fresh weight, relative to the control after 90 days (Jarret and Gawel 1991). However, at concentration above 18.2 gl^{-1} sorbitol induced callus formation at base of plants. In addition, sorbitol caused increased pigmentation of both stems and leaves (Jarret and Gawel 1991). In contrast, Desamero (1990) reported inability of 60-80gl^{-1} sorbitol to retard growth of two sweet potato genotypes, '*R*egal' and 'Jewel' for a period longer than one month. After one month, growth resumed and shoot height increased more than the control. She attributed this to metabolization of sorbitol by conserved sweet potato. In a another study, Lemos and Baker (1998) induced adventitious meristematic shoots from internode sections soursup (*Annona muricata*) with 1 % to 3 % sorbitol as the sole carbohydrate source. They showed that *in vitro* conserved plants, could utilise sorbitol for growth and development, in absence of sucrose.

Effects of sucrose on *in-vitro* conservation

Sucrose is usually used as a source of energy in tissue culture at a concentration of about 30 - 40 g l^{-1}. However, when used at sub-optimal concentrations growth retardation is achieved. For example, Jarret and Gawel (1991) reported that at 15 - 20 g l^{-1} sucrose caused reduction of shoot heights, number of roots and size of internodes of *in vitro* conserved sweet potatoes. Sucrose at 5 - 10 gl^{-1} resulted in

production of chlorotic plants, which had a reduced viability of 50%. They concluded that sucrose at 5 - 10 g l^{-1} was inadequate for *in vitro* conservation of sweet potato. In a related study, Villegas and Bravato (1991) were able to extend reculture time of cassava from 3 months to 12 - 18 months. They cultured single node cuttings of cassava on MS basal medium supplemented with sucrose at 120 g l^{-1} , BA at 0.02 ppm, 0.1 ppm gibberellic acid (GA_3), 0.01 ppm NAA, 100 ppm Inositol and 1 ppm thiamine - HCL. Similarly, Bannier and Steponkus (1972) reported the storage of callus tissue derived from internode section of *Chrysanthemum morifolium* for 28 days, in M.S. medium containing 10% sucrose. In addition, to reduced sucrose, temperature was raised to 27°C for 10 days after which it was reduced to 3 – 5°C for two weeks. A photoperiod of 16 hrs was provided throughout the conservation period.

Effects of reduced nutrients on *in vitro* growth

Auxillary shoot tips of tomato (*lycopersicum esculenta*) were successfully conserved in a sub-optimal MS basal medium of 75% for 1 year on MS medium containing 50g l^{-1} of sucrose. Temperature of 30/20°C (Day/night) was maintained (Schnapp and Preace; 1986). Similarly, the reculture time of *Xanthosoma sagithifoum* plantlets was extended from 3 months to 1 year by half strength medium. The cultures were incubated at 29°C (Acheampong and Henshaw, 1986). The cultures were raised on Whites (1934) medium.

Effects of glyphosate on plant growth

Glyphosate (N-Phosphomethyl glycine) is an organo-phosphorous derivative of amino acid glycine (Fletcher and Kirkwood, 1982). The term glyphosate is generally used to

indicate both the acid and its salts, since it is commonly recognized the salt and the ionic forms are biologically equivalent (Frantz, 1984). Glyphosate crystal is a zwitterion, $HO_3P\ CH_2\ NH_2+\ CH_2COOH$, whose molecules are connected by hydrogen bonds (Knuuttila and Knuuttila, 1984).

Glyphosate exhibits many unique biological activities. It is a broad - spectrum non-selective, post emergent herbicide (Frantz, 1984; Fetcher and Kirkwood 1982). Glyphosate is generally non-selective when applied to foliage (Sprankle *et al.*, 1975; Rueppel *et al.*, 1977) and when applied post- emergent, it shows no pre- emergent or residual soil activity, because it is tenaciously bound to soil particles. In soil it is readily metabolized to produce the plant nutrients; phosphoric acid, ammonia and carbon dioxide (Sprankle *et al.*, 1975; Rueppel *et al.*, 1977).

The most important characteristic of glyphosate is its rapid translocation from foliage of treated plants to the roots, rhizomes, and apical meristems. It is highly mobile in both xylem and phloem tissues. This systemic property results in total destruction of hard-to-kill perennial weeds (Frantz, 1984; Fletcher and Kirkwood, 1982).

Sprankle *et al.*, (1975) showed that application of 56 kg ha^{-1} glyphosate did not reduce fresh weight of wheat (*Triticum aestivum*) grown on either clay or muck soils. In contrast, Rodrigues *et al.*, (1982) reported that the exudates from roots of wheat that had received a foliar spray of glyphosate were able to inhibit root formation and also cause foliar injury symptoms in seedlings of maize (*Zea mays*) grown in the same soil. Similarly, Salazar and Appleby (1982) observed reduced growth in bent grass

(*Agrostes tenuis 'sibthhighland*'; Lucerne (*Mendicago sativa* (*L.*) *'Vernal'*) and red clover (*Trifolium pratense* (*L.*) *Kestar*) as a result of residual activity of glyphosate application at 1 - 3.4 kg/ha, applied in the field the previous season.

Quility and Geoghegan (1976) attributed the non-residual activity of glyphosate in peat soils to its degradation by fungal micro-organisms and they were able to stop this degradative activity by treating the soil with antifungal chemicals. Addition of chloramphenicol (an antibacterial compound) stimulated degradation of glyphosate. A strain of *pseudomona spp* that can utilize the phosphate moiety of glyphosate has been identified (Moore *et al.*, 1983).

Mode of action of glyphosate

Glyphosate is a competitive inhibitor of 5-enolpyruryl-shikimate-3-phosphate synthase (EPSP) (Armheim *et al*; 1980), an enzyme of shikimic acid pathway. This pathway is responsible for the synthesis of aromatic amino acids phenylalanine, tyrosine and trytophan. The pathway links primary metabolism and secondary metabolism initiated by condensation of phosphoenolpyruvate (PEP) with erythrose-4-phosphate (Cole, 1984). Phenylalanine and tyrosine products of EPSP feeds into phenylalanine-ammonia-lyase (PAL) to produce a diverse array of phenolic products such as lignin precursors, flavins and tannins (Cole, 1984).

Exogenous supply of phenylalanine, tyrosine and tryptophan demonstrated alleviation of glyphosate induced inhibition in *Arabidopsis thaliana* (Greshoff, 1979). This has also been demonstrated in micro-organisms and cultured plant cells (Jaworski, 1972; Davis and Harvey, 1979). There are a few exceptions to the above observation. For

example, Duke and Hoagland (1981) were able to reverse the damage caused by glyphosate on Soya beans (*Glycine max.*), though roots readily absorbed ^{14}C labelled phenylalanine and tyrosine. This suggests that, glyphosate may cause toxicity through other mechanisms too (Cole, 1984). Plants that appear tolerant to glyphosate allow limited translocation of same (Boerman *et al.*, 1990). Decomposition of glyphosate to amino phosphonate, glyoxylate, carbon dioxide and organic phosphate is possible (Coupland, 1985).

Sub-lethal levels of glyphosate have been implicated in inhibition of auxins. For example, Baur (1979) demonstrated inhibition of basipetal transport of C^{14} indole-3-acetic-acid (^{14}C-1AA) from auxin pre-treated blocks to excised maize shoots. In a separate study, tobacco calli pre-treated with 2×10^{-4} M l^{-1} glyphosate showed enhanced ability to metabolize ^{14}C - 1AA (Lee, 1982). In another study sub-lethal levels of glyphosate caused multiple tillering as a result of impaired auxin transport (Bovey, 1977). Destruction of auxin may also occur due to light induced photo-destructive processes (Cole, 1984).

Glyphosate has been reported to destroy chlorophyll in wheat and maize (Kitchen *et al.*, 1981). There seems to be a relationship between loss of chlorophyll and manufacture of aromatic amino-acids, though the mechanism is not understood well (Cole, 1984). The above inhibitory activities of glyphosate gives it wide applications in weed control and germplasm conservation. Xin, (1988) conserved sweet potato in MS basal medium containing glyphosate at 1 mg l^{-1}. The study reported a high survival rate of 70%.

Effects of abscisic acid on plant growth

ABA is a naturally occurring plant hormone, which has been implicated in initiation, and maintenance of embryo dormancy in seeds (Bewly and Black, 1994). It is also implicated in other physiological processes such counteracting gibberellic acid (GA_3) effects (Bewly and Black, 1994). In germplasm conservation, abscisic acid acts by slowing or curtailing cell division, depending on concentration used (Jarret *et al.*, 1992). Plants degrade ABA first to form two trans-isomers, which are further metabolized to glucose. The tetra-acetate glucose isomer of ABA, esterifies in acidic methanol to form abscisic acid, and glucose (Milborrow, 1974). There is evidence that dormancy resulting from ABA is dependent on its concentration in the plant and day-length conditions. For example, in onion plant ABA concentration increases under long- day conditions. Reversal of day length conditions has the opposite effect (Eagles and Wareing, 1964). Due to its inherent physiological properties, ABA is now widely used for *in-vitro* conservation of crop germplasm. Similarly, Jarret and Gawel (1991) used ABA at: 0, 0.01, 0.1, 0.5, 1.0 and 10 mgl^{-1} to conserve sweet potato *in vitro* over a three-month period. Plant growth retardation was dependent on concentration. Retardation of upto 82% was achieved with 0.01 mgl^{-1}. No new growth was observed with MS medium containing ABA at 10 mgl^{-1}. In another study, 2.5 - 5 mgl^{-1} ABA prolonged interval between sub-cultures from 3 months to 12 months in sweet potato genotypes, "Jewel" and "Regal" (Desamero, 1990). Similarly, ABA in the range of 5-10 mgl^{-1} prolonged *in vitro* storage time of Irish potatoes up to 12 months (Henshaw *et al.*, 1980). In addition, Roca *et al.*, (1983) reported improved storage of cassava genotypes, M Col 22 and M Col 1467 cultured on MS basal medium supplemented with 2. 5 mg/l -10 mgl^{-1} ABA.

Effects of reduced temperature on plant growth

Reduction in growth room temperature reduces frequency of reculturing in plants through its action on cell division (Chopra and Narasimulu, 1989). Jarret and Gawel (1991) established that low temperatures suppress auxiliary bud development in sweet potato. In their study, temperatures between 15.6 to 21.1°C resulted in growth reduction of 50 % in cultivar 'Jewel'. Temperatures lower than 15.6°C were injurious to conserved sweet potato. No recoveries were made on cultures conserved below 15.6°C, after 3 months. Their results confirmed earlier observations that temperatures lower than 15°C were lethal for growth of sweet potato (Withers, 1985).

Other tropical crops such as banana and plantain, have been successfully conserved *in vitro* for 12-15 months in MS basal media at 15°C (Banerjee and DeLanghe, 1985). Strawberries have also been continuously conserved at 4°C for 6 years without reduction in regenerative ability. In another study, Galzy (1969) reported conservation of plantlets of strawberry for 6 years, in liquid MS media at temperature of 1°C or 4°C in darkness. The plants were periodically replenished with 1 - 2 drops of similar medium. Dale (1980) conserved *Lolium spp, Dactylis glomerata, Festaca spp* and *Phleum spp* for 1 year instead of 2 months at incubation of 2 - 4°C. Light intensity of 300 lux and a photo-period of 8 hours were also provided. Shoot tips of *Beta vulgaris* have also been successfully conserved for more than 18 weeks, at temperature of 12°C (Miedena, 1982). In another study, Lundergan and Janick (1979) conserved apple shoot-tips for up to 12 months at a reduced temperature of 1 -

4°C. Similarly, leaf sheaths of sugarcane have been successfully maintained *in vitro* for 6 months, at temperature of 0 - 4°C (Withers, 1980).

Temperatures lower than 0°C have also been used to conserve germplasm. Freeze preservation, (cryopreservation), or storage of cultures of germplasm at - 196°C, is now commonly used. These sub-zero temperatures effectively suspend metabolism thus curtailing growth (Withers, 1990; Towill, 1991). Long term preservation of cultures at sub-zero temperature is possible if the cultures are not damaged during storage and removal from liquid nitrogen (Withers, 1990; Withers, 1993). To-date protoplasts, cell suspensions, meristems/shoot tips and embryos have been successfully been put under liquid nitrogen (Withers 1990, 1993; Towill, 1991). However, despite the potentials of the method, problems of cell injury are still common (Withers, 1990). Protoplasts and suspension cultures have less cell injury complications compared to meristems and shoot tips. Cell injury can be greatly reduced if cultures are properly pre-conditioned with cryoprotectants. Cultures are put in normal multiplication media with 5-10% proline or 3-6% mannitol for 7 days. Chances of survival are enhanced by subjecting preconditioned cultures to a treatment solution containing 1 molar dimethyl-sulphoxide (DMSO) and 2% sucrose and 2% proline (Withers, 1990). Cultures are cooled slowly at -1°C per minute up to -35°C after which they are held at this state for 40 to 80 minutes. The cultures are subsequently plunged into liquid nitrogen (Withers 1990; Withers 1985). Recovery is achieved by rapid warming in warm water at 40°C, followed by transfer into normal multiplication media (Withers, 1990).

There are variations to the procedure depending on culture type for example, Golmirzaie and Panta (1997) were able to conserve potato shoots using a vitrification protocol. In this protocol, shoot tips of 1.5 cm long derived from young *in vitro*

plants were excised and transferred into modified MS medium supplemented with 0.04 mg/l gibberellic acid and 0.09M sucrose for 24 hours. Shoot tips were later transferred to the same medium but with sucrose reduced to 0.06M and incubated for 5 hours at room temperature. The shoot tips were subsequently transferred into a medium containing 50: 15: 16 ethylene glycol: sorbitol: bovine serum albumin for 50 minutes, before transfer into liquid nitrogen. Recovery from liquid nitrogen was made possible by transfer of shoot tips to 1.50 molar solution of sorbitol for 30 minutes. Transfer of shoot tips thereafter into MS medium containing 0.04 mgl^{-1} kinetin, 0.1 mgl^{-1} gibberellic acid and 25 mg/l sucrose enabled full recovery.

Effect of ethylene on plant growth.

Ethylene is the principle cause of leaf abscission and culture die back, especially if gaseous exchange is limited (Garcia and Einset, 1982; 1983). Ethylene is produced during culture of all cells, tissues and organs. The rate is increased if cultures are subjected to stress for example mannitol or toxic levels of ammonia (Garcia and Einset, 1982; 1983). Ethylene production in cultured suspension cultures is reported to increase to reach maximum levels at stationary phase when nutrients become limiting (Gamborg and LaRue, 1971).

Genetic stability of *in-vitro* maintained sweet potato

The principal objective of a clonal germplasm collection is to maintain intact, specific gene combinations. Thus a good technique must ensure preservation of genetic and physiological integrity of material (Desamero, 1990). For this reason, conservation methods are thus judged valid if genetic and physiological stability is maintained. The type of ex-plant used can greatly affect the quantity and type of variation produced.

Culture of callus and protoplasts are known to induce varying degrees of genetic variability (Scowcroft and Larkin, 1982). Meristems and shoot tips are said to be much stable (Dodds *et al.*, 1991; Kuo; 1991). However, there are reports that plants regenerated from shoot apex may occasionally include variants (Lakhanpaul *et al.*, 1990). In view of the importance of genetic stability there is need to assess stability after storage. Genetic stability can be assessed by use of phenotypic or genotypic markers (Potter and Jones, 1991). Study of stability involves both morphological and biochemical markers. Assaying for changes at DNA level may involve study of Restriction Length Polymorphism's (RFLPs), Random Amplified Polymorphic DNA (RAPDs), amplified Fragment Length Polymorphism or microsatellites (small sequence repeats, SSR).

Morphological markers

Phenotypic markers have long been the main criteria used in variety identification. Morphological markers classifies plants based on quantitative and qualitative characters, such as plant height, leaf shape, flower colour, tuber shape, and pest resistance (Potter and Jones, 1991). Both cycocel (CCC) and mannitol cause detectable qualitative variations in leaf size and petiole sizes of *in vitro* conserved sweet potato (Jingyu *et al.*, 1989). However, these are not genetic changes because plants do not show these changes after several generations (Jingyu *et al.*, 1989). In contrast, Desamero (1990), did not report morphological abnormalities, in plantlets conserved MS media containing mannitol and sorbitol for 8 months. Similarly, Jarret and Gawel (1991) reported carry-over of morphological distortions (loss of apical dominance, shortened internodes) in sweet potato. Morphological markers are

disadvantageous because of environmental influence. Thus, there is need to use more reliable techniques to monitor genetic variation (Jarret and Gawel, 1991).

Biochemical and Molecular Markers

Protein composition is widely used as a biochemical marker (Denton *et al.*, 1977). Soluble proteins extracted from different genotypes or tissues are extracted and compared on basis of size (molecular weight) and conformation (Potter and Jones; 1991). Resolution is obtained by using electrophoresis in different buffer systems. Proteins are visualized by staining with stains such as coomassie blue, or silver stains in case of total proteins. Isozymes (specific proteins) are identified by specific stains, or specific antibodies. In sweet potato, isozyme of five enzyme systems: acid phosphatase, esterases, peroxidases, malate dehydrogenase and superoxide dismutase were investigated (Lakhanpaul *et al.*, 1990; Were and Gudu, 1999). Esterases and superoxides dismutase (SOD) gave identical patterns in *in vitro* regenerated plants regenerated with or without intervening callus phase. Banding patterns were also similar to those of *in vivo* propagated mother plants indicating that the loci coding for these isozymes were not changed by culture conditions. However, acid phosphatase, peroxidase and malate dehydrogenase (MDH) showed qualitative and quantitative differences. For example peroxidase gave similar pattern for both field grown sweet potato and those grown *in vitro* without intervening callus phases. In case of acid phosphatase, more bands we detected in field grown potato compared to *in vitro* regenerants. Likewise in MDH, field grown plants gave one band whereas *in vitro* regenerated plants 3 bands, with variation in number for those regenerated from the callus phase.

Assaying for stability of secondary metabolities such as alkaloid production, and gas evolution is more common in cryopreserved germplasm (Benson and Withers, 1987) and industrial plant cell culture (Fowler, 1984). Like other morphological markers, protein analysis is limited in application because resultant electrophoretic patterns represent a tiny fraction of certain gene products of the entire genome with good protein conservation (Dodds *et al.;* 1992). For this reason, the focus is on DNA finger- printing techniques based on Restriction Length Fragment Polymorphism (RFLP) and Random Amplified Polymorphic DNAs (RAPDs), amplified length Polymorphism (ALFP) and small sequence repeats (SSRs) or microsatelites. DNA fingerprinting offers opportunity to compare and contrast effects of growth retardant regimes as they affect/alter the physiology of tissue that make comparison by phenotypic methods not possible (Potter and Jones, 1991). Molecular approaches can allow marking of DNA sequences governing important plant characteristics providing hope that fingerprinting of accessions can be done routinely at gene level in future (Withers, 1993). DNA fingerprinting can only complement morphological and biochemical assessments (Withers, 1990).

Recent studies have shown that the greater the morphological differences in accessions, the greater the difference in DNA fingerprints. Zhang *et al.*, (1997) used RAPD to assay 66 suspected sweet potato duplicates in Brazil and Bolivia, used a set of 24 primers and produced 164 polymorphic fragments. The DNA finger- prints showed that accessions with great morphological differences equally differed in the DNA bands (Zhang *et al.*, 1997).

CHAPTER 3

MATERIALS AND METHODS

Introduction

Conservation of genetic diversity of crops may be through seed storage; or vegetative means either in field or *in vitro*. *In vitro* conservation may use organized parts of plants such as shoot tips, meristems or undifferentiated parts such as callus and embryos. *In vitro* storage involves cryopreservation or slow-growth. The reported study was geared to development of a slow-growth protocol using auxiliary shoots suitable for use in Kenya. The study was organized in form of two experiments. Two osmotica (mannitol and sorbitol), one growth regulator (Abscisic acid) and one herbicide (glyphosate) were studied.

Sweet potato varieties

The study used local and exotic clones. Four accessions: Lohafinjo, IITA-TIS-3290, NC-1582, and IRA-048 (CIP accessions 440391, 440068, 440094 and 440143) were sourced from International Potato Center (CIP) and three local varieties (KSP 20, Nyaluolo and CPT 560) were in included in the study. Based on preliminary study done at the Plant Quarantine Station, Muguga, all the seven clones were established to require sub culturing after every two months.

All the seven clones were confirmed virus free by both serological and indicator test plant techniques developed at Asian vegetable research and development center (AVDRC) (appendix 2).

Multiplication culture Medium.

The media consisted of Murashige and Skoog salts (Appendix 1) supplemented with 30 g/l sucrose, 100 mg/l inositol, 100 mg/l ascorbic acid, 100 mg/l calcium nitrate, 100 mg/l

Arginine, 0.4 mg/l Thiamine-HCL and 20 mg/l putrescine-HCL. pH was adjusted to 5.7 ± 0.01 with either 0.1 percent N HCL or 0.1 percent N NaOH. The media was solidified with 2.5 g/L phytagel. The medium was boiled until phytagel dissolved. 10 ml aliquots were dispensed into universal media bottles. The media was finally autoclaved at 15 pounds per square inch (Psi) for 15 minutes. Autoclaved media was allowed to cool in sterile environment after which it was ready for use.

Preparation of explants materials

Ten (10) centimeter cuttings of pathogen tested plants were prepared for *in vitro* culture as follows: - cuttings were taken from the field and immediately put into clear plastic bags pending their delivery to the tissue culture laboratory at the Plant Quarantine Station, Muguga. In the laboratory, leaves were removed with clean surgical blades. Disinfection of cuttings commenced thereafter. Cuttings were thoroughly washed in running tap water to remove any soil particles. Cuttings were further washed with tap water to which 5 drops of Tween 20 (20 Polyoxyethylene; 20 sorbitan monolaurate) from BDH laboratories was added. After rinsing off the soap, sterilization was continued by addition of 70 percent ethanol and shaken for one minute, after which the ethanol discarded. These cuttings were finally sterilized with 10 percent Sodium hypochlorite for 10 minutes. The explants were thoroughly washed to remove the disinfectant with distilled water under lamina flow hood. Ex-plants, thus prepared, were left in the fifth, final rinse.

***In vitro* culture and growth conditions**

Sterilized explants were carefully cut into single node cuttings under lamina flow hood at the tissue culture laboratory of Plant Quarantine Station, Muguga. Single node cultures were inoculated into sterile media. Each media bottle was inoculated with single node and

incubated in growth room fitted with cool Phillips® fluorescent bulbs. Light intensity (approximately 1000 lux units) was maintained. Photoperiod of 16 hours day length and 8 hours at night was provided. Growth room temperature averaged 26 - 32°C during the day and 18-22°C during the night.

Experiments and experimental combinations.

The study was organized as two separate experiments. For the first experiment effect of five genotypes and seven growth retardants were studied *in vitro* using a completely randomized design (CRD). Genotypes were used as the main plots and retardants as the sub plots. The five genotypes studied were: NC-1582 (440094), Lohafinjo (440391), IRA-048 (440143), CPT560, and KSP-20. The seven media treatments were designated: SW1, SW2, SW3, SW4, SW5, SW6 and SW7. The medium contained Murashige and Skoog (1962) basal medium components plus sucrose (30 g/1), myo- inositol (100 mg/l), ascorbic acid (100 mg/l), thiamine. HCL (0.4 mg/l), calcium nitrate (100 mg/l), putrescine. HCL (20 mg/l) and phytagel (2.5 g/l). SW2, SW3, SW4, SW5, SW6 and SW7 contained 4% mannitol, 6% mannitol, 2% sorbitol and 2% mannitol combined and ABA at 0.5, 1.0, and 2.0 mg/l) respectively while the control (SW1) contained no growth retardant.

Single node cuttings of sweet potato genotypes NC-1582 (440094), IRA-048 (440143), CPT560, Lohafinjo (440391) and KSP20 were cultured *in vitro* in SW1, SW2, SW3, SW4, SW5, SW6 and SW7 medium. Each genotype was replicated ten times in each of the seven medium treatments. A total of three hundred and fifty cultures were made (5 genotypes x 7 treatments x 10 replications).

In second experiment two genotypes namely; Nyaluolo (local) and IITA-TIS-3290(440068) and three medium treatments namely: SG1, SG2 and SG3 were utilized. The medium was same as experiment one but with different levels of glyphosate. SG1 did not contain any

growth glyphosate (control), whereas SG2 contained 5mg/l glyphosate, and SG3 contained 1mg/l glyphosate. All the tissue culture media were autoclaved at a temperature of 121°C and a pressure of 15 pounds per square inch (P.s.i.) for 15 minutes. Each genotype was replicated ten times in each of the three medium treatments. A total of sixty cultures were made (2 genotypes x 3 media treatments x 10 replications).

Data collection: Experiments one and two

Plant height:

Plant height of individual *in vitro* plants was measured at months 1,2,3, 4 and 5 using a ruler. Plant height was taken on all the cultured plants for all treatments.

Number of roots.

Number of roots in all the cultured plants were counted at month 1 and 2 of *in vitro* conservation. Number of roots per replicate were entered in the computer. Data analyzed as two-way analysis of variance. Where interactions of genotypes x growth retardation was significant, simple effects were used to present results.

Number of leaves and nodes

All replicates were used. Total number of leaves and internodes per replicate were counted after months 1 and 2 of *in vitro* culture.

Percentage leaf abscission

Abscised leaves in each replicate were counted after five months. Number of leaves were compared with the control. Abscised leaves were converted into percentage of total number of leaves in each replicate. The percentage data was transformed to arcsine prior to analysis.

For arcsine transformation, value of 0 % was replaced with ¼n and 100% with 100 - ¼ n where n was total number of units upon which percentage data was based (i.e., the denominator used in percentage (Gomez and Gomez, 1984).

Percentage survival of *in vitro* conserved sweet potato.

Number of surviving plants per treatment were recorded after eight months of *in vitro* culture and calculated as percentage of the initial plants.

Effect of growth retardants on % rejuvenation and phenotype.

Two plants of each genotype for every growth retardation medium were removed and cultured in retardant free medium for two months, and thereafter potted in sterile soil for an extra two months. A visual observation was done on size of leaves, petioles and pigmentation of stems.

Data analysis

Data for all parameters except the effect of retardants on the phenotype was analyzed using MSTAT computer package, for a two factor complete randomized design. Where genotype x growth retardation treatments were significant, simple effects rather than main effects were used and results presented. Mean separation was by Duncan Multiple Range Test ($p = 0.05$).

CHAPTER 4

RESULTS

Effects of genotypes and growth retardants on plantlet height

The effects of the genotypes on growth retardation was highly significant at months 1, 2, 3, 4, 5, $p = 0.01$ (Table 1). At months 4 and 5 clone KSP20 was the most retarded, while NC 1582 was least retarded. For example at month 5 clone KSP 20 had mean height of 17.49 mm, while NC 1582 had a height of 28.96 mm (Table 2).

Effects of growth retardant responses were also highly ($p = 0.01$) significantly different at months 1, 2, 3, 4, 5 (Table 1). The mean plantlet heights for the seven growth retardants were also significantly different, ($p = 0.05$) at month 5 (Table 3). After 1 month of *in vitro* culture ABA at 2.0 mg/l caused the most reduction in height, as it had mean plant height of 2.06 mm compared to control with 23.36 mm. After months 3, 4 and five, 4% mannitol, 6% mannitol, 1.0 mg/l ABA and 2.0 mg/l ABA caused lowest growth rates (Table 3). At five months ABA, at 2.00 mg/l with a mean height of 5.80 mm caused the greatest growth retardation. Mean heights for the treatments were: 7.86 mm (6% mannitol), 11.46 mm (4% mannitol), 12.20 mm (1.0 mg/l ABA), 30.88 mm (0.5 mg/l ABA), and 43.88 mm (2% mannitol + 2% sorbitol). The control with a height of 53.08 mm caused the least retardation (Table 3). Interactions between genotypes and growth retardants were highly significant ($p = 0.01$) throughout the study period (Table 1, Plates 1a and 1b). Because interactions between genotypes and growth retardants were significant the results were analyzed to separate main and simple effects.

Table 1: Two way analysis of variance with mean squares for height in five sweet potato genotypes as affected by seven growth retardant levels.

Source	Df	Time in months and mean squares				
		1	2	3	4	5
Genotypes(G)	4	366.20**	459.20**	855.81**	1017.44**	1478.48**
Growth Retardant(R)	6	2734.64**	8741.98**	13795.60**	16659.98**	19138.71**
G x R	24	106.33**	23.83**	454.8788	421.24**	461.92**
Error	315	50.23	110.99	165.23	178.90	183.28
	CV	100.44	82.19	68.64	61.44	56.69

- Data analyzed on monthly interval up to 5 months. Each treatment replicated 10 times

- Significant in LSD (p = 0.05)
- ** Highly Significant in LSD (p = 0.01)

- Table 2: Main effects of growth retardants on mean plantlet heights of five sweet potato clones 1, 2, 3, 4 and 5 of *in vitro* storage.

Clone	Mean plant height in millimeters, measured at end of each month up to five months				
	1	2	3	4	5
NC 1582 (440094)	9.56a	16.42a	23.54a	25.58a	28.96a
IRA 048(440143)	4.84b	9.99b	16.41b	20.69b	23.67c
CPT 560(LOCAL)	5.08b	7.28bc	11.12bc	20.79d	21.79d
LOHAFINJO(440391)	9.39a	14.27ab	21.17ab	25.69b	27.51b
KSP 20(LOCAL)	6.42b	12.29bc	15.22bc	16.66e	17.49e
LSD	3.50	4.28	5.26	5.26	5.45

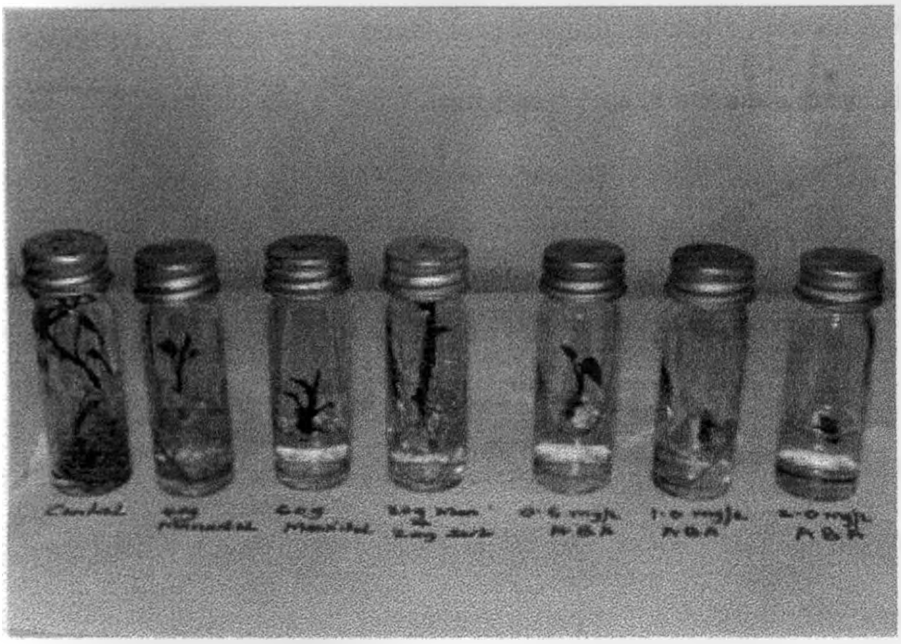
Means within a column with similar letters are not significantly different, LSD (p = 0.05).

Table 3. Main effects of growth retardants on mean plant height of five genotypes sweet potato after months 1,2, 3, 4 and 5 months of *in vitro* storage

Treatments	Storage time in months and plant height (mm)				
	1	2	3	4	5
Control	23.6a	39.84a	49.00a	53.22a	53.08a
4% mannitol	4.10cd	5.83d	8.82d	10.05d	11.46e
6% mannitol	3.01d	4.49d	6.17d	7.58d	7.86f
2% mannitol + 2% sorbitol	7.01b	19.60b	31.16b	39.00b	43.88b
0.5 mg/l ABA	6.16bc	11.61c	20.50c	25.86c	30.88c
1.0 mg/l ABA	3.60cd	5.59d	9.19d	11.63d	12.20d
2.0 mg/l ABA	2.06d	2.77d	5.34d	5.80d	5.80g
LSD (p= 0.05)	2.79	5.06	5.06	8.08	0.65

Means within a column with similar letters are not significantly different, LSD, p= 0.05.

Plate 1a: Effects of concentrations of growth retardants on mean plantlet height in clone Lohafinjo (440391), after 3 months of culture.



Left to Right Control, 4% mannitol, 6%mannitol, 2% mannitol combined with 2% sorbitol, 0.5mgL^{-1} ABA, 1.0mgl^{-1} ABA, 2.0mgl^{-1} ABA.

Plate 1b: Effect of concentrations of growth retardants on mean plantlet height in clone CPT 560 after 3 months of *in vitro* culture.

Left to Right Control, 4% mannitol, 6%mannitol, 2% mannitol combined with 2% sorbitol, 0.5mgL^{-1} ABA, 1.0mgl^{-1} ABA, 2.0mgl^{-1} ABA.

Simple effects of growth retardants on plant height in clone NC-1582

Simple effects of growth retardants on mean plantlet heights of clone NC-1582 (440094) after one month of *in vitro* storage ranged from 2.0 mm for ABA at 2.0 mg/l to 34.3 mm for the control (Table 4, Figure 1). All growth retardants significantly reduced growth relative to the control, p = 0.05. ABA at 2.0 mg/l was the best growth inhibitor, although the difference in height between 2.0 mg/l and 1.0 mg/l were not statistically different (p = 0.05). Similarly ABA at 0.5 mg/l did differ significantly from 6 % mannitol. Two percent mannitol combined with 2% sorbitol was the weakest inhibitor, followed by 4% mannitol.

Simple effects of growth retardants on plant height in clone NC-1582 after month 2 of *in vitro* culture ranged from 2.25 mm for ABA at 2.0 mg/l to 48.9 mm for the control. All growth inhibitors significantly retarded growth compared to the control. The weakest inhibitor was 2% mannitol combined with 2% sorbitol, whereas 2.0 mg/l ABA was the most effective. Except for 2% mannitol combined with 2% sorbitol which was a weak inhibitor the rest of the other retardants did not differ significantly (p = 0.05) from one another (Table 5, Figure 2).

The simple effects of growth retardants over NC 1582 on plant height after three months of storage ranged from 3.4 mm for ABA at 2.0 mg/l to 60 mm for the control. All growth retardants significantly retarded growth relative to the control, p=0.05. 2.0 mg/l ABA was the most effective inhibitor. However, 2.0-mg/l ABA, 1.0 mg/l ABA, 4% mannitol and 6 % mannitol were not significantly different from one another (Table 6, Figure 3).

After month 4 of conservation simple effects for height in clone NC-1582 ranged from 3.5 mm for ABA at 2.0 mg/l to 60 mm for the control. All growth retardants except 2 % mannitol combined with 2 % sorbitol significantly retarded growth compared to the control. ABA at a concentration of 2.0 mg/l was the most effective inhibitor. ABA at 2.0mg/l, 1.0 mg/l ABA, 4

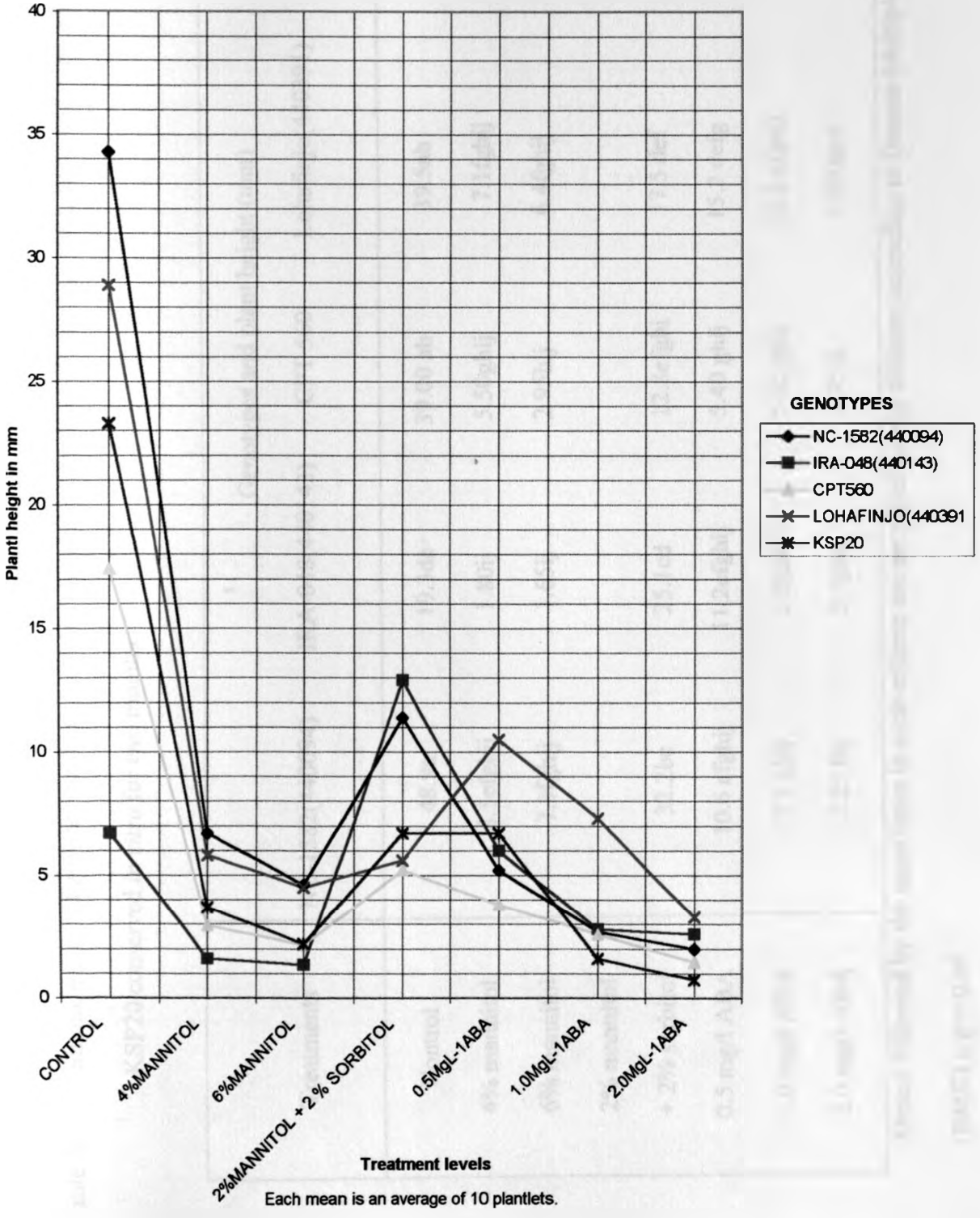
Table 4: Simple effects of growth retardants on mean plant height (mm) of clones NC-1582, IRA- 048, CPT 560, Lohafinjo and KSP20 conserved *in vitro* for one month.

Treatments	Genotypes and plant height (mm)				
	NC-1582(440094)	IRA-048(440143)	CPT 560	Lohafinjo(440391)	KSP 20
Control	34.3 a	6.6 hi	17.4 d	28.9 b	23.3 c
4% mannitol	6.7 hi	1.60 qr	2.95 nop	5.80 ij	3.7 mn
6% mannitol	4.6 kl	1.35qr	2.15 pq	4.50 klm	2.2pq
2% mannitol + 2% sorbitol	11.4 f	12.9 e	5.2 jk	5.6 j	6.7 hi
0.5 mg/l ABA	5.2 jk	6.0 ij	3.8 lmn	10.5 g	6.7 hi
1.0 mg/l ABA	2.7 op	2.8 op	2.6 op	7.3 h	1.60 qr
2.0 mg/l ABA	2.0 pq	2.6 op	1.45 qr	3.3 no	0.75 r

Means followed by the same letter in each column are not significantly different according to Duncan Multiple Range Test (DMRT), p = 0.05

Figure 1

Simple effects of growth retardants on mean plant height of five sweet potato genotypes: (NC-1582,IRA-048, CPT- 560, Lohafinjo and KSP20) maintained *in vitro* for one month.



Each mean is an average of 10 plantlets.

Table 5: Simple effects of growth retardants on mean plant height (mm) of clones NC-1582, IRA- 048, CPT 560, Lohafinjo and KSP20 conserved *in vitro* for two months

Treatments	Genotypes and plant height (mm)				
	NC-1582(440094)	IRA-048(440143)	CPT 560	Lohafinjo(440391)	KSP 20
Control	48.9a	19.3de	39.00 ab	39.5ab	46.7a
4% mannitol	8.5efghij	1.80ij	5.50ghij	7.1fghij	6.4fghij
6% mannitol	7.3fghij	1.65ij	2.95hij	6.4fghij	4.0ghij
2% mannitol + 2% sorbitol	32.2bc	25.1cd	12.8efghi	17.5 def	13.4 efgh
0.5 mg/l ABA	10.6 efghij	11.2efghij	5.40 ghij	15.2 defg	13.2 efgh
1.0 mg/l ABA	5.2 ghij	5.8ghij	5.35 ghij	10.2 efghij	1.35 ij
2.0 mg/l ABA	2.25 hij	5.1ghij	1.75 ij	4.00 ghij	0.75j

Means followed by the same letter in each column are not significantly different according to Duncan Multiple Range Test (DMRT), p = 0.05

Figure 2 Simple effects of growth retardants on height of sweet potato genotypes: NC-1582, IRA-048, CPT 560, Lohafinjo and KSP20 maintained *in vitro*.

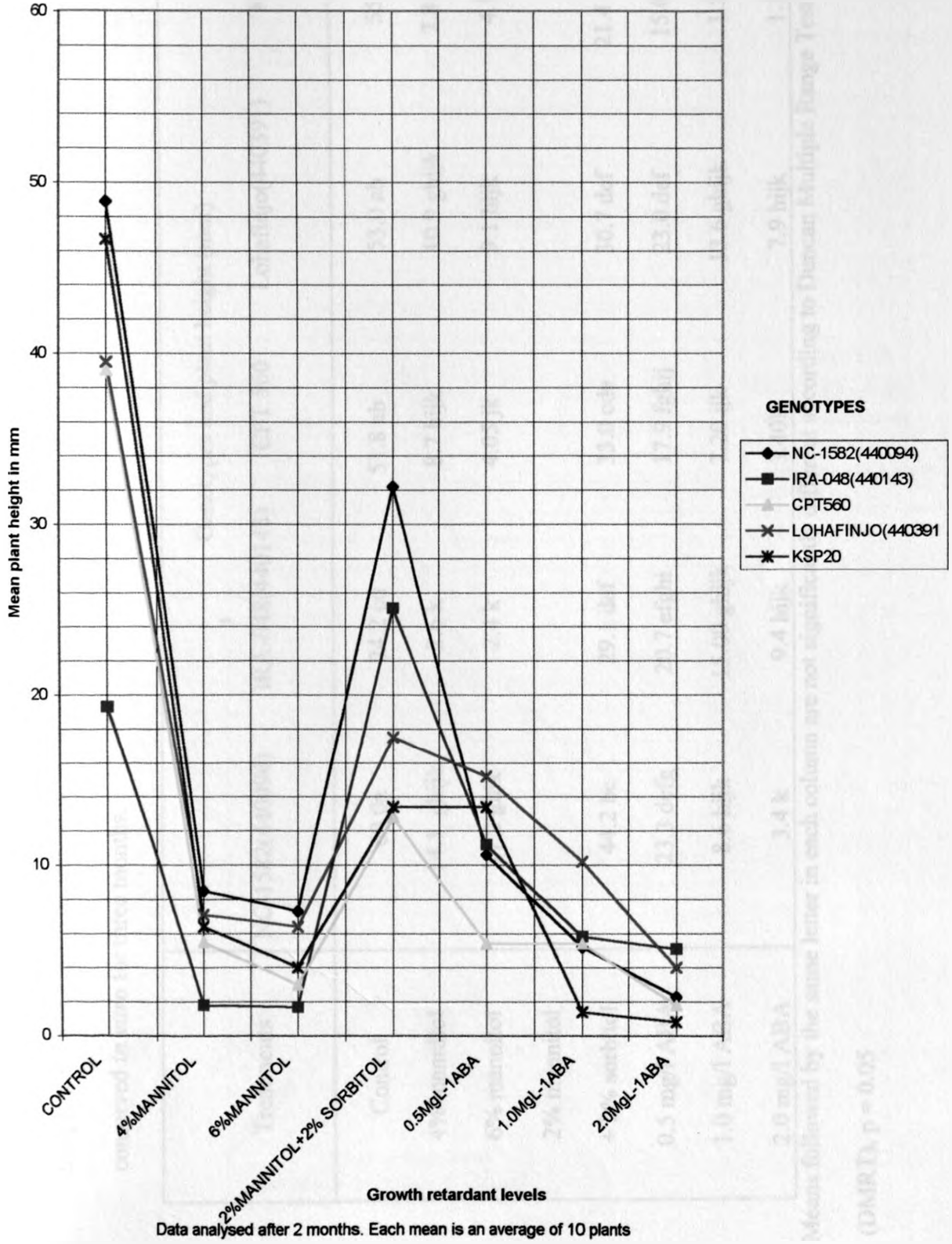
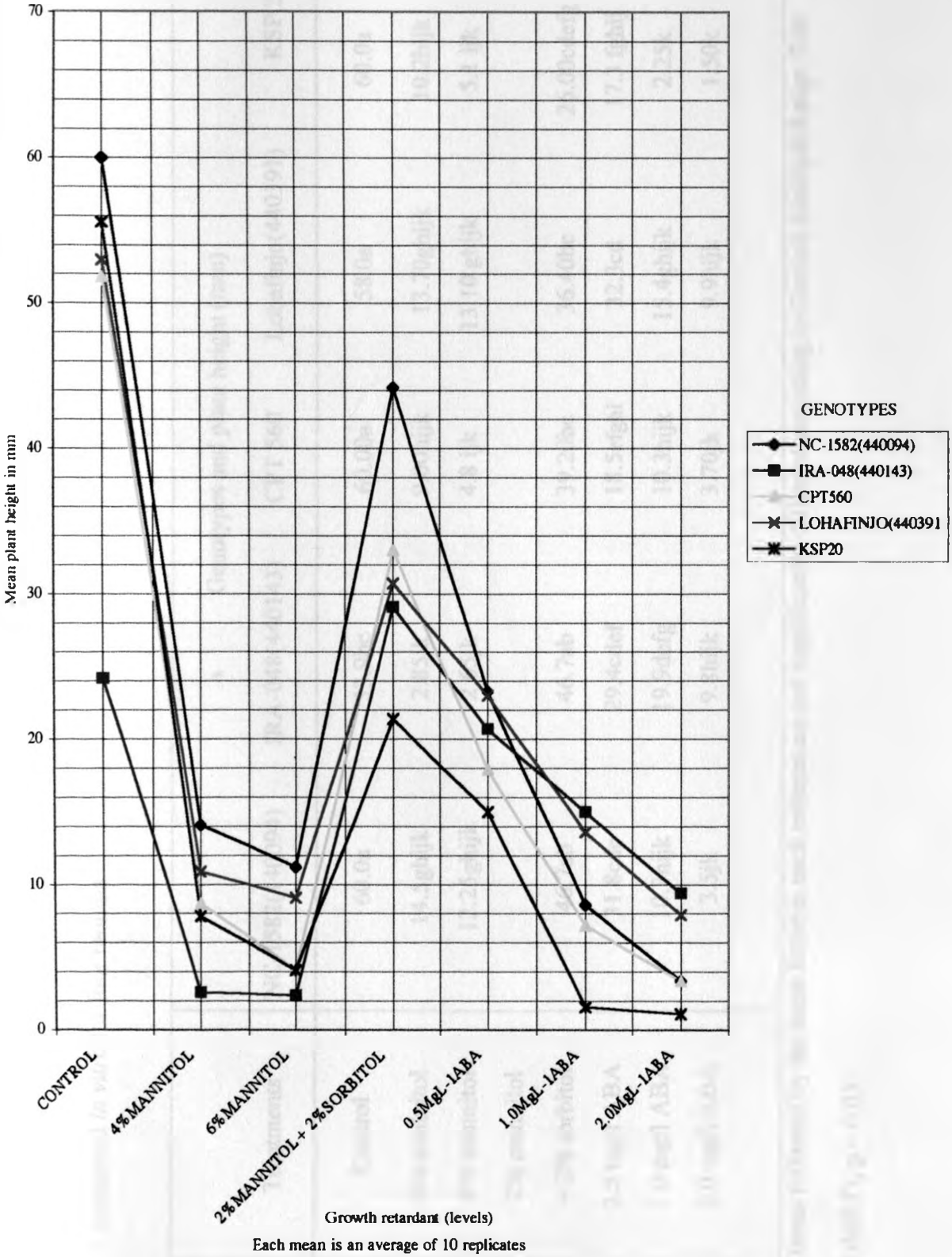


Table 6: Simple effects of growth retardants on mean plant height of clones NC-1582, IRA- 048, CPT 560 Lohafinjo and KSP20 conserved *in vitro* for three months.

Treatments	Genotypes and plant height (mm)				
	NC-1582(440094)	IRA-048(440143)	CPT 560	Lohafinjo(440391)	KSP 20
Control	60.0a	24.2 cd	51.8 ab	53.0 ab	55.6a
4% mannitol	14.1 ghijk	2.6 k	8.7 hijk	10.9 ghijk	7.8 hijk
6% mannitol	11.2 ghik	2.4 k	4.05 jk	9.1 hijk	4.1 jk
2% mannitol + 2% sorbitol	44.2 bc	29.1 def	33.0 cde	30.7 def	21.4 defgh
0.5 mg/l ABA	23.3 defg	20.7 efghi	17.9 fghij	23.0 def	15.0 ghij
1.0 mg/l ABA	8.6 hijk	15.00 ghijk	7.20 ijk	13.6 ghijk	1.55 k
2.0 mg/l ABA	3.4 k	9.4 hijk	3.40k	7.9 hijk	1.10 k

Means followed by the same letter in each column are not significantly different according to Duncan Multiple Range Test (DMRT), p = 0.05

Figure 3 Simple effects of growths retardants on plant height of five sweet potato genotypes: NC-1582, IRA-048,CPT560, Lohafinjo) and KSP20 maintained *in vitro* for 3 months.



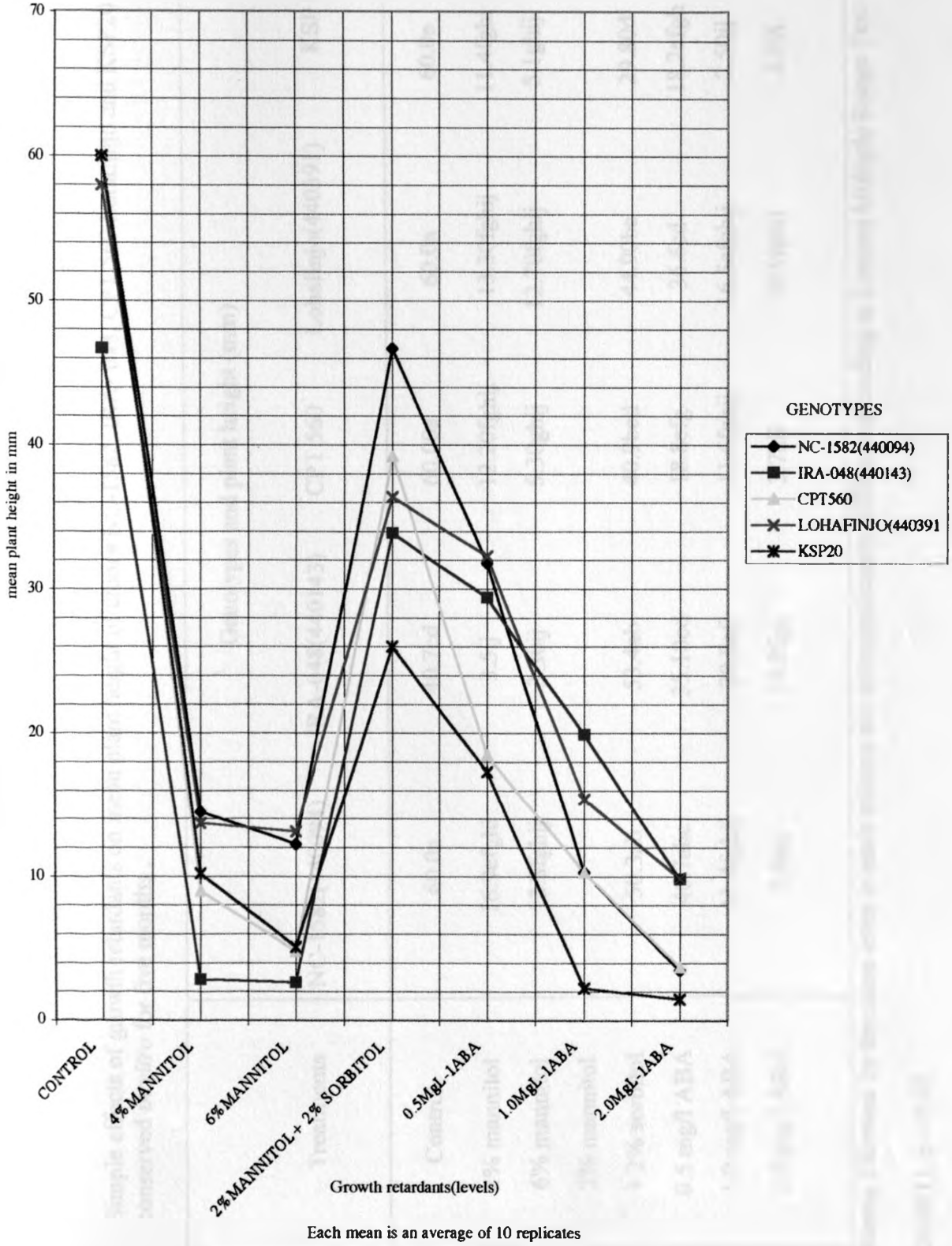
Each mean is an average of 10 replicates

Table 7: *Simple effects of growth retardants on mean plant height of clones NC-1582, IRA- 048, CPT 560 Lohafinjo and KSP20 conserved in vitro for four months.*

Treatments	Genotypes and plant height (mm)				
	NC-1582(440094)	IRA-048(440143)	CPT 560	Lohafinjo(440391)	KSP 20
Control	60.0a	33.9bc	60.00a	58.0a	60.0a
4% mannitol	14.5ghijk	2.85jk	9.00hijk	13.70ghijk	10.2hijk
6% mannitol	12.25ghijk	2.65jk	4.8 ijk	13.10 ghijk	5.1 ijk
2% mannitol + 2% sorbitol	46.7ab	46.7ab	39.20bc	36.40bc	26.00cdefg
0.5 mg/l ABA	31.8cde	29.4cdef	18.5efghi	32.3cd	17.3 fghij
1.0 mg/l ABA	10.3hijk	19.9defg	10.3hijk	15.4ghijk	2.25k
2.0 mg/l ABA	3.5jk	9.8hijk	3.70jk	9.9hijk	1.50k

Means followed by the same letter in each column are not significantly different according to Duncan Multiple Range Test (DMRT), p = 0.05

Figure 4 Simple effects of growth retardants on plant height of five sweet potato genotypes: NC-1582, IRA-048, CPT-560, Lohafinjo and KSP20 maintained *in vitro* for 4 months.



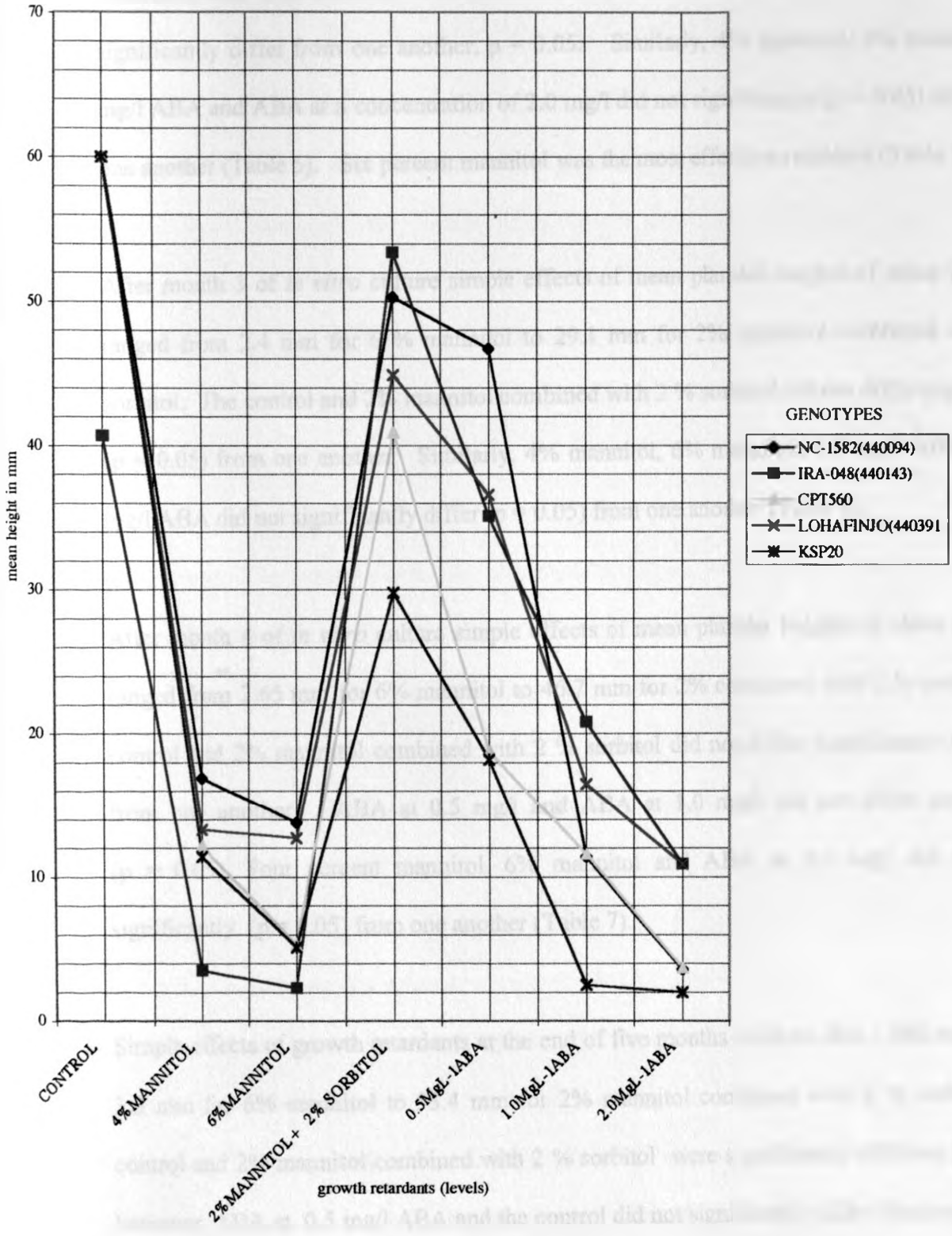
Each mean is an average of 10 replicates

Table 8: Simple effects of growth retardants on mean plant height of clones NC-1582, IRA- 048, CPT 560 Lohafinjo and KSP20 conserved *in vitro* for five months .

Treatments	Genotypes and plant height (mm)				
	NC-1582(440094)	IRA-048(440143)	CPT 560	Lohafinjo(440391)	KSP 20
Control	60.0a	40.7cd	60.00a	60.0a	60.0a
4% mannitol	16.9efghi	3.5ij	12.20fghij	13.30fghij	11.4fghi
6% mannitol	13.8fghij	3.30ij	5.30ghij	12.70fghij	5.1ghij
2% mannitol + 2% sorbitol	50.3ab	53.4ab	40.9bcd	44.90bc	29.80d
0.5 mg/l ABA	46.7abc	35.10cd	18.8efg	36.6cd	18.2efgh
1.0 mg/l ABA	11.4fghij	20.8ef	11.6fghij	16.5efghij	2.50ij
2.0 mg/l ABA	3.6hij	10.9fgij	3.7hij	10.9fghij	2.00j

Means followed by the same letter in each column are not significantly different according to Duncan Multiple Range Test (DMRT), p = 0.05

Figure 5 Simple effects of growth retardants on plant height of five sweet potato genotypes: NC-1582, IRA, CPT560, Lohafinjo and KSP20 maintained *in vitro* for 5 months.



Each mean is an average of 10 replicates

Simple effects of growth retardants on plant height in clone IRA- 048 continued

The simple effects of growth retardants on mean plantlet height of clone IRA 048, after month 2 of *in vitro* culture ranged from 1.65 mm for 6 % mannitol to 25.1 mm for 2% mannitol combined with 2 % sorbitol. The control and 2% mannitol combined with 2 % did not significantly differ from one another, p = 0.05. Similarly, 4% mannitol, 6% mannitol, 1.0 mg/l ABA and ABA at a concentration of 2.0 mg/l did not significantly (p = 0.05) differ from one another (Table 5). Six percent mannitol was the most effective retardant (Table 5).

After month 3 of *in vitro* culture simple effects of mean plantlet heights of clone IRA- 048 ranged from 2.4 mm for 6 % mannitol to 29.1 mm for 2% mannitol combined with 2 % sorbitol. The control and 2% mannitol combined with 2 % sorbitol did not differ significantly (p = 0.05) from one another. Similarly, 4% mannitol, 6% mannitol, 1.0 mg/l ABA and 2.0 mg/l ABA did not significantly differ (p = 0.05) from one another (Table 6).

After month 4 of *in vitro* culture simple effects of mean plantlet heights of clone IRA- 048 ranged from 2.65 mm for 6% mannitol to 46.7 mm for 2% combined with 2 % sorbitol. The control and 2% mannitol combined with 2 % sorbitol did not differ significantly (p = 0.05) from one another. ABA at 0.5 mg/l and ABA at 1.0 mg/l did not differ significantly (p = 0.05). Four percent mannitol, 6% mannitol and ABA at 2.0 mg/l did not differ significantly (p = 0.05) from one another (Table 7).

Simple effects of growth retardants at the end of five months in clone IRA - 048 ranged from 3.3 mm for 6% mannitol to 53.4 mm for 2% mannitol combined with 2 % sorbitol. The control and 2% mannitol combined with 2 % sorbitol were significantly different (p = 0.05). however, ABA at 0.5 mg/l ABA and the control did not significantly differ from one another ABA at 1.0 mg/l, ABA at 2.0 mg/l, 4% mannitol and 6% mannitol did not significantly differ

(p = 0.05) from one another (Table 8). Six percent mannitol was the most effective retardant (Table 8).

Simple effects of growth retardant on clone CPT 560

Simple effects of growth retardants on plant height of clone CPT 560 after one month of *in vitro* culture ranged from 1.45 mm for ABA at 2.0 mg/l to 17.4 mm for the control (Table 4). All growth inhibitors significantly reduced growth compared to the control. Four percent mannitol, 6% mannitol and 1.0 mg/l ABA did not significantly differ (p = 0.05) from one another (Table 4). ABA at 0.5 mg/l differed significantly from all other retardants including the control. Similarly, 2% mannitol combined with 2 % sorbitol differed significantly from all other treatments including the control. ABA at 2.0 mg/l was the most effective retardant (Table 4).

After month 2 of *in vitro* culture simple effects of growth retardants on mean height of clone CPT 560 ranged from 1.75 mm for ABA at 2.0 mg/l to 39.00 mm for the control. All growth inhibitors significantly (p = 0.05) reduced growth compared to the control (Table 5). Six growth retardation treatments namely: 4% mannitol, 6% mannitol, 2% mannitol combined with 2% sorbitol, ABA at 0.5 mg/l, ABA at 1.0 mg/l and ABA at 2.0 mg/l did not significantly differ (p = 0.05) from one another (Table 5).

Simple effects of growth retardants on plant height of clone CPT 560 after three months ranged from 3.4 mm ABA to 2.0 mg/l to 51.8 mm for the control. All growth retardants significantly retarded (p = 0.05) plant height compared to the control. Two percent mannitol combined with 2% sorbitol significantly (p = 0.05) retarded growth more than the control. ABA at 0.5 mg/l, ABA at 1.0, ABA at 2.0 mg/l, 4 % mannitol and 6% mannitol did not differ significantly (p = 0.05) from one another (Table 6).

After month four of *in vitro* culture, simple effects of growth retardants on height of clone CPT 560 ranged from 3.70 mm for ABA at 2.0 mg/l to 60.00 mm for the control. Four percent mannitol, 6% mannitol, 2% mannitol combined with 2% sorbitol, ABA at 0.5 mg/l, ABA at 1.0 mg/l and ABA at 2.0 mg/l significantly ($p = 0.05$) retarded plant height compared to the control. However, ABA at 0.5 mg/l, ABA at 1.0, ABA at 2.0 mg/l, 4 % mannitol and 6% mannitol did not differ significantly ($p = 0.05$) from one another (Table 7). ABA at 2.0 mg/l caused the greatest retardation.

After month 5 of *in vitro* culture simple effects of growth retardants on plant height of clone CPT 560 ranged from 3.7 mm for ABA at 2.0 mg/l to 60 mm for the control. Four percent mannitol, 6% mannitol, 2% mannitol combined with 2% sorbitol, ABA at 0.5 mg/l, ABA at 1.0 mg/l and ABA at 2.0 mg/l significantly ($p = 0.05$) retarded plant height compared to the control. However, ABA at 0.5 mg/l, ABA at 1.0, ABA at 2.0 mg/l, 4 % mannitol and 6% mannitol did not differ significantly ($p = 0.05$) from one another (Table 8). ABA at 2.0 mg/l caused the greatest retardation.

Simple effects of growth retardants on mean plantlet height of clone Lohafinjo

Simple effects of growth retardants on mean plantlet of clone lohafinjo after one month of *in vitro* culture ranged from 3.3 mm for ABA at 2.0 mg/l to 28.9 mm for the control (Table 4). All growth retardants significantly ($p =0.05$) reduced plant height compared to the control (Table 4). ABA at 2.0 mg/l resulted in the greatest retardation. 4% mannitol and 2% mannitol combined with 2% sorbitol did not differ significantly ($p = 0.05$) from one another. Six percent mannitol, ABA at 0.5 mg/l, ABA at 1.0 mg/l and ABA at 2.0 mg/l did not significantly ($p = 0.05$) differ from one another (Table, 4).

The simple effects for plant height after two months of *in vitro* ranged from 4.0 mm for ABA at 2.0 mg/l) to 39.5 for the control. All growth retardants significantly reduced growth compared to the control (Table 5). ABA at 2.0 mg/l resulted in the greatest retardation. However, 4% mannitol, 6% mannitol, 2% mannitol combined with 2% sorbitol, ABA at 0.5 mg/l, ABA at 1.0 mg/l and ABA at 2.0 mg/l did not significantly ($p = 0.05$) differ from one another (Table 8).

Simple effects of growth retardants on plant height of clone lohafinjo after three months ranged from 7.9 mm for ABA at 2.0 mg/l to 53.0 mm for the control. All growth retardants significantly ($p = 0.05$) reduced plant height compared to the control (Table 5). ABA at 2.0 mg/l resulted in the greatest retardation. Two percent mannitol combined with 2% sorbitol and 0.5mg/l ABA were not significantly different. Similarly, 4% mannitol, 6% mannitol, ABA at 1.0 mg/l and ABA at 2.0 mg/l did not significantly ($p = 0.05$) differ from one another (Table, 6)).

Simple effects of retardants on height of clone lohafinjo after four months of *in vitro* culture ranged from 9.9mm for ABA at 2.0 mg/l to 58.0mm for the control. All growth retardants significantly ($p = 0.05$) reduced growth compared to the control (Table 7). ABA at 2.0 mg/l resulted in the greatest retardation. Two percent mannitol combined with 2% sorbitol and 0.5 mg/l ABA were not significantly ($p = 0.05$) different. Four percent mannitol, 6% mannitol, ABA at 1.0 mg/l and ABA at 2.0 mg/l did not significantly ($p = 0.05$) differ from one another (Table, 7).

After five months of *in vitro* culture simple effects of growth retardants on plant height of clone lohafinjo, ranged from10.9 mm for ABA at 2.0 mg/l to 60 mm for the control. Similar to month four, ABA at 2.0 mg/l resulted in the greatest retardation. 2% mannitol combined

with 2% sorbitol and 0.5 mg/l ABA did not significantly (p = 0.05) differ from one another. Similarly, 4% mannitol, 6% mannitol, ABA at 1.0 mg/l and ABA at 2.0 mg/l did not significantly (p = 0.05) differ from one another (Table 8).

Simple effects of growth retardants on mean plantlet height of clone KSP 20

Simple effects of growth retardants on height of clone KSP 20 after one month of *in vitro* culture ranged from 0.75 mm for ABA at 2.0 mg/l to 23.3 mm for the control. All growth retardants significantly (p = 0.05) reduced growth compared to the control (Table 4). ABA at 2.0 mg/l caused the greatest retardation. However, ABA at 2.0 mg/l, ABA at 1.0 mg/l or 6 % mannitol did not differ significantly (p = 0.05) from one another (Table 4). 2% mannitol combined with 2% sorbitol and 0.5 mg/l ABA did not significantly (p = 0.05) differ from one another. All the other treatments significantly (p = 0.05) differed from one another (Table 4).

Simple effects for mean height, on clone KSP 20 after two months of *in vitro* storage ranged from 0.75 mm for ABA at 2.0 mg/l to 46.7 mm for the control. ABA at 2.0 mg/l resulted in the greatest retardation. However, ABA at 2.0 mg/l, 0.5 mg/l ABA, 1.0 mg/l ABA, 2% mannitol combined with 2 % sorbitol, 4% mannitol and 6% mannitol did not significantly (p = 0.05) differ from one another (Table 5).

After three months of culture mean height ranged from 1.10 mm for ABA at 2.0 mg/l to 55.6 mm for the control. Similar to month 2, the control significantly (p = 0.05) caused less retardation compared to all other growth treatments. ABA at 2.0 mg/l caused the greatest plant height retardation in clone KSP 20, after 3 months of *in vitro* culture. ABA at 2.0 mg/l 0.5 mg/l ABA, 1.0 mg/l ABA, 2% mannitol combined with 2 % sorbitol, 4% mannitol and 6% mannitol did not significantly (p = 0.05) differ from one another (Table 6).

Simple effects of growth retardants on plant height of clone KSP 20 after four months of *in vitro* culture ranged 1.5 mm for 2.0 mg/l ABA to 60 mm for the control. The control significantly (p = 0.05) caused less retardation compared to all other growth treatments. ABA at 2.0 mg/l resulted in the greatest retardation. Similar to months 2 and 3, ABA at 2.0 mg/l, 0.5 mg/l ABA, 1.0 mg/l ABA, 2% mannitol combined with 2 % sorbitol, 4% mannitol and 6% mannitol did not significantly (p = 0.05) differ from one another (Table 7).

Simple effects on mean plantlet height for month five ranged from 2.0 mm for ABA at 2.0 mg /l to 60 mm for the control. The control significantly (p = 0.05) caused less retardation compared to all other treatments. ABA at 2.0 mg/l resulted in the greatest retardation. ABA at 2.0 mg/l, 0.5 mg/l ABA, 1.0 mg/l ABA, 2% mannitol combined with 2 % sorbitol, 4% mannitol and 6% mannitol did not significantly (p = 0.05) differ from one another (Table 8). Two percent mannitol combined with 2% sorbitol significantly (p = 0.05) differed from the other treatments, including the control.

Effects of genotypes and growth retardants on number of roots

Effects of genotypes on mean of roots per *in vitro* plantlet was significant at months 1 and 2, p = 0.05, (Table 9). Effect of growth retardant was significant, p = 0.05 (Table 9). However, genotype x growth retardant interaction was not significant, p = 0.05 (Table 9). Because of insignificant genotype by retardant interactions main effects were used to interpret the results.

Mean number of roots per *in vitro* plantlet after month 1 of *in vitro* culture ranged from 0.72 for 6 % mannitol to 1.90 for 2% mannitol combined with 2 % sorbitol (Table 10). 2% mannitol combined with 2 % sorbitol, 4 % mannitol, 0.5 mg/l ABA and 1.0 mg/l ABA did no significantly (p = 0.05) differ from one another. Similarly, 6% mannitol and ABA at 2.0 mg/ did not significantly (p = 0.05) differ from one another (Table, 10).

Mean number of roots/ plantlet after month 2 of *in vitro* culture ranged from 1.32 for 6 % mannitol to 2.94 for 2% mannitol combined with 2 % sorbitol. The control and ABA at 0.5 mg/l did not significantly ($p = 0.05$) differ from one another. 2% mannitol combined with 2 % sorbitol significantly ($p = 0.05$) produced more roots compared to other treatments, including the control (Table 10). However, 4% mannitol, 6 % mannitol, 0.5 mg/l ABA, 1.0mg/l ABA and 2.0 mg/l ABA did not significantly ($p = 0.05$) differ from one another (Table 10; Figure 8).

Table 9 . Mean square variance for effects of genotypes and growth retardants on number of roots of sweet potato conserved *in vitro*.

Source	Df	Month 1	Month 2
Genotypes (G)	4	5.00*	8.66*
Growth Retardants (R)	6	9.48**	16.32**
G X R	24	2.1 ns	6.23ns
Error	315	1.56	2.69

Table 10. Effects of growth retardants on the mean number of sweet potato roots after months 1 and 2 of *in vitro* culture.

	Mean number of roots	
Treatment	1 Month	2 Month
Control	1.84ab	2.26 b
4% mannitol	1.32cd	1.66c
6% mannitol	0.72e	1.32c
2% mannitol+ 2% sorbitol	1.90a	2.94a
0.5 mg/l ABA	1.80abc	2.18b
1.0 mg/l ABA	1.40bcd	1.98c
2.0 mg/l ABA	1.12e	1.36c

Mean for individual month bearing same letters are not significantly (p = 0.05) different according to LSD.

Effect of genotypes and growth retardants on number of internodes

The effects of the genotypes, growth retardants, and their interactions after months 1 and 2 of *in vitro* culture were highly significant (Table 11). Because of significant interactions simple effects were used to report the results.

Simple effects of growth retardants on mean number of internodes of clone NC-1582.

Simple effects for number of internodes in clone NC-1582 after two months *in vitro* ranged from 1.7 for ABA at 2.0 mg/l to 6.8, for the control. 2.0 mg/l ABA, 1.0 mg/l ABA, 0.5 mg/l ABA, 4% mannitol and 6% mannitol did not significantly ($p = 0.05$) differ from one another (Table 12, Figure 6). Treatments of the control and 2% mannitol combined with 2% sorbitol did not significantly ($p = 0.05$) differ from one another (Table 12, Figure 6).

Simple effects of growth retardants on mean number of internodes of clone IRA- 048.

Simple effects for number of internodes in clone IRA-048, after month 2 of *in vitro* conservation ranged from 1.3 internodes/ plantlet for 6 % mannitol to 6.6 internodes / plantlet for 2 % mannitol combined with 2 % sorbitol. Six percent mannitol, 4 % mannitol, ABA at 0.5mg/l, ABA at 1.0 mg/l and ABA at 2.0 mg/l was not significantly different (Table12, Figure 6). Two percent mannitol combined with 2% sorbitol did not differ significantly from the control (Table 12, Figure 6).

Simple effects of growth retardants on mean number of internodes of clone CPT 560

The simple effects of growth retardants mean number of internodes per plant of clone CPT 560 after 2 months of *in vitro* culture ranged from 1.4 for ABA at 2.0 mg/l to 6.6 for the control. 2.0 mg/l ABA, 1.0 mg/l, 0.5 mg/l ABA, 4% mannitol and 6% mannitol did not significantly ($p = 0.05$) (Table 12, Figure 6). 2% mannitol combined with 2% sorbitol and the control did not significantly ($p = 0.05$) differ from one another (Table 12, Figure 6).

Table 11: Two factor analysis of variance for internodes and leaves of clones; NC-1582, IRA- 048, CPT 560, Lohafinjo and KSP20 after months 1 and 2 of culture.

Source	Df	Mean square internodes		Mean square leaves	
		1 month	2 months	1 months	2 months
Genotypes (G)	4	22.08**	6.14**	39.18**	57.05**
Growth Retardants (r)	6	43.77**	157..04**	43.57**	206.68**
G x R	24	1.87**	6.45**	2.38**	10.22**
Error	315	0.87	3.06	1.48	4.12

Data analyzed after months 1 and 2 of *in vitro* culture

* Significant (p=0.05)

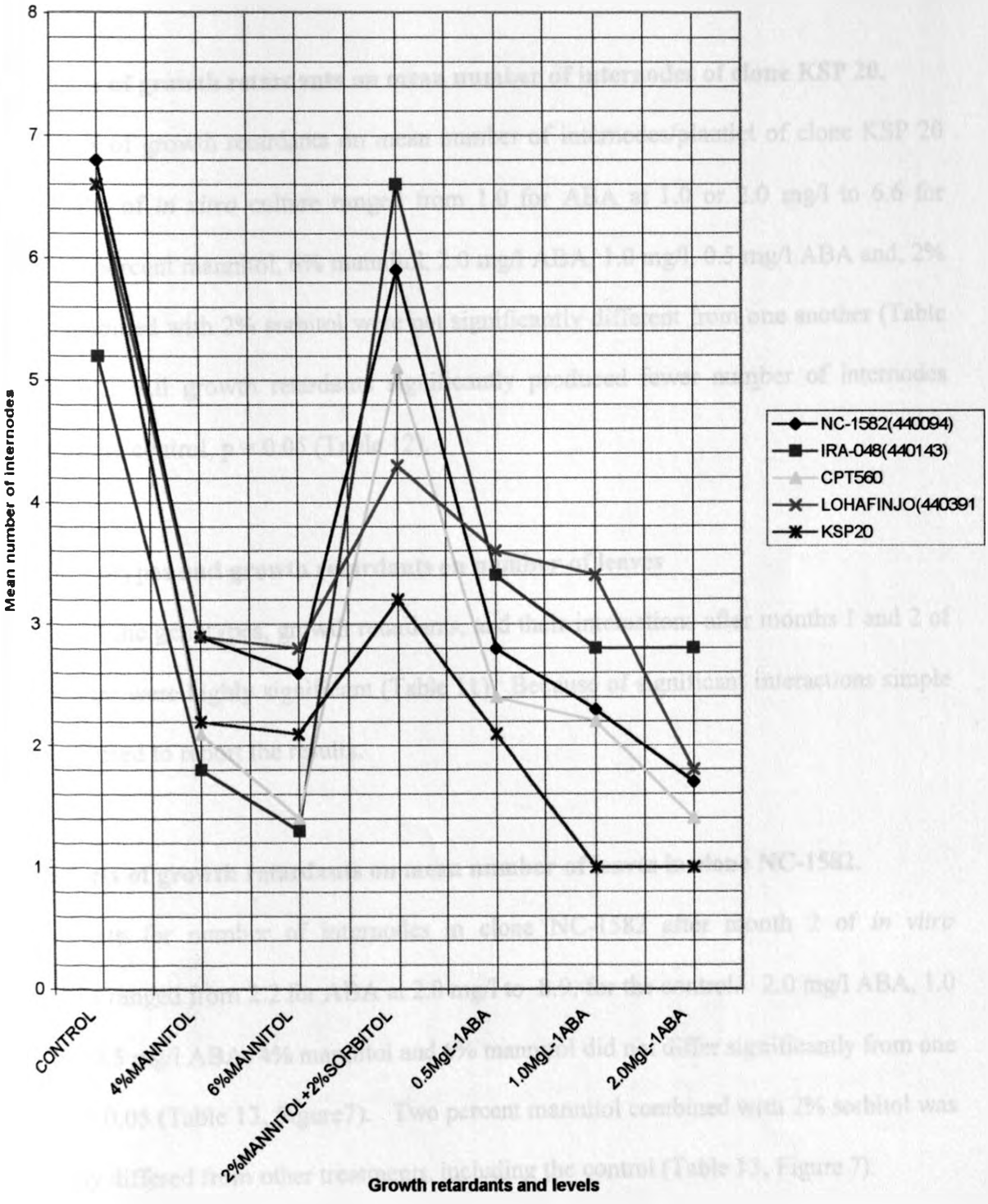
** Highly significant (p=0.01)

Table 12 Simple effects of growth retardants on mean number of internodes of five sweet potato genotypes conserved *in vitro* for two months.

Growth retardants	Genotypes				
Source	NC-1582	IRA-048	CPT 560	Lohafinjo	KSP 20
Control	6.8a	5.2abc	6.6a	6.6a	6.6a
4% mannitol	2.9def	1.8efg	2.1efg	2.9edf	2.2efg
6% mannitol	2.6defg	1.3fg	1.4fg	2.8def	2.1efg
2% man +2% sorb	5.9ab	6.6a	5.1abc	4.3bc	3.2def
0.5 mg/l ABA	2.8def	3.4de	2.4efg	3.6cde	2.1efg
1.0 mg/l ABA	2.3efg	2.8def	2.2efg	3.4de	1.0g
2.0 mg/l ABA	1.7efg	2.8def	1.4fg	1.8efg	1.0g

Means of each column followed by same letter are not significantly different ($p = 0.05$), according to Duncan Multiple Range Test (DMRT).

Figure 6 Simple effects of growth retardants on number of internodes of sweet potato genotypes NC-1582, IRA-048, CPT-560, Lohafinjo and KSP20 conserved *in vitro* for 2 months.



Each mean is an average of 10 plants

Simple effects of growth retardants on mean number internodes in clone Lohafinjo.

The simple effects of growth retardants on mean number of internodes/ plantlet of clone Lohafinjo ranged from 1.8 for ABA at 2.0 mg/l to 6.6 for the control. Four percent mannitol, 6% mannitol, 2.0 mg/l ABA, 1.0 mg/l, 0.5 mg/l ABA and , 2% mannitol combined with 2% sorbitol were not significantly different from one another (Table 12, Figure 6).

Simple effects of growth retardants on mean number of internodes of clone KSP 20.

Simple effects of growth retardants on mean number of internodes/plantlet of clone KSP 20 after 2 months of *in vitro* culture ranged from 1.0 for ABA at 1.0 or 2.0 mg/l to 6.6 for control. Four percent mannitol, 6% mannitol, 2.0 mg/l ABA, 1.0 mg/l, 0.5 mg/l ABA and, 2% mannitol combined with 2% sorbitol were not significantly different from one another (Table 12, Figure 6). All growth retardants significantly produced fewer number of internodes compared to the control, $p = 0.05$ (Table 12).

Effect of genotypes and growth retardants on number of leaves

The effects of the genotypes, growth retardants, and their interactions after months 1 and 2 of *in vitro* culture were highly significant (Table 11). Because of significant interactions simple effects were used to report the results.

Simple effects of growth retardants on mean number of leaves in clone NC-1582.

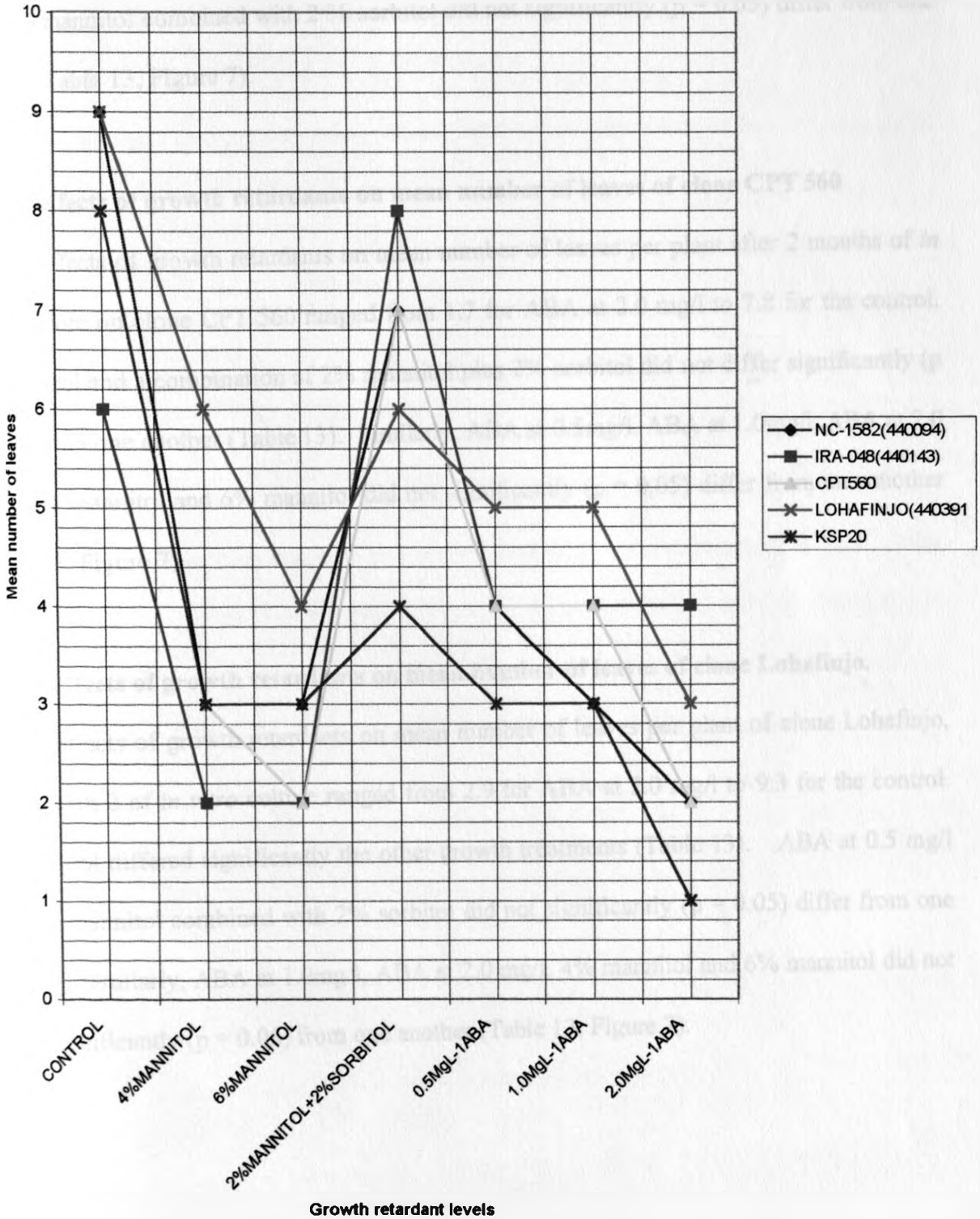
Simple effects for number of internodes in clone NC-1582 after month 2 of *in vitro* conservation ranged from 2.2 for ABA at 2.0 mg/l to 8.9, for the control. 2.0 mg/l ABA, 1.0 mg/l ABA, 0.5 mg/l ABA, 4% mannitol and 6% mannitol did not differ significantly from one another, $p = 0.05$ (Table 13, Figure7). Two percent mannitol combined with 2% sorbitol was significantly differed from other treatments, including the control (Table 13, Figure 7).

Table 13: Simple effects of growth retardants on mean number of leaves in five genotypes of sweet potato conserved *in vitro* for 2 months.

Treatments	Genotypes and mean number of leaves				
	NC-1582(440094)	IRA-048(440143)	CPT 560	Lohafinjo (440391)	KSP 20
Control	8.9a	6.2bcdef	7.8abc	9.3a	7.9ab
4% mannitol	3.3hijklmn	2.4jklmno	9.00hijk	13.70ghijk	10.2hijk
6% mannitol	3.2hijklmn	1.5no	1.9klmno	4.19hij	4.2fghij
2% mannitol + 2% sorbitol	6.6bcde	7.6abcd	6.7bcde	5.8cdefg	4.2fghij
0.5 mg/l ABA	3.8ghijkl	4.2fghij	3.8hijkl	5.2efgh	2.9ijklmno
1.0 mg/l ABA	2.6jklmno	4.00ghijk	3.7ghijklm	4.9efghi	1.60mno
2.0 mg/l ABA	2.2jklmno	4.00ghijk	1.7lmno	2.9jklmno	1.0o

Means of each column followed by same letter are significantly (p = 0.05) different, Duncan Multiple Range Test, DMRT.

Figure 7 Simple effects of growth retardants on mean number of leaves of five sweet potato genotypes: NC-1582, IRA-048, CPT-560, Lohafinjo and KSP20 maintained *in vitro* for two months



Each mean is an average of 10 replicates

Simple effects of growth retardants on mean number of leaves of clone IRA- 048.

Simple effects for number of leaves per plant of clone IRA-048 after 2 months of *in vitro* conservation ranged from 1.5 for mannitol at 6 % to 7.6 for 2 % mannitol combined with 2 % sorbitol. 2.0 mg/l ABA, 1.0 mg/l ABA, 0.5 mg/l ABA, 4% mannitol and 6% mannitol did not differ significantly (p=0.05) from one another (Table 13, Figure7). Similarly, the control and 2 % mannitol combined with 2 % sorbitol did not significantly (p = 0.05) differ from one another (Table 13, Figure 7).

Simple effects of growth retardants on mean number of leaves of clone CPT 560

Simple effects of growth retardants on mean number of leaves per plant after 2 months of *in vitro* culture on clone CPT 560 ranged from 1.7 for ABA at 2.0 mg/l to 7.8 for the control. The control and a combination of 2% mannitol plus 2% sorbitol did not differ significantly (p =0.05) from one another (Table 13). Similarly, ABA at 0.5mg/l, ABA at 1.0mg/l, ABA at 2.0 mg/l, 4% mannitol and 6% mannitol did not significantly (p = 0.05) differ from one another (Table 13, Figure 7)

Simple effects of growth retardants on mean number of leaves of clone Lohafinjo.

Simple effects of growth retardants on mean number of leaves per plant of clone Lohafinjo, after month 2 of *in vitro* culture ranged from 2.9 for ABA at 2.0 mg/l to 9.3 for the control. The control differed significantly the other growth treatments (Table 13). ABA at 0.5 mg/l and 2% mannitol combined with 2% sorbitol did not significantly (p = 0.05) differ from one another. Similarly, ABA at 1.0mg/l, ABA at 2.0 mg/l, 4% mannitol and 6% mannitol did not differ significantly (p = 0.05) from one another (Table 13, Figure 7).

Simple effects of growth retardants on mean number of leaves of clone KSP 20.

Simple effects of growth retardants on mean number of leaves per plant of clone KSP 20 after 2 months of *in vitro* culture ranged from 1.0 for ABA at 2.0 mg/l to 7.9 for the control (Table 13). The control significantly ($p = 0.05$) differed from all other treatments (Table 13). ABA at 0.5mg/l, ABA at 1.0mg/l, ABA at 2.0 mg/l and 4 % mannitol and 6% mannitol did not significantly ($p = 0.05$) differ from one another (Table 13, Figure 7).

Effect of genotypes and growth retardation percent leaf abscission of *in vitro* conserved sweet potato.

Effects of the genotypes, growth retardants, and their interactions on percent leaf abscission after month 5 of *in vitro* culture was highly significant (Table 14). Because of significant interactions, simple effects were used to report the results.

Simple effects of growth retardants on mean percent leaf abscission of clone NC-1582

Simple effects of growth retardants on mean percent leaf abscission on clone NC 1582, after month 5 of *in vitro* culture ranged from 2.9 percent for 6 % mannitol and 2.0 mg/l ABA to 41.4 percent for 2% mannitol combined with 2 % sorbitol. The control, ABA at 0.5mg/l and 2% mannitol plus 2 % sorbitol treatments did not significantly different from one another. Similarly, 4% mannitol, 6 % mannitol, ABA at 1.0 mg/l ABA and ABA at 2.0 mg/l ABA were not significantly different (Table 15, Figure 8).

Table 14 Mean square from the analysis of variances of leaf drop and tip drying of five sweet potato genotypes as affected by growth retardants.

Source	Mean squares for % leaf abscission and tip drying after five months of *in vitro* culture		
	Df	Percent abscission	% Tip drying
Genotypes (G)	4	3060.5**	475.20**
Growth Retardants (R)	6	6205.04**	528.59**
G X R	24	633.77**	398.59**
Error	315	286.18	61.11
LSD		6.66	3.08

* Significant according to, LSD, p = 0.05.

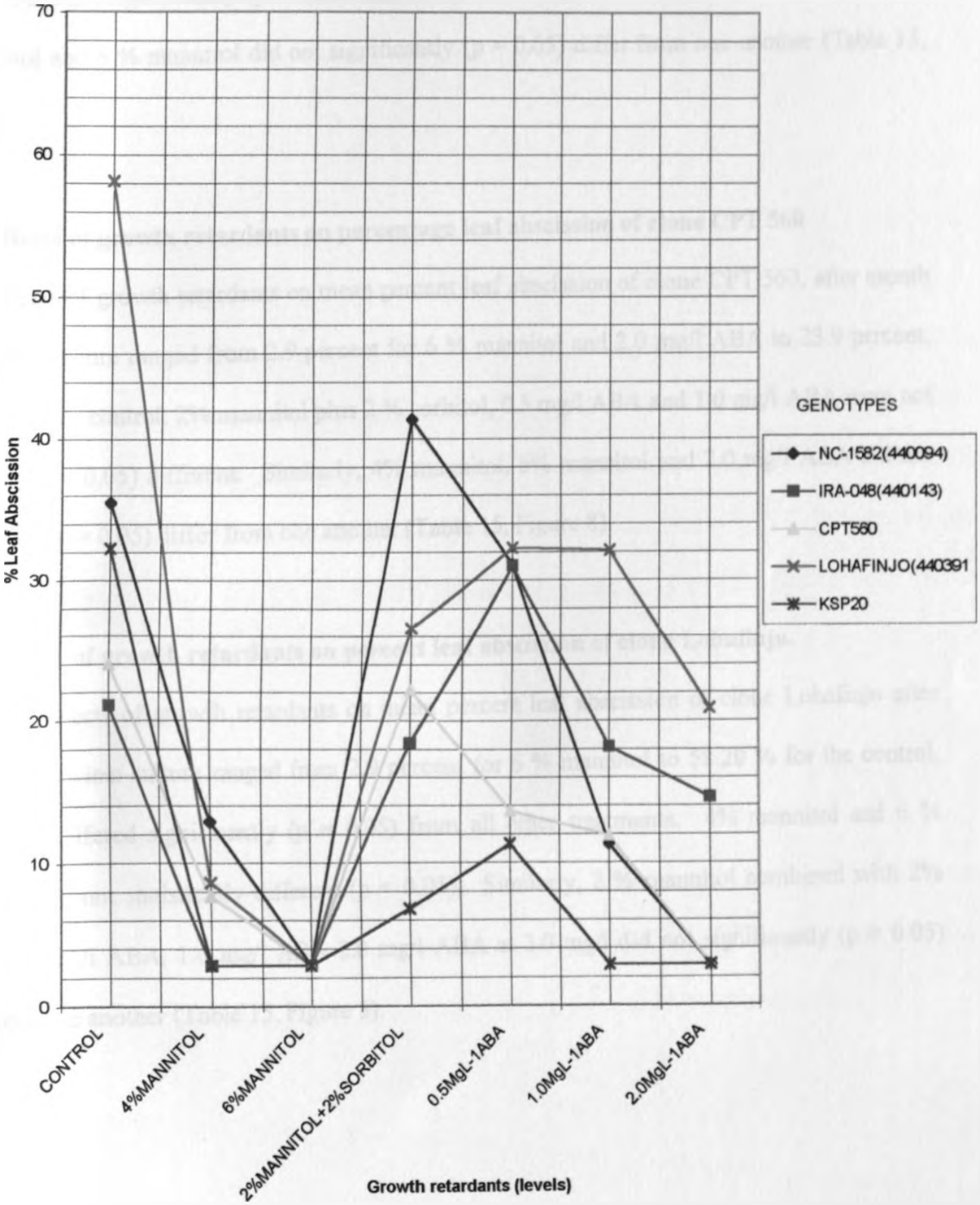
* * Highly significant according to, LSD, p = 0.05.

TABLE 15: *Simple effects of growth retardants on % leaf abscission after five months of in vitro conservation of five sweet potato genotypes*

Treatments	Genotypes and % leaf abscission				
	NC-1582	IRA-048	CPT 560	Lohafinjo	KSP 20
Control	35.5bc	21.1cdefg	23.9bcdefg	58.3a	32.3bcd
4% mannitol	12.9fgh	2.9h	7.6h	8.6gh	2.9h
6% mannitol	2.9h	2.9h	2.9h	2.9h	2.9h
2% mannitol + 2% sorbitol	41.4b	18.4cdefg	22.2cdefg	26.6bcdef	6.8h
0.5 mg/l ABA	31.1bcde	31.0bcde	13.6efg	32.3bcd	11.2fgh
1.0 mg/l ABA	11.3fgh	18.1cdefg	11.9fg	32.1bcd	2.9h
2.0 mg/l ABA	2.9h	14.6defg	2.9h	20.87cdef	2.9h

Means for each column followed by the same letter are not significantly different (p = 0.05)

Figure 8 Simple effects of growth retardants on percent leaf abscission of five sweet potato genotypes conserved *in vitro* Data taken after five months of *in vitro* culture



simple effects of growth retardants on mean percent leaf abscission of clone IRA- 048

ple effects of growth retardants on mean percent leaf abscission of clone IRA 048, after month *in vitro* culture ranged 2.9 percent for 4%mannitol and 6% mannitol to 31 percent for ABA at mg/l. The control, 2% mannitol plus 2 % sorbitol, ABA at 0.5mg/l, ABA at 1.0mg/l and ABA mg/l did not significantly (p = 0.05) differ from one another (Table 15, Figure 8). Similarly, mannitol and 6 % mannitol did not significantly (p = 0.05) differ from one another (Table 15, re 8).

ple effects of growth retardants on percentage leaf abscission of clone CPT 560

ple effects of growth retardants on mean percent leaf abscission of clone CPT 560, after month *in vitro* culture ranged from 2.9 percent for 6 % mannitol and 2.0 mg/l ABA to 23.9 percent, control. The control, 2% mannitol plus 2 % sorbitol, 0.5 mg/l ABA and 1.0 mg/l ABA were not stically (p= 0.05) different. Similarly, 4% mannitol, 6% mannitol and 2.0 mg/l ABA did not ificantly (p = 0.05) differ from one another (Table 15, Figure 8).

ple effects of growth retardants on percent leaf abscission of clone Lohafinjo.

simple effects of growth retardants on mean percent leaf abscission of clone Lohafinjo after nth 5 of *in vitro* culture ranged from 2.9 percent for 6 % mannitol to 58.20 % for the control. control differed significantly (p = 0.05) from all other treatments. 4% mannitol and 6 % nnitol were not statistically different (p = 0.05). Similarly, 2 % mannitol combined with 2% bitol, 0.5mg/l ABA, 1.0 mg/l ABA 2.0 mg/l ABA at 2.0 mg/l did not significantly (p = 0.05) fer from one another (Table 15, Figure 8).

Simple effects of growth retardants on percent leaf abscission of clone KSP 20

Simple effects of growth retardants on mean percent leaf abscission of leaves of clone KSP 20, after 5 months of *in vitro* culture ranged from 2.9 percent for 4% mannitol, 6 % mannitol, 1.0 mg/l ABA and 2.0 mg/l ABA to 32.3 % for the control. The control, differed significantly ($p = 0.05$) from all the treatments. 0.5mg/l ABA, 1.0mg/l ABA, 2.0 mg/l ABA, 2% mannitol combined with 2% sorbitol, 4% mannitol and 6% mannitol did not significantly ($p= 0.05$) differ from one another (Table 15, Figure 8).

Effects of genotypes and growth retardants on survival of five sweet potato genotypes eight months post-culture

The total number of surviving *in vitro* cultures were calculated as percentage of total *in vitro* cultures per treatment, after 8 months of culture ranged from 47.7% for 6% mannitol to 80.5% for ABA at 2.0 mg/l ABA (Table 16). Percentage survival increased with increased concentration of ABA, but decreased with increase of mannitol concentration (Table 16). 2 % mannitol combined with 2% sorbitol and 1.0 mg/l ABA did not significantly ($p = 0.05$) differ from one another. Similarly, treatments of 6% mannitol and 4% mannitol did not significantly ($p= 0.05$) differ from one another (Table 16).

Rejuvenation and appraisal of phenotypic characteristics.

Growth retardants did not significantly affect the regeneration of cultures upon transfer to retardant free media. However, plants initially conserved in media containing mannitol or sorbitol had slender roots, reduced petioles, short internodes and inwardly folded leaves (Table 17)

Effect of growth retardants (mannitol, sorbitol and Abscisic acid) on combined Percentage survival of sweet potato genotypes 'NC-1582', 'IRA-048', 'CPT 560' Lohafinjo and KSP 20, after eight months of *in vitro* culture.

Treatment	Combined % Survival of five sweet Potato genotypes
Control	65.88c
4% mannitol	51.49d
6% mannitol	47.73d
2% mannitol + 2% sorbitol	71.10b
0.5 mg/l ABA	67.58c
1.0 mg/l ABA	74.78b
2.0 mg/l ABA	80.52a

Notes: Each mean is an average of 70 plants from five genotypes with 10 replicates.

Table 17. Effects of growth retardants on phenotype and ease of rejuvenation.

Treatments	Rejuvenation	Remarks
Control (no growth retardant)	Rejuvenation possible	Robust plants. No morphological abnormalities. Strong roots, wide internodes, as well as broad green and open leaves.
4% mannitol	Rejuvenation possible	Plants carried over characteristics noted during slow growth. Weak slender roots, narrow petioles, short internodes, narrow inwardly folded leaves.
6% mannitol	Rejuvenation possible	Plants carried over characteristics noted during slow growth. These included; slender roots, reduced petioles, short internodes, and inwardly folded leaves.
2% mannitol combined with 2% sorbitol	Rejuvenation possible	Normal looking plants except leaves, stems, and roots had darker colour than those of control.
0.5 mg/l ABA	Rejuvenation possible	Robust plants with no morphological abnormalities. Strong roots and normal leaves.
1.0 mg/l ABA	Rejuvenation possible	Robust plants. No morphological abnormalities. Strong roots and normal leaves.
2.0 mg/l ABA	Rejuvenation possible	Robust plants with no morphological abnormalities. Strong roots and normal leaves.

Effects of genotypes and glyphosate on plant height of *in vitro* conserved sweet potato.

Effects of genotype on plant height was not significant (p = 0.05), but the effect of glyphosate was highly significant (p= 0.05) at months 1, 2, 3, 4, and 5 months of *in vitro* culture. However, genotype x glyphosate interaction was not significant (p = 0.05). Because interactions were insignificant main effects were used to present the results.

Effect of glyphosate on the two sweet potato genotypes "IITA-TIS-3290" and "Nyaluolo" were similar. Similarly, the effects of the two levels of glyphosate (5 mg/l and 10 mg/l) did not significantly differ from one another (Table 18). However, the two levels of glyphosate significantly (p= 0.05) reduced plant height compared to the control (Table 18).

Effect of genotypes and glyphosate on plantlet roots.

Effects of genotypes were insignificant (Table 19). Effects of glyphosate were however significant. Glyphosate at concentration of 5 mg/l or 10 mg/l, completely inhibited development of roots of sweet potato conserved *in vitro* (Table 19, Plates 2a and 2b).

Table 18: Effect of levels of glyphosate on height of sweet potato genotype Nyaluolo conserved *in vitro* for five months. Data analyzed at monthly interval for five months.

Levels of glyphosate in mg/l	Height (mm).				
	1 month	2 Month	3 Month	4 Month	5 Month
Control (0mg/l)	17.15a	48a	59a	60a	60a
5 mg/l	1.525b	1.75b	1.8b	1.925b	1.925b
10mg/l	1.025b	1.075b	1.15b	1.225b	1.225b

Means in every column followed by same letter do not differ significantly, LSD, p =0.05

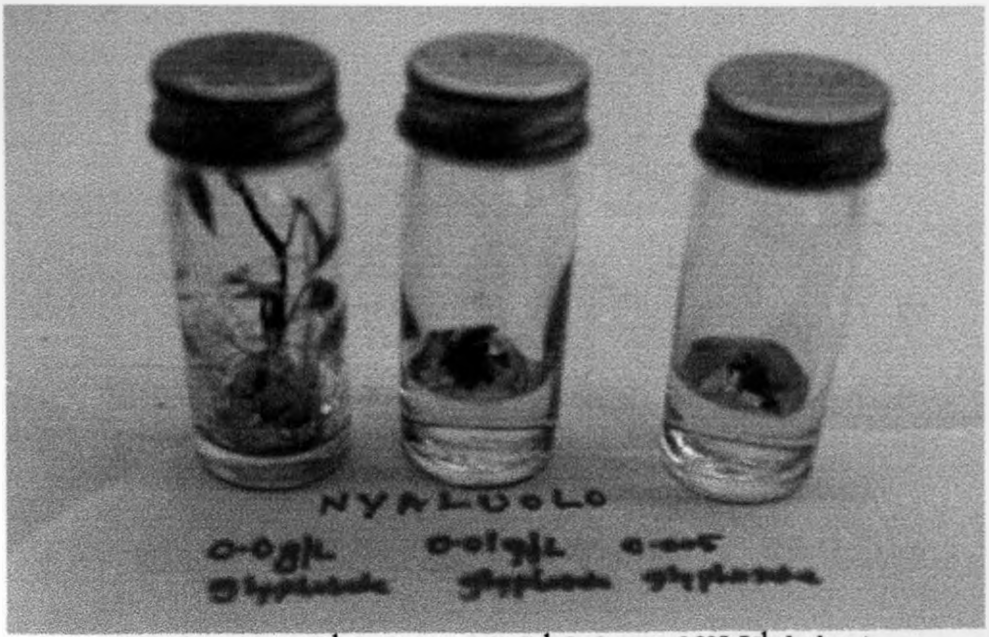
Table 19: Effects of different levels of glyphosate on mean number of roots, in clones IITA- 3290 (440068) and Nyaluolo, at one and two months post-culture.

Treatments (mg/l)	**Genotypes**	**mean number of roots and months of culture**	
		1 month	2 Months
Control (0 mgl-1 glyphosate)	TIS-3290(440068)	3.1	3.6
	Nyaluolo	1.5	1.9
5 mgl-1 glyphosate	TIS-3290(440068)	0	0
	Nyaluolo	0	0
10 mgl-1 glyphosate	TIS-3290(440068)	0	0
	Nyaluolo	0	0

Plate 2a: Effect of (0, 0.005g/l^{-1}, 0.01g/l^{-1}) glyphosate on roots of clones IITA-TIS-3290 (after 2 months of *in vitro* conservation.

Left to Right: Control, 0.0gL^{-1} glyphosate, 0.005gL^{-1} glyphosate, 0.1gL^{-1} glyphosate,

Plate 2 b: Effect of (0, 0.005, 0.01)g /l glyphosate on root development in clones Nyaluolo after 2 months of *in vitro* conservation



Left to Right: Control, 0.0gL^{-1} glyphosate, 0.01gL^{-1} glyphosate, 0.005gL^{-1} glyphosate,

Effects of genotypes and glyphosate on survival and rejuvenation of *in vitro* cultured sweet potato.

Percent survival was significantly higher for cultures conserved in glyphosate free medium. After eight months of *in vitro* culture percent survival for control was 70% and 80% for sweet potato genotypes 'IITA-TIS-3290'and 'Nyaluolo' respectively. However, percentage survival reduced to 40% and 50% respectively in treatment containing 5 mg/l. No plant survived with 10mg/l glyphosate after five months of conservation (Table 20).

Rejuvenation of cultures after eight month of *in vitro* conservation in glyphosate.

Glyphosate significantly reduced potential of cultures to resume growth upon transfer to glyphosate free medium compared to control (Table 20).

Table 20. Effect of glyphosate on mean percent survival, rejuvenation and morphology of recovered plants (8 months post culture).

Treatment	Clone	Percentage Survival	Rejuvenation	Remarks
Control.	IITA-TIS-3290-(440068) Nyaluolo	70 % 80 %	100 % 100 %	Recultured plants very robust and phenotypically similar to initial stock.
5 mgl-1 glyphosate	IITA-TIS-3290-(440068) Nyaluolo	40 % 50 %	None 50 %	normal plants with roots recovered on Nyaluolo after second transfer to flesh multiplication media
10 mgl-1 glyphosate	IITA-TIS-3290-(440068) Nyaluolo	0 % 0 %	None None	Recovery of plants not achieved

CHAPTER 5

DISCUSSION.

In vitro conservation of sweet potato using slow growth medium

Tissue culture is an important technology widely used for mass propagation of elite plant materials, development of new varieties, distribution of materials world wide and conservation of plant genetic resources. In the current study tissue culture was utilised to conserve seven sweet potato varieties *in vitro*. The genotypes included in the study were, NC-1582, IRA-048, CPT 560, Lohafinjo, KSP-20 Nyaluolo and IITA-TIS-3290. The medium environment was modified by adding osmoticums (mannitol and sorbitol), abscisic acid or sub-optimal levels of glyphosate. The results are discussed below.

Effects of Genotypes and Growth Retardants and Plant Height

Growth retardants had a significant (p= 0.05) effect on growth rates of five sweet potato genotypes NC-1582, IRA-048, CPT 560, Lohafinjo and KSP-20 maintained *in vitro*. Treatments of 4% mannitol, 6% mannitol, 1.0 mgL-1 ABA and 2.0 mgL-1 ABA. The four treatments did not significantly (p = 0.05) differ from one another after 5 months of *in vitro* culture. Two sweet potato genotypes, CPT- 560 and KSP 20 were more retarded by ABA at a concentration of 0.5 mg/l at 5 months, when all the five genotypes were compared.

ABA is used for germplasm conservation of various plants. It slows plant growth by curtailing cell division (Jarret *et al.*, 1992). Results of the current study show that ABA was able to significantly slow growth of sweet potato genotypes NC-1582, IRA-048, CPT 560, Lohafinjo and KSP-20. However, at low concentrations of 0.5 mg/l it was not able to

retard growth significantly (p =0.05) compared to the control for genotype NC-1582 after 5 months of conservation.

Sweet potato genotypes CPT 560 and KSP-20 were much slower degraders of ABA at the lowest concentration of 0.5 mg/l, compared to genotypes, NC-1582, IRA-048 and Lohafinjo

Plants are able to degrade ABA by breaking up the ABA molecule to methanol, and glucose (Milborrow, 1974). The rate of breakdown appears to be genotype dependent. The loss of inhibitory effect especially for genotype, NC-1582 show genotypic response differences. ABA at high concentrations above 2.5 mg/l has been reported to prolong the interval between sub-cultures of *in vitro* grown sweet potato (Desamero, 1990, Jarret *et al.*, 1991), cassava (Roca *et al.*, 1982) and irish potatoes (Henshaw *et al.*, 1980). For example, Desamero (1990) achieved complete growth retardation for 12 months with sweet potato genotypes, 'Regal' and 'Jewel,' maintained in MS medium supplemented with a high ABA concentration in the range of 20-40 mg/l.

For short term conservation of sweet potato genotypes ABA at 1 or 2 mgl^{-1} was suitable for storage of all the five genotypes of sweet potato. The inference to this is that for long term conservation, for one year and above, all five genotypes could be conserved longer with a higher concentration of ABA to take care of breakdown of the same. High concentrations of ABA cause total dormancy (Jarret and Gawel, 1991, Desamero, 1990; Spiegel-Roy and Kochba, 1980). For example, embryo formation in C*itrus sinensis* is suppressed by 11-21 mgl-1 ABA or higher (Spiegel-Roy and Kochba, 1980). At the above concentrations ABA arrests growth completely. However, the growth resumes once plants

are transferred to ABA free medium. Thus, ABA can be utilised to store plants *in vitro* for a long time (Amirato, 1973).

Growth retardation treatments of 4% mannitol and 6% mannitol significantly ($p = 0.05$) retarded plant growth of sweet potato genotypes NC-1582, IRA-048, CPT 560, Lohafinjo and KSP-20. However, growth retardation treatment of 2% mannitol combined with 2% sorbital was ineffective retarding growth of genotype, IRA-048 throughout the five months of *in vitro* storage. This was also true for genotype, NC-1582 at 5 months. Three genotypes namely Lohafinjo, CPT-560 and KSP-20 were significantly ($p=0.05$) retarded by treatment combining mannitol and sorbitol, each at 2%.

Mannitol and sorbital are metabolically inactive sugars and are used widely for conservation and as cryoprotectants (Jarret and Gawel, 1991; Desamero, 1990; Withers,1993; Ng and Ng ,1991 and Desamero ,1990). Mannitol at 20-40 mg/l has been used in sweet potato to extend the interval to 1 year (Aguilar and Lopez, 1993; Mandal and Chandel, 1990; Desamero, 1990; Guo *et al.*, 1995). Mannitol appears not to breakdown as quickly as sorbital hence the persistent retardation of growth by mannitol at either 4% or 6% in the current study.

Sweet potato plantlets can remain in 10-40 g/l sorbitol for 6-12 months (Acedo, 1993; Cubillas, 1997). Sorbital has been reported to only retard growth for short time after application (Desamero, 1990). Desamero (1990) was unable to retard growth *in vitro* of sweet potato 'Jewel', beyond one month when conserved with 6–8% sorbital. Lemos and Baker (1998) were able to utilise 1-3% sorbital as the sole carbon source to induce adventitious shoots from internode sections of soursop *(Annona muricata)*. The inability

treatment of 2% mannitol combined with 2% sorbitol significantly (p = 0.05) retard growth in sweet potato genotypes, NC-1582 and IRA-048 at month 5 was most likely due to breakdown of the sorbital fraction of the medium. These results however are at variance with those of Lizarraga *et al.*, (1989). They managed to conserve irish potatoes for one year on MS medium enriched with 4% sorbital combined with 2% sucrose and 0.8% agar, incubation temperature of 25°C. Recent research findings at CIP has shown that temperature of 16-18°C lengthen the storage period up to 1 year in cultures conserved in 2% sorbitol. However, some detrimental effects were noted in 25% of the accessions tested. More recently anew culture medium containing 2% sorbitol and 2% mannitol has been tested on 30 accessions. Their survival was 82% over a 16 month period (Cubillas, 1997). Arising from these results one would expect slightly different findings in the current study if room temperature was reduced from 28 – 32°C to 18°C.

Effect of Genotypes and Growth Retardants on Number of Roots

Four growth retardation treatments of 4% mannitol, 6% mannitol, 1.0 mg/l of ABA and 2mgl-1 of ABA significantly (p[0.05) reduced number of roots of sweet potato genotypes genotypes NC-1582, IRA-048, CPT 560, Lohafinjo and KSP-20 conserved *in vitro* after 2 months compared to the control. The lowest ABA treatment of 0.5 mg/l and the control did not significantly (p = 0.05) differ from one another. However, treatment of 2% mannitol combined with 2% sorbital significantly (p = 0.05) increased number of roots. These results are consistent in that root numbers were fewer with slow growing cultures.

The addition of osmoticums or growth retardants to the medium has proven to be an efficient way for reducing growth rate of different plant species. Mannitol reduces mineral uptake thereby reducing plant growth (Dodds and Roberts, 1985; Thompson *et al.*, 1986). The increased number of roots by the treatment of 2% mannitol combined with

2% sorbital was probably due to use of the sorbital fraction of the medium by the conserved sweet potato. A recent study by Lemos and Baker (1998) demonstrated ability of plants to use sorbitol as the sole source of carbon. They induced internode sections of soursop (*Anona muricata*) to produce adventitious shoots with, 1 – 3% sorbital as the sole carbon source. The current results indicate the conserved sweet potato benefited more to sorbitol as a source of carbon, hence the enhanced root numbers.

Two mannitol retardation treatments (4% and 6%) significantly ($p = 0.05$) reduced root mean numbers when data was analyzed after two months. Mannitol reduces mineral uptake resulting in reduced plant growth (Dodds and Roberts, 1985; Thompson *et al.*, 1986). This is possible because *in vitro* plants cannot use mannitol as a source of carbon for growth.

ABA at 1 and 2.0 mg/l significantly ($p = 0.05$) reduced number of roots of *in vitro* conserved sweet potatoes NC-1582, IRA-048, CPT 560, Lohafinjo and KSP-20. ABA acts in plants by reducing cell division. Its uptake into plant tissues appears to be by simple diffusion of the un-dissociated molecule, the anions being trapped upon entry into cells. However, it is less effective at pH levels above 5.5 (Minocha and Nissen, 1985; Patel *et al.*, 1986). In addition it is more effective at high concentrations (Spiegel-Roy and Kochba, 1980; Desamero, 1990).

Effect of genotypes and growth retardants on number of leaves and internodes

Five growth retardant treatments of 4% mannitol, 6% mannitol, 0.5 mg/l of ABA, 1.0 mg/l of ABA and 2.0 mg/l of ABA did not differ significantly on genotypes, NC-1582, IRA-048, CPT- 560 after two months of *in vitro* culture. However, treatment of 2% mannitol combined with 2% sorbital and the control did not significantly differ, for the 3 sweet

potato genotypes above. However, the treatment of 2% mannitol combined with 2% sorbital significantly reduced number of leaves and nodes in sweet potato genotype KSP-20 indicating genotype response differences. The results of this study indicate that growth retardants reduce number of leaves and internodes in a similar manner as their effect on shoot growth. However, on standpoint of conservation the most important consideration would be the ability of the nodes to resume growth after the plants are transferred to medium without growth retardants.

Effect of genotypes and growth retardants on leaf abscission

Treatments of 4% mannitol, 6% mannitol, 1.0mgl-1 ABA and ABA at 2 mgl-1 significantly ($p = 0.05$) reduced leaf abscission in sweet potato genotype NC-1582, CPT-560, IRA-048 and KSP-20 after five months of *in vitro* culture. The control and 2% mannitol combined with 2% sorbital treatments did not significantly ($p = 0.05$) differ from one another for genotypes NC-1582, IRA-048 and CPT-560. The increased leaf abscission in least retarded cultures was probably due to media depletion because of rapid plant growth. The fast growth rates, high room temperature (28-32°C) and high light intensity (1500 lux) and build-up of ethylene within the culture vessels may have contributed to abscission. Ethylene is produced during culture of all cells, tissues and organs. The rate is increased if cultures are subjected to stress for example mannitol or toxic levels of ammonia (Garcia and Einset, 1982;1983). Ethylene production in cultured suspension cultures is reported to increase to reach a maximum level when nutrients become limiting (Gamborg and LaRue, 1971). Other factors including flaming of the necks of culture vessels with alcohol/gas burner frame before transfer of cultures are known to increase the level of ethylene (Beaseley and Eaks, 1979).

Effect of growth retardants on plant survival

Mannitol treatments (4% and 6%) significantly ($p = 0.05$) reduced plant survival, relative to the control. In contrast, percentage survival increased with increased concentration of ABA. The reduction of percentage survival by mannitol was unique. Often high survival is reported with mannitol treatments (AVRDC, 1992). The reduced survival in the current study may have occurred due to media drying up as a result high incubation temperature (28-32°C) and ethylene accumulation. Mannitol can cause the onset of ethylene biosynthesis (Garcia and Einset, 1982, 1983). Low light in the range of 500-1000 lux in combination of low temperatures (18-25 °C) greatly lengthen interval between sub-cultures (AVRDC, 1992; Jarret and Gawel, 1991).

ABA significantly ($p = 0.05$) increased survival of *in vitro* cultures compared to the control. ABA at a concentration of 11-21 mgl^{-1} or more completely suppresses embryo formation in citrus (Spiegel-Roy and Kochba, 1980). Higher levels of ABA arrest growth, which resumes as soon as ABA is removed (Amirato, 1973). An ABA concentration of 10 – 20 mg l^{-1} completely suppresses growth in sweet potato *in vitro* (Desamero 1990, Jarret and Gawel, 1991). The findings of the current study are in agreement with those of Desamero, 1990). Her study reported survival rate of 70-85% of sweet potato conserved *in vitro* for eight months in growth medium supplemented with ABA in the range of 5-20 mg/l.

Effect of genotypes and growth retardants on rejuvenation and phenotypic integrity

Growth retardants did not significantly ($p = 0.05$) reduce regenerative ability of sweet potato genotypes NC –1582, IRA-048, CPT-560, Lohafinjo and KSP-20 after recall from growth limiting medium to retardant free medium. However, cultures initially maintained

in ABA, had a more uniform growth. High concentration of ABA can cause toxicity on conserved plants thus, affecting the genotype (Desamero 1990, Jarret and Gawel, 1991).

Growth retardation treatment of 4% mannitol, 6% mannitol or 2% mannitol combined with 2% sorbitol significantly ($p = 0.05$) affected the phenotype sweet potato genotypes NC-1582, IRA-048, CPT 560, Lohafinjo and KSP-20. The plants had inwardly folded leaves, short internodes, and narrow petioles. Previous studies have reported similar effects on the phenotype of sweet potato conserved in *vitro* on a media containing mannitol or sorbitol (Jarret *et al.*, 1992; Desamero, 1990). However, so long as callus is not formed, mannitol and sorbitol do not affect the genetic constitution of sweet potato (Jingyu *et. al.*, 1989; Lakhanpaul *et al.*, 1990). For example, Lakhanpaul *et al.*, (1990) did not report any quantitative or qualitative changes in isozymes of esterase, acid phosphatase, peroxide dismutase

Most crops are stable when stored on growth limiting medium containing mannitol. For example, Fernando *et.al.*, (1996) reported no phenotypic nor genotypic changes of cassava conserved for over 10 years in growth limiting medium containing mannitol.

Cost analysis of growth retardants

Growth retardants used for sweet potato conservation in the current study significantly differed from one another. ABA was cheapest, whereas mannitol was the most expensive. The cost in folds of 1.0 mg/l of ABA was 16.4: 24.7: 9.92:0.5:1.0: and 2.0 for 4% mannitol, 6% mannitol, 2% mannitol combined with 2% sorbitol, 0.5 mg/l of ABA, 1.0 mg/l of ABA and 2.0 mg/l of ABA, respectively (Sigma, 1997). Assuming all the retardants were readily available, it is advisable to use ABA rather than mannitol or

sorbitol, since it is cheaper. ABA was competitively superior to mannitol because % survival was higher than for the two best mannitol levels. This indicates that ABA was less stressful to the plants and hence, the higher survival compared to mannitol.

Effects of glyphosate on plant height.

Effects of genotype (Nyaluolo and IITA-TIS-3290) on plant height was not significant, ($p= 0.05$). However, effect glyphosate was significant ($p = 0.05$). Retardation of shoot growth has been reported previously (Appbley and Salazar, 1982). They reported substantial reduction in shoot growth of grass (*Agrostes tenuis*) and red clover (*Trifolium prantense*) grown in a field where 1-3.4 kg/ ha glyphosate was soil applied a season earlier. Glyphosate has many physiological properties. It has been reported to impair the synthesis of aromatic amino acids. This xylem and phloem translocated herbicide blocks the 5-enol-pyruvyl-3-phosphate synthase (EPSP) pathway. The EPSP pathway is responsible for the synthesis of aromatic amino acids tryptophan, tyrosine and phenylalanine, precursors of: flavinoids, anthocyanin, auxins and alkaloids (Armheim *et al.*, 1980). Lack of growth in the present study could have been contributed by the disruption of this vital pathway. This shows clones IITA-TIS- 3290 and Nyaluolo were not tolerant to glyphosate.

Effect of genotypes and glyphosate on mean number of roots.

Root formation was significantly ($p = 0.05$) affected by glyphosate. No roots formed in the conserved sweet potato on with glyphosate levels of 5 mg/l or 10 mg/l. Baur, (1979) reported complete inhibition of auxins from maize shoots treated with glyphosate. Lack of rooting in plants conserved in glyphosate in the current study probably results from impairment of auxin synthesis caused by the translocated glyphosate. Glyphosate activity

Summary and conclusions

Storage of sweet potato single node cultures requires sub-culturing every 2 to 3 months to maintain their vigour. This is not only labour intensive, but also predisposes cultures to contamination, and human error, which result in loss of plant materials. Manipulation of storage temperature, reduction of nutrients and addition of growth inhibitors/ retardants enables plant growth to occur in slower rate thus lengthening the period between sub cultures.

ABA at 1-2 mg/l and 4-6% mannitol significantly ($p = 0.05$) slow down growth of sweet potato conserved *in vitro*. Significant ($p = 0.05$) differences within genotypes studied were evident. Nevertheless, for short-term conservation of up to 1 year, the five genotypes can be successfully conserved with ABA in the range of 1 – 2 mgl^{-1}. However, due to breakdown of ABA with time, it may be necessary to increase the level to 5 mgl^{-1} or higher if longer duration storage is desired. Long term storage would also benefit if temperature and light intensity were reduced in the range of 20 – 25 ^{o}C and 500 lux respectively. Mannitol at a concentration of 4-6% was equally effective in arresting plant growth as ABA in the range of 1 – 2 mg/l The two levels of mannitol did not differ significantly ($p = 0.05$). However, mannitol significantly ($p = 0.05$) reduced percentage survival compared to the control and the best ABA treatments. Percent survival was: 65% (control), 51.49 % (4% mannitol), 47.73 % (6 % mannitol), 71.1% (2% mannitol +2% sorbitol), 67.5 % (0.5 mg/l of ABA), 74.8% (1.0 mg/l of ABA) and 80.5 for (2.0 mg/l of ABA). Mannitol also significantly ($p = 0.05$) reduced internode lengths, leaf sizes and root means of five sweet potato genotypes. The treatment combining sorbitol and mannitol (2% of each on w/w basis) partially reduced plant growth of three genotypes (CPT- 560, KSP-20 and

is significantly reduced if soil applied (Quility and Geoghegan, 1976). This is because soil micro-organisms break-down the herbicide to forms which are not injurious to plants. This is believed to be reason why glyphosate is ineffective as a pre-emergent herbicide. Total inhibition of rooting in the current study suggest that glyphosate was able to disrupt both auxin synthesis and translocation in clones IITA-TIS-3290 and Nyaluolo.

Effect of glyphosate on survival and rejuvenation.

Glyphosate significantly ($p = 0.05$) reduced the % survival of sweet potato genotypes: IITA-TIS-3290 and Nyaluolo. Survival did not exceed 50% in any of the cultures conserved with glyphosate. However, percent survival was higher than 60 % for those conserved in glyphosate free medium (control). Higher culture mortality occurred possibly due to inhibition of the aromatic acid biosynthetic pathway. Glyphosate is able to inhibit incorporation of shikimic acid into three important amino acids tryptophan, phenylalanine, and tyrosine, as well as protein, putrescine and lignin (Hollander and Armheim, 1980; Berlin and Witte, 1981).

In conclusion, glyphosate significantly ($p= 0.05$) reduced % survival of genotypes: IITA-TIS-3290 and Nyaluolo. These results suggest none of the two genotypes was tolerant to glyphosate at the levels used for conservation by the current study. The high mortality could also indicate concentrations studied were not optimal for conservation purposes.

Lohafinjo) throughout the study. However, plant height of genotypes, NC-1582 and IRA-048 maintained in the same treatment significantly ($p = 0.05$) outgrew the control after four months and one month of *in vitro* culture, respectively.

Incorporation of ABA into MS medium significantly ($p = 0.05$) reduced growth rates and increased percent survival of five sweet potato genotypes. The best retardation was achieved with 2 mg/l of ABA. At the concentrations studied ABA was gradually metabolized resulting in non-uniform culture sizes after 5 months of *in-vitro* culture. However, morphological characters of the five sweet potato (leaf shape, stem colour) were not significantly ($p = 0.05$) affected by ABA. Overall, ABA at concentration of 1-2 mg/l was the best retardant for the five genotypes studied due to ability to reduce plant growth and minimal changes on the phenotypic characteristics of the conserved sweet potato. Due to possible breakdown of ABA, long-term conservation would require higher concentrations than the levels used in the current study.

Glyphosate at (5 or $10mgl^{-1}$) significantly ($p= 0.05$) retarded growth of two sweet potato genotypes Nyaluolo and IITA-TIS-3290. Besides retarding growth glyphosate also significantly ($p = 0.05$) reduced mean survival of the two sweet potato genotypes compared to the control.

Recommendations for future Research

Arising from the results of this study the following are suggested: -

(a) Since none of the retardants was able to induce complete retardation it is prudent to undertake further studies combining growth retardants and reduced temperature.

(b) Owing to increased mortality as a result of glyphosate (5 or $10mgl^{-1}$), further studies with much lower levels may be necessary.

(c) More studies on effects of growth retardants on genetic integrity *in-vitro* conserved sweet potato may be necessary.

(d) A comparative economic study between *in vitro* and field based conservation is suggested.

CHAPTER 6

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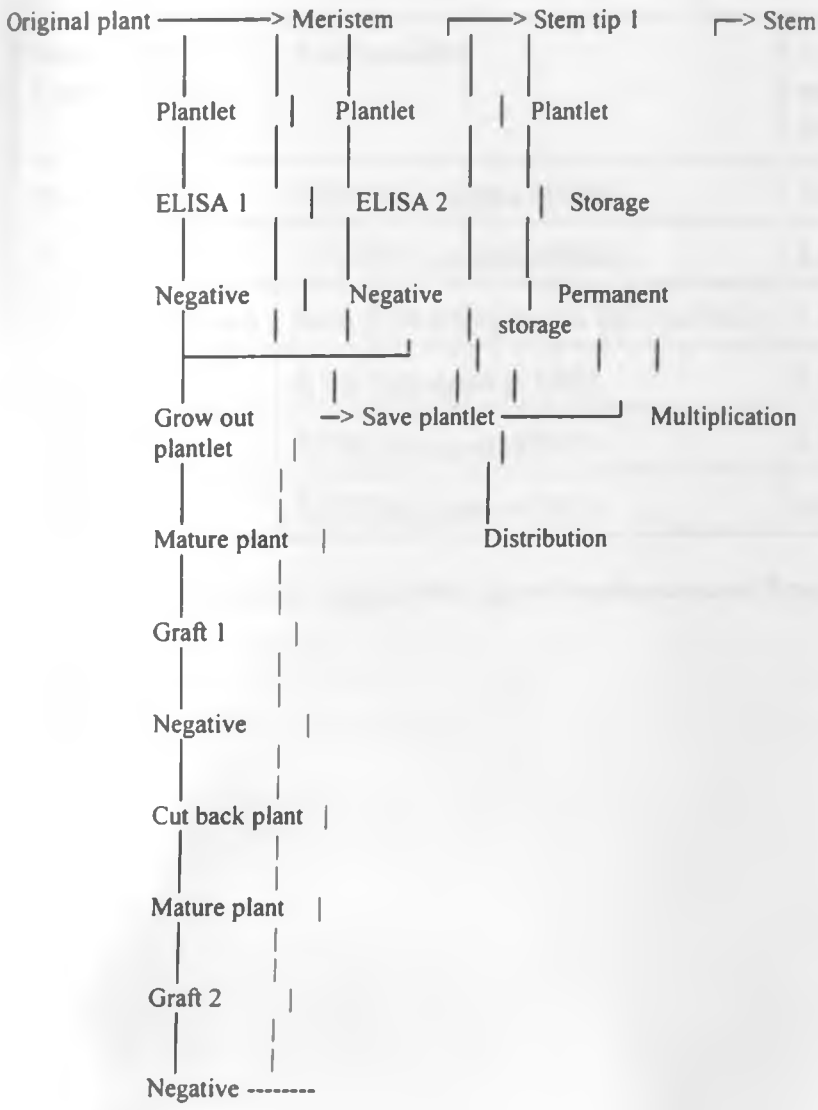
APPENDICES

Appendix 1: Murashige and Skoog (1962) basal medium (Without Vitamins and sucrose)

Constituents	Concentration in mg/l of medium
NH_4NO_3	1650.00
KNO_3	1900.00
H_3BO3	6.20
KH_2PO_4	170.00
KI	0.83
$Na_2MoO_4.2H_2O$	0.25
$CoC1_2.6H_2O$	0.025
$CaC1_2.2H_2O$	440.00
$MgSO_4.7H_2O$	370.00
$MnSO_4.4H_2O$	22.30
$ZnSO_4.7H_2O$	8.60
$CuSO_4.5H_2O$	0.025
Na_2EDTA	37.35
$FeSO_4.7H_2O$	27.85

Source: Murashige and Skoog (1962): A revised medium for rapid bio-assays with tobacco tissue culture, *Physiol. Plantarum 15: pp 473-497.*

Appendix 2: Protocol for virus eradication (AVRDC, 1992)Virus detection: A schematic presentation



Notes

(a) A first ELISA test for presence of feathery mottle virus (SPFMV) sweet potato Latent virus (SPLV) and sweet potato Yellow dwarf virus (SPYDV) done at 5 leaf stage.

(b) Plants showing negative results are recultured. Some planted in sterilized soil and subjected to two cycles of graft indexing. *Ipomea setosa* and *Ipomea nil*, two very sensitive indicator plants are grafted on sweet potato symptoms are observed for six weeks.
Diseased plants show spotting, vein clearing, Mottling, deformation, epinasty and stunting. These symptoms are more pronounced on leaves of indicator plant nearest to the graft. To ensure surety in terms of absence of viruses grafting is repeated once.

(c) Plants found negative ELISA and 2 Cycles of grafting are multiplied or kept under *in vitro* storage.

Adapted AVRDC catalogue, 1992.

Appendix 3: Cost /Benefit analysis of retardants used in experiment 1

Retardant/ Concentration	**Cost product**	**Cost of amount used**	**Cost in folds relative to 1.0 mg/l ABA**
4% mannitol	35 $/250 g (sigma M9546)	5.6 $	16.4
6% mannitol	35 $/250 g (sigma M9546)	8.4 $	24.7
2%man +2% sorb	Sorb @28.8 $/kg)+man @35 $/250 g	3.376 $	9.92
0.5 mg/l ABA	8.5 $/25g(sigma A7383)	0.17 $	0.5
1.0 mg/l ABA	8.5 $/25g(sigma A7383)	0.34 $	1
2.0 mg/l ABA	8.5 $/25g(sigma A7383)	0.68 $	2

Costs of growth retardants adapted from Sigma Biochemicals and Reagents catalogue for 1997

Appendix 4: World sweet potato production(By Regions).

Region	Cultivated Area (1000 Ha)		Yield Kg/ha		Yield Metric tonnes	
	1992	**1993**	**1992**	**1993**	**1992**	**1993**
World	9144	9111	13510	13582	123531	123750
Asia	7381	7332	15479	15622	114250	114530
Africa	1343	1368	4699	4620	6309	6321
South America	112	110	10486	10887	1177	1203
N&C America	183	175	6187	5929	1132	1040
Oceanic	17	17	12233	12056	210	206

Source FAO Year Book (1993).

Appendix 5: Sweet potato production (Major producing countries in Africa)

Country	Cropped Area 1000 Ha		Production (Yields) Kg/ha		Tonnes / ha	
	1992	1993	1992	1993	1992	1993
Uganda	442	451	4310	4200	1905	1894
Rwanda	160	163	4813	4294	770	700
Burundi	110	110	6370	6182	160	165
Kenya	61	64	9836	9844	600	630
Tanzania	198	200	2000	2000	257	260

Source FAO production Year book (1993).

Appendix 6: World production of leading food crops, 1984

Crops Production	Dry matter (M/tonnes)	Edible portion (M/tonnes)	Energy proteins (M/tonnes)	(Trillion kJ)
Wheat	530	463	5526	53.5
Paddy rice	478	421	4785	21.4
Maize	456	393	5760	35.8
Potatoes	317	64	804	5.4
Barley	175	155	1754	10.1
Cassava	131	53	461	0.5
S potato	119	35	452	1.6
Soya beans	91	82	1515	31.2
Sorghum	73	65	946	7.6
Bananas and Plantains	62	21	222	0.5
Tomatoes	60	4	46	0.6

Adapted from Woolfe, 1992.