EFFECTS OF NUTRIENT AND SOILLESS MEDIA ON GROWTH, SURVIVAL AND YIELD OF POTATO (*Solanum tuberosum* L.) APICAL ROOTED CUTTINGS

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DECLARATION AND RECOMMENDATIONS

Declaration

This thesis is my original work and has not been presented for examination in this university or any other institution for the award of any degree

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Recommendations

This thesis has been submitted for examination with our approval and recommendation as university supervisors according to Egerton University regulations**.**

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DEDICATION

This thesis is dedicated to my parents Richard Kitheka and Jane Makau for their selfless love. Special dedication to my sisters and brother Florence, Veronica, Mary and John.

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ABSTRACT

Good quality seed potato (*Solanum tuberosum* L.) is important for high productivity in all potato production systems. The major yield gap currently constraining potato productivity in Kenya is the poor quality of seed. Insufficient and untimely supply of quality seed has led to farmers depending on informal seed system (farm- saved, local markets or neighbours) resulting low tuber yields due to seed degeneration. Potato tubers are prone to accumulate and transmit pests and pathogens to the next generation and this weakens the plant production potential. Apical rooted cuttings have been introduced to increase the efficiency of seed production. With high productivity of apical rooted cuttings, it becomes economical for seed multipliers selling seed after two to three seasons. Seed tubers are known for a long dormancy period with some varieties going up to 4-5 months to be used as seed. This has led to a dely in timely planting.Two experiments were conducted to study the effects of nutrient (in vitro) and soilless media on growth, yield and survival of potato apical rooted cuttings. In the first experiment the explants from three local potato variety *Shangi, Unica* and *Wanjiku* were cultured in Murashige and Skoog (MS) media supplemented with different concentrations of sucrose (20, 30 and 40 g L^{-1}) and gibberellic acid (GA_3) (0.2, 0.5 and 1.0 mg L^{-1}). The study was laid out in a completely randomized design (CRD) using a 3×3×3 factorial arrangement. Analysis of variance was conducted by subjecting data to general linear model (GLM) to partition the variance component using SAS software version 9.0. After hardening plants were transferred to the greenhouse for experiment two where the plants were planted in polythene bags measuring 8×14×14cm filled with coco-peat + perlite, sand, coco-peat + pumice, coco-peat + vermiculite and soil. The study was laid out in a randomized complete block design (RCBD) with a 3×5 factorial arrangement. MS medium with sucrose and gibberellic acid 40gL^{-1} and 0.5 mgL^{-1} respectively significantly enhanced the shoot length of potato with *Wanjiku* being the tallest 10.3cm. The highest tuber yield (64.8g/plantlet) was recorded on *Unica* in coco-peat + pumice. This was followed by *Wanjiku* (59.8g/plantlet) and *Shangi* (40.8g/plantlet) in coco-peat + perlite. *Shangi* registered the highest number of mini tubers per plant (21) on coco-peat + perlite media. This, was however not significantly different from that of coco-peat +pumice treatment (19 tubers/plantlet). Results have shown that GA_3 at 0.5mgL^{-1} and Sucrose at 40gL^{-1} can be used in shoot elongation and as a carbon source respectively in growth of local potato varieties grown *in vitro*. Coco-peat +perlite can be used in tuber formation of the local potato varieties.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADC	Agricultural Development Corporation
ARCs	Apical rooted cuttings
AVCD	Kenya Accelerated Value Chain Development
BAP	6-Benzylaminopurine
CIP	International Potato Center
GDP	Gross Domestic Product
EDTA	Ethylene diamine tetra acetic acid
FAO	Food and Agriculture Organization of the United Nations
GA_3	Gibberellic acid
KEPHIS	Kenya Plant Health Inspectorate Services
MS	Murashige and Skoog
NPCK	National Potato Council of Kenya
RMTs	Rapid Multiplication Techniques
SDG's	Sustainable development Goals

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Potato (*Solanum tuberosum* L.) is the fourth most important food crop in the world after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and maize (*Zea mays* L.) (CIP, 2019a). It ranks first among root and tuber crops and by 2050, a global population of 9.7 billion people will demand 70% more than is consumed (FAO *et al*., 2018). In developing countries, production of the crop has increased with rising urban population where food security is a major problem (Muthoni *et al*., 2010). Potato (*Solanum tuberosum* L.) plays a major role in food security in Kenya and contributes to poverty relief through income generation and employment creation (Muthoni *et al.*, 2013).

Despite its importance, the potato sector is plagued by several problems that include; lack of quality seed planting material, lack of proper pest and disease management, a disorganized marketing system and a lack of clear policies on packaging (Riungu, 2011). The shortage of quality seed planting materials being the major problem has led to low yields, poor quality produce, and spread of diseases (GIZ-PSDA Kenya, 2011; Riungu, 2011). Kenya produces less than 3% of the national certified seed demand (Atakos & Parker, 2018).

Due to limited supply of certified seed, most farmers recycle seed tubers for many seasons causing an overall decline in seed quality due to accumulation of seed borne diseases through seed degeneration (Thomas-Sharma *et al*., 2015).The informal system has led to the use of poor quality seed planting material that hastens the spread of seed-borne diseases such as bacterial wilt (Kinyua *et al*., 2011). Several field multiplication generations of vegetatively propagated basic seed result in buildup of seed borne diseases which spread to new fields (Chindi *et al.*, 2014). To mitigate this, there is need for the use of rapid multiplication techniques such as *in vitro* propagation (Chindi *et al.*, 2014). Several seed production techniques are currently used worldwide to mitigate seed production problems (Otieno *et al.*, 2013). These include use of tissue culture (micro propagation) to produce plants for hydroponics, aeroponics systems and apical rooted cuttings (Chindi *et al.*, 2014).

Apical rooted cuttings are similar to a nursery grown seedling and are produced from tissue culture plants in a screen house, and are clean and free from diseases. They have several other advantages that include: No need to break dormancy hence faster propagation, it's economical and has a high rate of multiplication. With high productivity of apical rooted cuttings, it becomes economical to seed multipliers selling seed after two to three seasons of multiplication, as certified seed are sold normally after three seasons of multiplication.

The nutrient media is one of the most important factors for production of clean seed tuber material. The basic Murashige &Skoog (MS) medium (Murashige & Skoog, 1962) is the most widely used media in production of potato (*Solanum tuberosum* L.).The microtuber size and shape is under the control of many factors like sucrose level and growth regulators among others. The high sucrose level up to some concentration in association with growth regulator enhances microtuber productivity (Khan *et al*., 2018).

Soil is the main platform for pest and disease infections to spread, resulting in significant loss of yield and deteriorate tuber quality over seasons. In order to avoid and address this soil borne disease a media type has been introduced for producing healthy seed tuber-based on *in vitro* rapid multiplication of virus-free planting material (Altindal &Karadogan, 2010). Soil-less media has capability to grow plants in a conditioned, pest and disease free environment. Use of soilless media possibly is the most intensive culture system utilizing all the resources efficiently for maximizing yield of crops and the most intense form of agricultural enterprises for commercial production of greenhouse crops (Asaduzzaman *et al*., 2015).

Due to limited supply of certified seed potato, the Kenya Plant Health Inspectorate Service (KEPHIS), regulating seed certification, has endorsed apical rooted cuttings and is integrating the technology into seed potato certification protocol that is being finalized. To ensure success of this technology, there is need to build market demand for apical rooted cuttings, which will rely on diversifying end-user needs. The technology currently targets seed multipliers, however it is expected to expand to ware potato farmers who practice saving seed on farm. This will increase opportunities for private sector to invest in producing apical rooted cuttings of *Shangi*, *Wanjiku* and *Unica* varieties (Atakos & Parker, 2018).

1.2 Statement of the Problem

Lack of adequate supply of quality seed potato has been a major problem affecting yields. Certified seed can increase yield from ≤ 8 t ha^{-1} to 16-20 t ha^{-1} for smallholders, but currently less than 5% of the demand is satisfied (NPCK, 2019). About 92% of the Kenyan farmers store their own seed from the previous on farm harvest, which are prone to diseases and pests that lower tuber yields and thus total production. Seed tubers also having a long dormancy period with some varieties going up to 4-5 months to be used as seed has led to delay on planting time.

Apical rooted cuttings have the potential to transform potato seed systems by increasing the efficiency on seed production. The challenge, however, is in the development of ARCs from *in vitro* explants cut from the mother plant (petiole with leaf) and rooted in agar media and soilless media. The survival rate of this cuttings, their regrowth (shoot number and length) varies with variety, nutrition of agar and concentration of sucrose. Therefore, determination of the efficient nutrient and soilless media for survival and growth of the apical rooted potato cuttings is necessary.

1.3 Objectives

1.3.1 General Objective

To contribute to increased potato production through use of efficient nutrient and soilless media for potato apical rooted cuttings in Kenya.

1.3. 2 Specific Objectives

i. To determine the effects of nutrient media on growth and survival of potato varieties (*Shangi, wanjiku and Unica*). produced *in vitro*

ii. To determine the effects of soilless media (cocopeat+ perlite, cocopeat+pumice, cocopeat+ vermiculite, Sand and Soil) on growth of apical rooted potato cuttings in the green house.

iii. To determine the effects of soilless media on seed tuber yields of apical rooted potato cuttings in the green house.

1.4 Hypotheses

i. Nutrient media had no significant effects on growth and survival of potato varieties (*Shangi, wanjiku and Unica*) produced *in vitro*

ii. Soilless media (cocopeat+ perlite, cocopeat+pumice, cocopeat+ vermiculite, Sand and Soil) had no significant effects on growth of apical rooted potato cuttings in the green house.

iii. Soilless media had no significant effects on seed tuber yields of apical rooted potato cuttings in the green house.

1.5 Justification

Potato yields are affected by several factors of which the most important being lack of certified seeds. The average yield increase from the use of good quality seed ranges from 30 to 50% compared to farmers who use recycled seeds. Low seed production is due to challenges such as few numbers of certified seed multipliers, long dormancy of seeds and the cost involved in multiplying. However, demand for certified seed potato is growing with increasing awareness on the importance of using certified seeds.

If farmers could access early seed generation through apical rooted cuttings, harvested tubers can be multiplied on-farm for few seasons without the risk of significant seed degeneration, provided good agricultural practices and rotation are followed, making seed systems based on cuttings compatible with seed-saving smallholder farming systems. Apical rooted cuttings are profitable after two seasons of multiplication, rather than seed produced from minitubers that requires 3-4 seasons of multiplication to be profitable. The rapid and high rate of multiplication will significantly contribute to reducing shortage of seed potato. Furthermore, apical rooted cuttings are available on time for planting as seed dormancy requirement is limited. Water requirement is reduced as well as minimizing disease and pest infections that are prone to tubers grown in open fields. This implies that the ARCs innovation is a Climate Smart Solution with many benefits to the potato breeder and seed producer which will boost potato production to farmers with access to ARCs seedlings.

Investing in apical rooted cuttings for seed production could be interesting for the youths as little land is required, and profit margins are high. This research could enhance farmers potential to increase potato production for increased food and nutritional demand. This justification aims to contribute towards the Big Four Agenda No. 1, the National Potato Strategy (2021-2025) and Sustainable Development Goals (SDG's) No. 1&2

CHAPTER TWO

LITERATURE REVIEW

2.1 Potato Production in Kenya

Potato is a major food and cash crop in Kenyan highlands, widely grown by small scale farmers. It is produced mostly for local consumption and local markets in Kenya. Approximately half of the world's potato is produced in Asia, especially China, followed by Europe producing about a third (Scott & Suarez, 2012). Potato crop is highly produced in the temperate zone of the northern hemisphere during the summer frost-free period mainly as a cash crop (Campos & Ortiz, 2020). The crop is grown in the highlands of the Andes, African highlands, the volcanic mountains of West Africa and Southeast Asia, and the Rift valley where it is produced as a food and cash crop in the tropics (Muthoni & Kabira, 2010). In the subtropics, potato is grown as a winter crop in the Mediterranean region, North India and southern China during the heat-free period (Campos & Ortiz, 2020). Increased potato production in East African countries over the past years has highly contributed to the local food systems.

The world population is shown to be on the greatest rise in Africa, increased contribution of potato to local food systems is of high significance (Birch *et al.*, 2012). In Kenya, potato is mainly grown in the highlands by smallholder farmers for food and income and it performs better compared to maize which is a staple crop (Ireri, 2018). Potato is grown by about 800,000 farmers cultivating about 161, 000hectares per season with an annual production of about 3 million tonnes in two growing seasons. It is grown by approximately 800,000 smallholder farmers (AGRA, 2018) with an annual production of 2.06 tonnes valued at 279 million dollars annually (Komen *et al.*, 2017). The trend of production between the years 2010-2018 is as shown below (Table 2.1) where by there has been a decline in potato production yearly with increase in area harvested (FAOSTAT, 2020). There are 13 major potato producing counties which are: Meru, Nyeri, Nyandarua, Kiambu, Taita-Taveta, Nakuru, Narok, Bomet, Elgeyo-Marakwet, Trans-Nzoia, Bungoma, Uasin-Gishu and West-Pokot. Other potato producing counties include: Kisii, Nyamira, Kirinyanga, Muranga, Baringo, Nandi, Laikipia and Kericho. Other upcoming potato producing counties mainly in the hilly masses include: Machakos, Makueni, Embu, Kajiado, Tharaka-Nithi, Samburu, Kwale and Nairobi (NPCK, 2019).

Table 2.1: Potato area harvested, productivity and production trend between 2010 and 2018 in Kenya

Year	Area harvested (ha)	Yield (tonnes/ha)	Production (tonnes)
2010	121,542	22.4279	2,725,936
2011	123,390	19.1690	2,365,263
2012	143,325	20.3389	2,915,067
2013	152,007	14.4262	2,192,885
2014	115,604	14.0655	1,626,027
2015	133,532	14.7043	1,963,495
2016	145,967	9.1520	1,335,883
2017	192,341	7.9019	1,519,870
2018	217,315	8.6067	1,870,375

Source: FAOSTAT (2020)

2.1.1 Importance of Potato

Potato is a valuable and nutritious staple crop, driving both food security and Gross Domestic Product (GDP) globally (Devaux *et al*., 2014). The potato value chain employs over 3.5 million actors, contributing over Kshs 50 billion to the Kenyan economy. Potato has a critical role to play in developing nations facing hunger (Gebru *et al*., 2017). It supplements or replaces grain-based diets where rice, wheat, or maize availability has lessened or price has become unaffordable (Camire *et al*., 2009). It is a well-balanced major plant food with a good ratio between proteins and calories, and has substantial amounts of vitamins, especially vitamin C, minerals, and trace elements (Teshome, 2016). It has the correct balance of protein calories and total calories

Potato can give stable yield under conditions where other crops might fail, suitable to grow where land is limited and labour is plentiful (Lutaladio & Castaldi, 2009). The market is expanding rapidly as potatoes are increasingly becoming popular as a source of affordable food for growing urban populations with a preference for potatoes in processed forms such as fries and crisps providing a growing domestic market that presents a valuable opportunity for smallholder farmers (Abong' *et al*., 2010). Emerging markets for processed potatoes (e.g., chips, crisps, starch) furthermore, have increasingly focused attention on potatoes, with rising demand from the fast food industry and processing for added economic value (Abong & Kabira, 2013). Its plasticity to environmental conditions and yielding capacity also make it the best crop for food and nutrition security (Kyamanywa *et al*., 2010). It is also the most important tuber crop, ranking first in volume produced among root and tuber crops; it is followed by cassava (*Manihot esculenta* C.), sweet potato [*Ipomoea batatas* L.) and yam (*Dioscorea* spp.) (FAO, 2010). The potato crop has capacity to feed large populations and provide more food per hectare compared to other staples due to its short maturity period allowing two crops annually (Wang'ombe & Van Dijk, 2013). There is therefore need for it to be considered as a potential crop for the country's mission to attain food security.

2.1.2 Challenges Facing Potato Production in Kenya

Farmer's limited access to quality seed potatoes of the varieties in demand locally has become the main constraint of potato production (FA0, 2017). This has largely attributed to the high cost associated with access to quality seed, which finally limits the production of potatoes (Muthoni &Nyamongo, 2009). There is also limited storage facilities affecting the quality of available seed; with 52% of the farmers storing seed potato in conventional stores with seed potato being mixed with other produce. This is attributed to limited awareness and knowledge on good postharvest management practices when managing saved seed. Such poor seed storage practices lead to low yields of the potato crop (CIP, 2011) Despite the importance of potato in Kenya, yields have remained very low approximately between 9.0 to 10 t ha^{-1}against a potential of over 40 t ha^{-1} (Komen *et al*., 2017; VIB, 2019). The potato sector is plagued by problems such as lack of certified seeds (Riungu, 2011) and spread of diseases like bacterial wilt (Kinyua *et al*., 2011). The shortage of certified seeds (disease free) has led to low yields, poor quality produce, and spread of pests and diseases (GIZ-PSDA Kenya, 2011; Riungu, 2011) and the available certified seeds potato are highly priced (Ayieko &Tschirley, 2006).

Due to limited supply of certified seed potato, the main challenge facing potato production in Kenya is use of poor quality seed potato tubers that are highly contaminated with seed borne diseases (Mariita *et al.*, 2016; Okello *et al.*, 2017). The informal seed potato supply system made up of farm saved, seed exchanges by farmers and local markets is the main channel of acquiring seed potato tubers in developing countries and it accounts for over95% of seed potato volumes used (Hirpa *et al*., 2010; Kaguongo *et al*., 2013 ; Muthoni *et al*., 2013). In Kenya, over 95% of potato farmers use farm saved seed tubers which are of poor quality especially with accumulation of tuber borne diseases (Kagoungo *et al*., 2013). Seed borne diseases such as bacterial wilt (*Ralstonia solanacearum*), late blight of potato (*Phytopthora infestans*), dry rot (*Fusarium* spp.) and potato viruses are carried across cropping seasons leading to an overall decline in seed quality and causing high yield losses (Sharma *et al*., 2015 ;Waswa *et al* .,2017). Emerging initiatives and technologies have been used to address the challenge of unavailability of certified and quality declared seed potato in the Eastern and Central Africa (ECA) region, which include seed plots techniques, positive seed selection, and production of minitubers (Kinyua *et al*., 2011). However, these require additional supportive policies for recognition and regulation of seed production and distribution in the potato value chain since the available seeds have long dormancy leading to non availability during planting.

2.2 Rapid multiplication techniques (RMTS) vs conventional techniques of potato

The conventional method of propagation system is one of the slowest of seed multiplication rates of 1:10 ratio compared to RMTs which has a multiplication rates of 1:40 (Otazu, 2010). Conventional seed potato programs are the low multiplication rates of field-grown potato plants, resulting in a slow and inflexible system and the risk of catching viral, fungal or bacterial diseases with an increasing number of field multiplication (Aafia *et al*., 2007; FAO, 2008). Other negative impacts of conventional agriculture include the high and inefficient use of water, large land requirements, high concentrations of nutrients consumption, and soil degradation. To address these problems of bacterial wilt and viruses a new system has been established for producing healthy seed based on systemic virus testing and in vitro rapid multiplication of virus free planting materials.

RMTs are any type of manipulations that significantly increase tuber yield per plant. Rapid multiplication is very flexible and gives a high rate of multiplication. It also provides seed potato tubers free from seed borne diseases and it can solve some of the problems associated with the conventional multiplication system. Thus, different RMT's have been used for bulking up minitubers of released potato varieties and promising clones for distribution to growers. Many RMTs exist, but the most common in developing countries are micro-propagation (plantlets and micro tubers), cuttings (single-node, tuber-sprout, axillary, leaf-bud, apical), aeroponics, and hydroponics (Struik & Wiersema, 2012).

2.2.1 Micro propagation of potato

In vitro micropropagation is an alternative to conventional (vegetative) propagation of potatoes. In vitro propagation methods using nodal cuttings, meristem tips and micro tubers are more reliable to maintain genetic integrity of the multiplied clones since differentiation and the subsequent organogenesis/embryo genesis with the accompanying genetic have been reported (Xhulaj & Gixhari, 2018). Seed production technique of potato can be designed with in vitro multiplication through either plantlet regeneration or micro tuber production (Hossain, 2005). It has been proved to be very efficient technique to speed-up the production of high quality pathogen-free plantlets, in terms of genetic and physiological uniformities (Sathish *et al*., 2011; Supaibulwattana *et al*., 2011). The use of single-node cuttings excised from tissue cultured plantlets is more common and avoids the influence of tuber tissue from which sprout sections originate (Mohamed &Alsadon, 2010; Mohamed *et al*., 2009). Nodal cuttings have been reported for auxiliary shoot development and suggested to be the best explants source on either liquid or agar solidified medium (Mahapatra &Batra, 2017). Vanaei *et al*. (2008) studied the propagation of two commercial cultivars of potato (Marfona and Agria) using meristem tip as explants source. Potato tubers were also used as an explants source (Mutasim *et al*., 2010). Disease free healthy tubers were propagated on MS media with different concentrations of kinetin (Hoque, 2010). The sprouts were rinsed 3- times with sterile distilled water under the clean bench.

2.2.2 Use of cuttings to boost potato production

Integrating cuttings into seed systems increases efficiency of seed production as the productivity of cuttings surpasses that of minitubers. A cutting is similar to a nursery-grown seedling except that it is produced through vegetative means and does not originate from a seed (Parker, 2017). Rather than allowing tissue culture plantlets to mature and produce minitubers in the screen house, cuttings are produced from the plantlets by taking single or double node cuts from lateral shoots. Cuttings mature quickly in the field, with egg-sized tubers observed 30 to 40 days after planting (Atakos & Parker, 2018). Three techniques of potato multiplication i.e. stem cutting, nodal cutting, leaf bud cutting and apical rooted cutting are used to produce disease-free planting material. During the 1970s and 80s the use of stem cuttings became a rapid way of producing mini tubers in substrate in greenhouses. Propagation of potato seed stocks by stem cuttings was developed as a means of elimination of bacterial and fungal pathogens normally carried over by tuber propagation. Dahshan *et al*.

(2018) reported the possibilities to propagate potato through stem cuttings and enhancement of the growth capabilities using different plant growth regulators.

Potato apical rooted cuttings (ARCs) originating from juvenile simple rounded leaf mother plants are a significant new way of transplanting and field growing of seed potatoes under smallholder field conditions in the tropical highlands (Vanderzaag *et al*., 2021). Apical cuttings originate from tissue culture material, i.e. the mother plant is maintained in a juvenile state throughout the production cycle (Atakos & Parker, 2018). The high productivity potential is in the physiologically young tissue retained in a simple leaf stage. Stem cuttings generally implies that the mother plant has developed compound leaves, physiologically older. Normally mother plants for stem cuttings originate from a tuber, develop compound leaves, and sprouts and shoots are taken as cuttings. Stem cuttings originating from mother plants with compound leaves generally yield 2 tubers per stem.

Maximum productivity, vigor and mother plant juvenility has been associated with temperatures of about 18-25°C, relative humidity of 60–85%; regular fertigation with adequate nitrogen applied at 2–3 day intervals and use of clean media that exhibits low salt concentration (EC <0.5ds/m), pH 6.0-6.5 and can retain good moisture and nutrients (CIP, 2019b). Frequent cutting of the mother plant extends juvenility up to 9 months. Cuts should be obtained as soon as apical shoots have grown 5–7 cm high, or if the shoot has 4–7 complete leaves with 2–3 internodes. This is a key management protocol to attain prolific and juvenile mother plants. A mature mother plant literally gives stem cuttings with low multiplication ratio of 3–7 tubers/cutting; thus, only juvenile plants are propagated to result in high rates of ARCs production (CIP, 2019b).

2.2.3 Potato production in aeroponics

Aeroponics technique is a rapid multiplication technology (RMT) able to produce large numbers of minitubers in one generation, thus, allowing bulking of large number of potato seeds. It is a method, whereby the underground organs are enclosed in a dark chamber and supplied with a nutrient solution through a tight misting system under protective structures to produce minitubers (Farran & Mingo-Castel, 2006). The environment should be kept free from pests and diseases so that the plants may grow healthier and quicker than plants grown in a soil medium. As aeroponics is conducted in air combined with micro-droplets of water, almost any plant can grow to maturity because of an abundant supply of oxygen, water and nutrients. The ability to precisely control the root zone moisture levels and the amount of water delivered makes aeroponics ideally suited for the study of water stress

(Mbiyu *et al.*, 2012). Farran and Mingo (2006) observed that the field performance of aeroponically produced tubers to be similar to minitubers produced from the pots. Yields of over 100 tubercles /plant were obtained from plants grown in aeroponics at the International Potato Centre (CIP) in Peru (Otazu, 2010).

Aeroponics system offer several advantages over other methods. These include: (1) Aeroponics optimizes root aeration resulting in more yields than classical hydroponics; (2) Aeroponics uses little water that is, 1/10th to 1/30th of the water used in field production of the same amount of potatoes; (3) There is good nutrient recirculation, control of nutrients and pH; (4) No sterilization of growing media is required thus, minimizing the costs. There are no soil-borne diseases, weeds and nematodes in aeroponics systems; (5) An aeroponics system as a whole, allows for uniform water availability to plants; (6) Intensive production in a small space that is, more plants per unit area and hence, more yields; (7) No loss of fertility and so no crop rotation is needed in field grown crops; (8)) Extended growing season that is, crops can be grown all the year around and hence more yields. Aeroponics technique is up to ten times more effective than with the conventional techniques (CIP, 2010; Muthoni *et al.*, 2010).

2.2.4 Potato production in hydroponics technique

It is a technique for producing high-quality minitubers in troughs without electricity, as it does not require installing pumps (Mbiri *et al.*, 2015). Soilless production technologies are being an option for the production of quality early generation seed potato to fill the gap of seed demand. Hydroponic is the system of growing plants using nutrient solution dissolved in water without the use of soil as a growing medium. Plants can take all the required nutrients directly through their roots in dissolved form (Factor *et al*., 2007). It is the technique that involves the growing of plants in water mixed with all the necessary plant nutrients or in an inert medium such as gravel, block or cockpit. So many advantages could be counted for using hydroponics to grow plants; soil is not necessary; it's stability and high yields; no nutrition pollution is released into the environment; higher nutrient and water use efficiency due to control over nutrient levels (Barbosa *et al*., 2015).

Hydroponics crop production has significantly increased in recent years worldwide, as it allows a more efficient use of water and fertilizers as well as better control of climate change related pests. In addition to these, hydroponics production could boost the crop quality and productivity, which results in higher competitiveness and economic benefits to growers. A 1-acre (0.4ha) hydroponics greenhouse produces the same output as 10 acres (4ha) of field (Naik & Karihaloo, 2007).

2.3 Developing apical rooted cuttings of potato

Apical rooted cuttings were introduced to Stokman Rozen Ltd and Genetic Technologies International Limited (GTIL) by International Potato Center (CIP) (Harahagazwe *et al.*, 2018). Apical cuttings originates from tissue culture material, the mother plant is maintained in a vegetative state throughout the production cycle (Parker, 2017). Apical rooted cuttings have the potential to transform potato seed systems (Atakos & Parker, 2018). The rapid and high rate of multiplication reduces seed potato shortages since each cutting produces 10 to 15 tubers, which are multiplied a further season or two seasons (Parker, 2017). Two-nodal apical cuttings (4 to5 cm long) of potato are harvested and rooted in trays with a substrate. The cuttings are then transplanted in the field once they are fully rooted. Maintaining the juvenile stage of the mother plant is key to retaining productivity, whereby the mother plant remains with simple leaves (Vanderzaag, 2013). Rooted apical cuttings are transplanted right away in the field, thereby saving one generation, as minitubers are no longer needed (Parker, 2017). Farmers need to be informed of the practice of growing of potato ARCs with the same care as they do with tomato, kale or cabbage seedlings.

2.3.1 Yield potential of potato cuttings vs minitubers

Mini-tuber is an intermediate step of seed potato production between laboratory micro propagation and field multiplication (Naik & Khurana, 2003). Mini-tubers can be obtained from in vitro plantlets planted under in vivo condition after planting them in soil (Naik & Karihaloo, 2007; Otroshy, 2008). The use of high quality seed micro- tubers, produced using apical rooted cuttings (ARCs) has the potential to double productivity and thereby increasing food needs (Atakos & Parker, 2018).Given the high productivity of apical rooted cuttings, it is economical for multipliers to sell quality seed after 2 seasons of field multiplication, and after 3 seasons it’s highly profitable. This is compared to commercial seed produced from minitubers, which is usually sold after 3-4 seasons of multiplication. Apical cuttings produce 10 to 25+ tubers per cutting compared to 5 to 10 tubers per minitubers at a cost of USD 0.10 and USD 0.15 to 0.30 respectively as shown in figure below (Parker, 2020).

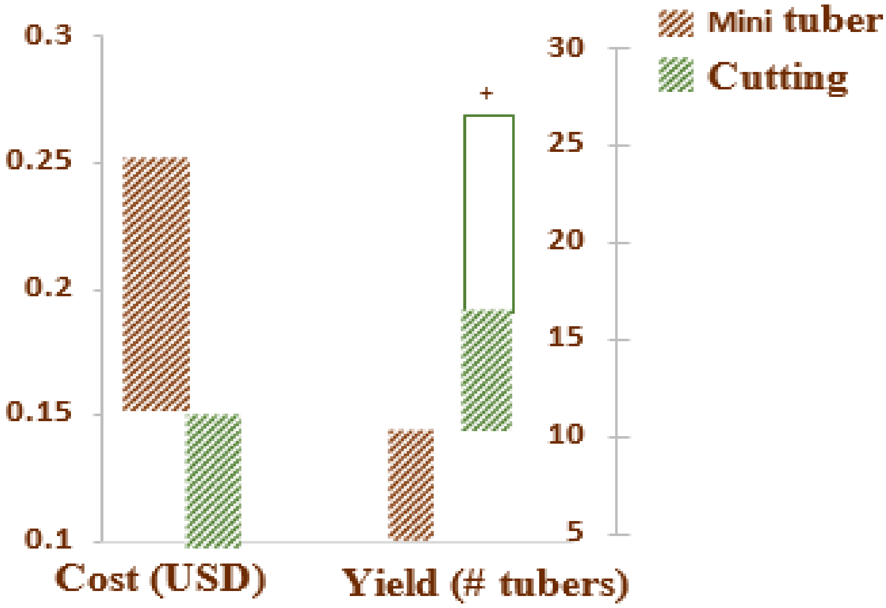

Figure 2.1: **Cost and Yield of cuttings verses minitubers**

Source: Parker (2020)

2.4 Effect of sucrose on growth and survival of potato.

One of the most important factors governing the *in vitro* shoots regeneration is largely determined by the composition of the culture medium. Plant growth media play a fundamental role in plant *in vitro* propagation as it provides water, nutrients, and support to plants. Consequently, its composition can have direct effects on plant performance (Kazemia & Mohorko, 2017). Carbon source in the medium; both its type and concentration have profound effects on tuber growth. Plant cell, tissue or organ culture normally requires the incorporation of a carbon source to the culture medium. Several tissue culture reports refer to the influence of the carbon source on the in vitro morphogenesis of different plant species. Among the many available carbon sources, sucrose has been the major one mostly used. The addition of sucrose to the *in vitro* culture medium provides an important source of carbon and energy for the plants (Cheong & An, 2015; Elazab & Shaaban, 2015). Sucrose has also been implicated in lateral bud outgrowth (Van den Ende, 2014). The axillary shoot outgrowth from the nodal segments of *Prunus africana* depended on the availability of sucrose in the medium (Komakech *et al*., 2020). However, different concentrations of sucrose in the culture medium have been observed to exhibit different effects on the shoots of plants (Joao *et al*., 2015).

Six percent sucrose concentration was much better for good shoot regeneration and root length and 3% sucrose was better for shoot length and average number of leaves for

PARS-70 cultivar while for Santa cultivar, 12% sucrose was found significantly better for shoot length (2.75cm), number of leaves (3.85), root formation (4.32%) and root length (2.04 cm) (Fatima *et al*., 2005). It was observed that when MS media was supplemented with 30g/l sucrose healthy shoots were produced. At higher levels of sucrose, the osmolarity of the medium increased and plants underwent stress (Hussain *et al*., 2006).

2.5 Effects of growth regulators (PGRs) on growth and survival of potato

The responses to exogenous PGRs vary according to the type of explant and the plant species, since the production of endogenous hormones varies among different explants and genotypes (Gana, 2011). 6-Benzylaminopurine (BAP) has shown a superior result in comparison to other cytokinins for enhancing shoot initiation in woody plant species (Siwach & Gill, 2011). This superiority may be due to the ability of BAP to trigger cell division and lateral bud development that plays a vital role in breaking axillary bud dormancy (Goyal *et al*., 2015; Sadeghi *et al*., 2015). Auxin hormones play a significant role in rooting initiation, growth, and development (Kang *et al*., 2018). These hormones are also important in root cell elongation and overall root growth (Pacheco-Villalobos *et al*., 2016). IBA and naphthalene acetic acid (NAA) are still the most widely used auxins for rooting stem cuttings and for rooting tissue culture–produced micro-cuttings. The best regeneration of meristem tips was obtained when MS medium was supplemented with 1.0 mgL^{-1} pantothenic acid + 0.5 mgL^{-1} gibberellic acid (Raza, 2011). GA_3 at the rate of 0.248 boosted all the morphological characteristics over control (0.00) and other treatments (0.12, 0.50 and 0.75mgL^{-1}) (Farhatullah *et al.,* 2007). Maximum number of shoots was observed on MS medium containing GA_3 at 1.0 mgL^{-1} producing 12 shoots/flask from 5 nodes. It was noted that higher concentration of GA_3 at 2.0 mgL^{-1} had no effect on shoot numbers but showed elongation in shoot size (Hussain *et al*., 2006).

When medium was supplemented with GA_3, multiple shoots of Zaubilati variety (2.87), Shadaguti variety (3.78) and Challisha variety (2.31) were recorded at a concentration of 0.2mgL^{-1} GA_3 (Parveen., 2011). Bergerac cultivar gave a higher number of shoots per explant (6.37) compared to Excuisita cultivar (3.75) when MS Media was supplemented with 0.25ppm GA_3 and 2ppm calcium pantothenic acid (Xhulaj & Gixhali, 2018). The longest roots were noted on Pasinler cultivar (9.98cm) on MS Media containing 0.25mgL^{-1} GA_3 followed by Granola cultivar (8.63cm) and Caspar (7.78cm) (Ahmet, 2014). The best regeneration potential of the meristem tips of variety Desiree was observed when MS medium was supplemented with 1.0 mgL^{-1} pantothenic acid and 0.5 mgL^{-1} GA_3 while in

Patrones variety it was observed when MS medium was supplemented with 2.0 mgL^{-1} pantothenic acid and 0.5mgL^{-1} GA_3 .Desiree showed higher mean plant height (11.1cm) in MS medium supplemented with 1.0 mgL^{-1} pantothenic acid and 0.5 mgL^{-1} GA_3 and Patrones gave higher mean plant height (8.3cm) in MS medium supplemented with 2.0 mgL^{-1} pantothenic acid and 0.5mgL^{-1} GA_3 (Yasmin & Jalbani, 2011).

From the foregoing review it is evident that growing of explant *in vitro* in MS media supplemented with rooting hormone at varied concentrations such as GA_3 (0.2-0.5mgL^{-1}) enhanced rooting and boosted morphological characteristics and micro tubers for different potato varieties. There is therefore, need to evaluate the response of Kenyan varieties to varied hormone concentrations for enhanced shooting and establishment of apical rooted cuttings (explants).

2.6 Effect of soilless media on growth of potato

The soilless media is one of the most important factors for production of clean seed tuber material. Different media can be used to grow *in vitro* potato plants in a greenhouse (Balali *et al.*, 2008). Potato varieties that were grown in Kalpeat plus (Coco peat: Perlite) in the ratio of 75:25 and soilrite mix media (perlite+ peat-moss + vermiculite) in the ratio of 1:1:1 had higher plant height of 160cm and 150cm for genotype 1005 respectively than that in soilrite mix TC (Peat moss+ perlite) in the ratio of 75:25 that had 100cm and control (black soil: sand) in the ratio of 75:25 that had 60cm (Awati *et al.*, 2019). It was reported that media composition of soil+ compost+ husk charcoal +coco-peat (2:1:1:1) recorded the highest plant height of 59cm followed by soil +compost +coco-peat (2:1:1) with 57cm and soil +compost +husk charcoal (2:1:1) with 48cm (Sutari *et al.*, 2018).

Rosseta and BP1 2007 genotypes of potato had the highest survival and establishment percentage of 100% on vermiculite media compared to sand (75 and 83%) and sawdust (83 and 56%) respectively than the up to date, magalabada and Rosita genotypes. Rosetta genotype gave the highest number of minitubers (21.46t/ha) followed by BP1 2007 genotype (21.40t/ha) and up to date genotype (20.12t/ha) when planted in vermiculite media than in sand and sawdust (Zimba *et al*., 2014).

Rivera variety gave the highest plant height (67.90cm, 63.11 and 61.97) when planted in both soilless media (sand+ horse manure in the ratio of 1:1, horse manure and sand) respectively, followed by Pekaro variety that gave a higher plant height of 61.33 in sand media, 59.22 in sand +horse manure (1:1) and 44.33cm in horse manure, while Arizona variety gave higher plant height of 59.22 in sand +horse manure, 54.78 in sand and lowest 46.11 in horse manure (Ahmed & Wafaa, 2018).

Higher plant survival and establishment percentage of 96% was observed in potato varieties planted in Kalpeat plus, followed by soilrite mix (89.6%) which was significantly more than in control (soil + sand) media, which recorded 79.2% of germination (Awati *et al.,* 2019). This could be due to improved aeration, soil water absorbing capacity, composition variables and characteristic of the soil-free medium than in control media.

The highest plant survival percentage of 93.33% was observed in mixture of loam soil, sand and compost soil in the ratio of 2:1:1 compared to individual media of loam soil alone, sand soil alone and compost soil alone (73.33%, 60.00%, 80.00%)respectively (Genene *et al*., 2018).

It was recorded that higher plant establishment percentage of 95% that was observed in genotypes planted in vermiculite media than in sand (77%) and sawdust (49%) (Zimba *et al.*, 2014). Review reports that mortality rates of potato varieties can be reduced from 36%% to 0% by establishing potato apical rooted cuttings in Kalpeat plus, soilrite mix, mixture of loam soil, sand and compost soil in the ratio of 2:1:1 and vermiculite media. There is therefore need to evaluate response of Kenya potato varieties to different soilless media combination available in the country.

From the review it shows that growth of potato varieties such as plant height, multiplication of tubers and number of stems is enhanced by different soilless media combinations. Kalpeat plus (Coco peat: Perlite), soilrite mix (perlite+ peat-moss +vermiculite), soil + perlite + compost treatments (enhanced plant height), sand +peat moss (multiplication of potato minitubers and river sand and pumice amended with coco-peat (plant height and stem length). There is therefore need to evaluate response of Kenya potato varieties to different soilless media combination available in the country to enhance the plant height, stem length and multiplication of tubers.

2.7 Effect of soilless media on mini-tuber yield of potato

Treatment of media composition of soil +compost+ coco-peat (2:1:1) and soil +compost +husks charcoal +coco-peat (2:1:1:1) increased the percentage of tuberization (93.33 and 90.86%) and the weight of tubers per plant (335.0 and 355.5g) compared to that of soil+ compost+ husk charcoal (2:1:1) that had percentage of tuberization of 86.33% and weight of tubers of 156.4% (Sutali *et al.*, 2018). Plantlets transplanted in perlite+ peat-moss +vermiculite in the ratio of 1:1:1 performed better with higher mini-tuber yield of 543.58

gm/container than in coco-peat + Perlite in the ratio of 75:25 (283.39), Peat- moss +perlite in the ratio of 75:25 (96.08) and in control (black soil: sand) in the ratio of 75:25 (52.61)grams per container(Awati *et al*., 2019). According to them plants planted in soilrite mix registered the highest number of tuber per plant (9), followed by 5 tubers per plant in Kalpeat plus media than in soil +sand (1).

It was reported that plantlets grown on vermiculite performed better with higher mini tuber yield of 17.4t/ha for all genotypes compared to sand and sawdust (8.5 and 2.92t/ha) respectively (Zimba *et al*., 2014).

In conclusion, it indicates that mini tuber yield (kg/ha) of potato varieties is enhanced by different soilless media combinations. Soil +compost +coco-peat (2:1:1), soil +compost +husks charcoal +coco-peat (2:1:1:1), perlite+ peat-moss +vermiculite (1:1:1) enhances mini-tuber weight and number of mini-tubers too. There is therefore need to evaluate response of Kenyan potato varieties to different soilless media combination for enhanced mini tuber numbers and yields.

2.8 Potato varieties in Kenya

There are 60 potato varieties grown in Kenya (NPCK, 2019). These varieties differ in use, tuber features, maturity period, yields, disease and pest resistance, size and color. Apical rooted cuttings of *Shangi*, *Wanjiku* and *Unica* potato varieties are done by Stokman Rozen, GTIL and CIP. More recently Aberdere farms are also growing ARCs indicating the growing demand for this technology.

Shangi was released in 2015 by Kenya Agriculture and Livestock Research Organization (KALRO) (Msyimi Ireri, 2019). It grows to a maximum of one metre high with broad leaves which are light green without anthocyanin pigmentation on the midrib (NPCK, 2019). *Shangi* has an upright growth with prolific flowering. The tubers are oblong and uniform. The tuber dormancy is very short (less than 1 month). *Shangi* takes 75-90 days to maturity yielding 30-40t ha^{-1} (NPCK, 2019). It is grown in attitude of 1500-2800 m.a.s.l.

Unica was released in 2016 by the International Potato Center (CIP) and KALRO. It takes 80-90 days to maturity. *Unica* is a medium tall variety with strong semi- erect stems and dark green medium- sized leaves. It produces pink flowers profusely. It is moderately resistant to late blight, highly resistant to Potato Virus X (PVX), resistant to Potato Leaf Roll Virus (PLRV) NPCK, 2019). It is a versatile variety recommended to all regions suitable for potato production. Tuber dormancy is long (2.5-3.5 months). It has a potential yield of >45 t ha^{-1}. Tubers are oblong, red skinned with shallow eyes and cream flesh (NPCK, 2019).

Wanjiku was released in 2017. It is a medium tall potato plant with strong semi erect stems and dark green medium size leaves with pinkish flowers (NPCK, 2019). It is tolerant to late blight, Potato Virus X & extremely tolerant to Potato Virus Y (NPCK, 2019). It takes 90-100 days to maturity. It has a potential yield of >40 tons ha^{-1}. Tuber dormancy is long (more than 3 months). Tubers are oblong shaped with white cream skin, shallow pinkish eyes and cream flesh.

CHAPTER THREE

EFFECTS OF SUCROSE AND GIBBERELLIC ACID ON GROWTH AND SURVIVAL OF POTATO VARIETIES GROWN *IN VITRO*.

Abstract

Tissue culture has been used for disease free seed potato production in many countries. Potato seed production using tissue culture technique can be designed with *in vitro* multiplication through either plantlet regeneration or micro tuber production. The objective of this study was carried out *in vitro* to determine the optimum concentration of sucrose and GA_3 for survival and growth of potato varieties. An experiment was conducted using explants from local varieties *Shangi, Unica* and *Wanjiku*. The explants were cultured in Murashige and Skoog (MS) media supplemented with concentrations of sucrose 20, 30 and 40gL^{-1} and concentrations of gibberellic acid 0.2, 0.5 and 1mgL^{-1} as a shooting hormone. The experiment was laid out in a completely randomized design (CRD) using a 3×3×3 factorial arrangement. Parameters measured include; days to shoot initiation, days to root initiation, number of leaves, number of roots, shoot length, number of shoots, leaf fresh weight and leaf dry weight. Data collected was subjected to general linear model (GLM) to partition the variance component using SAS software version 9.0 and means separated using Tukey's Honestly Significant Difference Test (HSD) at $P \leq 0.05$ level of significance. MS Medium with sucrose 40gL^{-1}and gibberellic acid 0.5 mgL^{-1} significantly enhanced the shoot length of varieties with *Wanjiku* variety showing higher length of 10.3cm. Treatment with 40gL^{-1} sucrose and 0.5 mgL^{-1} gibberellic acid showed the highest number of leaves of 11.3, 7.4 and 7.5 cm for *Wanjiku, Unica* and *Shangi* respectively. Highest plant survival was recorded under treatment with 0.5mgL^{-1} gibberellic acid + 40 gL^{-1} sucrose (90%) and this was significantly different from other treatments. The minimum days to shoot induction was observed in *Shangi* (5.11d) followed by *Wanjiku* (5.56d) and *Unica* (5.67d) on MS Medium containing 30 g L^{-1} sucrose. Effects due to interaction among gibberellic acid, variety and sucrose showed significant differences for number of leaves, number of roots, number of shoots and shoot length at $p \leq 0.001$. This study recommends that If farmers can use GA_3 at 0.5mgL^{-1} in combination with 40g L^{-1} sucrose in survival and growth of local potato varieties, then higher rates of rapid multiplication can be achieved for *Shangi, Wanjiku* and *Unica* varieties.

3.1 Introduction

Nutrient media is one of the most important consideration for production of clean tuber seed material. The basic Murashige &Skoog (MS) medium (Murashige & Skoog, 1962) is the most widely used media in production of potato (*Solanum tuberosum* L.). However, the amounts of various ingredients in the medium vary for cultures of different species. The size and shape of the microtuber are controlled by many factors such as sucrose level and growth regulators among others.

Potato (*Solanum tuberosum*) is the third most important food crop in the world after rice and wheat in terms of human consumption. It ranks first among root and tuber crops and by 2050, a global population of 9.7 billion people will demand 70% more than is consumed (FAO *et al*., 2018). In developing countries, production of the crop has increased with rising urban population where food security is a major problem (Muthoni *et al*., 2010). Potato plays a major role in food security in Kenya and contributes to poverty relief through income generation and employment creation (Muthoni *et al.*, 2013).

As a result of limited supply of certified seed, most farmers recycle seed tubers for many seasons causing an overall decline in seed quality due to accumulation of seed borne diseases through seed degeneration (Sharma & Ali *et al.*, 2015). The informal system has led to the use of poor quality seeds planting material that hastens the spread of seed-borne diseases such as bacterial wilt (Kinyua *et al*., 2011). To mitigate this, there is need for the use of rapid multiplication techniques such as *in vitro* propagation of disease free potato seed in a short period of time (Chindi *et al.*, 2014). Several seed production techniques are currently used worldwide to address seed production problems (Otieno *et al.,* 2013). These include use of micro propagation to produce plants for hydroponics and aeroponics systems (Chindi *et al.*, 2014).

Osmotically active solutes has shown that sucrose acts as a carbon source and as osmotic regulators. Sucrose and their concentration is important factor on potato microtuberization and has a profound effects on the tuber growth (Azar & Kazemiani, 2013). It acts as an energy for growth and biosynthetic processes, and may influence *in vitro* growth (Ferreira *et al*., 2011). Sucrose is also closely related to stomatal density and photosynthetic pigment content, as well as development induction in some plant tissues, such as vascular and support tissues, (Iarema *et al*., 2012; Mohamed & Alsadon, 2010). Increase in sucrose concentration in medium can enhance the microtuber potato production up to some extent

(Khan *et al.*, 2018). However, high sucrose concentrations in the medium may decrease the photosynthetic ability of *in vitro* potato plants (Fuentes *et al*., 2005)

The plant growth regulators (PGRs) modulate plant growth and development and mediate responses to both biotic and abiotic stress. They are of importance in regulating shoot and root development in potatoes *in vitro* (Badoni & Chauhan, 2010; Hoque, 2010). Previous studies show that micro propagation of potatoes depends on the biological value of cultivars, explant type (leaf, node, shoot tip, etc.), type of culture medium, season, temperature, photoperiod, and a balanced combination of plant growth regulators (PGRs) in the culture media (Akhtar *et al.*, 2006; Dhital *et al.*, 2010). GA_3 is involved in cell elongation and its addition in MS medium enhances shoot growth (Camara *et al.,* 2018; Rizza *et al.,* 2017). The successful *in vitro* multiplication of potatoes depends on the presence of a suitable combination of auxins with gibberellic acid (GA3) in the propagation medium (Farhatullah *et al.*, 2007).The objective of the research was to determine the optimum sucrose and GA_3 concentration on survival and growth of three potato varieties grown *in vitro*.

3.2 Materials and Methods

3.2.1 Experimental Site

Experiment was conducted at the Agricultural Development Corporation (ADC) farm in Molo Sub County, Nakuru, Kenya. The site lies at 0° 12' S latitude, 35 ° 41' E Longitude and at an altitude of 2200 m above sea level. Laboratory temperature of 18^0C and Photoperiod of 16 Hrs light and 8 Hrs darkness.

3.2.1 Varieties

Three local potato varieties, *Shangi*, *Unica* and *Wanjiku*, obtained from the Agricultural Development Corporation (ADC) Molo laboratory were used in this experiment. They were selected on the basis of their popularity among farmers due to their marketability and high yield.

3.2.2 Sterilization

All the glassware, culture vessels, test tubes, petri dishes, pipettes and small instruments (forceps, scalpel) needed for sterile dissection were autoclaved at 121 °C for 45minutes. Surface sterilization was carried out by first switching on the laminar air flow cabinet for 15 minutes. The Laminar air flow cabinet surfaces were wiped with 70% ethanol. All the apparatus were sanitized using 70% ethanol and kept inside the cabinet. The UV light in the laminar air flow was switched on for 20 minutes after which it was switched off. The laminar air flow cabinet remained on until culturing was completed. The conical flasks, test tubes and glass jars containing prepared media were sterilized by autoclaving at 121 °C for 20 minutes.

3.2.3 Stock solution in *In vitro* culture

Murashige and Skoog (MS) (Murashige & Skoog, 1962) medium was prepared by dissolving the appropriate amount of macro and micro nutrients and organic supplements in sterile distilled water. Single node cuttings of each potato variety were cultured *in vitro* on different media containing 3 different concentrations of sucrose (20, 30 and 40gL^{-}1) and GA_3 (0.2, 0.5 and 1.0mgL^{-1}) solidified with 7g L^{-1} of agar. One single node cutting was cultured in each test tube and sealed with parafilm.

3.2.4 Experimental Layout

The experiment was laid out in a 3×3×3 factorial in completely randomized design (CRD) with three replications. The treatments were standardized MS media with three levels of sucrose (20, 30 and 40gL^{-1}) and three levels of GA_3 (0.1, 0.5 and 1.0mgL^{-1}). Petiole with leaf from each variety were used as explants.

3.2.5 Data collection

The following parameters were measured;

Days to shoot initiation was counted from the day of culturing in the media to the appearance of the first shoots.

Number of shoots were counted from four samples two weeks after emergency till maturity and the average shoots number were recorded.

Length of shoots (cm) were measured after every two weeks till maturity. It was measured from the point of emergence to the tip using a ruler and the average length from four samples were used for analysis.

Number of leaves from four samples were counted and the average leaf number were recorded. Days to root initiation was done by counting the days from the day of culturing in the media to the emergence of first initial roots.

Number of roots were counted from four samples and the average root number were recorded. Percentage plants Survival was as reported by Parveen (2011).

Shoot fresh and dry weight. The haulms were weighed using an electronic weighing balance. weight was computed and recorded as shoot fresh weight at harvest. The haulm samples were taken into the laboratory and dried in the oven at $60^{o}C$ overnight to constant dry weight.

3.2.6 Data analyses

Data collected was subjected to Shapiro wilk test at probability P ≤0.05 for normality test using SAS software (SAS Institute, Cary. Inc, 2013). The data was subjected to analysis of variance at P ≤0.05 using PROC GLM code of Version 9.0 and means of significant treatments were separated using Tukey's Honestly Significant Different Test at P ≤ 0.05. Pearson's Correlation test at P ≤0.05 was also performed to determine the strength of linear relationships among the response variables. The research model was as given below:

$$Y_{ijk} = \mu + + var_i + G3_j + Su_k + VarG3_{ij} + VarSu_{ik} + G3Su_{jk} + VarG3Su_{ijk} + \varepsilon_{ijk}$$

Where: Y_{ijk}: observed response, μ= Overall mean level, var= Effect due to i^{th} level of variety, G3= Effect due to j^{th} level of GA_3, Su= Effect due to k^{th} level of sucrose,VarG3= Interaction between i^{th} level of variety and j^{th} level of GA_3,VarSu= Interaction between i^{th} level of variety and k^{th} level of sucrose, G3Su= Interaction between j^{th} level of GA_3 and k^{th} level of sucrose, VarG3Su= Interaction between variety, GA_3 and sucrose, ε_{ijk}= Random error term.

3.3 Results and Discussion

3.3.1 Days to shoot initiation.

The interaction effects on days to shoot initiation due to sucrose, GA_3 and varieties were highly significant at P≤0.001. The minimum days to shoot induction was observed in *Wanjiku* (5.11d) at a concentration of 0.2 mg L^{-1} ,GA_3 while in *Shangi* (5.56d) and *Unica* (6.22d) it was observed at the concentration of 1 mg L^{-1} GA_3 (Figure 3.1). Increasing GA_3 concentration resulted in delayed shoot induction. Ahmet (2014) reported that the minimum days to shoot induction were observed on cv. Granola (4.25 d) compared to other two varieties of seed potato on 1.0 × MS medium containing 0.25 mg L^{-1} GA3 + 1 mg L^{-1} NAA. The minimum days to shoot induction was observed in *Shangi* (5.11d) followed by *Wanjiku* (5.56d) and *Unica* (5.67d) on MS Medium containing 30 g L^{-1} sucrose (Figure 3.2**).** Optimum level of sucrose for days to shoot initiation was observed at a concentration of 30 gL^{-1} and at a higher concentration (40 g L^{-1}), shoot induction was delayed. However, these results contradicted those of Usman *et al.* (2012) who reported that 45 g L-1 of sucrose as the optimum level for faster growth and development of guava plants in vitro. This may be due to differences in varietal variations responding differently to different treatment levels.

3.3.2 Days to root induction

The minimum days to root induction was observed in *Unica* (11.3d) and *Shangi* (19.9d) at a concentration of 1 mg L^{-1} GA_3, while in *Wanjiku* (14.8d) at 0.2 mg L^{-1} GA_3 (Figure 3.1). This was as a result of differences in genotypes responding to different treatment levels. These results were in agreement with those of Iarema *et al*. (2012) and Ullah *et al*. (2012) who observed that GA_3 at a concentration of 0.25 mg L^{-1} resulted in minimum days to root initiation in Desiree compared to Pasinler potato varieties. Root induction was delayed the most in *Wanjiku* (29.3d), followed by *Unica* (24.6d) at a concentration of 0.5 mg L^{-1} GA_3 and *Shangi* (21.9d) at 0.2 mg L^{-1} GA_3 (Figure 3.1). Minimum days to root induction was recorded on *Unica* (7d) at 0.2 mg L^{-1} GA_3 + 30 g L^{-1} sucrose, followed by *Shangi* (10d) and *Wanjiku* (12d) at 0.5 mg L^{-1} GA_3 + 40 g L^{-1} sucrose (Figure 3.3). Days to root induction were minimum at low concentrations of GA_3 (0.2 mg L^{-1}) and optimum concentration of sucrose (30 g L^{-1}). High sucrose concentration may not determine root induction but it may induce better growth. Similar results were reported by Martins *et al*. (2015) who recorded that high sucrose concentration (41 g L^{-1}) inhibited roots growth.

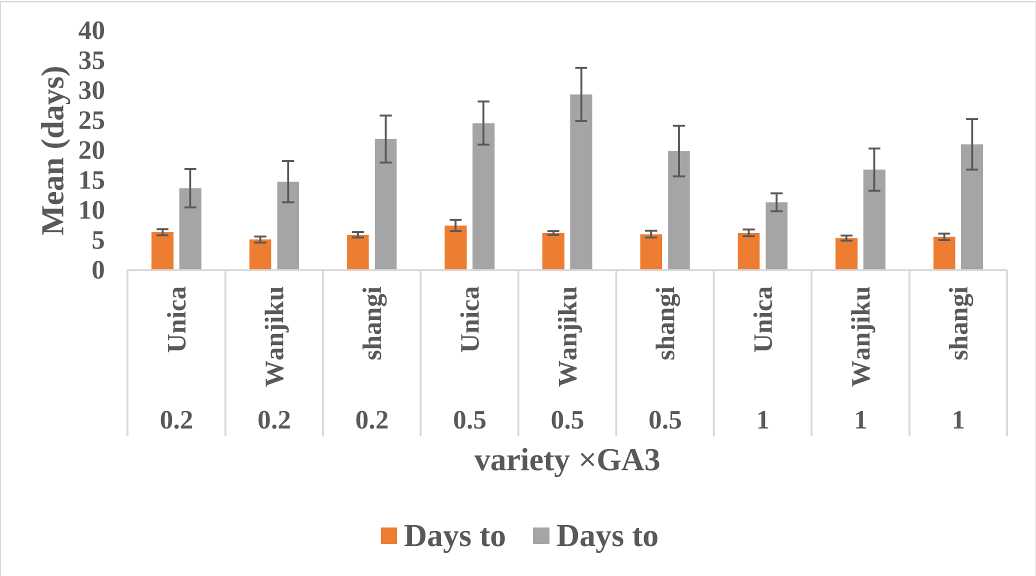

Figure 3.1: Effects of GA_3 on shoot and root initiation of *shangi*, *wanjiku* and *unica* varieties grown *in vitro* in Kenya

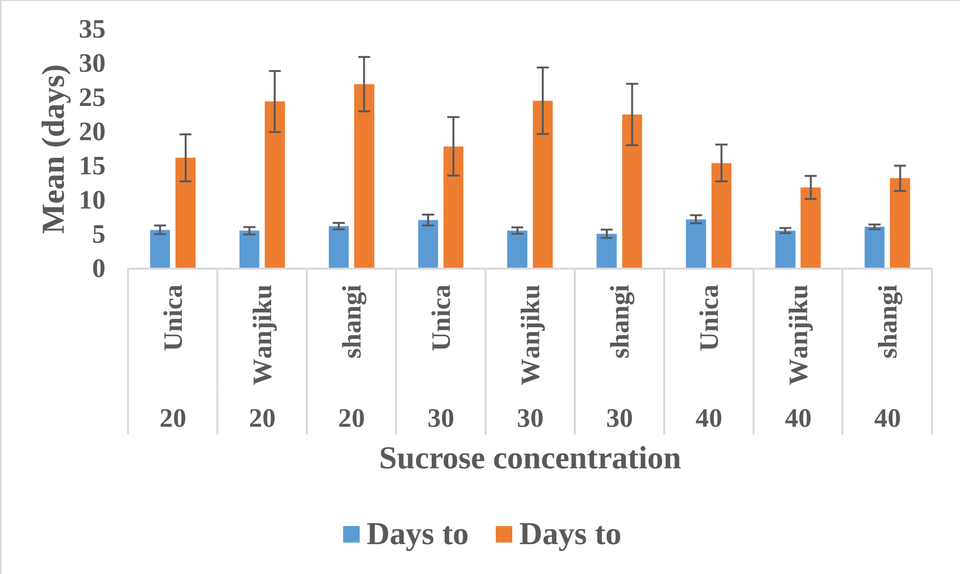

Figure 3.2: Effects of Sucrose on the shoot and root initiation *shangi*, *wanjiku* and *unica* varieties grown *in vitro* in Kenya

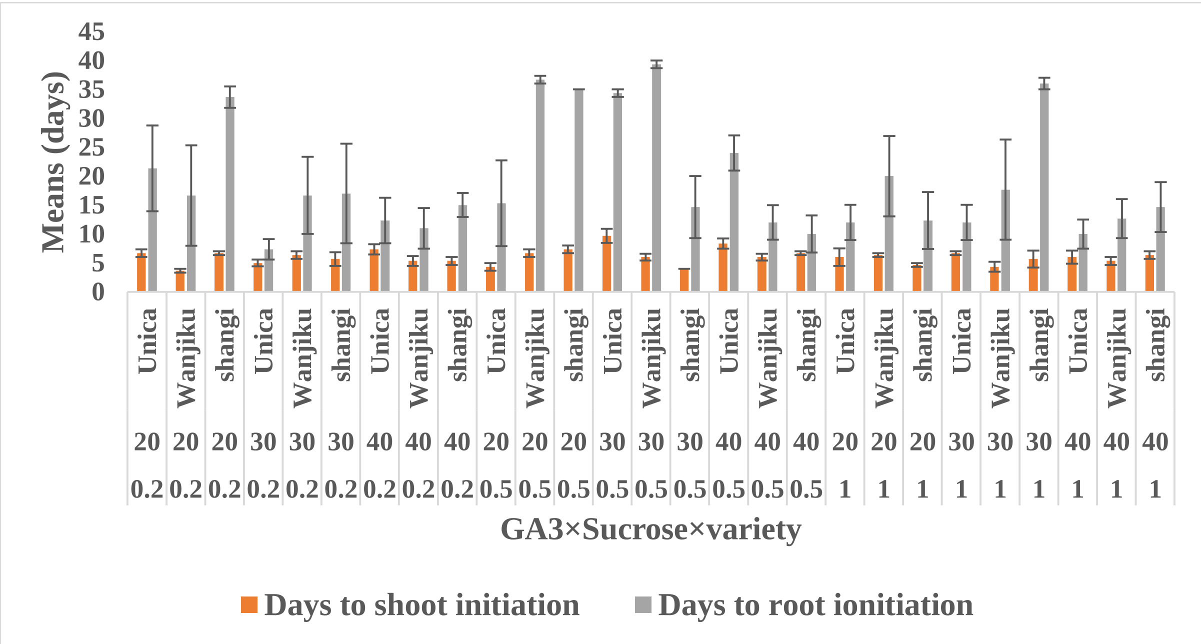

Figure 3.3: Interaction effects of GA3, sucrose and variety on days to shoot and root initiation *shangi*, *wanjiku* and *unica* varieties grown *in vitro* in Kenya

3.3.3 Effect of GA_3 and Sucrose on the Survival of in vitro

High plant survival was observed on materials treated with 0.5 mg L^{-1} GA_3 + 40 g L^{-1} sucrose (90%) and this was significantly different from other treatments (Table 3.2). *Shangi* showed the highest survival (75.56%) at 0.5 mg L^{-1} GA_3 but this was not significantly different from *Wanjiku* and *Unica* (68.89%) at the same GA_3 level (Table 3.3). Lowest survival was observed for those entries treated with 0.5 mg L^{-1} GA_3 + 20 g L^{-1} sucrose (61.11%). However, this was not significantly different from those treated with 0.2 mg L^{-1} GA_3 + 20 g L^{-1} sucrose (62.22%). *Wanjiku* showed the highest survival (77.8%) followed by *Shangi* (73.3%) and *Unica* (73.3%) at 40 g L^{-1} of sucrose (Table 3.1). Optimum concentration of sucrose (40 g L^{-1}) and GA_3 (0.5 mg L^{-1}) gave the highest survival of *in vitro* (Plate 3.1, 3.2 and 3.3). Sucrose at 40 g L^{-}1 increased the osmotic pressure and therefore nutrients and water retention forces in the medium decreased thus higher survival recorded compared to other treatments. In addition, number of leaves decreased with an increase in GA_3 concentration thus reducing the photosynthetic area of the plant. These results are in agreement with that of Mazri (2014) who reported that explants cultured MS medium supplemented with 40 g L^{-1} sucrose showed the highest survival rate.

Plate 3.1: 14 days after culturing

Plate 3.2: 20 days after culturing

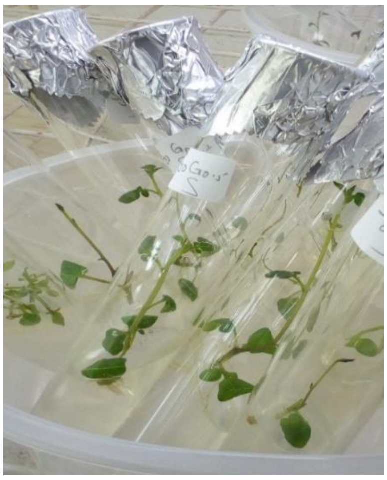

Plate 3.3: 30 days after culturing

3.3.4 Number of leaves as affected by GA_3 and Sucrose

The sucrose × GA_3 × variety interaction effects on number of leaves was highly significant at $P \leq 0.001$ (Appendix 2). The maximum number of leaves (8.5) was recorded in *Shangi*, followed by *Wanjiku* (8.0) and *Unica* after treatment with 40 g L^{-1} sucrose (Table 3.1). The highest number of leaves was observed on 0.5 mg L^{-1} GA_3 + 40 g L^{-1} (Table 3.2). The highest number of leaves was observed in Shangi (8.2) at a concentration of 0.5 mg L^{-1} GA_3, followed by *Wanjiku* (7.9) at a concentration of 0.2 mg L^{-1} GA_3 and unica (7.2) at 1 mg L^{-1} GA_3 (Table 3.3). Genotypes were found detrimental for *in vitro* growth responses; it is not possible to micro-propagate both cultivars on the same combination of $GA_{3.}$ However, the optimized concentration of GA_3 gave different responses into different varieties in this study.

Similar responses of GA_3 concentration was observed by Farhatullah *et al*. (2007) and Fatima *et al.* (2005) who reported that different concentration of treatments varied among varieties.

3.3.5 Effect of GA_3 and Sucrose on the number of roots

The interaction effects of GA_3 by sucrose by variety were highly significant on the number of roots at $P \leq 0.01$ (Appendix 2). The maximum number of roots were observed in *Unica* (4.6/plantlet), followed by *Shangi* (4.4) and *Wanjiku* (3.4) at 1 mg L^{-1} GA_3 (Table 3.3). Sucrose at 40 g L^{-1} produced the maximum number of roots in *Shangi* (8.5), followed by *Wanjiku* (8.0) and *Unica* (7.2) (Table 3.1). Minimum number of roots were observed in *Wanjiku* (2.0) at 20 g L^{-1} sucrose. At higher levels of sucrose, the osmolarity of the medium increased enabling plants to undergo stress that resulted to production of more roots. These results were in agreement with the findings of Mazri (2014) who reported that plantlets of date palm (*Phoenix dactylifera* L.) cultured on MS medium containing 40 g L^{-1} sucrose had the best rooting response (4.8roots/plantlet). He also observed that lower concentrations of sucrose (10, 20, or 30 g L^{-1}) resulted in fewer roots. The highest number of roots (8.8) was observed at a concentration of 0.5 mg L^{-1} GA_3 +40 g L^{-1} sucrose (Table 3.2).

3.3.6 Shoot length as affected by GA_3 and Sucrose

The sucrose × GA_3 × variety interaction effects on the shoot length were highly significant for at $P \leq 0.001$ (Appendix 2). A longer shoot length was observed in *Shangi* (8.1cm), followed by *Wanjiku* (7.0cm) and *Unica* (6.5) at 40 g L^{-1} sucrose (Table 3.1). Increasing the sucrose concentration promoted an increase in shoot length. These results were in agreement with the findings of Mazri (2014) who reported that plantlets of date palm (*Phoenix dactylifera* L.) cultured on MS medium containing 40 g L^{-1} sucrose produced the longest shoots (average = 14.9 cm). The longest shoot (8.0cm) was observed in 0.5 mg L^{-1} GA_3 +40 g L^{-1} sucrose (Table 3.2). *Shangi* produced the longest shoot length (7.4cm) at 0.5 mg L^{-1} GA_3, followed by *Wanjiku* (6.8cm) and *Unica* (6.2cm) at 0.2 mg L^{-1} GA_3 (Table 3.3). GA_3 at 0.5 mg L^{-1} and 0.2 mg L^{-1} increased shoot length by extending the internodes of growing shoots. These results were in agreement with the findings of Yildirim (2019) who reported that GA_3 at 0.5 mg L^{-1} gave the best results in terms of shoot length and shoot number on micro propagation of lentisk (*Pistacia lentiscus* L.).

3.3.7 Effect of GA_3 and Sucrose on the number of shoots

The interaction effects of GA_3 by Sucrose by variety significantly influenced the shoots number $P \leq 0.001$ (Appendix 2). The maximum number of shoots were observed on *Shangi* (1.9), followed by *Wanjiku* (1.8) at 0.2 mg L^{-1} GA_3 and finally *Unica* (1.4) at 1 mg L^{-1} GA_3 (Table 3.3). Number of shoots tends to decrease as GA_3 concentration increases in the culture media. GA_3 at high concentration prevented shoot formation. These results also may have occurred due to genotypic variations interacting with different treatment levels. These results were in agreement with the findings of Padron *et al*. (2020) who observed that increasing the supply of GA_3 in the medium reduced the number of shoots of *Alpinia purpurata* (Zingiberaceae). *Shangi* recorded a maximum number of shoots (2.0), followed by *Wanjiku* (1.8) and *Unica* (1.5) at 40g L^{-1} sucrose (Table 3.1). Shoot induction increased with increase in sucrose concentration resulting to an increase in osmotic pressure that hastens shoot induction. Iarema *et al*. (2012) and Usman *et al*. (2012) also observed that shoot induction increased with increase in sucrose concentration 45 g L^{-1} and 50 g L^{-1} in guava plants *in vitro* and fig (Ficus carica L.) respectively. However, these results contradicted the findings of Taha *et al*. (2001) who reported more shoot bud formation on medium containing 30 g L^{-1} sucrose than on media containing 10, 20 or 40 g L^{-1} sucrose in the date palm cultivar 'Zaghlool'. This shows that treatment levels varies among the plant species. The highest number of shoots (1.9) was observed on 0.5 mg L^{-1} GA_3 +40 g L^{-1} sucrose (Table 3.2).

3.3.8 Leaf fresh and dry weight as affected by GA_3 and Sucrose

The interaction effects of GA_3 by Sucrose by variety significantly influenced the leaf fresh weight at $P \leq 0.05$ (Appendix 2). The highest leaf fresh weight was observed on 1.0 mg L^{-1} GA_3 +40 g L^{-1} sucrose, whereas the lowest was observed on 0.2 mg L^{-1} GA_3 +40 g L^{-1} sucrose (Table 3.2). *Unica* recorded the highest leaf fresh weight (0.83g) from the treatment with 30 g L^{-1}, followed by *Shangi* (0.81g) at 40 g L^{-1} and *Wanjiku* (0.38) at 20 g L^{-}1. However, results obtained in *unica* for leaf fresh weight were not significantly different from that of *shangi* (Table 3.1). This was due to difference in genotypic variations. Similar results were reported by Haida *et al.* (2020) who recorded that treatment of 30 g L^{-1} sucrose in clinacanthus nutans (Sabah Snake Grass) produced the highest leaf fresh weight. Although the highest number of leaves were recorded in *unica* and *wanjiku* at 40 g L^{-1} sucrose, the leaves produced were smaller than that of the sucrose concentration at 30 g L^{-1} and 20 g L^{-1} for *unica* and *wanjiku* respectively.

Table 3.1: Interaction effects of sucrose by variety on the number of leaves, number of roots, shoot length (cm), number of shoots, leaf fresh weight and leaf dry weight (grams) (Mean ±SE) of seed potato in Kenya.

Level of sucrose	Level of Variety	Survival (%)	Leaves number	Roots number	Shoots length	Shoots number	Leaf fresh weight	Leaf dry weight
20	Shangi	62.22±3.85ab	6.93±0.43b	2.77±0.36bc	5.53±0.42a	1.44±0.12b	0.65±0.11cd	0.62±0.11c
20	Unica	63.33±6.45ab	7.18±0.38b	3.37±0.31d	6.18±0.43b	1.16±0.05a	0.76±0.05de	0.74±0.04d
20	Wanjiku	60.00±5.00a	6.96±0.37b	1.99±0.21a	5.73±0.39a	1.58±0.12c	0.38±0.13b	0.35±0.13b
30	Shangi	73.33±5.77c	7.49±0.38c	3.86±0.45e	7.36±0.54c	1.49±0.11bc	0.65±0.10c	0.62±0.10c
30	Unica	65.56±5.85b	6.36±0.37a	2.90±0.27c	5.31±0.47a	1.44±0.10b	0.83±0.13f	0.82±0.12e
30	Wanjiku	61.11±3.47a	7.00±0.44b	2.61±0.28b	5.56±0.42a	1.47±0.09b	0.30±0.08ab	0.27±0.08ab
40	Shangi	73.33±10.00c	8.51±0.52e	5.43±0.46g	8.11±0.59d	1.98±0.15e	0.81±0.22ef	0.78±0.22de
40	Unica	73.33±8.66c	7.24±0.42bc	4.83±0.40f	6.49±0.46b	1.49±0.08b	0.54±0.16c	0.52±0.16c
40	Wanjiku	77.78±5.61c	8.02±0.44d	4.54±0.32f	6.98±0.48c	1.82±0.11d	0.28±0.07a	0.25±0.07a

- Means within a column followed by the same letters are not significantly different ($P \leq 0.05$) according to Tukey's HSD test

Table 3.2: Interaction effects of GA_3 by sucrose on the number of leaves, number of roots, shoot length (cm), number of shoots, leaf fresh and leaf dry weight (grams) (Mean ±SE) of seed potato in Kenya.

Level of GA_3	Level of Sucrose	Survival (%)	Leaves number	Roots number	Shoot length	Shoots number	leaf fresh weight	Leaf dry weight
0.2	20	62.22±3.85a	7.20±0.45b	2.30±0.22a	6.00±0.36b	1.73±0.14e	0.66±0.10d	0.64±0.10d
0.2	30	67.78±6.31bc	7.24±0.45b	4.12±0.31d	7.04±0.51c	1.49±0.11cd	0.44±0.05b	0.41±0.05b
0.2	40	68.89±4.51c	7.64±0.48b	3.92±0.32cd	6.78±0.45c	1.84±0.13e	0.13±0.05a	0.11±0.05a
0.5	20	61.11±5.36a	6.58±0.39a	2.12±0.34a	5.31±0.43a	1.29±0.08b	0.50±0.12c	0.47±0.12c
0.5	30	62.22±5.61ab	6.22±0.36a	2.37±0.40a	5.20±0.49a	1.49±0.11cd	0.63±0.16d	0.61±0.16d
0.5	40	90.00±4.08d	8.78±0.53c	5.06±0.40e	8.04±0.62d	1.87±0.15e	0.52±0.06c	0.50±0.05c
1	20	62.22±6.31ab	7.29±0.33b	3.70±0.31c	6.13±0.43b	1.16±0.05a	0.63±0.12d	0.60±0.12d
1	30	70.00±4.50c	7.38±0.37b	2.88±0.28b	5.98±0.45b	1.42±0.07c	0.72±0.13d	0.69±0.13d
1	40	65.56±6.53abc	7.36±0.33b	5.83±0.43f	6.76±0.45c	1.58±0.07d	0.97±0.21e	0.95±0.21e

- Means within a column followed by the same letters are not significantly different ($P \leq 0.05$) according to Tukey's HSD test

Table 3.3: Interaction effects of GA_3 by variety on the number of leaves, number of shoots, shoots length (cm), number of shoots, leaf fresh and leaf dry weight (grams) (Mean ±SE) of seed potato in Kenya.

Level of GA_3	Level of Variety	Survival (%)	Leaves Number	roots Number	Shoots Length	Shoots Number	Leaf fresh Weight	Leaf dry Weight
0.2	Shangi	68.89±5.36b	7.22±0.50b	3.50±0.41c	6.76±0.53c	1.89±0.14f	0.49±0.10cd	0.46±0.10cd
0.2	Unica	67.78±3.85ab	6.96±0.39ab	3.41±0.25bc	6.24±0.40b	1.38±0.10bc	0.41±0.09bc	0.40±0.09bc
0.2	Wanjiku	62.22±5.61a	7.91±0.48d	3.43±0.25bc	6.82±0.40c	1.80±0.13ef	0.33±0.11ab	0.30±0.11ab
0.5	Shangi	75.56±9.18c	8.16±0.53d	4.17±0.50d	7.42±0.63d	1.71±0.16de	0.54±0.10d	0.51±0.11d
0.5	Unica	68.89±10.58bc	6.64±0.44a	3.10±0.38b	5.78±0.52b	1.29±0.08a	0.77±0.10e	0.76±0.10e
0.5	wanjiku	68.89±8.39bc	6.78±0.39a	2.28±0.34a	5.36±0.46a	1.64±0.11d	0.34±0.11ab	0.31±0.11ab
1	Shangi	64.44±6.53ab	7.56±0.29c	4.39±0.44d	6.82±0.45c	1.31±0.07ab	1.07±0.16g	1.04±0.16g
1	Unica	65.56±6.53ab	7.18±0.36b	4.59±0.38d	5.96±0.44b	1.42±0.07c	0.95±0.10f	0.93±0.10f
1	wanjiku	67.78±5.61ab	7.29±0.37bc	3.43±0.33bc	6.09±0.44b	1.42±0.07c	0.30±0.07a	0.27±0.07a

- Means within a column followed by the same letters are not significantly different ($P \leq 0.05$) according to Tukey's HSD test

3.3.9 Correlation analysis

There was strong positive correlation between number of leaves and length of shoots (r=0.76***), number of leaves and number of roots (r=0.65***), number of roots and length of shoots (r=0.67***), number of leaves and number of shoots (r=0.58***) and length of shoots and number of shoots (r=0.57***). An increase in root numbers resulted in an increase in shoot length and leaf numbers. Also, increase in shoot length and shoot numbers led to an increase in the number of leaves (Table 3.4). Number of roots and number of shoots showed weak correlation of 0.45*** at P>0.001. This may have been due to the limited space for growth in the test tubes that led to fewer number of roots thus leading to fewer number of shoots.

There was also strong positive correlation between leaf fresh weight and leaf dry weight (r=0.99***), days to root initiation and days to shoot initiation (r=0.71*), days to root initiation and leaf dry weight (r=0.69*) and days to root initiation and leaf fresh weight (r=0.66*). Leaf dry weight increased with increase in leaf fresh weight. An increase in leaf fresh and dry weight as a result of early root initiation can be explained by earlier root development which led to increased nutrient uptake, increased shoot growth and maturity of the shoots/leaves. (Table 3.5). Similar results were reported by Nasir and Toth (2021) who recorded that above ground biomass positively correlated with plant height, number of leaves, leaf area index and foliage dry matter.

Table 3.4: Pearson correlation coefficients on the response variables of number of leaves, number of roots, length of shoots, number of shoots

Response variables	Number of Leaves	Number of roots	Length of shoots
Number of roots	0.65***	-	-
Length of shoots	0.76***	0.67***	-
Number of shoots	0.58***	0.45***	0.57***

***Correlation is significant at P<0.001

Table 3.5: Pearson correlation coefficients on the response variables of days to shoot initiation, days to root initiation, leaf fresh weight and leaf dry weight.

Response variables	Days to shoot initiation	Days to root initiation	Leaf fresh weight
Days to root initiation	0.71*	-	-
Leaf fresh weight	0.51ns	0.66*	-
Leaf dry weight	0.49ns	0.69*	0.99***

* Correlation is Significant at P<0.05, ***significant at P<0.001

3.4 Conclusion

Using MS Medium and sucrose as a carbon source in growth of *in vitro* potato production at the rate of 40g L^{-1} resulted highe number of leaves number, number of shoots, number of roots and longer shoot length. Supplying gibberellic acid (GA_3) at a concentration of 0.5mg L^{-1} combined with sucrose at a concentration of 40g L^{-1} significantly resulted in better shoot growth on local potato varieties cultured *in vitro*. Potato varieties responded differently to different treatment levels as a result of genotype by environment effects. These results showed that 0.5mg L^{-1} GA_3 along with 40g L^{-1} sucrose had better effect on survival and improvement of shoot growth.

CHAPTER FOUR

EFFECTS OF MEDIA TYPE (GREEN HOUSE) ON GROWTH AND YIELD OF POTATO (*Solanum tuberosum* L.) VARIETIES APICAL ROOTED CUTTINGS IN KENYA

Abstract

To address soil-borne virus diseases; a soilless media has been introduced for producing healthy seed tuber based on *in vitro* rapid multiplication of virus-free planting material. The objective of this study was to determine the effect of different media on the growth of potato variety using apical rooted cuttings. A greenhouse study was carried out where five media types; coco-peat + perlite, sand, coco-peat + pumice, coco-peat+ vermiculite, and soil were tested along with three local potato varieties (*Shangi*, *Unica* and *Wanjiku*). The study was a 3×5 factorial arrangement laid out in a randomized complete block design (RCBD). Data collected was subjected to a general linear model (GLM) to partition the variance component using SAS software version 9.0 and means separated using Tukey's Honestly Significant Difference Test (HSD) at $P \leq 0.05$. The interaction between varieties and different media had a significant effect on the number of tubers per plant. *Shangi* registered the highest number of mini tubers per plant (21tubers) on coco-peat + perlite which was not significant different from that of coco-peat +pumice mixture (19 tubers). Plant height of 53cm was recorded under coco-peat + perlite mixture. Highest shoot fresh weight was recorded on *Shangi* (24.86g/plantlet) cultivated on coco-peat + pumice mixture, followed by *Unica* and *Wanjiku* (24.64 and 21.17g/plantlet respectively) cultivated on coco-peat + perlite mixture. *Unica* produced the highest yield (64.8g/plantlet) in coco-peat + pumice, followed by *Wanjiku* (59.8g/plantlet) and *Shangi* (40.8g/plantlet) in coco-peat + perlite. This study recommends that for farmers to overcome spread of pests and diseases, use of Coco-peat + perlite and coco-peat+pumice as a soilless media should be promoted to enhanced seed potato growth and tuber formation.

4.1 Introduction

Globally, potato is estimated to be grown on 19 million hectares with a production of 378 million tonnes in 2017 (Campos & Ortiz, 2020). Between 2015 and 2017, potato production in Africa was 6.5% of the world's production. Kenya produced a total of 1.6 million tonnes on 0.4 million hectares which is 7.5% of Africa's harvested area (FAOSTAT, 2020). The current potato production in Kenya is 7-10 t ha^{-1} against the demand of 20-40 t ha^{-1} .The yield gap between what is currently produced and what is achievable is often greater than 20 t/ha (Mutegi *et al*., 2021). This gap is attributed to virus replicons that tend to increase in the seed material during field multiplication cycles, and therefore leading to low yield.

In addition, multiplication of seed potato take place in field (soil), thereby exposing the seed potatoes to soil-borne disease. Soil is the main platform for pest and disease infections to spread, resulting in significant loss of yield and deteriorate tuber quality over seasons To avoid and address this soil-borne disease, soilless media has been introduced for producing healthy seed tuber based on *in vitro* rapid multiplication of virus-free planting material (Altindal &Karadogan, 2010).

A soilless media is any sterile medium for growing plants that doesn't involve soil. The physical properties of soilless media comprises of particle size distribution, porosity and pore distribution, water content and water potential. Soil-less media has capability to grow plants in a conditioned, pest and disease free environment. A soilless has gained popularity, as they eliminate or reduce the need for soil disinfestations. Use of soilless media possibly is the most intensive culture system utilizing all the resources efficiently for maximizing yield of crops and the most intense form of agricultural enterprises for commercial production of greenhouse crops (Asaduzzaman *et al*., 2015). They are considered as important technologies for better water use efficiency as well as high good quality and quantity products. Number of organic and inorganic materials such as gravel, sand, peat, sawdust, pumice, tuff, coir, vermiculite, perlite, and rock wool pure or in mixture are used as solid growing media in addition to hydroponics (Hussain *et al.,* 2014; Putra & Yuliando, 2015). Such substrates replace the soil because the natural soils are often poorly suited for cultivation and contain chemical, physical, or biological contaminations (Asaduzzaman *et al.,* 2015).

Apical rooted cuttings are similar to a nursery grown seedling and are produced from tissue culture plants in a screen house, and are clean and free from diseases. They have several other advantages that include; no need to break dormancy hence faster propagation, it's economical and has a high rate of multiplication. With high productivity of apical rooted cuttings, it becomes economical to seed multipliers selling seed after two to three seasons of multiplication, as certified seed are sold normally after three seasons of multiplication. This study was conducted to determine the growth and yield response of apical rooted potato cuttings of local Kenya varieties in different media to produce good quality clean planting materials.

4.2 Materials and Methods

4.2.1 Experimental Site

The study was carried out in a greenhouse at Egerton University Agronomy Field, Seven, Njoro campus, Kenya. The site lies at a latitude of 0^o 22\`11.0\`\`S, longitude of $35^o$ 55\`58.0\`\`E and altitude of 2670 m.a.s.l. The site is in agro-ecological zone III with an average annual rainfall range of 800-1500 mm with two seasons. The green house maximum temperature was 22.4 ^oC and the minimum temperature was 7.8 ^oC. The soils are well-drained, dark reddish clays, slightly acidic, and contain medium levels of organic carbon and low levels of phosphorus classified as Mollic Andosols (Jaetzold *et al*., 2006).

4.2.2 Experimental design and treatment application

The experiment was laid in a factorial randomized complete block design (RCBD). Treatments included media at five levels, i.e., coco-peat + pumice, coco-peat + perlite, sand, soil and coco-peat +vermiculite, and three local potato varieties (*Shangi*, *Unica* and *Wanjiku*). Each treatment was replicated three times.

4.2.3 Establishment of mother plant

In *vitro* well-rooted shoots (plantlets) obtained from experiment 1, were taken out gently from the culture media and washed with sterile water to remove any traces of agar on the roots and dipped in fungicide solution for 20 minutes (soil-borne disease). The plantlets were transferred to containers filled with sterilized sand for establishment. The plantlets were covered for 25 days with clear polythene to acclimatize to the ambient growth environment. Plants were watered with water until they attained 3-5 nodes (maturity stage). After cutting the apical tips, mother plants were supplied with nutrient solution to enhance the sprouting of new shoots.

Plate 4.1: Mother plants 14 days after establishment

4.2.4 Hardening of apical rooted cuttings

Before cutting the apical tips from the established mother plant, hands and scissors were disinfected with 70% ethanol. Apical tips of the established mother plants were cut at one node after the mother plant developed ≥ 3 nodes. After cutting the apical tip of the cutting, each mother plant provided 1-3 shoots for cutting. The cuttings were rooted in trays filled with sterilized coco-peat. The cuttings took two to three weeks to root after which they were taken to the different media to produce seed tubers.

Plate 4.2: Apica rooted cuttings that are ready for planting

4.2.5 Preparation of media type

Sand, coco-peat + perlite, coco-peat + pumice, coco-peat +vermiculite and soil media types were prepared. Sand was sterilized by washing with water several times until water became clean. After that it was soaked with hot water for 24 hours to kill germs. Coco-peat was soaked in a mixture of water and calcium nitrate for twelve hours to remove high potassium (K^+) and sodium (Na^+) ions so that plant roots could absorb water efficiently (Marock, 2021). Sand, vermiculite, pumice and perlite were soaked with hydrogen peroxide at a rate of 500ml in 1000litres for 24 hrs.

4.2.6 Data collection

Several plantlet growth parameters were determined. Plant height was measured from the surface of media until the tip of the apical meristem.

Number of leaves from three samples was counted and the average leaf number was recorded and used for analysis.

Number of branches was counted two weeks after planting until the maturity of the crop. The number of branches was recorded. Data for plant height, number of leaves, and number of branches commenced two weeks after planting at an interval of 14 days.

Plant biomass at harvest was done by weighing (g) the above ground biomass. Number of mini tubers was counted after harvesting and calculated per treatment. Weight of tubers (g) was calculated using a weighing balance.

Number of mini tubers was counted after harvesting and calculated per treatment. Weight of tubers was calculated using a weighing balance

4.2.7 Data analysis

The collected data was subjected to Shapiro wilk test at probability P ≤0.05 for normality test using SAS software. For any data not normally distributed, data transformation was performed. The data was subjected to analysis of variance (ANOVA) at p ≤0.05 using PROC GL M code of Version 9.3 (2010) and means of significant treatments was separated using Tukey's Honestly Significant Different Test at P ≤ 0.05. Correlation analysis was also performed among the response variables: plant height, number of leaves, number of branches, plant biomass, tuber weight and number of minitubers. The RCBD model to fit for the experiment was as follows:

$$Y_{ijk} = \mu + var_i + Tj + varTij + B_k + \varepsilon_{ijk}$$

Where, μ = overall mean, var_i = effect due to the i^{th} level of potato varieties, Tj = effect due to the j^{th} level of media, varTij = effect due to interaction of potato varieties and media, B_k =effect of the k^{th} block and ε_{ijk} = random error term.

4.3 Results and Discussion

4.3.1 Plant height as affected by media and variety

The interaction between varieties and different media was significant on shoot height at P<0.01 **(**Appendix 3**).** Highest plant height was recorded in *Shangi*, followed by *Unica* and *wanjiku* (53.0, 31.6, and 31.0cm), respectively, in coco-peat + perlite (Table 4.1). However, the result recorded in coco-peat + perlite was not significantly different from that of coco-peat + pumice for plant height recorded on *Shangi*. This could be because of coco-peat +perlite and coco-peat +pumice composition which retain nutrients better and has a high water holding capacity that boosts plant height under greenhouse conditions. This shows that coco-peat and perlite composition is a good media for potato mini-tuber production as tall healthy plants had more photosynthetic area translating to higher yield. The observed results are in consonance with the findings of Awati *et al.* (2019) and Dwelle and Love (2000) who reported that a well-established root and shoot system, is important for subsequent growth which, in turn, influences tuber bulking in potato. The shortest shoot height was observed on *Wanjiku* (24.1cm) grown in soil followed by *Unica* (24.5cm) grown in sand.

4.3.2 Number of leaves and branches as affected by media and varieties

Maximum number of leaves were observed on *Wanjiku* (43.53), followed by *Unica* (42.20) and *Shangi* (41.73) in coco-peat + perlite. However, the results were not significantly different from that of coco-peat + pumice for *Wanjiku* and *Unica*, which recorded a mean of 43.40 and 37.87, respectively. Lowest number of leaves was noted on *Unica* in sand. Most numbers of branches were noted on coco-peat + perlite media with *Wanjiku* having the highest, followed by *Unica* and *Shangi* (7.27, 6.53, and 6.33) respectively. However, the number of branches recorded on *Wanjiku* in coco-peat +perlite was not significantly different from that of coco-peat+pumice (6.40). Least number of branches was noted in *Shangi* on sand media (Table 4.1). This could be due to media type aeration, soil absorbing water capacity and composition variables. Similar findings were reported by Abouzari *et al*. (2012) who recorded that the largest number of lateral shoots (8.593) of Benjamin Tree (*Ficus benjamina*) were observed in cuttings cultivated in composted tea wastes + rice husks compared to other 4 substrates due to higher water retention capacity and draining components which were the best for vegetative plant growth.

Table 4.1: Effects of media type and varieties on plant height (cm), number of leaves, and number of branches (Mean ±SE) on potato apical rooted cuttings in Kenya

Variety	Soilless Media	Shoot Height	Number of leaves	Number of branches
Shangi	Coco-peat + pumice	52.93±8.55f	30.67±4.76cd	5.93±0.81fg
	Coco-peat + perlite	53.00±9.25f	41.73±6.74fg	6.33±1.05gh
	Coco-peat + vermiculite	42.07±8.00e	30.20±4.20bcd	4.00±0.60bc
	Sand	31.60±4.93d	27.67±4.46b	2.33±0.25a
	Soil	44.47±7.02e	28.67±4.27bc	3.67±0.61b
Unica	Coco-peat + pumice	31.40±4.61cd	37.87±5.66efg	5.73±0.83f
	Coco-peat + perlite	31.60±4.63d	42.2±6.12f	6.53±0.79gh
	Coco-peat + vermiculite	30.07±4.48cd	36.80±6.55e	3.87±0.56b
	Sand	24.47±3.97ab	20.80±2.43a	3.67±0.44b
	Soil	28.33±3.71b	34.40±4.85de	4.53±0.72cd
Wanjiku	Coco-peat + pumice	28.67±4.49cd	43.40±6.90g	6.40±0.96gh
	Coco-peat + perlite	30.93±4.35cd	43.53±7.07g	7.27±0.96h
	Coco-peat + vermiculite	28.67±4.27cd	35.20±4.51e	5.47±0.74ef
	Sand	27.33±4.20bc	26.27±4.36b	3.87±0.49b
	Soil	24.13±2.96a	37.00±5.39ef	5.00±0.67de

- Means within a column followed by the same letters are not significantly different ($P \leq 0.05$) according to Tukey's HSD test

4.3.3 Effect of media and Variety on the weight of minitubers per plant

Variety of media interaction was significant on the weight of minitubers. *Unica* produced the highest yield (64.8g) in coco-peat + pumice, followed by wanjiku (59.8g) and shangi (40.8g) in coco-peat + perlite (Table 4.2). The variation in yield per variety could be attributed to the influences boosted by media type and varietal genetic composition. These results are similar to those of Awati *et al*. (2019) and Khurana *et al*. (2003). Yields were lower in sand and soil media which correlated with poor plant growth. This could be due to photosynthetic area differences that affects tuber formation of potato apical rooted cuttings. The main role played by physical characteristics of coco-peat + perlite mixture and coco-peat + pumice mixture can be attributed as suitable air-filled porosity, for effective oxygen diffusion and maintaining favorable water content for supplying water, nutrient, and respiration of plant roots unlike sand media that has low water and nutrient holding capacity and can exacerbate deficiencies (Dharti *et al.*, 2021).

4.3.4 Number of Mini-Tuber per Plant as Affected by Media and Varieties

The interaction between varieties and media was significant with respect to the number of tubers per plant (Table 4.2). *Shangi* registered the highest number of mini tubers per plant (21tubers) which was not significant different from that of coco-peat +pumice mixture (19 tubers). *Wanjiku* recorded a higher number of mini-tubers (20 tubers) in coco-peat +perlite mixture. However, the results were not significantly different from that of coco-peat + vermiculite. *Unica* produced a higher number of mini-tubers (15tubers) in coco-peat +perlite and coco-peat + pumice mixtures. The variation in the number of tubers per variety could be because of interaction between the genetic constitutions of varieties responding differently to different environments. It could also be as a result of nutrient uptake of plants as it is determined by the media (Asghari *et al.*, 2009). Coco-peat + perlite mixture and coco-peat + pumice mixture indicates their superiority among the other media used. This could be attributed to their ability to hold and retain nutrients which are available to the plants.

4.3.4 Effects of media and varieties on the shoots fresh weight and shoots dry weight

Variety by media was significant with respect to the shoots fresh weight and shoot dry weight at $p<0.05$ **(**Appendix 4**)**. Highest shoot fresh weight was recorded on *Shangi* (24.9g) cultivated with coco-peat + pumice mixture. This results were not significantly different from that of coco-peat +perlite and coco-peat vermiculite mixtures (20.3g and 20.0g), respectively. *Unica* and *Wanjiku* gave a shoot fresh weight of 24.6g and 21.2g, respectively, in coco-peat +

perlite mixture. Highest shoot dry weight was observed on *Unica*, followed by *Wanjiku* (17.2 and 14.8g respectively) cultivated on coco-peat + perlite mixture and *Shangi* (14.4g) cultivated on coco-peat +pumice mixture. However, the shoot dry weight recorded on *Shangi* in coco-peat +pumice mixture treatment was not significantly different from that of coco-peat +perlite and coco-peat +vermiculite mixtures. Lowest shoot fresh and dry weight was noted on sand media among all varieties (Table 4.2). These results are in agreement with the findings of Haman and Izuno (2003) who reported that plant yields such as biomass production could positively be affected depending on the specific combination of potting mix and water retention capacity.

Table 4.2: The effects of media type and variety on the number of minitubers, weight of minitubers (grams), fresh and dry weight of shoots (grams) (Mean ±SE) on potato apical rooted cuttings in Kenya

Variety	Soilless Media	Number of minitubers	Weight of minitubers	Shoot fresh weight	Shoot dry weight
Shangi	Coco-peat + pumice	19.33±7.31fg	40.67±11.67d	24.86±4.75e	14.40±2.33f
	Coco-peat + perlite	21.00±2.08g	28.33±1.76b	20.28±5.12de	14.17±3.21fg
	Coco-peat + vermiculite	16.00±2.65e	23.00±9.02b	19.98±9.03de	13.09±2.46f
	Sand	4.67±0.88a	10.67±2.33a	8.39±1.81a	3.81±0.53a
	Soil	6.00±0.58b	10.67±2.33a	11.87±2.47b	6.75±0.88c
Unica	Coco-peat + pumice	15.33±3.53e	64.67±12.98e	16.90±5.80cd	9.96±2.31de
	Coco-peat + perlite	15.33±3.53e	41.33±12.39d	24.64±4.32e	17.19±1.92g
	Coco-peat + vermiculite	13.33±1.76d	47.00±2.00d	16.40±1.91c	9.19±0.64d
	Sand	11.67±2.19d	11.67±1.45a	12.08±3.13b	6.07±1.39c
	Soil	8.00±1.53c	33.00±7.21c	11.78±0.74b	5.23±0.27b
Wanjiku	Coco-peat + pumice	18.67±1.86ef	43.67±14.17d	19.41±2.48d	11.43±1.11e
	Coco-peat + perlite	20.33±3.33fg	59.67±10.27e	21.17±8.16de	14.79±3.17fg
	Coco-peat + vermiculite	16.00±6.03efg	39.67±16.42cd	16.79±4.39cd	10.03±1.02e
	Sand	3.67±1.20a	10.67±1.86a	11.42±1.63b	6.04±0.92c
	Soil	6.67±1.67c	23.67±5.33b	12.38±2.56b	6.77±0.63c

- Means within a column followed by the same letters are not significantly different ($P \leq 0.05$) according to Tukey's HSD test

4.3.5 Correlation analysis

There was a significant positive correlation (r=0.97) between the shoots fresh weight and shoots dry weight (Table 4.3). An increase in shoot fresh weight resulted in an increase of shoot dry weight. There was a positive correlation between the number of leaves and number of branches (r=0.72). The number of leaves increased with increase in number of branches. Shoot fresh and shoot dry weight positively influenced the number of minitubers (r=0.69 and r=0.66). Similar to these results, Otroshy *et al*. (2013) observed that shoot dry weight positively influenced number of minitubers (r= 0.43).

Table 4.3: Pearson correlation coefficients on the response variables of number of leaves, height, number of branches, number of minitubers, weight of minitubers, leaf fresh weight and leaf dry weight

Response variable	Height	No. of leaves	No. of branches	No. of minitubers	Weight of minitubers	Shoot fresh weight
No. of leaves	0.09ns	-	-	-	-	-
No. of branches	0.03ns	0.72***	-	-	-	-
No. of minitubers	0.32*	0.52***	0.46**	-	-	-
Weight of minitubers	-0.08ns	0.65***	0.55***	0.49***	-	-
Shoot fresh weight	0.32*	0.40**	0.40**	0.66***	0.36*	-
Shoot dry weight	0.35*	0.45**	0.43**	0.69***	0.35*	0.97***

**Correlation is significant at the 0.001 level, **Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level

4.4 Conclusion

The performance of potato varieties varies with propagating media. Coco-peat + perlite was found to be superior in supporting plant growth and tuber formation, followed by coco-peat + pumice. The study has provided valuable information on the media type and the role it plays in increasing the productivity of seed tuber in perspective of commonly used local varieties in Kenya.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The rapid and high rate of multiplication in production of apical rooted cuttigs can reduce seed potato shortages since each cutting produces 10 to 15 tubers, which are multiplied a further season or two seasons (Parker, 2017). This will help farmers to access readily available seed materials.

Carbon source concentration in the medium has profound effects on *in vitro* growth and tuber formation. The addition of sucrose to the *in vitro* culture medium provides an important source of carbon and energy for uptake and utilization by plants (Cheong & An, 2015). However, different sucrose concentration in the culture medium have been observed to exhibit different effects on the shoots of plants. An increase in sucrose concentration resulted to higher survival, highest shoot length and number of shoots recorded (Elazab & Shaaban, 2015). Increasing the sucrose concentration promoted an increase in shoot length and enhanced rooting. Low sucrose concentration produced fewer and smaller shoots per expant. Optimum sucrose concentration promotes photosynthetic activity and increases the speed of plantlets adaptation producing heathier plants.

GA_3 is involved in cell elongation and its addition in MS medium enhances shoot growth (Camara *et al.*, 2018; Rizza *et al.*, 2017). The successful *in vitro* multiplication of potatoes depends on the concentration of GA_3 in the propagation medium. Supply of GA_3 in the culture media of explants from different species has proved to increase culture size and contribute to a better performance of seedlings when transferred to ex vitro conditions. GA_3 has a positive effect on water uptake and germination of *in vitro* culture. Increasing gibberellic acid (GA_3) concentration upto (1.0 mg L^{-1}) resulted in delayed shoot length and growth of *in vitro* potato. GA_3 at 0.5 mg L^{-1} increased shoot length by extending the internodes of growing shoots (Yildrim, 2019), while at 1.0 mg L^{-1} the shoots produced were slender with the absence of internodes.

The supply of GA_3 at a concentration of 0.5mg L^{-1} combined with sucrose at a concentration of 40g L^{-1} significantly recorded a better shoot length, the number of shoots, number of roots, number of leaves, root initiation, shoots fresh weight and shoots dry weight on local potato varieties cultured *in vitro.* This combination also gave a better effect on survival and improvement of shoot growth.

Soil-less media has capability to grow plants in a conditioned, pest and disease free environment. Cocopeat + perlite was the best for vegetative potato growth, followed by coco peat+ pumice. This was due to their composition which retain nutrients better and has a high water holding capacity that boosts growth under greenhouse conditions (Abouzari, 2012). Sand reported the poorest with stunted growth due to its very low water and nutrient holding capacity. Plants grown in soil were susceptible to blight and required frequent weeding. Well-established root and shoot system, is important for subsequent growth which, in turn, influences tuber bulking in potato growth. However, the performance of potato varieties varies with propagating media.

Coco-peat + perlite composition was a good media for potato mini-tuber production as tall healthy plants had more photosynthetic area translating to higher yield. A well-established root and shoot system, is important for subsequent growth which, in turn, influences tuber bulking in potato (Awati *et al.*, 2019). Yields were lower in sand and soil media which correlated with poor plant growth. This could be due to photosynthetic area differences that affects tuber formation of potato apical rooted cuttings. The main role played by physical characteristics of coco-peat + perlite mixture can be attributed as suitable air-filled porosity, for effective oxygen diffusion and maintaining favorable water content for supplying water, nutrient, and respiration of plant roots unlike sand media that has low water and nutrient holding capacity and can exacerbate deficiencies (Dharti *et al.*, 2021).

5.2 Conclusions

i. GA_3 at 0.5mg L^{-1} in combination with sucrose at 40g L^{-1} significantly resulted to higher shoot length, number of shoots, roots, leaves, root initiation, shoots fresh and dry weights on local potato varieties cultured *in vitro*.

ii. Coco-peat + perlite was the best soilless media in supporting potato growth in terms of plant height, no of branches and number of leaves

iii. Coco-peat + perlite, followed by coco-peat + pumice were the best soiless media for tuber formation that resulted in better tuber yield

5.3 Recommendations

i. Farmers could achieve rapid multiplication, survival and growth of local potato varieties of seed potato by using GA_3 at 0.5mgL^{-1} in combination with 40g L^{-1} sucrose.

ii. Farmers could overcome the potato seed degeneration by using Coco-peat + perlite and coco-peat+pumice as a soilless media as they enhanced seed potato growth. This will result in ready availability of planting materials and farmers will stop recycling seed.

iii. Coco-peat +perlite can be used in tuber formation of the local potato varieties

Further research should be carried out in the field to determine the performance of the local potato varieties that are propagated through apical rooted cuttings

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APPENDICES

Appendix A: MS Media nutrient concentration

Stock solution	Salts/Vitamins	G/L	Amount of stock
1	NH_4NO_3	33g	50ml
	KNO_3	38g	
2	$MgSO_4 .7H_2O$	18.19g	10ml
	$MnSO_4.4H_2O$	1.7g	
	$ZnSO_4.7H_2O$	0.86g	
	$CuSO_4.7H_2O$	0.0025g	
3	$CaCl_2.2H_2O$	33g	10ml
	KI	0.083g	
	$CoCl_2.6H_2O$	0.0025g	
4	KH_2PO_4	17g	10ml
	H_3BO_3	0.62g	
	$NaMoO_4.2H_2O$	0.025g	
5	$FeSO_4.7H_2O$	2.8g	10ml
	Na_2-EDTA	3.725g	
6	Inositol	0.05g	10ml
	Glycine	0.2g	
	Nicotinic acid	0.05g	
7	Pyridoxine-HCl	0.05g	10ml
	Thiamine-HCl	0.5g	
	$NaH_2PO_4.H_2O$		$0.17gL^{-1}$
8	Sucrose		$2gL^{-1}$
	Agar		$7gL^{-1}$
	Total for media		**1000ml**

Appendix B: Analysis of variance for number of leaves, number of roots, length of shoots, shoot initiation, root initiation, number of shoots, leaf fresh weight and leaf dry weight as affected by sucrose, GA_3 and variety.

Source of variation	df	Leaves	Roots	Length	Shoots	Shoot initiation	Root initiation	Leaf Fresh weight	Leaf Dry weight
Replicate	2	12.81	19.94	105.68	1.21	0.60	125.27	0.02	0.02
Sucrose	2	40.01***	189.83***	72.37***	5.19***	1.72ns	669.83***	0.03ns	0.02
Gibberellic acid (GA_3)	2	1.16ns	32.82***	6.54ns	3.12***	6.01ns	579.79***	0.91***	0.90***
Variety	2	17.50***	33.03***	41.72***	3.21***	9.12*	153.42ns	1.32***	1.39***
GA_3 × Sucrose	4	24.51***	39.52***	32.65***	1.11**	0.33ns	235.12*	0.48***	0.48***
Ga_3×Variety	4	13.32***	12.30***	13.11***	1.33**	0.85ns	218.88*	0.36***	0.35***
Sucrose × Variety	4	8.11**	8.39**	25.22***	0.86*	4.22ns	133.20ns	0.14ns	0.14
GA_3×Sucrose ×Variety	8	13.55***	28.61***	20.45***	1.19***	9.72***	282.77***	0.18*	0.19*
Error	372	2.28	1.96	2.18	0.30	1.94	65.03	0.07	0.07
R^2		0.74	0.73	0.81	0.51	0.57	0.69	0.74	0.75
CV (%)		20.69	38.99	23.21	35.29	23.16	41.90	44.38	46.14

- *Significant at P<0.05, **significant at P<0.01, ***significant at P<0.001

Appendix C: Analysis of variance for the height, number of leaves and number of branches as affected by soilless media and variety.

Source of Variation	df	Height	Number of leaves	Number of branches
Replicate	2	6.16	1372.80	27.77
Treatment	4	846.50***	1922.87***	85.12***
Variety	2	6632.48***	311.11**	25.29***
Treatment ×Variety	8	279.11**	141.35**	18.39
Error	204	82.16	65.81	2.23
R^2		0.86	0.87	0.78
CV (%)		26.68	23.27	30.02

- *Significant at P<0.05, **significant at P<0.01, ***significant at P<0.001

Appendix D: Analysis of variance for the number of tubers, weight of tubers, shoots fresh weight and shoots dry weight

Source of Variation	df	Number of tubers	Weight of tubers	Shoot fresh weight	Shoot dry weight
Replicate	2	128.96	595.62	18.68	12.31
Treatment	4	361.58***	2029.98***	225.43***	155.01***
Variety	2	38.69ns	911.36*	3.10ns	3.30ns
Treatment× Variety	8	19.49ns	270.41ns	23.37ns	10.44ns
Error	28	21.79	217.60	20.61	9.45
R^2		0.76	0.69	0.66	0.74
CV (%)		37.92	44.49	27.42	30.97

- *****Significant at P<0.05, **significant at P<0.01, ***significant at P<0.001

Appendix E: Research Permit

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Appendix F: Publication

RESEARCH ARTICLE

European Journal of Biology and Biotechnology
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Effects of Sucrose and Gibberellic Acid on Growth and Survival of Local Potato (*Solanum tuberosum* L.) Varieties *in vitro* in Kenya

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Anthony Mwangi Kibe and Judy Oggema

ABSTRACT

Tissue culture techniques' have become useful technologies for producing disease & pest free seed potato (*Solanum tuberosum* L.) in the developed world. However, these techniques have yet to be standardized for locally produced potato varieties in Kenya. Developing countries can also use these innovations for rapid multiplication of popular local seed material through rooted apical cuttings generated from either plantlets or micro tubers. *In vitro* experiments were therefore conducted to determine the optimum concentration of sucrose and gibberellic acid for growth and survival of local potato varieties, namely, *Shangi, Unica* and *Wanjiku*. The explants were cultured in Murashige and Skoog (MS) media supplemented with sucrose at a concentration of 20, 30 and 40 gL^{-1}, while gibberellic acid was applied at a concentration of 0.2, 0.5 and 1.0 mgL^{-1}. The study was laid out in a completely randomized design (CRD). MS Medium with sucrose 40 $gL^{-1}$and gibberellic acid 0.5 mgL^{-1} significantly enhanced shoot length, with the longest shoot (10.3 cm) being recorded for *Wanjiku*. The same treatment also gave the highest plant survival of 90%. Murashige and Skoog media, added with 0.5 mgL^{-1} gibberellic acid along with 40 gL^{-1} sucrose is recommended for generating *wanjiku, unica and shangi* apical rooted cuttings because it gave the best improvement of *in-vitro* clonal growth.

Keywords: Gibberellic acid, *in-vitro*, growth, nutrient, potato, sucrose, tissue culture.

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