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COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

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GENERAL BIOLOGY PROGRAM

Micropropagation of *Oreosyce africana* from shoot tips

Thesis submitted to the school of graduate studies, Addis Ababa University in partial fulfilment of the requirement for the Degree of Master of Science in Biology

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Abstract

Oreosyce africana *('manabasi' in Afan Oromo) is the formulated medicinal plant that kills Anopheles arabienses at both larvae and adult stage. The availability of this plant species is very limited and scarcely distributed in Ethiopia. Hence, this study is aimed to develop efficientmicro propagation protocol for this plant speciesusing shoot tip explants. Shoot tips were excised from mother plant and sterilized with 70% alcoholfor fifteen seconds followed by20% sodium hypochlorite (NaOHCl for 20 min and rinsed three times in sterile distilled water. The sterilized shoot tips were cultured on Murashige and Skoog (1962) (MS) medium supplemented with 0.05mg/l BAP. The initiated shoot tipswere transferred to MS medium supplemented with different concentrations of BAP alone, KIN alone, BAP in combination with IBA and KIN in combination with IBA for shoot multiplication. For rooting, half strength MS medium supplemented with various concentrations of IAA alone, IBA alone and IAA in combinations with IBAwere used. MS medium without PGRs was used as control. The result showed that highest mean shoot number (36.66±2.03) per explants was obtained on a medium supplemented with 0.5 mg/l BAP and the highest mean shoot height (4.26±0.10 cm) was obtained on growth regulators free medium.The highest mean root number (7.77±.44) and mean root length (5.42±.45 cm) were obtained on 1/2 strength MS medium containing 0.5mg/l IAA. Up on acclimatization, 98% plants survived. The results showed that, this micro propagation protocol can be used for mass propagation and ultimate conservation of this medicinal plant.*

Key words: *Oreosyce africana, Micro propagation, Medicinal Plant, Larvicidal, Adulticidal, Plant Growth Regulator*

List of abbreviations

ANOVA	$Analysis\ of\ Variance$
BAP	$6-Benzyl\ Amino\ Purine$
EDTA	$EthyleneDi-AmineTetraaceticAcid$
IAA	$Indole\ 3-AceticAcid$
IBA	$Indole\ 3-ButyricAcid$
KIN	$Kinetin$
LSD	$Least\ Significance\ Difference$
MS	Murashige and Skoog
PGRs	Plant Growth Regulators
SE	Standard Error

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1. Introduction

Oreosyce africana (‘manabasi’ in Afaan Oromo) belongs to family Cucurbitaceae and genus *Oreosyce*. The plant species has tendrils at its node, broad leaves, creeping or prostrating or climbing nature and has a length of 3-4m. *O. africana* region as medicine, food, ornaments, and poultry feed (Nmom, 2017). Extracts from *Oreosyce africana* is mainly used as larvicidal and adulticidal to the larvae and adult of *Anopheles arabienses* is distributed in Cameron, Zimbabwe, Botswana, South Africa, and east Africa. Plant species in Cucurbitaceae family have different values throughout the world especially in tropical that is the major malaria vector in the world, especially Sub-Saharan countries including Ethiopia (Damtew Bekele *et al*., 2016). Plants in the family ofCucurbitaceae, momordicaare also used in treatment of malaria, as antifungal, anti-inflammatory, antiparasites, antiseptic, and act as digestive stimulant, febrifuge, menstrual stimulator, and wound healing (Choudhury, 2010 and Ross, 1999). *Oreosyce africana* plays a vital role to eradicate malaria vectors (*Anopheles arabienses*) (Damtew Bekele *et al*., 2016). The plant material was collected from Oromia regional state, Akaki district, Eastern Addis Ababa (Damtew Bekele *et al*., 2016). However, its distribution is scarce in Ethiopia indicating the propagation and conservation of this species is important.Plant tissue culture is a technique for the production of large numbers of genetically identical plantlets under aseptic condition. Plant micropropagation has a great advantage in agriculture, horticulture, forestry, selective crop production, to multiply medicinal and commercial plants.

Therefore, the aim of this study was to develop micro propagation protocol for *Oreosyce africana* which is further used to eliminate *Anopheles mosquito* at their larval and adult stage. Since the values of plant species were not known by societies, they don’t give a care foe it and simply on eliminating it.

2. LITERATURE REVIEW

2.1. Description and taxonomy of *Oreosyceafricana*

Oreosyce afriana is small scandent perennial herb plant that has simple netted leaves, which are alternative, petiolate, tendrils at its node, fibrous root and few flowers with sessile fascicles. The plant is prostrating plant that can climb 3-4m length and belongs to Cucuritaceae family. This family comprises more than 130 genera and have beyond 800 species. These are native in most countries of the world, especially in tropics and sub-tropic, where they are cultivated in every country, state, and province (Nmom, 2017). Of above 800 species of this family, *O. africana*is distributed in horn of Africa including Ethiopia, Cameron, Botswana, Zimbabwe and some other parts of Africa. The plant family mostly occur in wet or moist *Pouteria (=Aningeria) adolji-friederic-Syzygium guineese* forest margins, grassland and in plantation at altitude between 1650-2000m (Jeffery and Edward, 1995). Cucurbitaceae plant family has multi-purpose use as food, ornament, animal feed, medicine, antifungal, anti-inflammatory(Choudhury, 2010 and Ross, 1999).

2.2. Morphological Characteristics of *Oreosyceafricana*

Cucurbitaceous plant family is mostly characterized by having annual weak stemmed trailing or climbing with its tendrils. E.gacanthiosicyos of South Africa and debdrosicyos in India are cultivated for use as vegetable. The vegetative nature of cucurbitaceous reveals us some plants are perennials and others are annuals. This characteris shown by having branched taproot. Sometimes their root ismodified into monoliform root, for example in the case of momordica.

Plants like, acanthosiryos has root that can extend to 15m which is very thick and used tostore food. The root of *Oreosyce africana* exhibits adventious, which arise from axial of stem. Cucurbitaceae has stem, which is characterized with herbaceous, and climbs by tendril.

Likewise, *Oreosyce africana* has perennial, soft and climbing stem. The plant stem is not of woody nature rather it is hollow. *Oreosyce africana* has triangular, ovate or broadly ovate leaf.

Plants in Cucurbitaceae family have solitary flower with panicle inflorescence. The flower may be monocious or dioecious in which male and female flower found on different or same plants. Most of their flowers are regular, unisexual, actinomorphic, showy, large, white and yellow colour. Imbricate calyx with sepal five, showy colored corolla with five petals, free or combined form of stamens that depends on their genera and twisted transverse anther are some of the characteristics of male flower of Cucurbitaceae. The female part of the flower contains soft fleshy fruit, none endospermic seed, androecium that is reduced to rudiments of staminodes, tricarpellary gynoecium and calyx and corolla with five-sepal and petal respectively.

2.3. Economic importance of Cucurbitaceae

Cucurbitaceae plant family has valuable economic importance for our environment and us. It is used in nutrition, medicine, commercial and nutrient recycling within ecosystem.

2.3.1. Nutrition

In far eastern part of the world, china, India, Bangladesh, most vegetation are edible. Plants like pumpkins, squashes, gourds, marrows, and courgattes are the group of cucurbit used as a major source of food (Ajuru, 2014)**.** Nmom(2017) said that melons, cucumbers, water melons are also cultivated for food.

Some species of Cucurbitaceae plant family are cultivated in tropical regions of Asia and Africa. Species grown in this region exhibit rapid growth and plants like *Benincasa hispida* or winter melon are grown recumbent means its growth looks like the growth of pumpkins or cucumber. The matured fruits and seeds of *Benincasa hispida* plant species are harvested,

fried and eaten by humans in the form of soup. The white, chalky wax of *Banincasa hipsida* is used to kill microorganisms. Winter melon fruits can be stored for a year without refrigeration.

The plant *Citrullus colocynth is* drought tolerant and its productivityis increased during dry and sunny period. This plant is native to Africa that is mainly used to obtain balanced diet(Fokou, 2004). The seed powder of Egusi, *Citrullus colocynthis's* is used for human nutrition in the form of soup thickener or flavouring agent (Ogunsua, 1991).

2.3.2. Medicinal use

Most cucurbit plants are used in treatment of various types of disease because species of Cucurbitaceae family contain a phytochemical substance which has wide range in biological activities of both plants and animals. Highly toxic and bitter substancesthat exist in the plant family make it to be candidate in different pharmacopoeias (Miro, 1995and Abdel-Rahman, 2006)**.**Choudhury (2010) demonstrated that whole *Momordica charantia* (bitter melon or bitter gourd) fruit is very important in treatment of malaria.Das(2006),Omoloso(1998) and Ross(1999)explained that bitter melon possess antifungal, anti-inflammatory, antiseptic properties, purgative, digestive stimulant, anti-parasite, and wound healing characteristics. Gurbuz (2000) and Dhiman(2012) also reported that traditionally, mature fruits are used for wound healing and immature fruits used in treatment of diabetes. The authors reported that the fruits of bitter gourd used to treat a lot of ailment such as cholera, anaemia, and jaundice.

The fruits of *Cucurbita pepo* (pumpkins) are very useful in purification of blood, astringent effect on bowel, treatment of sore chest, treatment of human leprosy, fever, bronchitis, haemoptysis and antiulcer (Gill, 2011 and Roman-Ramos, 1992).

Acosta-Patino (2001) and Abou Zaid (2001) reported that various part of Cucurbitaceae family, leaves, seeds and fruits are used in treatment of different types of disease such as tapeworm, prostrate glands in men, diabetes type 2. similarly Acosta-Patino, (2001), Abou Zaid, (2001) and Vouldoukis, (2004) described anthelmintic nature of Cucurbitaceae and taken as tonic.In addition Cucurbitaceae also used as anti-inflammatory, antipyretic and treatment of liver ailments (Marzouk, 2010; Adam and Ahmed, 2001).

The root of snake gourd used as a cure for boil, headache, anti-diabetes, anti-inflammatory, anti-tumour promoters (Kolte, 1996; Mohan, 2009and Gordon, 2000). Plants like Schism educe, *Trichosanthes tricuspidata*, *Lagenaria sicenaria* (bottle gourd), *Benincasa hispida* (wax gourd), *Cucumeropsis mannii* and *Telfairia occidentalis* are medicinally and traditionally used as blood tonic, to treat sudden attack of convulsion, cardiovascular protection, and to treat kidney related disease(Dire, 2003; Moon, 2009; Akorode, 1990;Davis, 1991; Shah and Gill 2010).

Secondary metabolities of *Oreosyce africana* contain various compounds that play a vital role in their biological activities (Damtew Bekele *et al*., 2014). The presence of unsaturated fatty acid such as linoleic acid compound in *Oreosyce africana* is used as adulticidal and larvicidal of Anopheles arabiences. Evidence from different source(Z, 2014) and Abbott (1925)showed that linoleic acid extracted from *O. africana* is used as anti-inflammatory, anticancer, hepatoprotective, nematicidal, insectifuge, antihistaminic, 5-αreductase, antiandrogenic and enhance immune system.

Generally, purified extract from the leaf of *Oreosyce africana* Hook f. is an excellent medicine to kill *Anopheles arabiences* at their larvae and adult stage (Damtew Bekele *et al*., 2014). Therefore, the use of the products of *O. africana*, an indigenous plant to Ethiopia for the impregnation of mosquito nets must be seriously considered in malaria control and elimination effort currently in place. This is particularly relevant because of the ever-

increasing failure of the conventional residual sprays with DDT or other insecticides for malaria vector control in Ethiopia. People give valuable place for their cattle's and poultries. Especially domestication of animals and life with their livestock has long history for Ethiopians. To keep the healthcare of their livestock, traditional healthcare practices provides as veterinary medicines. The traditional ethno medicines are available easily around local area and not as expensive as western type of veterinary medicine (Tadeg, 2005). Ethno veterinary medicine offers medicines that are cheap and locally available than pharmacotherapy. Farmers can prepare and use homemade remedies.Yirga(2012) reported that plant remedies are still the most important and sometimes the only source of therapeutics for nearly more than 90% livestock population.

2.4. Cucurbitaceae as ornamental and energy source

Various species of cucurbitaceous plant family has a valuable importance to produce ornamental materials. Some of the plants that have a crucial importance to make different materials are *Lagenaria siceraria* and *Luffaa egyptiaca*. Jimoh(2013)reported the use of *Lagenaria siceraria* in the production of ladles, boxes, water jugs, planters, flutes, sitars and other musical instrument. In addition, *Lagenaria siceraria*is usedto make beads, shells, gunpowder, and palm wine and metals. Fruits of gourds are excellent in cosmetic industry because fruits are good for rubbing skin for softness and whiteness; it is cooling, soothing to the skin irritated by sun, in soap making and healing skin.

The mature fruits of *Luffaa egyptiaca* are the source of the spongy reticulated material (domestic loofah). Loofahs are used for sponges, filters, stuffing pillows, saddles, and slippers. These species can also be used for insulation and are attractive sources for packing materials.USA imports the product of loofahs from Asia in millions of tonnes each year. This encourages Asians to increase the domestic product from loofahs.

From above 300 species of melon, *Citrullus colocynthis* is cultivated for its seeds, and rich in oil (53%) and protein (28%). Different studies have reported that egusi melon seed oils contain high linoleic fatty acid. Because of unsaturated fatty acid composition of its oil, it resembles sunflower, cotton seed, maize, soybean and sesame oil that have already been used for biodiesel production.

Likewise, *O. africana* contains high linoleic fatty acid that has the characteristics of medicine to kill insects including *Anopheles mosquito* which is a vector of malaria.

2.5. Micro propagation

Micro propagation is the application of tissue culture technique for propagation of plants starting with very small parts grown aseptically. It is one of the key tools of biotechnology, which is important to meet the growing demands for elite planting material. Micro propagation is the backbone in agriculture for the demand of disease free and quality of plants in ornamental, horticultural and agroforestry sectors.

2.6. Development of Plant Growth Regulators

Different authors had developed plant growth regulators in last 20th century. During this period, several plant growth hormones and their substitutes were discovered. Fritz

(2012) discovered Indoleacetic Acid (IAA). Addition, coconut milk can cause a drastic increase in the growth of plant embryos and tissue cultures that was identified by Van Overbeek, J. (1968). Skoog, F. and Tsui, C. (1948) revealed that the induction of cell division and bud formation is due to adenine administration and Gamborg*et al.* (1968) isolated kinetin, adenine derivative (6-furyl amino purine). They proposed the concept of hormonal control for organ formation. Some hormones grouped together in one classification such as kinetin and many similar functioning compounds, which show bud-promoting activities, collectively classified as cytokinins. Skoog and Miller (1957) proved that the ratio of

cytokinin to auxin in nutrient media greatly influences the morphogenesis of roots and shoots in plant tissue culture.

3. OBJECTIVES

3.1. General objective

To develop micro propagation protocol for *Oreosyce africana* from shoot tip explants

3.2. Specific objectives

- To initiate shoot tips of *Oreosyce africana* using BAP
- To determine the optimum plant growth regulators concentration for shoot multiplication
- To identify optimum growth regulators concentrations and combinations for *in vitro* rooting

4. MATERIALS AND METHODS

4.1. Plant material

The only existing single pot plant of *O. africana* at Addis Ababa University was obtained from Prof. Beyene Petros, Bio-Medical Laboratory.

4.3.1. Preparation of stock solutions

Macronutrients, EDTA, micronutrients, and vitamins of Murashige and Skoog (1962) nutrient media components were usedin preparation of stock solution. Stock solutions of vitamins and plant growth regulators (PGRs) were prepared by dissolving each in required quantity of chemicals in distilled water. The stock solutions of nutrients were prepared freshly every month as required while vitamins and PGRs were prepared every two/three weeks and all of the MS stocks were stored in refrigerator at +4 $^{\circ}$C.

4.3.2. Plant growth regulators stock solution preparation

Plant growth regulators (PGRs) were prepared in 1mg/ml of double distilled water concentration. 6- Benzyl aminopurine (BAP) and kinetin (KIN) were used as cytokinins (for shoot initiation and multiplication) and indol-3- butyric acid (IBA) and indol acetic acid (IAA) as auxin, which induces root. To prepare PGRs, 0.10mg powdered crystal of each hormone was weighed and dissolved in 3-4 drops of NaOH by stirrer in small glass bottle. Upon complete dissolution, 10ml of double distilled water was added to it and stirred gently and stored at a temperature of +4 °C for two/three weeks.

4.3.3. Culture media preparation

The culture media prepared for shoot initiation and shoot multiplication of this study contained full strength MS basal medium (50 ml/l macronutrient, 5ml/l of EDTA, 5 ml/l micronutrient, and 5 ml/l vitamin), 30 g/l sucrose and with or without (for control) PGRs. The media prepared for rooting was half strength MS basal medium (25 ml/l macronutrient, 2.5ml/l micronutrient, 5ml/l vitamin, 5ml/l EDTA) and with IAA or IBA at different concentrations and without IAA or IBA for control group. In addition to this, pH of all media was adjusted to 5.72 by using 1N HCL and/or 1N NaOH before addition of agar. Lastly, 7.0 g/l agar was used as a solidifying agent throughout the experiment.

The prepared mediawas boiled on a hot plate until the agar melted and then, 50 ml of the prepared mediumwas dispensed into 10 x 6.5 cm size Magenta GA-7 culture vessels. The culture vesselswere covered with caps immediately after dispensing the medium and autoclaved at a temperature of 121°C and 105-kpa pressure for 15 minutes. Immediately after autoclaving, the medium was taken and kept in lamina-air-flow-cabinet bench until the media was cooled and solidified.

4.3.4. Culture Initiation

About 2.0 cm long shoot tips were excised from the mother plant, collected in beaker and washed with running tap water. This was followed by surface sterilization with 70% ethanol for 15 seconds followed by washing in sterile distilled water. The shoot tips were then sterilized with 20% sodium hypochlorite for 20 min followed by three times washing with sterile distilled water. The sterilized shoot tips were trimmed to about 1.0 cm and cultured on MS medium containing 0.05mg/l BAP, 30 g/l sucrose and 7.0 g/l agar. The cultures were

maintained under light intensity of 22 µmol m^{-2} s^{-1} and 16 h photoperiod provided by white fluorescent lamps at 25±2ºC.

4.3.5. Shoot Multiplications

Shoot tips from initiated shoots were excised and transferred onto MS medium supplemented with different concentrations of BAP or KIN (0.0, 0.5,0.75, 1.0, mg/l) in combination with IBA at various concentration (0.0, 0.25, 0.5, 0.75) for shoot multiplication. Growth regulators free MS medium was used as control. A total of 16 treatmentswere done and for each treatment, five culture vessels, each with six shoot tips, were used. Cultureswere maintained under the same culture conditions for shoot initiation. The number of shoots per explant and shoot lengthwere recorded after four weeks.

4.3.6. Rooting and acclimatization

The shoots multiplied on multiplication medium were excised and transferred on to half strength MS media supplemented with different concentrations of IAA or IBA (0.0,0.25, 0.5, 1.0, mg/l) and the combination of IAA with IBA at different concentrations (0.05,0.25,0.5 mg/l) were used for rooting. Growth regulators free half strength MS medium was used as control. Number of roots per shoot and root length were recorded after four weeks. Plantlets with well-developed rootswere transferred to 10 cm diameter pots containing sterilized soil and placed in the greenhouse.The pots were coveredwith polyethylene bags. The polyethylene bags were removed after 14 days of acclimatization. Thirty days after transplanting the plantlets to the pots, the number of survived plants was recorded.

4.3.7. Data analysis

The study was conducted at Plant Tissue Culture and Molecular Biology Laboratory, Addis Ababa University. Data were subjected to analysis of variance (ANOVA) and least significant difference (LSD) test using statistical data analysis software SPSS 20.0 version at 0.05 probability level.

5. Results

5.1. Shoot Initiation

From five shoot tips of *Oreosyce africana* excised from mother plant and supplemented with 0.05ml/l BAP, all of them survived and new growth of shoots emerged.

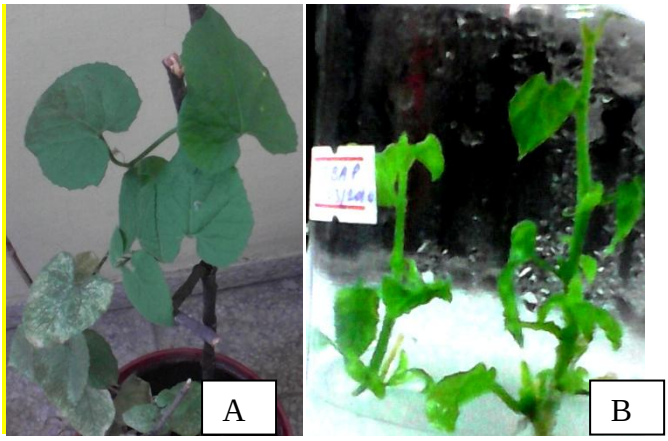

Figure 1: Shoot initiation from shoot tip explants of *O. Africana* on MS medium containing 0.05mg/l BAP. A) Mother plant B) Initiated shoot

5.2. Multiplication

5.2.1. The effect of BAP and KIN alone on shoot multiplication of *Oreosyceafricana*

The result of shoot multiplication with different concentrations of BAP alone is presented in table 1. After three weeks of culture on MS medium containing various concentration of BAP alone, different number of shoots/explant were emerged (fig2). The number of shoot was highly affected by different concentrations of BAP. The maximum mean number of shoots perexplant (36.66±2.03) was obtained on MS medium supplemented with 0.5 mg/l BAP (fig 2B). Maximum mean shoot length (4.26±0.10 cm) was observed on growth regulators free MS medium. This result describes that BAP PGRs have a great role in inducing of shoot by activating its cell division. As BAP hormone concentrationdecrease activation of cell division

decrease and the plant increase in length, however the shoot number increase with increasing of BAP concentration up to its saturation level which is 1.

There was no significant difference in rate of shoot multiplication among MS medium supplemented with different concentration of KIN at $P<0.05$ after three weeks. The maximum mean number of shoots per explant (7.67 ± 0.63) was obtained on MS medium supplemented with 0.5 mg/l KIN. Shoots on MS culture media supplemented with 0.25 mg/l BAP and 0.5 mg/l BAP had high quality appearance. On medium containing KIN alone, the proliferated shoots had poor quality and were very slow in growth.

In both KIN and BAP, the mean number of shoots per explant was greater than the shoot number per explant on growth regulators free MS medium.

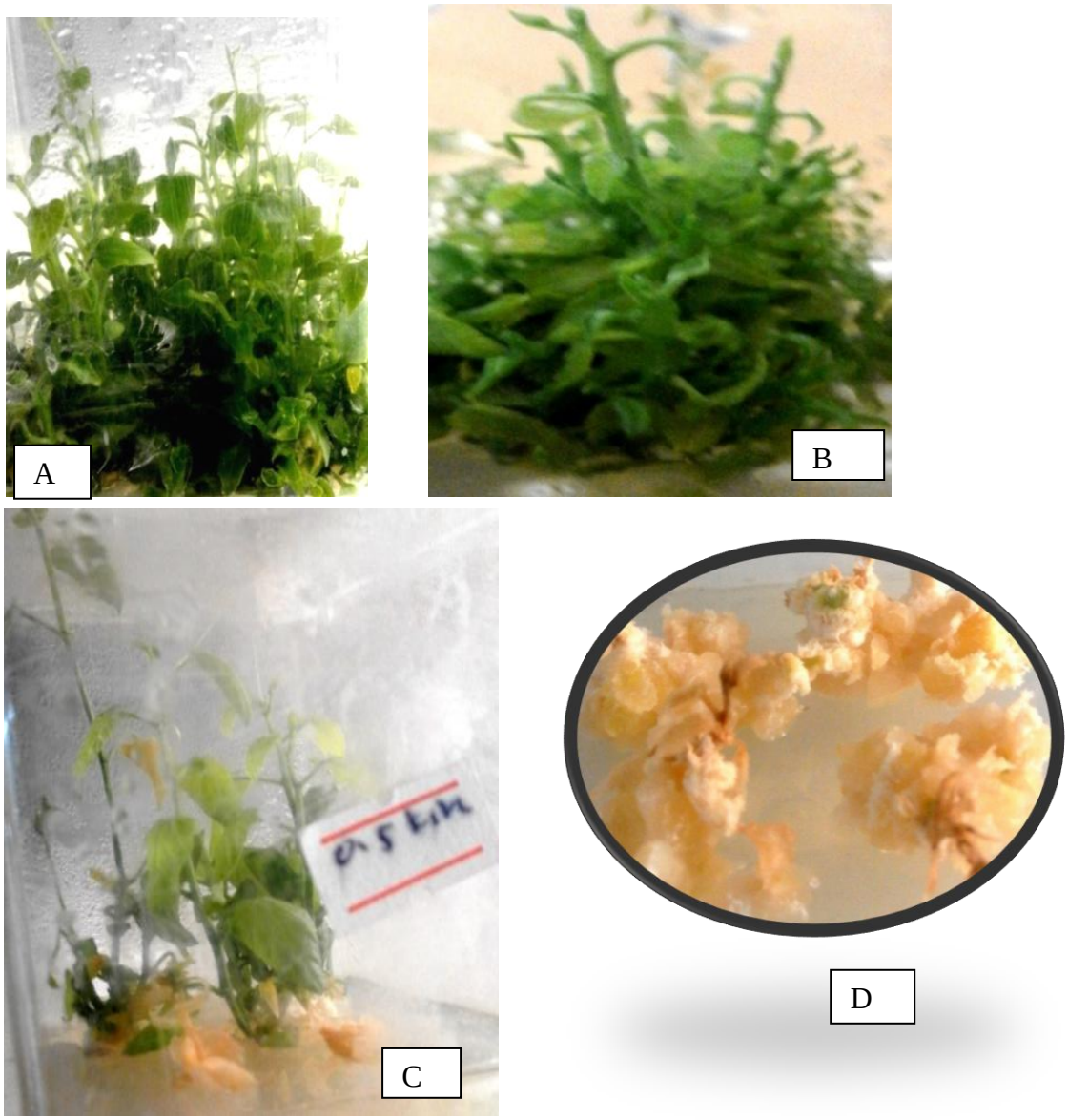

Figure 2: Shoot multiplication from shoot tip explants of *O. Africana* on MS medium containing BAP alone at various concentrations (A) 0.25mg/l and 0.5mg/l (B), KIN alone (C) 0.5mg/l) and combinations of BAP with IBA at different concentrations (D).

Table 1:Effect of different concentrations of BAP and KIN on shoot multiplications of *Oreosyceafricana*

PGRs (mg/l) BAP	No of shoots/ explant Mean ±SE	Shoot length (cm) Mean ±SE
0.00	1.53 ±0.33[c]	4.26 ±0.10[a]
0.25	19.20±1.00[b]	3.31 ±0.15[b]
0.5	36.66 ±2.03[a]	1.94±0.15[c]
1.00	1.33 ± 0.03[c]	1.53±0.33[d]
KIN		
0.25	3.60±0.37[b]	4.19±0.25[a]
0.5	7.67±0.63[a]	2.17±0.16[b]
1.00	1.00±0.22[b]	1.42±0.29[c]

Mean with the same letter within the same column are not significantly different at P< 0.05. The values represented as mean ± SE. The number of explants cultured on each treatment was 30.

5.2.2. The effects of different concentration of BAP and KIN in combinations with IBA on shoot multiplications of *Oreosyceafricana*

In all treatments of shoots, multiplication on MS medium supplemented with different concentrations of KIN and BAP in combination with IBA, callus formation in all treatment rather than shoot formation were observed. This means at the base of cultured explants showed callus formation All shoots cultured on medium containing different concentrations of BAP and KIN (0.5, 0.75, 1.0 and1.5 mg/l) in combination with IBA (0.15, 0.5, 0.75 and1.0 mg/l) formed callus. However, above stalk of callus, weak and very little shoot were also present.

Table 2: Effects of different concentrations of KIN in combination with IBA on shoot multiplications of *Oreosyceafricana*

PGRs (ml/l)		No. of shoots/ explant	Shoot length (cm)
BAP	IBA	**Mean ± SE**	**Mean ± SE**
0.00	0.00	1.17±0.14[d]	3.07±0.29[b]
0.5	0.25	1.10±0.15[d]	1.30±0.17[d]
1.5	1.0	1.40±0.18[c]	1.49±.156[d]
KIN	IBA		
0.00	0.00	2.17±0.18[b]	4.07±2.93[a]
0.5	0.25	3.90±0.25[a]	3.30±2.37[b]
1	0.75	2.40±0.28[b]	1.89±.256[c]

Mean with the same letter within the same column are not significantly different at P< 0.05. The values are represented as mean ± SE. The number of explants cultured on each treatment was 30

5.3. Rooting and acclimatization

5.3.1. Rooting

After multiplication, shoots cultured on half strength MS medium supplemented with different concentrations of IAA (0, 0.25 and 0.5 mg/l) produced roots. The maximum mean number of root (7.77±.44) and highest mean length of root (5.42±.45 cm) was obtained on MS medium supplemented with 0.5 mg/l IAA. As concentration of IAA increased, the root number and root length increased. However, the shoots became thin with increasing of concentration.

In all treatments of rooting on MS medium supplemented with IBA alone or IBA in combination with IAA, callus formation was observed.

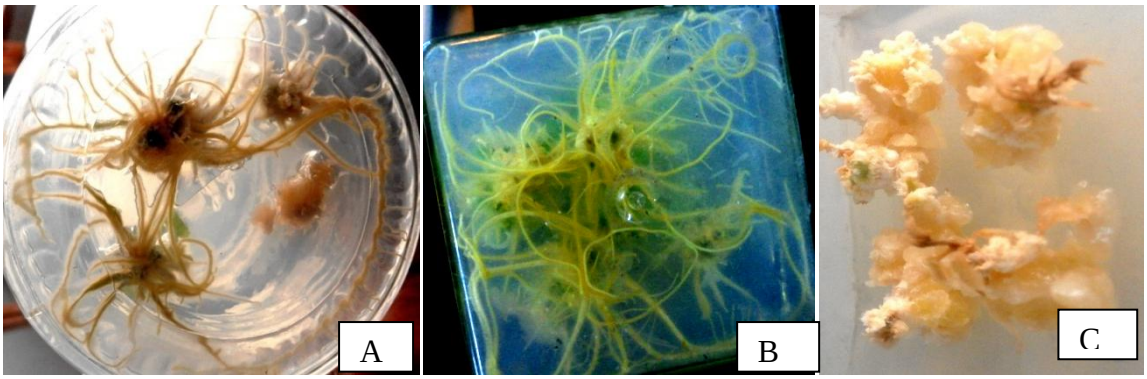

Figure 3: *In vitro* rooting of *Oreosyceafricana* onhalf strength MS medium containing different concentrations of IAA (A) 0.25 B) 0.5) and IBA alone(C).

Table 3: Effect of different concentrations of IAA, IBA and their combinations on *in vitro* rooting of *Oreosyceafricana*

PGRs (ml/l)		No of Roots/ explant Mean ±SE	Root length (cm) Mean ±SE
IAA alone			
0.00		0.00±.00[c]	0.00±0.00[c]
0.25		5.67±.39[b]	3.94±.39[b]
0.5		7.77±.44[a]	5.42±.45[a]
IBA alone			
0.25		0.00±.00[c]	0.00±0.00[c]
0.5		0.00±.00[c]	0.00±0.00[c]
IAA	IBA		
0.5	0.25	0.00±.00[c]	0.00±0.00[c]
0.75	0.5	0.00±.00[c]	0.00±0.00[c]

Mean with the same letter within the same column are not significantly different at (P< 0.05). The values represented as mean ± SE. The number of explants cultured on each treatment was 30

5.3.2. Acclimatization

The plantlets were carefully removed from culture vessels and the roots were washed under running tap water and then planted in potted soil immediately. From the total number of plants transferred to potted soil, 98% survived.

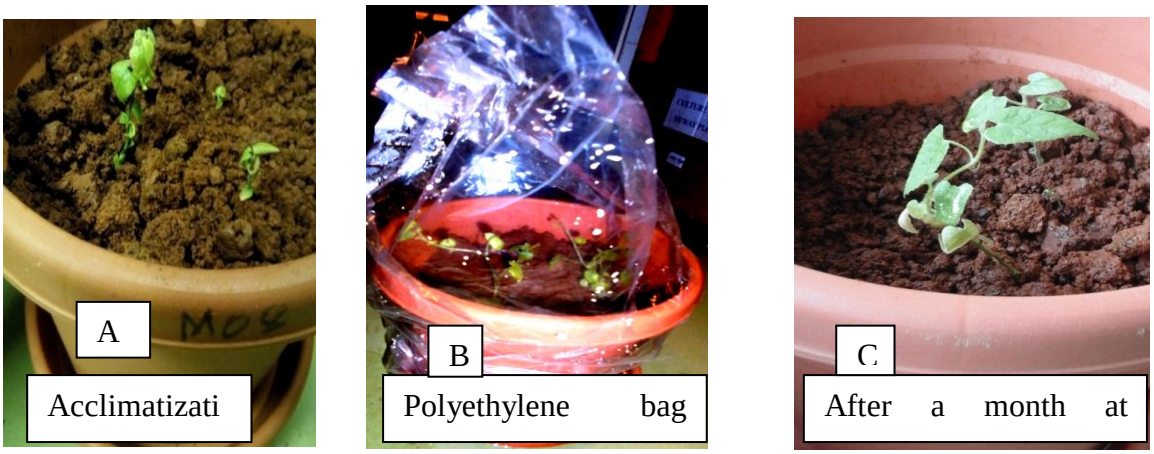

Figure 4: Acclimatization of *in vitro* rooted shoots of *O. Africana* in glasshouse. (A) Plants transferred from the medium to the pots; (B) Plants covered in polyethylene bag and placed in greenhouse for two weeks and (C) Plants in a garden after four weeks.

6. Discussion

The results of the present study showed that the performance of BAP on shoot induction was more effective than KIN, which was also reported by Venkateshwarlu (2012) and Kathal (1988) on *Cucumis sativus*. Vižintin, and Bohanec (2004) reported, about 87% of *Cucumis stivus* treated with 0.5 BAP mg/l polireferated shoot, which was almost similar with the present study (100%). From 12 different treatments, 0.5mg/l BAP was better in shoot number than other growth regulators. The highest mean number of shoots per explant (36.66±2.03) of *Oreosyce africana* was obtained on MS medium supplemented with 0.5 mg/l BAP followed by 19.20±1.00 shoots per explant on MS medium supplemented with 0.25 mg/l BAP. The results of this study showed that generally KIN is less effective than BAP in shoot multiplication of *Oreosyce africana*. Although there was no significant difference in number of shoots per explant among different concentrations of KIN used for shoot multiplication, the highest mean number of shoots per explant (7.67±0.63) was obtained on MS medium supplemented with 0.5 mg/l KIN.

In earlier study on cucurbits, Venkateshwarlu (2012) realized that most of different concentrations of BAP were more effective than KIN. Froz (2015) described that the combinations of cytokinin (BAP/KIN) with auxin (IBA and IAA) for shoot induction on *Cucumis sativus* was about 60%. However, in present study the mean number of shoot induced from shoot tips of *Oreosyce africana* supplemented with various concentrations of cytokinin (BAP and KIN) in combination with different concentrations of auxins (IBA and IAA) were very low. The present study showed that micro propagation of *Oreosyce africana* on MS medium supplemented with 0.5mg/l BAP resulted in better shoot number per explant than other PGRs BAP (0.25, 1.0), KIN (0.25,0.5,1.0) and their combinations with IBA.

However, application of 0.25 mg/l BAP showed remarkable result in respect to shoot length. The mean shoot number per explant on MS medium supplemented with 0.25 mg/l BAP (19.20±1.00) was less than that of 0.5mg/l BAP (36.66±2.03). However, the result of mean shoot length per explant was the reverse of shoot multiplication on medium containing these growth regulators. The length and quality of shoot determines the efficiency of rooting and acclimatization. Therefore, in micro propagation of *Oreosyce africana,* to get large shoot number per explant, 0.5mg/l BAP is recommendable. Moreover, for further study, it is better to use 0.25mg/l BAP to have medium shoot number per explant but have good length, which is very suitable for acclimatization.

Contrary to the present study result, the physiological response to shoot induction for *Cucumis sativus* was better on MS medium supplemented with 0.5 mg/l KIN than the other cytokinin hormone(0.25mg/l KIN and 1.0 mg/l KIN) and its combinations with IBA. Saeid and Abu-Romman (2015) reported that the maximum mean shoot number per explant on MS medium supplemented with 0.5 mg/l KIN for *Cucumis sativus* was 7.93. The result was very low when it is compared with the present study on *Oreosyce Africana* with maximum mean shoot number per explant of 36.66 on MS medium supplemented with 0.5mg/l BAP. From the above result, KIN is better for *Cucumis sativus* while BAP was excellent in physiological response of *Oreosyce africana* in their shoot multiplication.

Different concentrations of BAP or KIN (0.5, 0.75, and 1.0 mg/l) in combination with IBA (0.25, 0.5 and 0.75 mg/l) on MS medium resulted in callus formation. Some earlier studies (Saeid M.Abu-Romman, 2015) reported that shoot regeneration of *Cucumis sativus* with various concentrations of BAP or KIN in combination with IBA resulted in relatively few

number of shoots per explant. However, in the present study shoots cultured on MS medium containing BAP or KIN in combination with IBA resulted in callus formation regardless of the concentration of IBA. Increasing the concentration of cytokinin, results in increasing plant physiological response to shoot regeneration until the saturation level reaches. Beyond their saturation level, the physiological responses of plant shoot regeneration decreases (Saeid and Abu-Romman, 2015). Similarly, the present study showed, at the concentration of 1.0 mg/l BAP, the mean number of shoots /explant was decreased.

Concerning rooting, shoots cultured on half strength MS medium supplemented with IAA or IBA resulted in different response. Among nine treatments, only shoots cultured on different concentrations of IAA produced roots whereas shoots cultured on medium containing IBA or IAA in combination with IBA resulted in callus formation without producing any roots. The highest mean number of root produced per plantlet was 7.77±.44 on half strength MS medium supplemented with 0.5mg/l IAA followed by 0.25mg/l IAA that produced5.67±.39 mean number of root per plantlet. Study on *Coccinia abyssinica* showed that medium supplemented with 0.5mg/l IBA and 0.5mg/l IAA resulted in high root efficiently (Folla Bekele*et al*., 2013).According to Folla and Bekele*et al*. (2013), IBA and IAA were efficient at about more than 90% in root induction.In present study however, application of IBA on root regeneration formed callus while IAA resulted in 100% rooting. Moreover, root regeneration of *Oreosyce africana* supplemented with IAA was best ant takes only few weeks (3-4).Hence, it is not recommended to use IBA for root induction on *Oreosyce africana*. There was also variation in mean shoot length among shoots cultured on medium containing different concentrations of IAA. The highest mean root length (5.42±.45 cm) was obtained on medium containing 0.5mg/l IAA.

7. Conclusion

From this study, in general the role of PGRs on micro propagation of *Oreosyce africana* from shoot tips is very valuable to get enormous plantlets. The present study demonstrates that application of 0.5mg/l BAP on MS medium resulted in 36.66±2.03 mean number of shoots per explant. As concentrations of PGRs increase, the rate of shoot multiplication increased until the supra-optimal concentration is reached, this is beyond 1.0 mg/l BAP that resulted in callus formation rather than shoot multiplication.Maximum mean numbers of roots per shoot (7.77±.44) was produced on MS medium supplemented with 0.5mg/l IAA. About 98% of the acclimatized plantlets survived. After its propagation it is possible to prepare larvicidal and adultcidal sprays from *O. africana* to kill *Anopheles mosquito*.

8. Recommendations

The following are recommended based on the present study

- ♣ The effect of PGRs on shoot regeneration on other medium other than MS-medium requires further study.
- ♣ To test the significance effect of sub-culturing on shoot multiplication, there is a need for further study on sub-culturing.
- ♣ Developing *in vitro* regeneration experiments using different explants from multiplied shoots for future genetic transformation is recommended
- ♣ Only one genotype was used in the present study and using different genotypes in the future for micro propagation is recommended

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APPENDIX

Components	Concentration(g/L)	ml/L during media preparation
Micronutrients		5ml/L
$ZnSO_4.7H_2O$	1.72	
H_3BO_3	1.124	
$MnSO_2.4H_2O$	3.38	
$MnSO_4.H_2O$	0.05	
KI	0.166	
$NaMoO_4.2H_2O$	0.05	
$CoCl_2.6H_2O$	0.05	
Na_2EDTA	7.472	
$FeSO_4.7H_2O$	5.56	
Macronutrients		50ml/L
NH_4NO_3	33	
KNO_3	38	
$CaCl_2.2H_2O$	8.8	
$MgSO_4.7H_2O$	7.4	
KH_2PO_4	3.4	
Vitamins		5ml/L
Myo-inositol	20	
Glycin (glycocoll)	0.4	
Nicotinic acid (NaOH)	0.1	
Pyridoxin (B6)	0.1	
Thiamin (B1)	0.02	

Appendix 1 stock solution for MS (Murashige and Skoog 1962)