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Micro propagation of Egyptian taro using tissue culture technique

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Abstract

The present study was carried out during the period from 2016 to 2018 for two parts of the experimental work. The first part was *in vitro* culture and the second part was *in vivo* culture using the Egyptian local taro cultivar (*Colocasia esculenta* var. *esculenta*, Schott.).

In vitro experiments were conducted at the plant tissue culture laboratory , Horticulture Department, Faculty of Agriculture, Benha University , to improve number and growth of plantlets *in vitro* propagation and then to evaluate the field performance of in well adapted taro plants obtained by meristems culture as compared with the traditional propagation methods, i.e. by apical meristems ,leaf pieces ,stem segments and cormal pieces Results indicated that leaf pieces induced 90 % of callus, while stem segments formed 80% of callus .The apical meristem and cormal pieces did not induce callus after 2 weeks of culturing on the (Murashige and Skoog, 1962) MS medium supplemented with (Benzyl Amino Purine) BAP and (Naphthalene acetic acid) NAA and only explants of apical meristem produced shoots (98%). As for the production of callus, apical meristem (zero%), leaves (90%), stems (80%) and cormel (zero %). As for the production of shooting, apical meristem (98%) and the other parts (zero%). These results clearly indicate that among the tested growth regulators types at different concentrations, the apical meristem was the most effective ones to produce the highest number of shoots and leaves explant and the cormel pieces was the least. The treatments BAP (0.20mg/L) and NAA (0.1mg / I) and (40g) sugar gave the highest values of roots number (3.16 and 4.01 roots), respectively .The lowest treatments in this regard were BAP at (0.2 mg/l), NAA at (0.1mg /L) and 30g sugar as well as control treatment which scored (2.74 and 3.02 roots), respectively. The rest treatments gave roots number values between these two extremes. The results illustrated that the best media for acclimatization was peat moss which produced 100% of survival plants.

Keyword: *in vitro*, tissue culture, shoot multiplication, taro, Kinetin, BAP, NAA.

Introduction

Taro is a herbaceous, perennial tuberous crop belonging to the Araceae family. It is taxonomically classified into two different varieties, *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta,* with hundreds of cultivars (Pursglove, 1972)(Macharia ,et al ., 2014).

Colocasia esculenta var. *antiquorum* is triploid (2n = 3x) with 42 chromosomes, whereas *Colocasia esculenta* var. *esculenta* is diploid (2n = 2x) with 28 chromosomes (Plucknett, 1983) (Das, et al ., 2015) . The latter is more widely preferred by consumers in certain regions of the world as a major food staple (Wang, 1983) and the Egyptian taro is belonging to this botanical variety.

Taro is a very common crop for wet soils in the humid tropics, especially in Southeast Asia, the Pacific Basin, wet tropical Africa and Egypt, the West Indies, and certain areas of South America.

Young taro leaves are used as a main vegetable throughout Melanesia and Polynesia. Thus, taro is one of the few major staple foods where both the leaf and the underground parts i.e. corms and cormels are equally important in the human diet.(Nip,2023)

Taro has been known in Egypt from over 2500 years (Morishita, 1988). Where, it occupies considerable acreage especially in Menoufia, Qalubia and Assuit Governorates. The total area was 3893,88 hectare and produced tons with an average of 7.7 ton /feddan (FAO2017).

Vegetative propagation of taro using the traditional methods, i.e corm-sections and cormels makes this crop especially sensitive to viruses and bacteria, which are transmitted with the propagation material and greatly reduce the quality and yield of taro production (Cao and Chen, 1990; Zettler et al., 1991; Rosemary and Chong, 1994; Valverde et al., 1997 and Yongwei et al, 2002).

Large application of fertilizers has been practiced on soils to improve crop production and recover inherent and induced soil nutrient deficiency. Contamination of agricultural soils with heavy elements such as cadmium, lead and iron, usually Occurs as these elements are transferred during manufacturing from organic and inorganic fertilizers, especially phosphate fertilization. Cadmium and lead are nonessential elements. They are considered toxic elements, whears iron as a micronutrient could become toxic when exceeding certain limit. Such heavy metals present in the roadside soils and grasses may be transported through the food chain on the human body. Heavy metal pollution in agricultural areas owing to traffic emissions may contaminate the crops growing near the roadways; cadmium and lead are the typical metal pollutants due to traffic activities. (Hou, et al., 2020)

Removing heavy metals from soil matrix is an energy intensive and time consuming process. On the other hand, reducing the mobility of heavy metal species by means of (stabilization solidification technique) a chemical additive is a much more cost

effective solution for heavy metal contaminated soils. Taro is an important root crop in the family.

Araceae ,(Kreike, et al., 2004), The crop ranks among the most important root and tuber crops in terms of production offer potato, cassava, sweet potato and yam (FAO 2018). The crop is particularly important in the poor regions of the world, which boost the immune system and help human body to resist disease (Soudy, et al., 2010). It is essential to study the relationship between status of heavy metals in soil. and correspondent status in grown plant, the pattern of distribution and the transition of these elements should be in consideration, The metal contents in taro plants seemed to be dependent on both physical and chemical nature of the soil as well as absorption capacity of each metal by taro plant, which is altered by various factors like environmental and human it reference as well as the nature of the concerned plant. Also, being vegetatively propagated and extremely heterogeneous as a group, conservation of taro germplasm is an urgent task. However, taro germplasm is not actively exchanged due to the difficulty of ensuring virus-free material. Hence, recommendations by the International Board for Plant Genetic Resources (IBPGR) are for the exchange of *in vitro* materials only (Zettler ,et al., 1989). Furthermore, taro seeds are only viable for about 30 days after harvest (Kikuta, et al., 1938) and the *in vitro* conservation of taro is thus necessary. As well as, there are other problems that result in increase of production costs, such as using a large amount of corm seeds, occupying area of the land up to the next cultures, problem of the low standing after cultivation which reach to 40%. These problems make the taro producers search for new methods that can provide the growers with the required number of plants and overcome the previous disadvantages.

Another propagative technique for taro is the production of plantlets through meristem tissue culture. The multiplication of taro by tissue culture has several distinct advantages as follows:

- It provides an extremely rapid means for multiplying elite clones.
- It affords a phytosanitary method for producing disease-free material.
-The tissue culture technique provides a handy yet phytosanitary method for international and inter-regional transfer of germplasm.

<u>Materials and methods</u>

<u>The first part, *in vitro* culture experiments</u>

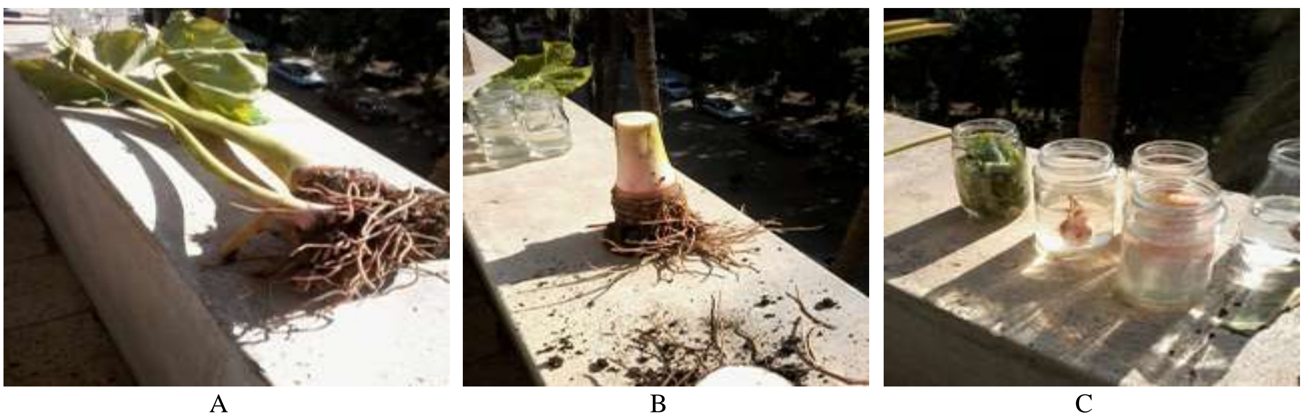
In vitro culture experiments were conducted at the Plant Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Benha University, to improve number and growth of plantlets *in vitro* propagation and then to evaluate the field performance of well adapted taro plants obtained by meristems culture as compared with the traditional propagation methods i.e. by apical meristem, leaf pieces, stem segments and cormal pieces .

<u>1. *In vitro* culture experiment</u>

This part of the present study was achieved into the Plant Tissue Culture
following steps:-

<u>1.1. Plant materials.</u>

Taro corms had been obtained from a farmer in shanawan village, El-Menoufia Governorate. Corms (about 0.75-1.0 kg, 15-20 cm high and 10-12 cm in diameter) of the local Egyptian cultivar. Then, they were thoroughly scraped to remove any soil parts on the corm prior to washing in running tap water. In PlantTissue Culture Laboratory, apical meristem ,leaf pieces, stem segments and cormal pieces which were subtended by corm. Tissues were excised using a pointed knife and used as explants. The explants are then trimmed with a small and sharp knife so that the final explant was approximately 3 cm and 1.5 cm long apical meristem ,leaf pieces, stem segments and cormal pieces respectively with about 1 cm2 of corm tissue.fig.1.



A B C

Fig . (1) Preparation of starting sterilization of different explants types
A - Starting explants of taro for tissue culture cultivation .
B – Taro plants as a source establishment tissue culture cultivation (cormes explants).
C- Leaves pieces _ stem segments

2.1. Surface sterilization.

The excised explants were thoroughly washed with running tap water containing a few amount of household detergent for 30 minutes (outside the culture cabinet). Thereafter, they are transferred to inside the culture cabinet since, they were surface sterilized by immersion in 70 % (v/v) ethanol for 2 minutes followed by a treatment with 2 % sodium hypochlorite solution with 5 drops of tween 20 for 5 minutes, and during this step of surface sterilization, the container which contained the explants was shaken as much as possible. Then, the explants were thoroughly rinsed in sterile distilled water for 4 times for 3 minutes each. After surface sterilization, the explants were trimmed to about 1-2 mm long by removing the outer tissues with instruments which had been sterilized by dipping in 95 % ethanol then flamed using a gas burner. Then the explants were sterilized (M.C)for15 minutes each. Finally, the explants were kept in sterile antioxidant solution containing ascorbic and citric acid at 1000 ppm each, until they were inoculated on the culture media (Metwaly, 2003). (Fig 2)

3.1. Culture media.

Apical meristem (about 1-2 mm long) ,leaf pieces ,stem segments and cormal pieces were cultured aseptically into 200 ml sterilized glass jars, contained 40 ml of MS cultured medium (Murashige and Skoog, 1962) supplemented with $NaH_2PO4.\ H_2O$ (50 mg/l), L-tyrosine (100 mg /1), Myo-inositol (100 mg /1), Adenine- hemi-sulphate (40 mg /l),three different amount sucrose (20,30,40g /l) and 7 g Agar agar /1 (w/v). The basal constituents of MS medium presented in Table (1).

The pH of all media was adjusted to 5.8 and all growth regulators added to the media prior to autoclaving. All the tested media *in vitro* culture of taro experiments were autoclaved at 121 ^oC and 1.2 kg /cm^2 for 20 minutes.

A

B

Fig. (2) Surface sterilization of different explants .
A- Pretreatment of explants with Uniform390 suspension emulsion
B- After of sterilization Surface sterilization of different explants type of taro plants.

Table (1) Constituents of Murashige and Skoog basal nutrient medium

Constituents	Concentration
Macroelements	
NH4NO3	1650 mg /l
KNO3	1900 mg /l
CaCL2. 2H2O	440 mg /l
MgSO4. 7 H2O	370 mg /l
KH2PO4	170 mg /l
Microelements	
H3BO3	6.2 mg /l
MnSO4. 2H2O	16.9 mg /l
ZnSO4. 7H2O	8.6 mg /l
KI	0.83 mg /l
NaMoO4. 2H2O	0.25 mg /l
CuSO4. 5H2O	0.025 mg /l
COCL2. 6H2O	0.025 mg /l
Na2 EDTA (2H2O)	37.3 mg /l
Myo-inositol	80.0 mg /l
Glysine	2.0 mg /l
Nicotinic acid (B5)	0.5 mg /l
Pyridoxine- Hcl (B6)	0.5 mg /l
Thiamine- Hcl (B1)	0.1 mg /l

4.1. Culture conditions.

In vitro culture, all experimental treatments were represented by 10 cultured jars per each treatment and all the cultured jars were incubated at 25 ± 2 °C under 16 h /day photoperiod produced in white fluorescent light lamp with light intensity (1500 lux) in establishment and multiplication stages and (2500 lux) in rooting and acclimatization stages.

5. Studied experiments.

This part of the present study had been contained various stages which were carried out *in vitro* for obtaining well-adapted plantlets of local taro cultivar by meristem tip cultures. Each stage included one or more than experiment.

5. 1. Establishment stage

This stage contained one experiment which aimed to establish successful and aseptic tissue culture ,it was constituents of MS basal nutrient medium.

5. 1. 1. Explant type.

Apical meristem as explants (about 1-2 mm long), leaf pieces, stem segments and cormal pieces were cultured as described above in item (3). Comparison study between the four explant types was done to determine the best one for *in vitro* taro propagation.

Number of shoots, leaves and roots and length of shoots and roots were recorded after 6 weeks of culturing date on MS medium, in addition to the other additives as previously mentioned in item (3).

5. 2. Proliferation stage.

The experiments of this stage were conducted on well established explants which produced from the previous stage. The tested experiments were as follows

5. 2. 1. Effect of various growth regulators at different concentrations.

This experiment was done to study the best type and concentration of growth regulators BAP and NAA ,concentrations of each one were added to the basal MS medium plus the other additives that mentioned above in item (3).

The used treatments were as follows:

1- Control (suger30 g+4.4agr)
2-suger(20g)+BAP (0.2mg\l)+NAA(0.1mg\l)
3-suger(30g)+BAP(0.2mg\l)+ NAA(0.1mg\l)
4-suger(40g) + BAP(0.2mg\l)+ NAA(0.1mg\l)
5- suger(30g)+BAP (0.1 mg\l)+NAA(0.1mg\l)
6- suger(30g)+BAP (0.5 mg\l)+NAA(0.1mg\l)
7- suger(30g)+BAP (0.3 mg\l)+NAA(0.1mg\l)
8- suger(30g)+BAP (0.2mg\l)+NAA(0.5 mg\l)
9- suger(30g)+BAP (0.3 mg\l)+NAA(0.5 mg\l)

Each of the previous treatments were represented by 10 cultured jars and all the planted jars included 40 ml of the MS medium plus other additives in item (3). All the cultured jars were incubated as previously described in item (4).

Multiplication rate characteristics were recorded after 8 weeks from culturing date as number of shoots, leaves and roots and length of shoots and roots.

5.2.2. Effect of subcultures number on multiplication rate.

This experiment was conducted to indicate the effect of subcultures number on improvement of taro shoots multiplication rate. Therefore, the taro shoots regenerated on the best multiplication medium were sub cultured individually in jars contained 40 ml of MS medium supplemented with materials indicated in item (3), plus the best
treatment of the previous multiplication experiment. The number of shoots and leaves and roots and length of shoots and roots were recorded .

5.3. Rooting stage.

An experiment was carried out to determine the optimum type and concentration of auxins on rooting of taro shoots produced from the second subculture medium. Firstly, the shoots (about 2.34 cm long) derived from second subculture were isolated and cultured on hormones free-MS medium (control treatment) for 2 weeks (60mg "Phloroglucinol" PG\ l and the auxin treatments) . Thereafter, they transferred to jars (40 x 125 mm), each of them contained 40 ml of MS medium plus previously mentioned additives in item (3).

The tested experiment was as follows:

5.3.1. Effect of different growth regulators types and its concentrations.

In this experiment, two types of auxins i.e, BAP and NAA were used
Each treatment represented by 10 cultured jars. The cultured jars contained 40 ml of MS medium supplemented with(0.00, 0.50, 1.00, 2.00 and3.00 mg /l . All the cultured jars were incubated as mentioned above in item (4). In regard to rooting stage ,data were recorded 8 weeks after culture date as plantlets, shoots and roots fresh weight; length of shoot sand roots and roots number.

5.4. Acclimatization stage.

To standardize the hardening procedure, healthy and well-rooted plantlets resulted from culturing on MS medium supplemented with 1.0 NAA /l mg were taken away from the culture jars. Their roots were thoroughly washed in warm tap water to get quit of any medium residues to remove a potential source of contamination, then trimmed well. Thereafter, the plantlets were transplanted into black plastic pots (5 cm in diameter) filled with the tested agricultural media

The objective of this stage was obtaining well-adapted taro plants for the following evaluation of field performance *ex vitro*. The tested agricultural media were peat moss, perlite, vermiculite and sand.

During this period, the plants were watered every 2 days with tap water and once per week with compound Rosasol fertilizer which consisted of N.P.K (19.19.19) + 1 MgO + micro elements (as 260 ppm Fe , 75 ppm Cu , 230 ppm Zn , 320 ppm Mn and 100 ppm B) at 1.0 g /l of irrigation water. Plantlet and roots fresh weigh; number of roots /plantlet; length of shoot and root and leaf area (cm^2) /plantlet were recorded after 6 weeks. After 6 weeks of *ex vitro* growth, plants were transferred to the plastic house, since they were

repotted to black plastic pots (16 cm in diameter) contained peat moss : sand : soil (1 : 1 : 2, v/v/v). They were watered every 3-4 days with tap water and once per week with compound Rosasol fertilizer at 1.5-2.0 g /l of irrigation water. After another 4 weeks of growth in plastic house, plants were transferred to the external ambience for another 14 days and accordingly, they were become fully acclimatized and ready to cultivate in the field

- The technique provides an economical, space-saving, and labour-saving method for the preservation of germplasm over long periods of time. Rather than repeatedly growing germplasm collections in the field, they can be stored as tissue culture in nutrient media. Only occasionally (once in several months) does the material need to be re-cultured; and even then, the space, time and labour consumption for the exercise are minimal.

The main objectives of this study were regeneration of the Egyptian taro plants by plant tissue culture technique and aimed to find the optimum explant types (apical meristem ,leaf pieces, stem segments and cormel pieces), the optimum dose of different growth regulators (BAP and NAA) required to obtain the best growth and number of shoots during proliferation stage, determine the best rooting medium. Added to that plantlets obtained from tissue culture were grown in different media under controlled light, humidity, and temperature to be acclimatized and ready for growing in pots under greenhouse.

Results and Discussion

1.First Part:

1.1. Establishment of aseptic plant cultures:

For establishing aseptic cultures of taro growing *in vitro* , four explant types were surface sterilized. A common disinfectant used to surface sterilize plant tissues is sodium hypochlorite. Four explant types of taro plants(apical meristem , stem segments ,leaf pieces and cormel pieces)
were immersed in 2.5% sodium hypochlorite for (apical meristem , stem segments ,leaf pieces and cormel pieces) which is present in commercial bleach solutions (Clorox) then they were rinsed five times with sterile distilled water for 10 min.

Pesticide fungi (Uniform390) was 2g for 30 min and the ethanol concentration was 70% for all treatments, cl(Clorox) and Mc(HgCl2).

-In order to compare between of different explant types of taro plants for *in vitro* regeneration ,four explant types(apical meristem, leaf pieces, stem segments and cormel pieces)were used for this experiment (Table3) . leaf pieces induced 90% of callus ,while stem segments formed 80%of callus. The apical meristem and cormal pieces did not induce callus after 2 weeks of culturing on MS medium supplemented with BAP or NAA only explants of apical meristem produced shoots(98%).

At the beginning of the basic stage of agriculture, (10)explants as starting materials different of the taro ex plants were cultured on MS medium .These parts are as follows(Apical meristem, Leaves pieces, Stem segments and Cormel pieces).

As for the production of callus, apical meristem (zero)%, leaves (90)%, stems(80)% and cormel(zero)%. As for the production of shooting, apical meristem(98)% and the other parts (zero)%.(Fig.3).

Table (2) Effect of ratio of sterilization, material used and duration of sterilization on different explant types of taro.

Types of explant	Ratio of sterilization and material used			Duration of sterilization			
	Mc	**cl**	**Sterilize water**	**Ethanol alchdol**	**mc**	**Cl**	**Sterilize water**
Apical meristem	0.1\ L	25%	Sterilize water	5 min	25 min	20 min	15 min
Leaves pieces	0.1\ L	10%	Sterilize water	1 min	10 min	5 min	5 min
Stems segments	0.1\ L	15%	Sterilize water	3 min	15 min	10 min	10 min
Cormel pieces	0.1\ L	20%	Sterilize water	4 min	20min	15 min	12 min

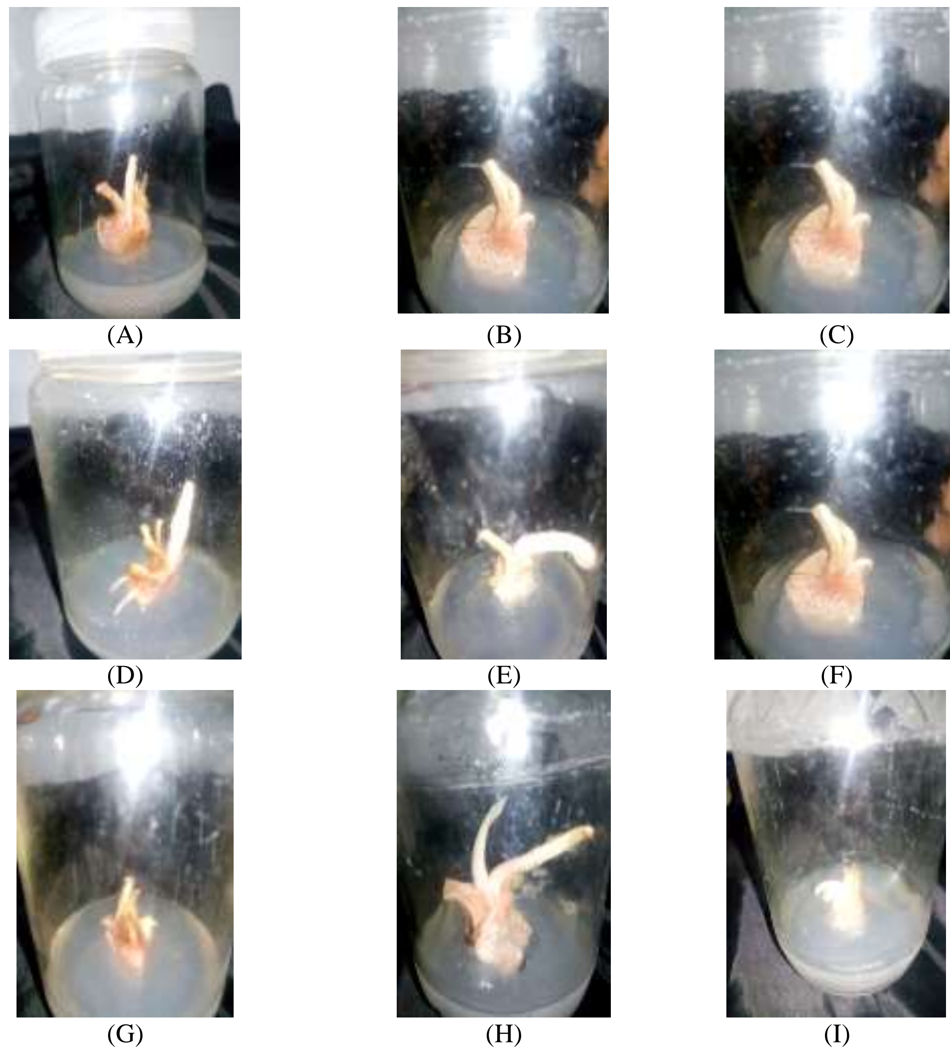


Fig. (3) Initation of shoot formation after 2 weeks of culturing on MS media supplemented with different growth regulators.

The concentrations should be MS medium;

A- Control (suger30 g+4.4agr)
B-suger(20g)+BAP (0.2mg\l)+NAA(0.1mg\l)
C-suger(30g)+BAP(0.2mg\l)+ NAA(0.1mg\l)
D-suger(40g) + BAP(0.2mg\l)+ NAA(0.1mg\l)
E- suger(30g)+BAP (0.1 mg\l)+NAA(0.1mg\l)
F- suger(30g)+BAP (0.5 mg\l)+NAA(0.1mg\l)
G- suger(30g)+BAP (0.3 mg\l)+NAA(0.1mg\l)
H- suger(30g)+BAP (0.2mg\l)+NAA(0.5 mg\l)
I- suger(30g)+BAP (0.3 mg\l)+NAA(0.5 mg\l)

Table (3) Effect of explant on callus and shoot formation types after 2 weeks of culturing on MS media supplemented with different growth regulators.

Explant types	Starting material	survival	Callus induction%	Shoot formation%
Apical meristem	10	10	0	98%
Leaf pieces	10	10	90%	0
Stem segments	10	10	80%	0
Cormel pieces	10	10	0	0

2. Proliferation stage.

2. 1. Effect of different cytokinin types and concentrations

Results on the effect of different growth regulators types at different concentrations on number of shoots, leaves and roots as well as length of shoots and roots of culturing on MS medium were recorded in Table (4). These results clearly indicate that among the tested growth regulators types at different concentrations, the apical meristem was the most effective ones to produce the highest number of shoots and leaves\ explant and the cormel pieces was the least.

The apical meristem gave(9.85) shoots, the production rates of other parts (leaves, stems and cormel) were less than Apical meristem .leaves(6.31),Stems(3.21)and cormel had no response to growth because there are no live buds ,So all growth parameters(zero)in all treatments of cormel. In succession for leaves number /explant, followed by apical meristem(13.45),leaves(10.30)and stems(5,28).

Concerning the shoot length, results obtained obviously revealed that growth regulators within all concentrations ,shoots obtained from apical meristem were(2.67)cm comparing with (1.34)cm and (0.68)cm from leaves pieces and stem segments, respectively(Table 4). Results in (Table 4) showed that number of roots induced from apical meristem was the highest value (2.8)cm comparing with explant from leaf pieces , stem segments and cormel pieces , respectively .Moreover, results showed that the highest root length of apical meristem explants(2.45)cm and the root length of explants of stem segments (0.67)cm (Table 4).(Fig.4).

Fig. (4) Shows the elongation stage of explants after 2 weeks of culturing on MS media supplemented with different growth regulators.

Control (suger30 g+4.4agr)
B-suger(20g)+BAP (0.2mg\l)+NAA(0.1mg\l)
C-suger(30g)+BAP(0.2mg\l)+ NAA(0.1mg\l)
D-suger(40g) + BAP(0.2mg\l)+ NAA(0.1mg\l)
E- suger(30g)+BAP (0.1 mg\l)+NAA(0.1mg\l)
F- suger(30g)+BAP (0.5 mg\l)+NAA(0.1mg\l)
G- suger(30g)+BAP (0.3 mg\l)+NAA(0.1mg\l)
H- suger(30g)+BAP (0.2mg\l)+NAA(0.5 mg\l)
I- suger(30g)+BAP (0.3 mg\l)+NAA(0.5 mg\l)

Table (4) Effect of 4 explant types on the development of taro after 8 weeks of culturing on MS medium supplemented with BAP and NAA.

Explant types	Growth parameters				
	Shoots number	Leaves number	Shoot length (cm)	Roots number	Root length (cm)
Apical meristem	9.85±0.19	13.41±0.52	2.67±0.05	2.8±0.13	2.45±0.03
Leaves pieces	6.31±0.27	10.30±0.26	1.34±0.02	1.3±0.06	1.21±0.05
Stem segments	3.21±0.15	5.28±0.13	0.68±0.01	0.67±0.04	0.612±0.01
Cormel pieces	0.0	0.0	0.0	0.0	0.0

3. Rooting stage.

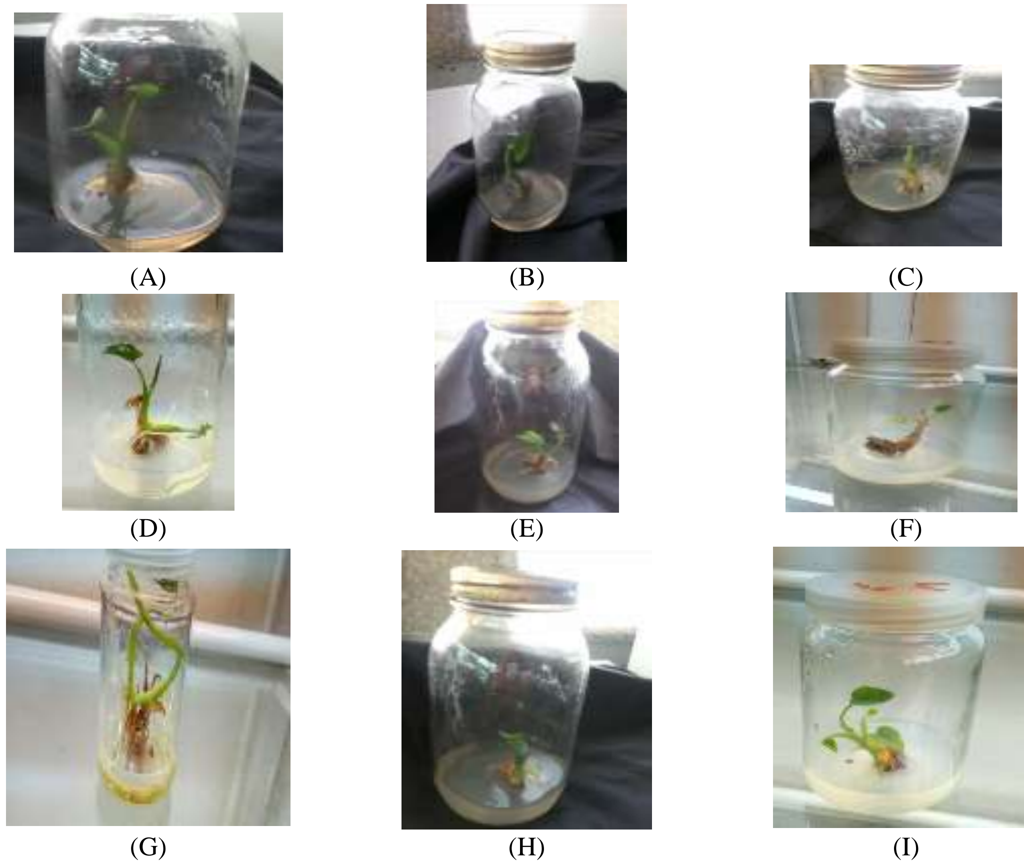
3. 1. Effect of different auxin types and concentrations on taro rooting

This experiment was conducted to test the effect of various concentrations(0.00,0.1,0.2and0.3 mg\l) of different auxins ;NAA and BAP on plantlet, shoots and roots fresh weight; roots number and length of shoots and roots. Results were recorded after 8 weeks of culture on MS medium plus 20, 30and 40 sugar. *Concerning the roots number*, data in Table (4) clearly indicate that the treatments BAP (0.2)mg) \l and NAA (0.1)mg \l and (40)g sugar gave the highest values of roots, Number(3.96 and 4.01roots). The lowest treatments in this regard were BAP at (0.2 mg\l) ,NAA at (0.1mg\l) and 30g sugar as well as control treatment which scored (2.74 and 3.02roots) respectively. The rest treatments gave roots number values between these two extremes. *As for roots length*, the best treatments in this connection were NAA (0,1),BAP(0,2) at mg/l and (40) g sugar which gave the highest roots length(4.01)cm. On the contrary, the medium contained NAA at(0.1) ,BAP(0.2) mg /l and(20) g sugar scored the shortest roots length(1.39), respectively. The rest treatments gave roots length values ranged from(1.34 to 3.90)cm. The control treatment recorded an average of (2.75 cm) for roots length. In respect to plantlet fresh weight, data obviously revealed that the medium augmented with NAA at(0.1) ,BAP(0.2) mg /l and (40) g sugar gave the highest values of fresh weight(7)g. The least values of plantlet fresh weight were distinctly observed with NAA(0,1), BAP(0,2)mg\ l and (30) g sugar . The control treatment scored (5.7g) for this character.

3.2. This experiment was conducted to test the effect of growth regulators types and concentrations on shoots, leaves and roots number and length of shoot and root of taro shoots .Results were recorded after 6 weeks of culture on MS medium plus (20,30.40)g sugar concerning the roots number, data in table(5) clearly indicate that treatments BAP(0.2mg\l) and NAA(0.1mg\l)and sugar (40)g gave the highest values of root number (11.6 and 26.2) leaves number(31.1) . the lowest treatments in this regard were BAP at(0.1 mg\l), NAA(0.1mg\l) and sugar(30g). as well control treatments gave roots which scored (3.4 and 3.1 2roots) and leaves (10.4)respectively. The rest treatments gave roots number values between these two extremes *As for roots length*, the best treatments in this connection were NAA (0,1),BAP(0,2) at mg/l and (40) g sugar which gave the highest roots length(4.55)cm. On the contrary, the medium contained NAA at(0.1) ,BAP(0.2) mg /l and(30) g sugar scored the shortest roots length(2.24), respectively. The rest treatments gave roots length values ranged from(2.2to 3.90)cm. The control treatment recorded an leaves number(9).(Fig.5).

Table (5): Effect of the concentrations of BAP NAA and sugar amount on number of roots; length of shoot and root and plantlets fresh weight (FW) after 8 weeks of culturing on MS medium.

Treatments				Measurements			
	sugar	BAP	NAA	Root length (cm)	Shoot length (cm)	Rooting number%	Plantlets f w (g)
Control	30g	-	-	3.02	2.74	80.6%	5.74
1	20g	(0.2mg\l)	(0.1mg\ l)	1.39	1.64	68.2%	4.64
2	30g	(0.2mg\l)	(0.1mg\l)	1.70	1.34	84.18%	3.15
3	40g	(0.2mg\l)	(0.1mg\l)	4.01	3.96	91.24%	7.00
4	30g	(0.1mg\l)	(0.1mg\l)	1.80	2.56	78.54%	5.30
5	30g	(0.5 mg\l)	(0.1mg\l)	2.20	1.98	75.34%	2.53
6	30g	(0.3mg\l)	(0.1mg\l)	3.16	2.48	62.36%	4.22
7	30g	(0.2mg\l)	(0.5 mg\l)	3.45	2.24	65.24%	6.86
8	30g	(0.3 mg\l)	(0.5 mg\l)	2.88	1.75	60.33%	4.14



(A) (B) (C) (D) (E) (F) (G) (H) (I)

Fig. (5) Shows effect of different growth regulators types and concentrations on shoots, leaves and roots number and length of shoot and root of taro shoots cultured on MS medium.

A-Control (suger30 g+4.4agr)
B-suger(20g)+BAP (0.2mg\l)+NAA(0.1mg\l)
C-suger(30g)+BAP(0.2mg\l)+ NAA(0.1mg\l)
D-suger(40g) + BAP(0.2mg\l)+ NAA(0.1mg\l)
E- suger(30g)+BAP (0.1 mg\l)+NAA(0.1mg\l)
F- suger(30g)+BAP (0.5 mg\l)+NAA(0.1mg\l)
G- suger(30g)+BAP (0.3 mg\l)+NAA(0.1mg\l)
H- suger(30g)+BAP (0.2mg\l)+NAA(0.5 mg\l)
I- suger(30g)+BAP (0.3 mg\l)+NAA(0.5 mg\l)

Table (5) Effect of different growth regulators types and concentrations on shoots, leaves and roots number and length of shoot and root of taro shoots cultured 6 weeks on MS medium.

Treatments				**Measurements**				
	Sugar	**BAP**	**NAA**	**Shoots Number**	**Leaves number**	**Roots number**	**Shoot length (cm)**	**Root length (cm)**
Control	30g	-	-	2.9	10.4	13.4	3.4	3.12
1	20g	(0.2mg\l)	(0.1mg\ l)	4.6	19.8	14.7	2.32	2.24
2	30g	(0.2mg\l)	(0.1mg\l)	8.5	23.5	16	2.59	2.74
3	40g	(0.2mg\l)	(0.1mg\l)	11.6	31.1	26.2	4.90	4.55
4	30g	(0.1mg\l)	(0.1mg\l)	7.9	25.5	22.2	3.17	2.11
5	30g	(0.5 mg\l)	(0.1mg\ l)	5.4	21.1	24.8	2.20	2.85
6	30g	(0.3mg\l)	(0.1mg\l)	6.9	15.8	15	3.32	3.56
7	30g	(0.2mg\l)	(0.5 mg\l)	4.4	19.9	18	1.91	3.55
8	30g	(0.3 mg\l)	(0.5 mg\1)	5.7	22.4	19.6	2.11	3.09

Acclimatization:

In order to obtain taro plants from tissue culture techniques, the plantlets of taro produced from different explant types , the plantlets transferred into different media such as:-

Media Sand - Clay media- moss peat- The results illustrated that the best media for acclimatization of taro was peat moss which produced 100% of survival plants. (Fig.6).

Fig. (6) A Shows acclimatization of taro plants produced from tissue culture.

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