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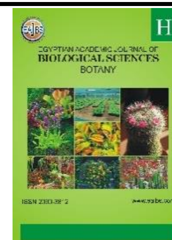

Influence of Different Plant Growth Regulators on Micropropagation of Egyptian Native Cultivar of Taro (*Colocasia Esculenta Var. Esculenta*)

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ABSTRACT

This investigation was conducted at the plant tissue culture laboratory of the plant production department, Faculty of Agriculture Saba-Basha, Alexandria University, Egypt, during the period from 2019 to 2023. This study was conducted to establish the best technique for micropropagation of the Egyptian native cultivar of taro (*Colocasia esculenta var. esculenta*). The traditional method of propagation could not produce the high demand and quality planting materials due to its low productive capacity and viral disease transmission. Therefore, this study was carried out to develop a micropropagation protocol for taro using auxiliary buds. At the initiation stage, MS (Murashige and Skoog media) was supplemented with different types and concentrations of plant regulators such as benzylamino purine (BAP)), indole-acetic acid (IAA), and Naphthalene acetic acid (NAA) for initiation, shoot multiplication and root induction. There was a significant difference among growth regulators at the initiation, multiplication and rooting stages. The highest culture initiation growth was obtained on MS medium supplemented with 0.50 mg/l of BAP and NAA. Also. the maximum number of shoots was obtained on MS + 0.50 mg/l of BAP and 0.50 mg/l of NAA. Roots and root length per plantlet were significantly recorded on MS media supplemented with 2 mg/l of IAA and 3 mg/l of NAA, respectively. Furthermore, at the acclimatization stage, the highest survival percentage was recorded at 100% and 86 % after 45 and 60 days respectively, with plants that transferred on a cultivated substrate containing peat moss+ vermiculate at a ratio (1:1). This micropropagation protocol can be used to produce quality and true-to-type taro planting material for large scale for commercial production.

INTRODUCTION

Taro (*Colocasia esculenta var.esculenta*) is a perennial, aquatic and semi-aquatic herbaceous species that belongs to the family Araceae, originated in North Eastern India and Asia and then gradually spread worldwide (Macharia *et al.,* 2014; Oladimeji *et al.,* 2022). The world production of taro is around 11 million tons per year over an area of around 2

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million hectares in 2018. The top producers in West Africa were Ghana (3,303,118 tones), Nigeria (1,460,938 tones), Togo (16,620 tones) and Benin (1,069 tons) (FAOSTAT 2018).

Taro contains high amounts of vitamins while it is low in fat and protein, but its carbohydrate content was higher than that of other root and tuber crops such as yam (*Dioscorea alata* L.), cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* L.). Taro leaves provide plenty of vitamin A, which was necessary for good growth, eye health and disease prevention. They also contained vitamin C (Acedo *et al.,* 2018). Besides its nutritional value, taro was used as a medicinal plant and provides bioactive compounds used as anticancer drugs (Kundu *et al.,* 2012 and El-Sayed *et al.,* 2016). Taro is mainly cultivated for corm production, taro corms are a good source of digestible starch, crude fiber, minerals, vitamin C, thiamin, riboflavin, and niacin (Kidanemariam, 2018 and Boampong *et al.,* 2019). Taro plant contains bioactive proteins which used as cancer therapeutics. (Kundu *et al.*, 2021).

Plant tissue culture techniques have become a powerful tool for the propagation of taro to overcome many problems facing traditional methods of propagation. Different explants were used to produce disease-free planting materials (Behera and Sahoo, 2008; Bogale 2018; Akplogan *et al.,* 2018).

The success of this technique involves certain exogenous factors including an adequate combination of auxin and cytokinin; and endogenous hormone, including the type of taro explant (Ahanhanzo *et al.,* 2010 and Cacaï *et al.,* 2013). For this purpose, (Minas 2002) used MS medium supplemented with benzylamino purine (BAP) and Naphthalene acetic acid (NAA) to establish the protocol for micropropagation of taro from auxiliary buds.

I*n vitro* cultivation the balance of growth regulators depends on the objective such as shoot and root, callus or suspension culture (Legesse and Bekele 2021) and on the micropropagation phase considered as initiation, multiplication, or rooting (Manju *et al*., 2017). In the multiplication phase, the level of cytokinin should be normally higher than the auxin. In the rooting phase, in turn, the use of cytokinin, in some cases, is not necessary and higher levels of auxins can be supplemented to the culture medium (Torres *et al*., 2001). Moreover, Acedo *et al.,* (2018) established a protocol for micropropagation of taro from auxiliary buds with MS medium supplemented with benzyladenine (BA) and indole-acetic acid (IAA) as culture media.

So, the goals of this study are to look at the possibility of reaching to protocol for the propagation of the Egyptian taro cultivar under laboratory conditions. Also, evaluating in vitro micropropagation using auxiliary buds' culture to produce plants free of pathogens.

MATERIALS AND METHODS

The present study was carried out at the plant tissue culture laboratory of the Plant Production Department, Faculty of Agriculture Saba-Basha, Alexandria University, Egypt, during the 2019 to 2023 seasons.

Source of Plant Material:

The source of explants (corms) was obtained from Shanawan, Menofiya Governorate, Egypt. The cultivar was Egyptian native cultivar of taro; *Colocasia esculenta var. esculenta* (L.) Schott. Mature corms were used at the initiation stage.

preparation and Sterilization Of Explants:

The corms were first washed with tap water and liquid soap to remove soil and root residues. Then, a sharp knife was used to excise the Axillary buds from dry corms (about 0.75 kg., 1.5 cm. height and Ca. 12 cm in diameter). Radial cuts, 1cm apart, were made into the outermost layer of cut petiole bases and these pieces were discarded to expose the axillary buds close to the base of petioles. The axillary buds were also excised and treated similarly. Rectangular and sunken cuts were made around the axillary buds with the assistance of an

appointed scalpel blade leaving ca.1.0 mm of the interior tissue around each bud following the excision of axillary bud explants; they were rinsed rapidly with distilled water, then immersed into 70% (v/v) ethanol for 1 minute, then washed twice with distilled water. Under operating the air flow cabinet, the excised axillary buds were surface sterilized with different concentrations of mercuric chloride (HgCl2) at 0.05 and 0.1% (v/v) with a few drops of wetting agent "Tween-20" (surfactant agent) for 5 and 10 minutes (Ilahi et al., 2007). A similar procedure was repeated sodium hypochlorite (commercial bleaching compound, Clorox) + two drops of tween 20 in different concentrations (30% for 15 minutes, 50 % for 10 minutes and 70 % for 5 minutes). Dead tissues were removed after each concentration and then rinsed thrice with double sterile distilled water. After the surface sterilization of explants with mercuric chloride and sodium hypochlorite solution were decanted and the explants were rinsed with sterile double distilled water four times, to lower the toxic effects of HgCl2 and NaOCl and became ready for culturing. Determination of the contamination ratio for each sterilant agent used in the sterilization of culture explants was done.

Micropropagation Stages:

- **Initiation Stage (establishment of *in vitro* cultures):**

At the establishment stage, auxiliary buds were cultured on MS (Murashige and Skoog, 1962) strength basal medium with 3% sucrose and 0.8 % agar. Explants that had been removed were used for growing at 25 ± 2ºC for 45-50 days to form the first leaves. The contamination and survival percentages were recorded weekly. For initiation auxiliary buds were cultured in the basal medium (MS) supplemented with various levels of the cytokinin BAP (6-Benzylamine-purine) (0, 0.125, 0.250, 0.50 mg/L) in combinations with auxin NAA (α-naphthalene acetic acid) (0, 0.250, 0.50, 1.00 mg/L).

- **Multiplication Stage**:

The individual shoots, which formed at the end of the establishment stage, were cultured on MS media having 0, 0.50, 1, 2, 4, mg/l, of (BAP) in combinations with NAA at concentrations: 0, 0.50, 1, 2 mg/L. Each treatment had 6 jars, containing 40 ml medium. The pH of all media was adjusted to 5.6-5.8 with 1M HCl or 1M NaOH, while they were autoclaved at 121ºC under a pressure of 1.5 kg/cm^2 for 20 mins. The duration of the multiplication stage was five subcultures. Shoots obtained were separated and individually subcultured after 30-45 days. The shoots, which were cultured on MS medium with different concentrations of BAP, were extended to the fifth subculture. The number of shoots at the end of each multiplication subculture was recorded. In addition, the multiplication rate was calculated. Multiplication rate is defined as the ratio of the number of shoots to the number of meristem tips after the first subculture or the ratio of the number of new shoots to the number of initial shoots after each subculture (Du *et al.,* 2006).

- **Rooting Stage:**

The obtained shoots from the multiplication stage were separated and cultured on a rooting stage. MS media in addition to two types of auxin were tested; whereas IAA was at four concentrations: (0, 0.50, 1, and 2 mg/l), in combinations with NAA at three concentrations: 0., 0.50, 1 and 2, mg/l.

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number of roots, and number of leaves were recorded. Also, shoot length, root length and number of shoots were recorded after 21 days of culture.

- **Acclimatization Stage:**

The objective of this stage was to adapt the plantlets obtained from *in vitro* before culture to the open field. Three types of substrate mixtures, namely, vermiculate + perlite + peat moss (1:1:1), vermiculate + peat moss (1:1) and sand + peat moss (1:1) were tested at the acclimatization stage. First, the mixtures were autoclaved at 121°C under a pressure of 1.5 kg/cm^2 for 20 mins. Then, the plantlets were washed with tap water to remove the agar residues. Previously, the plantlets' roots were in distilled water for several hours and, fungicide (Rozolex) 1 g/l for 2 mins. the plantlets were cultured in plastic pots filled with the various substrates mixtures covered with polyethylene bags. For 16 hours, the plantlets were kept in a growth room at 24 ± 2°C. For 16 hours, the plantlets were kept in a growth room at 24 ± 2°C.. photoperiod. Subsequently, the polybags to they were removed totally after 15 days. The surviving plantlets were cultured on the same substrate mixtures in plastic pots after 45 days of acclimatization and transferred to a net house, The plantlets were irrigated whenever needed and fertilized with nutrients solution containing macro and micronutrients (Stimufol Amino, Shoura Chemicals Co.). The survival percentage was measured as the following equation: -

$$\text{survival percentage } (\%) = \frac{\text{survival plantlets}}{\text{total number}} \times 100$$

The plantlet height and leaves numbers were recorded at 45 and 60 days after transferring plantlets into the acclimatization medium.

Statistical Analysis:

Data were, statistically, analyzed as Complete Randomized Design (CRD) with several replicates depending on the stage of micropropagation. R computerized statistical analysis using MSTAT package for analysis of variance (ANOVA) and means of treatments were compared using LSD at 0.05 according to Snedecor and Cochran (1985).

RESULTS AND DISCUSSION

A) Initiation Stage:

Effect of Different NAA and BAP Concentrations on Micropropagation of Taro:

Table (1) and figure (1) showed the interaction between the different concentrations of the two tested hormones NAA with concentrations as (0, 0.25, 0.50 and 1 mg /l) and BAP with concentrations (0, 0.125, 0.250 and 0.500 mg /l) and their combinations on number of shoots, shoot length (cm), number of leaflets and roots. Significant differences emerged. in the number of shoots, shoot length, number of leaflets and number of roots among the different concentrations of NAA. All the NAA concentrations (0.25, 0.50 and 1 mg /l) obtained a higher average number of shoots, shoot length, number of leaflets and no. of root as compared to control (0 .00 NAA). Also, NAA at 0.500 mg l$^-$ recorded the highest significant values of all previous parameters as compared with all the different NAA concentrations.

Similar observations regarding the role of exogenous levels of plant growth regulators in determining the shoot forming-capacity of taro had reported by (Manju *et al.,* 2017, and. Oladimeji *et al.,* 2022) In addition (Matthews and Ghanem 2021) demonstrated that a critical endogenous level of growth regulators has to be attained before cell division and organogenesis. Also, the addition of BAP, increased the number of shoots, shoot length, no. leaflets and no. root by increasing the concentration of BAP (0.250 and 0.500 mg /l), its concentration at 0.250 mg /l recorded a higher number of shoots (1.64 cm) and shoot length (1.97cm), as compared to the control (0 .00 BAP), while BAP at 0.500 mg /l recorded

significantly the highest number of leaflets and number of the root as compared to all other concentrations,

The interaction between NAA and BAP was highly significant on the numbers of shoots, shoot length (cm), number of leaflets and the highest values obtained at (0.5 NAA and 0.5 BA mg /l), although there was no significance on the number of roots.
This can be supported by the study of (Manju *et al*., 2017) who reported that the addition of BAP into a culture medium enhanced the growth and development of shoot auxiliary buds of taro.

On the other hand, the lower percentage of shoot induction with increasing levels of BAP above optimal concentration is in accordance with the research of other root crops such as anchote (*Coccinia abyssinica L.*) (Bekele *et al.,* 2013) and cassava *(Manihot esculenta L.*) (Beyene *et al.,* 2010). According to these studies, using BAP above a certain optimum level decreased the shoot proliferation rate either by direct inhibition of the produced shoots or by encouraging callusing in the in-vitro culture plant. This was very obvious from our findings.

Table 1: Interaction between NAA and BA (mg/l) different concentrations and their combinations on the initiation stage of *Colocasia esculenta* cultured *in vitro*

Treatments		No. shoots	Shoot length (cm)	No. leaflets	No. root
NAA	BA				
0	0	0.22	0.24	0.45	0.00
	0.125	0.56	0.88	1.33	0.44
	0.250	0.89	1.66	1.89	0.44
	0.500	0.56	0.89	1.33	0.78
0.25	0	0.78	1.07	1.00	0.00
	0.125	1.00	1.48	2.11	0.33
	0.250	1.78	1.85	2.67	0.33
	0.500	1.44	1.48	2.89	1.11
0.50	0	1.67	1.59	2.00	0.00
	0.125	1.67	1.87	2.89	0.67
	0.250	2.33	2.66	2.55	1.22
	0.50	3.11	2.92	4.22	1.34
1	0	0.67	1.13	1.56	0.00
	0.125	1.11	1.42	2.11	0.33
	0.250	1.56	1.72	2.11	0.78
	0.500	1.22	1.33	1.78	1.33
LSD $_{(0.05)}$		**0.65**	**0.21**	**0.39**	**0.38**

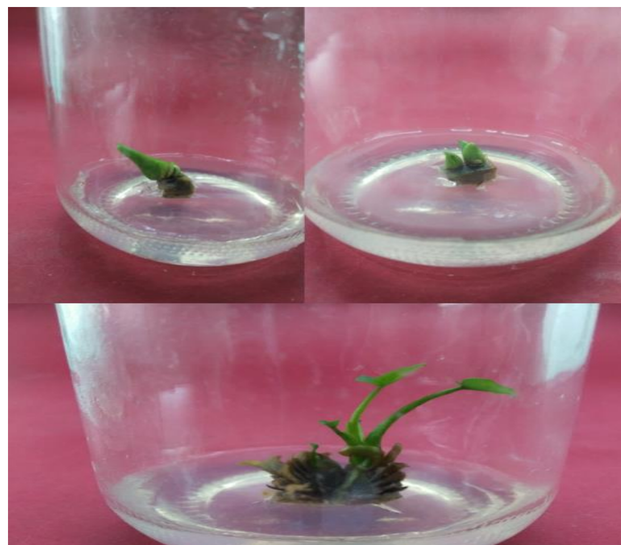

Fig. 1: Taro shoot Initiations at the establishment stage.

B) Multiplication Stage:

Effect of Different Concentrations of NAA and BAP on Micropropagation Of Taro:

Data illustrated in Table (2) and Figure (2) show the effect of different concentrations of NAA (0, 0.50 and 1 and 2 mg l^{-1}) and BAP concentrations (0, 0.50, 1, 2 and 4 mg l^{-1}) on number of shoots, length of shoot, number of leaves per shoot and number of roots.

The highest rate of multiplication of *in-vitro* shoot tips of taro was recorded in MS media supplemented with either NAA at 1 and 2 mgl^{-1} or BA at 1 and 2 mgl^{-1}. However, the highest values of shoots numbers were recorded at the low concentration of NAA/ (1 mg/l^{-1}) therefore, the highest values of shoot length and number of leaflets recorded with NAA concentrations at (2 mg/l^{-1}), as compared with control and other concentrations Figure (2).

On the other hand, MS media supplemented with BAP at 1 mg/l obtained the highest number of roots as compared to other concentrations of BAP moreover, the explants cultured at MS medium at 1 mg/l recorded the highest values of shoots length and the number of leaflets (5.33 and 5.70) respectively, furthermore, the concentration 2 mg/l recorded the highest number of the shoot (7.53), as compared to control.

Interactions between NAA and BAP showed highly significant for all studied previous parameters and the highest values were obtained at (1 NAA and 1 BA mg l).

It is known that the multiplication stage requires cytokines during the micropropagation of various plants. This requirement appears to differ depending on the type of plants, the source of explants, the phase of development, the concentration of plant growth regulators, the interaction between plant growth regulators and environmental conditions (Yokoya and Handro, 1996).

According to these studies using BAP they mentioned that a certain optimum level decreased the shoot proliferation rate either by direct inhibition of the produced shoots or by encouraging callusing in the *in-vitro* culture plant. This might be due to the effect of cytokinin (BAP) in releasing lateral bud shoots by breaking apical dormancy by inhibiting the effect of high levels of endophytic auxins.

Seetohul *et al.* (2008) It was also reported that in vitro shoot tips have the highest rates of multiplication of taro were recorded in MS medium supplemented with BAP at 2 mg/l. but no significant variation was detected among cultivars on the number of shoots, open leaf and corm diameter when cultured in rooting media. This result may be attributed to the role of auxin in the enhancement of rooting and not shooting, where the development of lateral buds is inhibited by auxin produced at the apical meristem and transported down the stem (Bhuiyan *et al.,* 2011). However, our data is slightly in contrast to earlier reports by Ngetich *et al.* (2015) in *C. esculenta*. This difference might be because of the genotypic variation of the plant. On the other hand, with further increases in BAP concentration to 10 and 12 mg/L, shoots become few in number, very short and start to develop the abnormal morphological appearance of shoots it might result from the use of higher levels of cytokinin (BAP) above to the optimum levels. The main observation of inhibition of *in-vitro* growth of crop plants was observed by Bekele *et al.* (2013) and Beyene *et al.* (2010). From combination tested medium which contain 1 mg/l^{-1} of BAP with 1 and 2 mg/l^{-1} NAA elicited an optimal response in terms of average shoot numbers and length as compared to media containing separately.

A comparatively lower response in shoot number, average height and bad-looking shoots were observed when BAP at higher concentration combined with higher auxins concentration. This might be related to the interacting effect of the two growth regulators at this level of concentration or due to the use of a supra-optimal concentration of BAP. Here, the combination of a relatively lower auxin with a relatively higher cytokinin promotes the shot number and shoot height at this stage. The average number of shoots and length obtained from this study were in agreement with the work of Chien-Ying *et al.* (2008) who recorded

an average of 5.9 shoots per explants using MS + 8 mg/L of BA + 3 mg/L IAA while in this work, using BAP instead of BA in combination with 3 IAA mg/L an average number of shoots 8.53 with an average length of shoot 6.7 cm was recorded. A medium with free growth regulators and a medium with a high concentration of BAP alone and in combination with IAA resulted in a low multiplication rate. The length of shoots that were obtained from sprout tip explants was slightly shorter than from corm base explants. The good-looking response in shoot numbers and length on MS multiplication media containing IAA was also reported on other cocoyam species (*C. esculenta* and *X. sagittifolium*) by Kambaska and Sahoo (2008) and Chien-Ying *et al.* (2008).

Table 2: Interaction between NAA and BA (mg/l) different concentrations and their combinations on the multiplication stage of *Colocasia esculenta* cultured *in vitro*

Treatments		No. shoots	Shoot length (cm)	No. leaflets	No. root
NAA	**BAP**				
0	**0**	0.56	1.77	2.11	0.00
	0.50	1.22	2.36	3.33	0.22
	1.	2.44	2.78	3.11	0.33
	2	4.67	2.50	4.11	0.00
	4	2.78	2.10	2.33	0.00
0.50	**0**	1.67	2.77	3.55	1.55
	0.50	1.89	2.92	3.89	2.22
	1	3.56	2.69	4.78	1.67
	2	4.44	2.75	6.67	1.78
	4	3.11	3.21	3.22	1.55
1	**0**	2.00	6.38	6.11	3.00
	0.50	4.78	7.91	6.67	3.78
	1	13.33	4.89	5.00	4.22
	2	13.00	3.97	5.00	3.44
	4	8.22	3.27	3.56	3.33
2	**0**	1.66	7.83	7.00	2.78
	0.50	3.89	9.71	7.45	2.89
	1	7.33	10.97	9.89	1.22
	2	8.00	5.86	6.00	0.33
	4	4.00	4.41	4.78	0.33
LSD (0.05)		0.66	**0.46**	**0.62**	**0.30**

Fig. 2: Taro shoots multiplication at the multiplication stage.

C) Rooting Stage Effect Of Different Concentrations of IAA and NAA on Micropropagation Taro:

Results presented in Table (3) and Figure 3 showed the effect of IAA and NAA at different concentrations on the number of roots, number of shoots after 15 and root number, number of leaflets, shoot length (cm) root length (cm) and No. of shoots, after 21 days from shoot culture The analysis indicated that the all-previous in vitro traits under study were highly significantly influenced by differences in concentrations of IAA and NAA except for the differences of concentrations of NAA for the number of leaflets after 21 days from shoot culture. Data showed that the highest number of roots after 15 and 21 days of culture was obtained by the shoots cultured on MS medium with low concentrations of IAA or NAA (0.00 and 0.5 mg/ L). The number of leaflets after 15 and 21 days of culture show the same response under the low concentration of IAA or NAA (0.00 and 0.5 mg/ L). On the other hand, 2.0 mg/l of IAA or NAA showed the highest Shoot length and root length after 21 days of culture among all treatments.

Previously, numerous investigators have pointed out that NAA (naphthalene acetic acid) and IAA (Indole –3-acetic acid) are an important auxin used in root induction (Behera and Sahoo 2008; Hutami and Purnamaningsih 2013; Evelyn *et al.,* 2017; Alam and Abdul Kadir 2022). Behera and Sahoo (2008) observed that when in vitro excised shootlets were inoculated onto the half-strength MS basal medium supplemented with 2.0 mg NAA the rooting response was 95%. Hutami and Purnamaningsih (2013) reported that in MS + IAA 0.5 mg/l, the root was grown and developed better than that in MS + NAA 0.5 mg/l. Evelyn *et al.* (2017) reported that at the rooting stage, taro shoots were cultured on MS media supplemented with 10 ml of 0.1 mg/ml NAA. Roots were produced on all the cultivars with an excellent mean growth rate of 14.7 ± 0.69 recorded in cultivars with dark green petiole and small leaves. Recently, Alam and Abdul Kadir (2022) reported that at the rooting stage, the highest number of roots per shoot was achieved on a half-strength MS medium containing 0.5 mg/L IAA after 30 days of culture.

Table 3: Effect of different concentrations of NAA and IAA (mg/l) on the rooting stage of *Colocasia esculenta* cultured *in vitro*

Treatments	After 15 days		After 21 days				
	No. of Root	No. of leaflet	No. of Root	No. of leaf	Shoot length (cm)	Root length (cm)	No. of shoots
	IAA (mg/l)						
0	5.25a	3.00b	9.00a	5.75a	3.21b	7.49b	2.25a
0.50	5.88a	3.00b	7.08b	3.88b	5.98a	11.75a	0.38b
1	4.17b	2.33c	4.50c	3.83b	4.90a	6.51b	0.00b
2	1.83c	4.67a	9.17a	6.50a	5.51a	10.50a	2.00a
	NAA (mg/l)						
0.0	5.25a	3.oob	9.50a	5.75a	3.21b	7.49b	2.25a
0.50	5.00a	3.00bc	9.50a	4.50ab	3.19c	7.27b	1.75b
1	2.33b	2.00c	4.08b	3.08b	2.61c	7.25b	0.29c
2	2.00b	3.33b	4.50b	3.50ab	6.61b	11.91a	1.67b

*Means followed by the same letter (s) are not significantly different at 0.05 level of probability

Fig. .3: Root induction of taro shoot at the rooting stage

E) Acclimatization Stage:

According to Kozai and Zobayed (2000), acclimatization is the method used to an organism, particularly a plant that has been relocated to a new area, adapts to its climatic or environmental conditions.

The analysis indicated that the plantlets' survival % was highly significantly influenced by differences in days of acclimatization and substrate mixture. The interaction between days of acclimatization and substrate mixture was not significant and the results indicated that plantlets could be survived on all tested media with a high percentage of survival after 45 days of acclimation. Substrate mixture containing peat moss+ vermiculate in the ratio (1:1) showed the highest survival percentage it was (100%) and (86 %) after (45 and 60 days) respectively as compared to all other mixtures Table and Figure, (4).

Data showed that the lowest surviving percentage was at Peat moss + Vermiculate (1:1) across the different days of acclimatization (Table 4-) which was significantly different from the other three substrate mixtures. On the other hand, 45days of acclimatization recorded the highest surviving percentage across the four-substrate mixture
Approximately 90% of the plantlets survived ex vitro acclimation.

The above result might be a good indication that taro needs quite fertile soil as the requirement of fertile soil (soil with dung) and such as sandy soil for hardening of taro during acclimatization is also stated by Chien-Ying *et al.* (2008). Moreover, Fujimoto (2009) also reported the need for such soil combination during the cultivation of taro under natural field conditions.

Previously, Nath *et al.* (2012) found that after 2 weeks, well-developed plantlets were hardened in plastic cups in a potting mixture (vermiculite + sand, 1:1, v/v). Acclimated plants were transferred to 30 cm plastic pots containing topsoil and vermicompost (3:1, v/v) where they grew well. El-Sayed *et al.* (2016a) reported that at the acclimatization stage, vermiculate + peat moss substrate recorded the highest surviving percentage. Anupama *et al.* (2018) observed that well-developed plantlets were acclimatized in a potting mixture of sand and cocopeat in a ratio of 1:1. Nearly 94% of the regenerated shoots survived after transferring them to the field.

Table 4: Taro plantlets survival percentage (%) after 45- and 60-days acclimatization of different substrate mixtures

Days of acclimatization	Substrate mixture	Surviving (%)
45 days	Vermiculate + Sand + Peat moss (1:1:1)	90
	Sand + Peat moss ++ Perlit (1:1:1)	95
	Sand + Peat moss (1:1)	60
	Peat moss+ Vermiculate (1:1)	100
60 days	Vermiculate + Sand + Peat moss (1:1:1)	85
	Sand + Peat moss ++Perlit (1:1:1)	83
	Sand + Peat moss (1:1)	30
	Peat moss+ Vermiculate (1:1)	86

Fig.4: Acclimatization of newly formed plantlets *ex vitro*: in *vivo* of taro for 45 and 60 days.

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ARABIC SUMMARY

تأثير منظمات النمو المختلفة على الإكثار المعملى الدقيق لنبات القلقاس المصرى

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تم إجراء التجربة في معمل زراعة الأنسجة النباتية بقسم الإنتاج النباتي، كلية الزراعة سابا باشا، جامعة الإسكندرية، مصر، خلال الفترة من 2019 إلى 2023. أجريت هذه الدراسة لتطوير بروتوكول الإكثار المعملى الدقيق لنبات القلقاس المصرى باستخدام البراعم الجانبية حيث تم استخدام بيئة موراشيج وسكوج فى توليفات مع أنواع وتركيزات مختلفة من المنظمات النباتية (BAP وIAA وNAA) للإنبات والتضاعف وتطورالجذور. تم الحصول على أعلى مستوى لبدء الاستزراع على وسط بيئة موراشيج وسكوج معزز بـ 0.500 ملجم / لتر من BAP وNAA للنبتيات على التوالي. وتم الحصول على الحد الأقصى لعدد البراعم على MS + 0.500mg / l BAP و0.50mg / l NAA. تم تسجيل أعلى متوسط لعدد الجذور وطول الجذر لكل نبتة على بيئة موراشيج وسكوج بـ 2 مجم / لتر IAA و3 مجم / لتر NAA، على التوالي. تم الحصول على أعلى كفاءة للبقاء (100٪) بعد 45 يومًا و (86٪) بعد 60 يومًا لوحظت على بيئةالتربة المحتوية على البيتموس + الفيرميكوليت بنسبة (1: 1). نستطيع القول من خلال هذة الدراسة أنه يمكن استخدام بروتوكول الإكثار المعملى الدقيق بإستخدام البراعم الجانبية لإنتاج وزراعة القلقاس المصرى ذات الجودة الأعلى والخالى من الأمراض من أجل الإنتاج التجاري على نطاق واسع في ظل الظروف المعملية المعقمة.