Middle East Journal of Applied Sciences
ISSN 2077-4613

Volume : 06 | Issue :01 | Jan.-Mar. 2016
Pages: 241-248

In vitro Microcorms Induction of Egyptian Native Cultivar of Taro, *Colocasia esculenta* var. *esculenta*

[1]El-Sayed, S. F., [1]A. A. Gharib, [2]A. M. El-Sawy, and [1]Omaima S. Darwish

[1]Vegetable Crops Department, Faculty of Agriculture, Cairo University, Egypt.
[2]Plant Biotechnology Department, National Research Center, Egypt.

Received: 14 February 2016 / Accepted: 15 March 2016 / Publication date: 30 March 2016

ABSTRACT

Three experiments were conducted to study the stimulation of *in vitro* microcorms formation of taro shoots using sucrose, maltose and activated charcoal (AC) in different concentrations. These experiments were conducted in the tissue culture laboratory, Vegetable Crops Dep., Fac. Agric., Cairo Univ. In the first experiment, *in vitro* shoots were cultured on MS medium +2 mg/l BAP with 3, 6, 8 or 10 % sucrose. There was no microcorm produced in all sucrose concentrations. In the second experiment, *in vitro* shoots were cultured on hormone free MS medium with 3, 4, 6, 8, 10, or 12 % sucrose or maltose. The results showed that MS medium with 3, 10 or 12 % sucrose or maltose did not produce any microcorms. Concerning maltose concentration, MS medium with 6% maltose was the only medium that formed microcorm. The highest microcorm diameter and length were obtained on MS medium with 6% sucrose. In the third experiment, *in vitro* shoots were cultured on hormone free MS medium with 3% sucrose supplemented with 0.1, 0.5 or 1% AC. MS medium with 1% AC produced the highest microcorm diameter and length.

Key words: Taro, micropropagation, microcorms, sucrose, maltose, activated charcoal

Introduction

Taro *(Colocasia esculenta*) is a tropical and subtropical crop belonging to the monocotyledonous family Araceae. It is cultivated for the edible starchy corms (underground stems) (Tindall, 1983). Taro is one of the major root and tuber crops worldwide, i.e., potato, sweet potato, taro or dasheen, cassava and yam. These crops are in the second level in importance to cereals as a global source of carbohydrates. The Egyptian native cultivar of taro is belonging to *Colocasia esculenta* (L.) Schott var. *esculenta* variant (Matthews, 2014). *Colocasia esculenta* (L.) Schott var. *esculenta,* which possesses a large cylindrical central corm with few cormels, is classified to the 'dasheen' type of taro (Purseglove, 1972; Tindall, 1983 and Lebot & Aradhya, 1991).

Every 100 g of taro corms possess 112 kcal, 26.46 g carbohydrate, 0.20 g total fat, 1.50 g protein and 4.1 g fiber (USDA National Nutrient data base). Besides its nutritional value, taro is used as a medical plant and provides bioactive compounds which used as an anti cancer drugs (Kundu *et al*., 2012).

Probably, taro originated in India but possibly from other parts of South-east Asia. It was distributed from India to Egypt about 2000 years ago, from where it was introduced into Europe. From Spain, the cocoyam was taken to the new world and then into West Africa from tropical America (Tindall, 1983).

Worldwide, the top producers of taro are Nigeria, China, Ghana and Cameroon (FAOSTAT, 2013). In Egypt, the harvested area was 8200 feddan at 2013, while the production was 120000 tons in the same year with average yield 14.6 tons per feddan (FAO statistics division, 2016). The top governorates producers at Egypt are Al Menofiya, Al Sharqia, Al Qalyubia , Assiut and Al Minia .

The cormels or divided corms are the methods of propagation of taro. The seed quantities reach about 1000 kg of corms or 800-900 kg of cormels. These methods of propagation are not always suitable due to the large quantities of seed, high percentage of seed rotting and susceptibility to pathological agent. Taro is affected by viral diseases which decrease the yield (Ooka, 1994). Among other Araceae plants, Taro plants are highly sensitive to Dasheen mosaic virus (DMV), which significantly reduces yield (Keolanui *et al.,* 1993).

Meristem culture technique is used to produce virus free plants especially at the vegetative propagated plants (Abo El-Nil and Zettler, 1976). Application of plant tissue culture techniques has been found very useful for rapid multiplication, safe exchange and conservation of many vegetative propagated crops (Hussain and Tyagi, 2006). Furthermore, induction of *in vitro* storage organs has been found useful for conservation of germplasm of many vegetative propagated crops. In addation, *in vitro* microcorms formation exhibited high plantlets survival at acclimation stage and during open field growth (Hussain and Tyagi, 2006).

Corresponding Author: Omaima S. Darwish, Vegetable Crops Department, Faculty of Agriculture, Cairo University, Egypt.
E-mail: Omaima.Darwish@agr.cu.edu.eg

Exogenous growth substance, including sugar (sucrose or maltose), constituted a key factor in the formation process of storage organs in taro, potato and yam. High concentration of sugar is needed for the initiation of storage organs, as they constitute the main component of the stored organs (Zhou *et al*., 1999).

Activated charcoal is commonly used in tissue culture media due to its structure, and its addition may have either beneficial or harmful effects. The effects of activated charcoal could be attributed to a) providing a dark environment in the medium; b) adsorption of certain inhibitory substances in culture, produced by either media or explants; c) adsorption of plant growth regulators and other organic compounds; and d) the release of substances naturally present in or adsorbed by activated charcoal, which are beneficial to growth of *in vitro* culture (Pan and Van, 1998). Besides, many reports showed that using activated charcoal in tissue culture medium stimulated stored organ formation (Lajayer *et al.*, 2011).

The present research was aimed to study the effect of different concentrations of sucrose, maltose and activated charcoal to enhance *in vitro* micro corms formation .

Material and Methods

Plant materials

Three experiments were conducted to study the stimulation of *in vitro* microcorms formation of taro shoots by using different concentrations of sucrose, maltose and activated charcoal. These experiments were conducted in tissue culture laboratory, Vegetable Crops Dep., Fac. Agric., Cairo Univ. The source of explants (corms) was from Shanawan, Menofiya Governorate, Egypt. The cultivar was Egyptian native cultivar of taro *C. esculenta var. esculenta* (L.) Schott. Mature corms were used at initiation stage.

Preparation and sterilization of explants

The corms were first washed with tap water and liquid soap to remove soil and roots residues. Then, a sharp knife was used to excise the tip meristems, which were surrounded by leaf primodia and large part of corm. The excised explants were washed three times with tap water. After each washing time, the outer parts of corms and leaves bases were cut off. At laminar air flow hood, the excised explants were surface sterilized with sodium hypochlorite (commercial bleaching compound, Clorox) + two drops of tween 20 in sequent steps with different concentrations 100% for 15 minutes, 75 % for 10 minutes and 50% for 5 minutes. Dead tissues were removed after each time. The excised meristems (0.5-1 cm) were washed with sterilized distilled water five times.

Establishment and multiplication stages

At establishment stage, meristems were cultured on ½ MS (Murashige and Skoog, 1962) strength basal medium with 3% sucrose and 0.8 % agar. The excised explants were incubated at 24±2C^{o} for 30-45 days or up to form the first true leaf. At multiplication stage, taro shoots were cultured on MS medium + 2 mg/l BAP.

Taro microcorms production experiment

An initial experiment (first experiment) was conducted at mid March 2014 for 90 days to form taro microcorms. Taro shoots, which formed on MS medium with 2 mg/l BAP, were used in this experiment. They were cultured on MS media with 2 mg/l BAP and 3, 6, 8 or 10 % sucrose. Each treatment consisted of 6 jars containing 40 ml of solid medium. The pH of all media was adjusted to 5.6-5.8 with 1M HCl or 1M NaOH, while they were autoclaved at 121^{o}C under a pressure of 1.5 kg/cm^2 for 20 min. The survival and contaminated percentages were recorded monthly. After 90 days, the sign of micro corms and number of shoots were recorded.

The second and third experiments were conducted at January 2015 for 90 days. Taro shoots, which formed on MS medium with 2 mg/l BAP, were used at these experiments. At second experiment, *in vitro* shoots were cultured on hormone free MS medium with 3, 4, 6, 8, 10 or 12 % sucrose or maltose. At third experiment, hormone free MS medium was supplemented with 0.1, 0.5 or 1 % AC. Each treatment consisted of 6 jars containing 40 ml of medium. The pH of all media was adjusted to be 5.6-5.8 with 1M HCl or 1M NaOH, while they were autoclaved at 121^{o}C under a pressure of 1.5 kg/cm^2 for 20 min. Plantlet height**,** number of leaves, number of roots, shoots number and microcorm formation were recorded after 60 days of cultivation in both experiments. In addition to the previous measurements, microcorm length and diameter were recorded after 90 days of cultivation. Plant fresh weight was recorded after 90 days at the third experiment.

Statistical analysis

Regular analyses of variance of Completely Randomize Design (CRD) were performed on obtained data. $LSD_{0.05}$ was calculated for comparing means (Snedecor and Cochran, 1980).

Results and Discussion

First experiment

Data in Table 1 show the response of taro shoots to different sucrose concentrations in MS medium supplemented with 2 mg/l BAP, after 90 days of culture. From visual observation, there was no microcorms formation at all concentrations of sucrose. The only response was a weak shoots formation at all concentrations of sucrose. Number of shoots after 90 days of culture differed according to sucrose concentration. The highest number of shoots was recorded in MS medium +2 mg/l BAP + 3 % sucrose. However, there was significant difference between MS medium with 3 or 4 % sucrose. Number of shoots was declined by increasing the sucrose concentration. MS medium + 2 mg /l BAP + 3% sucrose recorded number of shoots 5.87 fold than MS medium + 2 mg/l BAP+ 10% sucrose. Similar results were obtained by Yuping *et al*. (2003), who found that by increasing the sucrose concentration in MS medium, taro lateral bud number and plant height were decreased. Hussain and Tyagi (2006) obtained maximum higher number of taro shoots (5.8 shoots /culture) and longer shoots on M1 medium (MS with 2.2 μ*м* BAP + 0.6 μ*м* NAA + 0.8 % agar + 3% sucrose) as compared to shoots regenerated on M2 medium (8% sucrose) and M3 medium (10% sucrose) in both 3 and 6 months old cultures. The unformed corms in the present experiment may be attributed to the presence of BAP in MS medium. Alizadeh *et al.* (1998) reported that the microtuberization in some yam species was shown to occur only when shoots were grown on culture media containing sucrose at 8-10% in the absence of growth regulators. In their experiment, of the cytokines tested, all evaluated concentrations of BAP decreased, at least by half, the percentage of microtuberization as compared to the control. On the contrary, Zhou *et al*. (1999) reported that no taro microcorms were formed when medium devoid of sucrose, irrespective of BAP concentration. They added that increasing sucrose concentration to 5–10% promoted corm formation, but the effect became significant when 22–44 μM BAP was added simultaneously. In the combinations of 22–44 μM BAP and 8–10% sucrose, corm induction rate was 100%.

Table 1: Effect of different concentrations of sucrose on shoots number 90 days after culture

Sucrose concentration	Number of shoots
MS + 2 mg /l BAP + 3 % sucrose	1.88
MS + 2 mg /l BAP + 6 % sucrose	0.82
MS + 2 mg /l BAP + 8 % sucrose	0.44
MS + 2 mg /l BAP + 10 % sucrose	0.32
LSD 0.05	1.13

Second experiment

Because no microcorms were formed in the first experiment that tested the effect of using different concentrations of sucrose in MS medium on the microcorm formation in the presence of 2mg/l BAP. So that, two experiments were conducted at January 2015 for 90 days. In the second experiment, 6 concentrations (3, 4, 6, 8, 10 and 12 %) of sucrose or maltose were added to hormone free MS medium. In the third experiment, *in vitro* taro shoots were cultured on hormone free MS medium with 3% sucrose and supplemented with 0.1, 0.5 or 1% AC) to study their effects on corm formation.

Data in Table 2 show the response of taro shoots to such concentrations of maltose and sucrose after 60 days of culture. Irrespective of concentrations, mean of plantlet height and number of roots were higher in all sucrose treatments. However, mean of number of leaves and number of shoots recorded higher values in maltose treatments. On the other hand, irrespective of type of sugar, MS medium with 3 % sucrose or maltose recorded the tallest plantlet, the highest number of leaves and the highest number of roots. In contrast, MS medium with 6% sucrose or maltose showed the highest number of shoots. It was noticed that with increasing the concentration of sugar the plantlet height and number of leaves and roots were decreased.

The interaction between sugar types and concentrations was significant. In this regard, MS medium with 3% sucrose gave the tallest plantlet and the highest number of roots. On the other hand, the highest number of leaves was obtained by using MS medium with 3% maltose. The same concentration of maltose (3%) showed the tallest plantlet and the highest number of leaves and roots (as compared with different maltose concentrations). Contradicting, the highest concentration of sucrose (12 %) showed no response for all recorded parameters, even though; the same concentration of maltose did not exhibit the same trend. It was noticed that by increasing the concentration of sucrose or maltose, the plantlet height was decreased. Similar results were obtained by Yuping *et al*. (2003); who found that increasing the sucrose concentration in MS medium led to a decrement in the plantlet height. They added that 3 % sucrose gave the highest plantlet (6.38 cm), while the highest sucrose concentration (14%) exhibited the lowest plantlet height (0.51 cm). Hussain and Tyagi (2006) reported that the maximum shoot length was regenerated on M1 medium (MS with 2.2 μ*м* BAP + 0.6 μ*м* NAA + 0.8 % agar + 3% sucrose) in

compared with shoots regenerated on M2 medium (8% sucrose) and M3 medium (10% sucrose) in both 3 and 6 month old cultures.

Table 2: Effect of sucrose and maltose concentrations on plantlet height, number of leaves, number of roots and number of shoots 60 days after culture

Concentration (%)		Plantlet height (cm)	Number of leaves	Number of roots	Number of shoots
3		5.69	5.00	6.00	0.30
4		3.94	1.10	5.40	0.20
6		2.63	3.30	3.50	0.70
8		1.03	2.50	2.60	0.40
10		0.62	0.90	0.90	0.20
12		0.47	0.70	0.50	0.00
Maltose	3	4.88	6.00	5.40	0.60
	4	2.82	4.20	4.40	0.40
	6	2.52	3.40	3.60	0.40
	8	1.10	3.80	1.00	0.80
	10	0.94	1.20	1.20	0.40
	12	0.78	1.40	1.00	0.00
Mean		2.17	3.33	2.77	0.43
Sucrose	3	6.50	4.00	6.60	0.00
	4	5.06	4.00	6.40	0.00
	6	2.74	3.20	3.40	1.00
	8	1.28	1.20	4.20	0.00
	10	0.14	0.60	0.60	0.00
	12	0.00	0.00	0.00	0.00
Mean		2.62	2.17	3.53	0.17
LSD 0.05	Concentration	2.49	2.68	3.64	ns
	Sugar × Concentration	3.52	3.79	3.23	ns

The shoots formation was rare in the different concentrations of maltose and sucrose. There was insignificant difference among all concentrations of maltose and sucrose in the number of shoots (Table 2). The weak shoots formation might be due to the absence of cytokines in MS medium. It is well known that cytokines play an important role in plants on growth and development. It is also required at micropropagation for shoot formation by stimulating cell division (Kianamiri and Hassani, 2010). This finding agrees with Hussain and Tyagi (2006),who reported that the maximum number of taro shoots (5.8 shoots /culture) was regenerated on M1 medium (MS with2.2 μ*м* BAP + 0.6 μ*м* NAA + 0.8 % agar + 3%) as compared to shoots regenerated on M2 medium (8% sucrose) and M3 medium (10% sucrose) in both 3 and 6 month old cultures. It was noticed that the MS medium contained BAP.

Data presented in Table 3 show the effect of sucrose and maltose concentrations on plantlet height, number of leaves, number of roots and number of shoots 90 days after culture. Irrespective of concentrations, mean of plantlet height and number of roots mean were higher in sucrose treatments. On the contrary, number of leaves mean and number of shoots mean were higher in maltose treatments.

On the other side, irrespective of type of sugar, MS medium + 3% sucrose or maltose gave the tallest plantlets and the highest number of leaves in compared to all concentrations. It was found that when the concentration of sugar was increased, the plantlet height and number of leaves were decreased. The highest number of roots was recorded on MS medium + 4% sugar, while the maximum shoots number was recorded on MS medium + 6% sugar. Similar results were obtained by Yuping *et al*. (2003), who found that increasing the sucrose concentration in MS medium decreased plantlet height. Also, sucrose concentration at 3% exhibited the highest plantlet (6.38 cm). Hussain and Tyagi (2006) reported that the maximum shoot length was regenerated on M1 medium (MS with 2.2 μ*м* BAP + 0.6 μ*м* NAA + 0.8 % agar + 3% sucrose) in compared to shoots regenerated on M2 medium (8% sucrose) and M3 medium (10% sucrose) in both 3 and 6 month old cultures.

Concerning the interaction between sugar type and concentration, MS medium with 4% sucrose recorded the tallest plantlet (9.96) and the highest number of roots (15.60) as compared to concentrations of sucrose and maltose. However, there were significant differences between MS medium with 3 or 4 % sucrose at plantlet height. On the other hand, the highest number of leaves was exhibited by MS medium + 3% maltose, without a significant difference with 4% maltose. The same concentration of maltose (3%) produced the tallest plantlet and the highest number of roots, exceeding in this regard all maltose concentrations. The best sucrose concentration for the number of leaves was MS medium + 3% sucrose.

It was found that the highest concentration of sucrose (12 %) exhibited no response for all recorded parameters. This agrees with the results of Yuping *et al*. (2003) who found that the highest sucrose percentage (14%) exhibited the shortest plantlet (0.51cm) as compared to all sucrose concentrations. In the present experiment, the same

concentration of maltose (12%) did not show the same response. By increasing the concentration of maltose, the plantlet height and number of leaves declined.

Table 3: Effect of sucrose and maltose concentrations on plantlet height, number of leaves, number of roots and number of shoots 90 days after culture

Concentration (%)		Plantlet height (cm)	Number of leaves	Number of roots	Number of shoots
3		8.09	5.90	11.10	0.20
4		6.92	4.70	11.50	0.20
6		3.94	4.60	7.10	0.60
8		1.54	2.50	3.10	0.30
10		0.73	0.70	1.10	0.20
12		0.54	1.10	1.00	0.00
Maltose	3	7.02	7.00	11.20	0.40
	4	3.88	5.00	7.40	0.40
	6	3.42	4.60	7.40	0.00
	8	2.00	3.80	2.60	0.60
	10	1.26	1.20	1.60	0.40
	12	1.08	2.20	2.00	0.00
Mean		3.11	3.98	5.37	0.30
Sucrose	3	9.16	4.80	11.00	0.00
	4	9.96	4.40	15.60	0.00
	6	4.46	4.60	6.80	1.20
	8	1.08	1.20	3.60	0.00
	10	0.20	0.20	0.60	0.00
	12	0.00	0.00	0.00	0.00
Mean		4.14	2.53	6.27	0.20
LSD 0.05	Concentration	3.38	3.02	3.02	ns
	Sugar × Concentration	4.78	4.27	7.58	ns

There were insignificant differences among all concentrations of maltose and sucrose on the number of shoots. Generally, the shoot formation was very weak in the different concentrations. The weak shoots formation might be due to the absence of cytokines in MS medium. It is well known that cytokines play an important role in plants at growth and development. It also required at micropropagation for shoot formation by stimulating cell division (Kianamiri and Hassani, 2010).

Data in Table 4 show the effect of sucrose and maltose concentrations on microcorm diameter and microcorm length after 90 days of culture. Irrespective of concentrations, microcorm diameter and microcorm length were higher in MS medium with sucrose than maltose. On the other side, irrespective of sugar, MS medium with 6% sucrose or maltose recorded the highest microcorm diameter and microcorm length as compared to all concentrations. On the other hand, MS medium with 3, 10 or 12 % sucrose or maltose did not show any corm formation. These findings agree with those of Altindal and Karadogan (2010), who found that the low (20 g/l) and high concentration (120 g/l) of sucrose or maltose decreased potato microtuber number. Also, Dodds *et al*. (1992) mentioned that low and high sucrose concentrations retarded the beginning of microtuberization and produced less microtubers numbers in potato. However, it was reported that high carbon doses stimulated tuber formation (Welander and Pawlicki, 1994; Khuri and Moorby, 1996).

MS medium with 6% maltose was the only medium that formed microcorm as compared to MS medium having any other maltose concentration. This medium produced microcorms with 1.22mm diameter and 1.32mm length. On the other hand, all other maltose concentrations did not produce any microcorms.

The highest microcorm diameter and length were recorded on MS medium with 6% sucrose. MS medium with 4, 6 or 8 % sucrose gave microcorms with 5.80, 6.30 and 3.82 mm diameter and 6.45, 7.22 and 3.87 mm length, respectively. In contrast, MS medium with 3, 10 or 12 % sucrose did not form microcorms. Yuping *et al*. (2003) mentioned that MS medium with 8% sucrose exhibited the highest corm length and diameter as compared with all other concentrations of sucrose.

There are many factors affecting corm or tuber formation as a storage organ in taro, potato and yam. Exogenous growth substance, including sugar, constituted a key factor in the process of storage organ formation (Hussain and Tyagi, 2006). According to obtained results, it could be concluded that using very low or high carbon concentration for microtuber formation was unsuitable. This may be referring to that the low carbon concentrations aren't enough for tuber formation. On the other hand, the high concentrations of carbon source increased osmotic concentrations which ruined the pH and nutrient balance in medium (Altindal and Karadogan, 2010).

Table 4: Effect of sucrose and maltose concentrations on microcorm diameter and microcorm length 90 days after culture.

Concentration		Microcorm diameter (mm)	Microcorm length (mm)
3		0.00	0.00
4		2.90	3.23
6		3.76	4.27
8		1.91	1.94
10		0.00	0.00
12		0.00	0.00
Maltose	3	0.00	0.00
	4	0.00	0.00
	6	1.22	1.32
	8	0.00	0.00
	10	0.00	0.00
	12	0.00	0.00
Mean		0.20	0.22
Sucrose	3	0.00	0.00
	4	5.80	6.45
	6	6.30	7.22
	8	3.82	3.87
	10	0.00	0.00
	12	0.00	0.00
Mean		2.65	2.92
LSD 0.05	Concentration	3.11	ns
	Sugar × Concentration	4.34	5.47

Third experiment

Data in Table 5 show the effect of different AC concentrations in MS medium on plantlet height, number of leaves and number of shoots after 60 and 90 days and number of roots after 90 days. There were insignificant differences among the different concentrations of AC on plantlet height, number of leaves and number of shoots after 60 days. The same trend was observed with number of shoots after 90 days of culture. Contrariwise, the MS medium with 0.5% AC recorded the tallest plantlet (13.02 cm) as compared with 0 and 0.1 % AC. The highest number of leaves (7.50) was obtained by 0.5% AC as compared with all other concentrations after 90 days of culture. On the other hand, MS medium with 0% AC (control) showed the lowest plantlet height (8.07 cm) and number of leaves (4.25). Also, the values of plantlet height and number of leaves were increased by raising AC concentration in MS medium up to 0.5%. The highest AC concentration (1%) in MS medium declined plantlet height and number of leaves. Lajayer *et al*. (2011) reported a similar trend, where the addition of activated charcoal in culture medium influenced shoot growth and tuberization of potato plantlets under *in vitro* condition. In present experiment, the highest concentration of AC (1%) exceeded all concentrations of AC in number of roots (19.75) after 90 days of culture, while, MS medium without AC (0%) had the lowest number of roots (8.75). It was observed that number of roots increased by raising AC concentration in MS medium. One of the positive effects of using activated charcoal in the tissue culture media might be attributed to exclusion of light from root medium and inhabitation of auxin degradation by light and improve rooting ability of explants (Nissen and Sutter, 1990 and Lajayer *et al.,* 2011).

Table 5: Effect of activated charcoal concentration on plantlet height, number of leaves, number of roots and number of shoots 60 and 90 days after culture

Activated charcoal (%)	After 60 days			After 90 days			
	Plantlet height (cm)	Number of leaves	Number of shoots	Plantlet height (cm)	Number of leaves	Number of shoots	Number of roots
0	6.55	4.00	0.00	8.07	4.25	0.00	8.75
0.1	6.05	4.00	0.00	9.07	6.00	0.00	13.25
0.5	8.25	5.00	0.50	13.02	7.50	0.50	15.50
1	8.80	4.25	0.50	12.57	5.25	0.50	19.75
LSD0.05	ns	ns	ns	3.73	1.13	ns	3.37

Data in Table 6 show the effect of different activated charcoal concentrations in MS medium on microcorm diameter, microcorm length and plant fresh weight after 90 days of culture. MS medium with 1% AC produced the highest microcorm diameter (10.49 mm) and microcorm length (17.14 mm). On the other hand, MS medium without AC (0 %) did not form any microcorms. The microcorm diameter was increased by raising AC

concentration in MS medium. However, microcorm length did not show the same trend. MS medium with 0.1% AC recorded the second microcorm length (15.51 mm) but without a significant difference with 1% AC. Lajayer *et al*. (2011) reported that activated charcoal (0.1, 0.5 or 1%) significantly stimulated microtuber formation in potato. Activated charcoal not only increased potato microtuber numbers, but also microtuber size. The largest microtubers achieved from plantlets grown on media containing 0.5 % of activated charcoal.

The highest plant fresh weight (8.56 g) was recorded for MS medium with 0.5 % AC, while, the lowest value (2.95 g) was recorded for MS medium without AC (0%). The plant fresh weight was increased by rising AC concentration in MS medium up to 0.5%. AC at 1% in MS medium recorded lower plant fresh weight than 0.5% AC. In contrast with the present study, Lajayer *et al*. (2011) obtained the highest shoot fresh and dry weight of potato plantlets in media containing 0.1 % of AC.

Table 6: Effect of activated charcoal concentrations on microcorm diameter, microcorm length and plant fresh weight 90 days after culture

Activated charcoal (%)	Microcorm diameter (mm)	Microcorm length (mm)	Plantlet fresh weight (g)
0	0.00	0.00	2.95
0.1	9.09	15.51	5.23
0.5	9.36	9.04	8.56
1	10.49	17.14	5.20
LSD0.05	1.25	3.15	1.14

Charcoal is a form of carbon which characterized by a high adsorptive capacity for gases, vapors and colloidal solids. Activated charcoal is commonly used in tissue culture media. Use of the charcoal can induce either positive or negative effects on cultures (Pan and Van Staden, 1998). In general, the positive effects of activated charcoal might be due to inhibition of sucrose hydrolysis, adsorption of plant growth regulators, adsorption of undesirable or inhibitory substance (such as ethylene), reduction of light in root medium and releasing macro and microelements (Nissen and Sutter, 1990 and Pan and Van Staden, 1998).

References

Abo El-Nil, M. M. and F.W. Zettler, 1976. Callus initiation and organ differentiation from shoot tip cultures of *Colocasia esculenta.* Plant Science Letters*,* 6: 401-408.

Alizadeh, S., S. H. Mantell, and A. MariaViana, 1998. *In vitro* shoot culture and microtuber induction in the steroid yam *Dioscorea composita* Hemsl. Plant Cell, Tissue and Organ Culture, 53: 107–112.

Altindal, D. and T. Karadoğan, 2010. The effect of carbon sources on *in vitro* microtuberization of potato (*Solanum tuberosum* L.). Turkish journal of Field Crops,15:7-11.

Dodds, J. H., D. Silva-Rodriguez, and P. Tovar, 1992. Micropropagation of potato (*Solanum tuberosum* L.) In: Biotechnology in Agriculture and Forestry: High-Tech and Microprogation III, (Ed. Bagaj, Y.S.P), Springer, Berlin, Heidelberg, New York, 19: 91-106.

Hussain, Z. and R.K. Tyagi, 2006. *In vitro* corm induction and genetic stability of regenerated plants in taro *(Colocasia esculenta* (L.) Schott). Indian Journal of Biotechnology, 5:535-542.

Keolanui, R., S. Sanxter and J. R. Hollye, 1993. Handbook for Commercial-Scale Taro (*Colocasia esculenta*) Tissue Culture in Hawai‘i. Hawai‘i Agricultural Experiment Station. HITAHR, College of Tropical Agriculture and Human Resources, University of Hawai‘i at Manoa, 22 pp.

Khuri, S. and J. Moorby, 1996. Nodal segments or microtubers as explants for in vitro microtuber production of potato. Plant Cell, Tissue and Organ Culture,45 (3):215-222.

Kianamiri, S. and M. Hassani, 2010. The effect of benzyl adenine (BA) hormone and explants type on establisment and proliferation of Iranian dwarfing apple rootstock ˊAzayesh’ under *in vitro* condition. Acta Hort, (ISHS), No.865:135-140.

Kundu, N., P. Campbell, B. Hampton, C. Lin, X. Ma, N. Ambulos, X. F. Zhao, O. Goloubeva, D. Holt, and A. M. Fulton, 2012. Antimetastatic activity isolated from *Colocasia esculenta* (taro). Anti-Cancer Drugs, 23 :200-211.

Lajayer, H.M., B. Esmaielpour and E. Chamani, 2011. Hinokitiol and activated charcoal influence the microtuberization and growth of potato (*Solanum tuberasum* cv. Agria) plantlets *in vitro*. Australian journal of Crop Science, 5:1481-1485.

Lebot, V. and K. M. Aradhya, 1991. Isozyme variation in taro (*Colocasia esculenta* (L.) Schott) from Asia and Oceania. Euphytica, 56: 55-66.

Matthews, P.J., 2014. On the Trail of Taro: An Exploration of Natural and Cultural History. National Museum of Ethnology Senri Expo Park, Suita, Osaka, Japan. 430 pp.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue

cultures. Physiol. Plant., 15: 473- 497.
Nissen, S.J and E.G. Sutter, 1990. Stability of IAA and IBA in nutrient medium to several tissue culture procedures. HortScience, 25: 800–802.
Ooka J.J.,1994.Taro Diseases: A Guide for field identification. University of Hawaii, Hawaii Inst. Tropical Agricultural Human Resources, Research Extension Series 148, 13 pp.
Pan, M.J. and J. Van Staden, 1998. The use of charcoal in vitro, a review. Plant Growth Regulators, 26: 155-163.
Purseglove, J. W., 1972. Tropical Crops, Monocotyledons. Longman Limited, London. 607 pp.
Snedecor, G. W. and W. G. Cochran, 1980. Statistical Methods.7 th Edition, Iowa State Univ., Press, Ames, Iowa. U.S.A., pp.325-330.
Tindall, H.D., 1983.Vegetables in the Tropics. The Macmillan press LTD , London and Basingstoke. pp.187
Welander, M. and N. Pawlicki, 1994. Carbon compounds and their influence on *in vitro* growth and organogenesis. pp. 83-93. In: P. J. Lumsden, J. R. Nicholas and W. J. Davies (eds.). Physiology, Growth and Development of Plants in Culture. Kluwer Academic publisher.
Yuping, L., K. W. Dong, H.X. Fang and P. Jing, 2003. Induction of *in vitro* corms of taro *Colocasia esculenta* Schott. Acta Horticulturae Sinica, 30: 43-46.
Zhou, S. P., He, Y.K. and S.J. Li, 1999. Induction and characterization of *in vitro* corms of diploid- taro. Plant Cell, Tissue and Organ Culture, 57:173-178.