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Micropropagation protocol of Egyptian native cultivar of taro, *Colocasia esculenta* var.*esculenta*

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Abstract

Experiments were conducted to establish a protocol for taro micropropagation through meristem culture. These experiments were conducted at Faculty of Agriculture , Cairo University . The tip meristems were excised from corms of Egyptian native cultivar of taro *Colocasia esculenta* var.*esculenta*. The excised explants were surface sterilized with sodium hypochlorite in sequent steps with different concentrations. Meristems were cultured at establishment stage on Murashige and Skoog (MS) medium with 3% sucrose and 0.8 % agar. Shoots proliferation was induced in MS with 0, 1.0, 2.0, 3.0, 4.0, 5.0 or 6.0 mg/l of benzyl amino purine (BAP) or kinetin (Kin). The best number of shoots at the end of third and fourth subculture was obtained with MS with 2 mg/l BAP. At rooting stage, taro shoots were cultured on MS with 0, 0.5, 1.0 or 2.0 mg/l of IAA, IBA or NAA. Supplementation of MS media with IBA 2.0 mg/l gave the highest number of roots after 15 and 21 days. Three types of substrate mixtures, namely, vermiculate & perlite (1:1), vermiculate & peat moss (1:1) and sand & peat moss (1:1) were used at acclimatization stage. The highest surviving percentage (69.7%) was recorded in vermiculate & peat moss substrate mixture after 60 days of acclimatization.

Keywords: Taro, Micropropagation, Auxins ,Cytokinins, Substrate mixtures.

Introduction

Taro, *Colocasia esculenta* (L.) Schott 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. There are two commonly cultivated variants of *Colocasia esculenta,* which of them is *C. esculenta* (L.) Schott var. *antiquorum,* which has a small globular central corm with several relatively large cormels arising from the main corm, this variety is referred to as the 'eddoe' type of taro. The other variant is *C. esculenta* (L.) Schott var. *esculenta,* which possesses a large cylindrical central corm with only few cormels classified as the 'dasheen' type of taro **(Purseglove, 1972; Tindall, 1983 and Lebot and Aradhya, 1991)**. The Egyptian native cultivar of taro is belonging to *C. esculenta* (L.) Schott var. *esculenta* variant.

Every 100 g of taro corms possess 112 kcal, 26.46 g carbohydrate, 1.50 g protein, 0.20 total fat 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 used as an anti cancer drugs (**Kundu *et al*., 2012**).

Worldwide, the top producers of taro are Nigeria, China, Ghana and Cameroon (FAOSTAT, 2013). In Egypt, the harvested area was 8200 feddan at 2012, while the production was 120000 tons in the same year with average yield 14.6 tons per feddan (FAO statistics division, 2013). The top governorates producers at Egypt are Menofiya, Al Sharqia, Qalyubia , Assiut and Al Minia .

The cormels or divided corms are the methods of propagation of taro. The seed quantities reach to 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 agents. Taro is affected by viral diseases which decrease the yield **(Ooka, 1994).** Dasheen mosaic virus (DMV) reported as the most substantial viral pathogen infect the wild and cultivated Araceae plants **(Li *et al.,* 2002)**. Among other Araceae plants, Taro plants are highly sensitive, to DMV, which significantly reduces yield **(Keolanui *et al.,* 1993).** Prominent mosaic pattern, dwarfing and malformation are the visual symptoms of DMV **(Li *et al.,* 2002)**. Meristem culture technique is used to produce plants free of virus especially in the vegetative propagated plants **(Abo el-nil and Zettler, 1976)**. Plant tissue culture techniques have become a powerful tool for propagation of taro to overcome many problems facing traditional methods of propagation. Different explants were used to produce disease free planting material **(Hartman, 1973; Chng and Goh, 1994 and Behera and Sahoo, 2008).** The present research aimed to develop a complete protocol for micro propagation of Egyptian native cultivar of taro using shoot tip culture.

Materials and Methods

Plant materials

Experiments were conducted at tissue culture laboratory of Vegetable Crops Dep., Fac. Agric., Cairo Univ., during the period from Nov. 2012 to April 2015 . 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 residues and roots. 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 leaf 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 15mintues, 75 % for 10 minutes and 50% for 5 minutes. Dead tissues were removed after each time. The excised meristems (0.5-1 cm) (Fig. 1) were washed with sterilized distilled water 5 times.

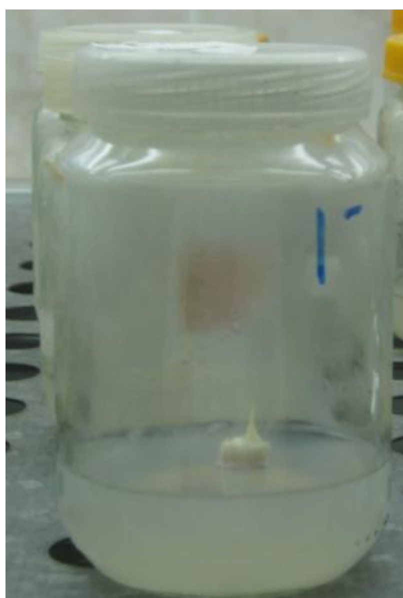

Fig. 1. Taro explants (Tip meristem).

Establishment stage

At establishment stage, meristems were cultured in ½ (MS) strength medium with 3% sucrose and 0.8 % agar **(Murashige and Skoog, 1962).** The excised explants were cultured at 24±2C° for 30-45 days or up to forming the first true leave. The contaminated percentage and dead percentage were recorded weekly.

Multiplication stage

The individual shoots, which formed at the end of establishment stage, were re- cultured on MS media having 0, 1.0, 2.0, 3.0, 4.0, 5.0 or 6.0 mg/l of benzyl amino purine (BAP) or kinetin (kin.). Each treatment had 6 jars, containing 35-40 ml of medium . The pH at all media was adjusted to be 5.6-5.8, while they were autoclaved at 121ºC under a pressure of 1.5 kg/cm^2 for 20 min. The duration of multiplication stage was four subcultures. Each subculture period ranged from 30-45 days. Number of shoots at the end of each multiplication subculture was recorded.

Rooting stage

Taro shoots, which formed on MS medium with 2 mg/l BAP, were used at this stage. They were re cultured on MS media with 0, 0.5, 1.0 and 2.0 mg/l of IAA, IBA or NAA. Each treatment consisted of 6 jars containing 40 ml of medium. The pH of all media was adjusted to be 5.6-5.8. Number of roots, number of leaves, plantlet length and root length were recorded, 15 and 21 days after shoots culture. The period of rooting stage ranged from 30-35 days.

Acclimatization stage

At the end of the rooting experiment, all survived plantlets were adapted. Three types of substrate mixtures, namely, vermiculate & perlite (1:1), vermiculate & peat moss (1:1) and sand & peat moss (1:1), were used at acclimatization stage. Firstly, the three types of substrate mixtures were autoclaved at 121ºC under a pressure of 1.5 kg/cm^2 for 20 min. Secondly, the plantlets were washed with tap water to remove the media residues. In addition, the plantlets roots were soaked in distilled water + fungicide 1 g/l for 2 minutes. After all of these steps, the plantlets were cultured in plastic cups full with the different substrate mixtures. The plastic cups were covered with poly bags. Subsequently, holes were made gradually in poly bags up to they were removed totally. The survived plantlets were cultured on the same substrate mixtures after 60 days of acclimatization and transferred to net house. Survival percentage, plantlet length and number of leaves were recorded 45 and 60 days after plantlets transferring into the acclimatization medium.

Statistical analysis

Regular analyses of variance of Complete Randomize Design (CRD) were performed of obtained data. $LSD_{0.05}$ was calculated for comparing means **(Snedecor and Cochran, 1982).**

Results and Discussion

1. Effect of different benzyl amino purine and kinetin concentrations on taro multiplication

The response of taro shoots cultured at different shoot proliferation media over the period of 4-5 months during 4 subcultures is shown in Table 1. Data showed that BAP produced significantly higher number of shoots as compared to Kin. There was an increment in the number of shoots at all concentrations of BAP or kin. with increasing number of the subcultures, so the highest shoots number was noticed at the end of the fourth subculture. However, such increment differed according to plant growth regulators and their concentrations. On the other hand, the free hormone MS medium (0 mg BAP or kin/l) showed stability in the number of shoots during four subcultures. The mean of number of shoots was only 1.00 in the control treatment. At first subculture, the highest number of shoots (2.80 shoots) was obtained by using MS medium + 5 mg kin/l followed by MS + 4 mg BAP/l (2.20 shoots). At the second subculture, MS medium + 4 mg kin/l recorded the highest number of shoots among all concentrations of BAP or Kin. At third subculture, the MS medium + 2 mg BAP/l showed the highest number of shoots (8.20) exceeded, in this respect, all other BAP and kin. concentrations. This medium (MS medium + 2 mg kin/l) showed the same superiority at fourth subculture compared with the other BAP and kin. concentrations. At the fourth subculture, all the concentrations of BAP showed higher values of number of shoots than the same concentrations of Kin. The raise of number of shoots reached 4.1, 3.29, 2.08, 3.68, 2.28 and 2.59 fold at 1, 2, 3, 4, 5 or 6 mg BAP/l compared with the same concentrations of kin. In addition, starting from the second subculture, the MS + 2 mg BAP/l showed superiority in number of shoots among all treatments either BAP or kin. concentrations. It recorded 2.40, 8.20 and 24.40 shoots at the second, third and fourth subculture, respectively **(Fig.2)**. It is well known that

Table 1. Effect of BAP and kin on number of shoots at the end of each multiplication subculture.

Plant growth regulators (PGR)	Concentration (mg /l) \ Subculture	1	2	3	4	Mean
Benzyl amino purine (BAP)	**0**	1.00	1.00	1.00	1.00	1.00
	1	1.60	2.00	5.60	16.40	6.40
	2	1.40	2.40	8.20	24.40	9.10
	3	1.80	1.80	4.40	15.40	5.85
	4	2.20	2.20	5.60	18.40	7.10
	5	2.00	2.00	4.80	16.80	6.40
	6	1.40	1.60	2.60	11.40	4.25
Mean		1.62	1.86	4.60	14.83	5.73
Kinetin (kin.)	0	1.00	1.00	1.00	1.00	1.00
	1	1.00	1.40	2.80	4.00	2.30
	2	1.60	2.20	4.80	7.40	4.00
	3	2.20	2.20	5.00	7.40	4.20
	4	2.00	2.80	4.80	5.00	3.65
	5	2.80	1.60	4.20	5.00	3.40
	6	1.80	2.20	4.00	4.40	3.10
Mean		1.77	1.91	3.80	4.89	3.09
LSD 0.05	**PGR × Conc.**	2.80				
	PGR × Subculture	2.12				
	Conc. × Subculture	3.97				
	PGR × Conc. × Subculture	5.62				

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Fig. 2. Cluster of taro plantlets produced from single meristem was cultured at MS medium+ 2mg/l BA after fourth subculture.

cytokines play an important role in plants at growth and development. It also regulate flowering and fruit formation by stimulating cell division (**Kianamiri and Hassani, 2010)** .It is known that the multiplication stage requires cytokines during the micro propagation of various plants. This requirement appears to differ depends on type of crop plants, explant type, phase of development, the concentration of plant growth regulators, the interaction between plant growth regulators and environmental conditions **(Yokoya and Handro, 1996).** Cytokines is a group of plant growth regulators such as thidiazuron (TDZ), benzyl amino purine (BAP), kinetin (Kin) or zeatin. The high concentration of BAP or Kin. (6 mg/l) produced low number of shoots in comparison with the lower concentration in the present study. This may be due to the toxicity of the high concentration of cytokines which caused a delay in shoot formation. The present results are in agreement with those of **Chang and Goh (1994)** who reported that the addition of BAP into culture medium enhanced growth and development of shoot auxiliary buds of *C. esculenta* var *esculenta* (L.) Schott. Also, **Seetohul *et al.* (2008)** showed that the highest multiplication rate of *in vitro* shoot tips of taro was recorded in MS medium supplemented with either BA at 2 mg/l or TDZ at 0.9 mg/l. However, many reports showed that the high multiplication rate and the number of shoots demanded the combination between auxins and cytokinins **(Jing *et al.*, 2004; Lin *et al.*, 2004; Behera and Sahoo, 2008).** On the other hand, **Hutami and Purnamaningsih (2013)** used two types of cytokines at multiplication stage. They found that, after three months of culture, the highest shoot number was obtained in MS media with 2 mg BA/l+ 1 mg of thidiazuron (TDZ). Data also showed that the means of total number of shoots at the MS free of hormone media was the worst media between all the concentrations. Also, MS +1mg kin/l ranked as second at low number of shoots.

2. Effect of different concentrations of IAA, IBA and NAA on rooting of taro shoots.

The response of taro shoots cultured on MS medium with different concentrations of IAA, IBA or NAA on root number after (15 & 21 days) and root length after 21 days is shown at Table 2. 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 high concentration of IBA (2 mg /l). The number of roots at this concentration was 8.50 and 14.50 after 15 and 21 days, respectively. However, the number of roots did not show the same response under the same concentration of IAA or NAA.

Table 2. Effect of different concentrations of IAA, IBA and NAA on root number after 15 & 21 days and root length after 21 days from shoot culture.

Auxins	Conc. (mg/l)	No. roots (15 days)	No. roots (21 days)	Root length (cm)
IAA	0	5.50	10.25	3.13
	0.5	5.80	8.40	6.60
	1	4.00	4.00	5.40
	2	2.50	9.50	3.13
Mean		4.45	8.04	4.56
IBA	0	5.50	10.25	3.13
	0.5	6.00	11.75	4.73
	1	4.25	8.00	2.70
	2	8.50	14.50	2.25
Mean		6.06	11.13	3.20
NAA	0	5.50	10.25	3.13
	0.5	0.75	2.75	1.88
	1	1.33	4.33	7.00
	2	5.40	10.00	7.72
Mean		3.24	6.83	4.93
$LSD_{0.05}$		**7.01**	**9.12**	**4.85**

Similar result was obtained by **Nandwani (2009)** as the basal MS medium containing IBA with concentration (1.0 mg/l) was the best medium for rooting of regenerated taro shoots. On the other hand, **Behera and Sahoo (2008)** found that the rooting of taro shoots was better in the ½ MS medium + 1.5 or 2.0 mg NAA/l than the ½ MS medium + 1.5 or 2.0 mg IBA/l. After 15 days of culture, MS + 0.5, 1 and 2 mg IAA/l recorded higher number of roots than the same concentrations of NAA. The same result was obtained by MS +0.5 mg IAA after 21 days of culture. This may be due to that IAA is a natural auxin more likely to be recognized by the receptors in plants. However, NAA is a synthetic auxin, so that if it was added at the same concentration may have an inhibition effect (**Hutami and Purnamaningsih, 2013**). MS + 1 mg/l or 2 mg/l of NAA showed, respectively, the longest roots (7.00 cm and 7.72 cm) after 21 days of culture among all treatments. On the other hand, 0.5 mg/l of NAA showed the lowest root length and the lowest number of roots after 15 and 21 days of culture among all treatments. The same concentration (0.5 mg/l of NAA) showed hardly any rooting of cultured taro shoots during 4 weeks **(Behera and Sahoo, 2008).**

Data in Table 3 show that MS+ 2 mg/l of IAA, IBA or NAA recorded the highest number of leaves (5.50, 5.50 and 4.60, respectively) after 15 days of culture. After 21 days, MS medium with 2 mg/l IAA produced more leaves (6.25), while that with 2 mg NAA/l recorded a lower number of leaves and 2 mg IBA/l showed in leaf number stability value (5.50). The active effect of IAA on increasing leaf number may be due to one of physiological effects of IAA, where the concentration of IAA in cells was near or within the abscission zone appears to delay the abscission process. NAA at 0.5 mg/l showed the lowest number of leaves among all treatments after 15 days and after 21 days. The previous results are compatible with the results from Table 2 with root length and number of roots after 15 and 21 days. No significant difference was detected among treatments on plant length of shoots and number of shoots after 21 days. These results may be attributed to the role of auxins for enhancement of rooting and not shoot formation, where the development of lateral buds is inhibited by auxins produced at the apical meristem and transported down the stem (**Yokoya and Handro, 1996 ; Bhuiyan, *et al.,* 2011**). Fig.3 shows Taro shoot after rooting stage.

Table 3. Effect of different concentrations of IAA, IBA and NAA on number of leaves after 15 & 21 days and shoot length and number after 21 days from shoot culture.

Auxins	Conc. (mg/l)	No. leaves (15 days)	No. leaves (21 days)	Plant length (cm)	No. Shoots
IAA	0	3.00	4.75	7.20	1.75
	0.5	3.20	3.80	11.62	0.00
	1	2.80	3.67	7.03	0.00
	2	5.50	6.25	10.78	1.25
Mean		3.62	4.62	9.16	0.75
IBA	0	3.00	4.75	7.20	1.75
	0.5	4.25	5.50	9.73	1.50
	1	4.00	5.75	9.05	1.25
	2	5.50	5.50	11.60	1.00
Mean		4.18	5.37	9.39	1.37
NAA	0	3.00	4.75	7.20	1.75
	0.5	1.50	2.50	7.25	0.50
	1	3.67	3.67	11.87	1.33
	2	4.60	4.75	7.20	1.75
Mean		3.19	3.92	8.38	1.33
$LSD_{0.05}$		**3.71**	**3.85**	**NS**	**NS**

Fig.3. Taro shoots after rooting

3. Effect of substrate mixtures on acclimatization of *in vitro* taro plantlets

Table (4) shows the effect of substrate mixtures on plantlets surviving % after 45 and 60 day of adaptation. Data showed that the lowest surviving percentage was at vermiculate+ perlit (1:1) substrate after 45 and 60 days from adaptation (16 % and 0%, respectively). On the other hand, vermiculate + peat moss (1:1) substrate recorded the highest surviving percentage after 45 (86.04 %) 60 days (69.70 %). These results agree with those of **Ping *et al.* (2003)** who reported that the survival ratio was affected by the culture media of acclimatization and the vermiculate was the best. In addition **Chand *et al.* (1999)** showed that the plants grown in vermiculate developed a better root system than those in soil potting mixture but both recorded good growth during acclimatization stage. The vermiculate + peat moss substrate and sand+ peat moss substrate showed little difference in surviving percentage. The surviving percentage was 82.5 % in sand + peat moss substrate after 45 days of acclimation, while this percentage went down after 60 days to be 67.5 %. Although, when **Minas (2002)** adapted the micropropagated taro transplants in sphagnum peat moss in plastic pots, the survival rate recorded 50% after 4 weeks, while the survival rate reached 95% under the foggy greenhouse conditions. Also, in an experiment in East of Africa, **Seetohul *et al.*, (2008)** adapted a regenerated taro shoots on different combinations of substrates. They reported that using peat moss only as a substrate recorded 0 % mortality, while the combination between peat moss and vermiculate increased the mortality to 20 %. Fig. (4) illustrate taro transplants during first week of acclimatization at laboratory. Table (5) shows insignificant differences among the three types of mixture substrates in both plantlet height and number of leavers after 45 days of acclimation. However, there were significant differences among treatments after 60 days of acclimation. Vermiculate + perlit substrate mixture had the lowest values of plantlet height and number of leavers. In addition, vermiculate + peat moss recorded the highest value of plant height and number of leaves without significant differences between this treatment and vermiculate + sand. This is in agreement with results of **Seetohul *et al*. (2008)** who showed that the 50% peat moss + 50 % vermiculate substrate was in the second level on number of leaves and length of plantlets between seven substrate mixtures. It is noted that the number of leaves were decreased after 60 days due to defoliation during hardening. The interpretation of the low surviving percentage at vermiculate + perlit substrate is that this substrate provides low support for the small transplants. Meanwhile, water flowed through this substrate quickly in comparison with using the two other substrates. In addition, using peat moss in the two other substrates provided a steady water supply to the confined roots of transplants. Also, the fibrous nature of peat moss lends structure and physical stability to both mixture substrates. Fig (5) illustrate Taro transplants after 60 days of acclimatization.

Table 4. Effect of acclimatization substrate mixtures on taro plantlets survival after 45 and 60 days.

Days of acclimatization	Substrate mixture	% surviving
45 days	Vermiculate +Perlit (1:1)	16
	Vermiculate + Peat moss(1:1)	86.0
	Sand + Peat moss (1:1)	82.5
60 days	Vermiculate +Perlit (1:1)	0
	Vermiculate + Peat moss(1:1)	69.7
	Sand + Peat moss (1:1)	67.5

Table 5. Effect of acclimatization substrate mixtures on taro plantlets height and number of leaves after 45 and 60 days.

Days of acclimatization	Substrate mixture	Plant let height (cm)	No. leaves
45 days	Vermiculate +Perlit (1:1)	12.3	2.5
	Vermiculate + Peat moss(1:1)	12.7	2.9
	Sand + Peat moss (1:1)	12.5	2.7
	$LSD_{0.05}$	NS	NS
60 days	Vermiculate +Perlit (1:1)	00.0	0.0
	Vermiculate + Peat moss(1:1)	13.0	2.7
	Sand + Peat moss (1:1)	12.9	2.5
	$LSD_{0.05}$	1.8	0.4

Fig.4. Taro transplants during first week of acclimatization at laboratory

Fig. 5. Taro transplants after 60 days of acclimatization

Conclusion

At Multiplication stage, the MS medium + 2 mg BAP/l showed the highest number of shoots at the fourth subculture. At rooting stage, the highest number of roots after 15 and 21 days of culture was obtained by the shoots cultured on MS medium + IBA (2 mg /l). At Acclimatization stage, vermiculate + peat moss substrate recorded the highest surviving percentage.

References

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

Behera, K. K. and Sahoo, S. 2008. *In vitro* micropropagation of *Colocasia esculenta* (L.) Schott.(cv local-jhankhri) through corm sprouts. The Orissa journal of Horticulture, 36: 50-54.

Bhuiyan, M.K.R, Hossain, M.J.; Rahman,M.S; Rahman, S.M.L and Sattar, M.A.2011. Root initiation in mukhikachu (*Colocasia esculenta)* as influenced by IAA and NAA. Bangladesh.J.Agril.Res.36:487-494.

Chand, H., Pearson, M. N. and Lovell, P.H. 1999. Rapid vegetative multiplication in *Colocasia esculenta* (L.) Schott (taro). Plant Cell Tissue & Organ Culture, 55: 223-226 .

Chng, R. C. and Goh C. J., 1994. High frequency direct shoot regeneration from corm axillary buds and rapid clonal propagation of taro, *Colocasia esculenta* var. *esculenta* (L.) Schott (Araceae). Plant Science , 104: 93-100.

Hartman, R.D. 1973. Dasheen mosaic virus and other phytopathogens eliminated from caladium, taro and cocoyam by culture of shoot tips. Phytopathology, 64:237-240.

Hutami, S.and Purnamaningsih, R. 2013. Shoot multiplication of taro (*Colocasia esculenta* var. *antiquorum)* through in vitro culture . Proceeding International Conference.The 4th Green Technology Faculty of Science and Technology Islamic of University State Maulana Malik Ibrahim Malang. 34- 40.

Jing, M., Fu, B.X., Man, J.X. and Ping, Z.H.2004. Effects of growth regulators on rapid propagation of virus free plantlets of taro. Journal of Anhui Agricultural University . 31:466-468.

Keolanui , R., Sanxter, S. and Hollye, J. R. 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**.**

Kianamiri, S. and Hassani, M. 2010. The effect of benzyl adenine (BA) hormone and explants type on establisment and proliferation of iranian dwarfing apple rootstock Azayesh' under *in vitro* condition. Acta Hort, (ISHS), 865:135-140.

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

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

Li, Y.,XU.C. and Chen, J. 2002. Establishment of virus-free taro (*Colocasia esulenta* cv. Fenghuayunaitou) by meristem-tip culture combined with thermotherapy. Plant Pathology Journal, 1: 40 -43.

Lin, T.Q., Yu, L., Rong, L., Ming, S. and Fu, L.J.2004. Optimization of *in vitro* rapid propagation system for taro. Journal of Southwest Agriculture University , 26:686-688.

Minas, G.J. 2002. *In-vitro* micro-propagation of kolokasi from apical meristem. .Miscellaneous reports. Nicosia, Cyprus. 84: 8pp.

Murashige, T. and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.

Nandwani, D. 2009. PGRs and agriculture development in the American pacific. Proceedings of the 36th Annual Meeting of the Plant Growth Regulation Society of America, Asheville, North Carolina, USA, pp.162- 168.

Ooka J.J.1994. Taro diseases: A guide for field identification. University of Hawaii, Hawaii Inst. Tropical Agricultural Human Resources, Research Extension Series.148 pp 13.

Ping, L.Y., Dong, K. W., Fang, H.X. and Jing, P. 2003. Induction of *in vitro* corms of taro *Colocasia esculenta* Schott. Acta Horticulturae Sinica, 30: 43-46.

Purseglove, J. W. 1972. Tropical Crops, Monocotyledons. Longman Limited, London. 607 PP.

Seetohul, S., Puchooa, D. and Ranghoo-Sanmukhiya, V.M.2008. Genetic improvement of Taro (*Colocasia esculenta* var *esculenta*) through *in-vitro* mutagenesis. Uom Research Journal, 13:79-89.

Snedecor, G. W. and Cochran, W. G.(1982). 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.187 pp.

Yokoya, N.S. and Handro, W. 1996. Effects of auxins and cytokinins on tissue culture of *Grateloupia dichotoma* (Gigartinales, Rhodophyta). Hydrobiologia, 326/327: 393–400.

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