IN VITRO MULTIPLE SHOOT REGENERATION IN AQUA AROIDS, *COLOCASIA ESCULENTA* L.SCHOTT STOLONIFEROUS USING STOLON BUD EXPLANTS

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Abstract

In this research study *in vitro* regeneration protocols were established for *Colocasia esculenta* L. Schott (stoloniferous) using stolon bud explant. MS supplemented with different concentration and combinations of auxins and cytokinins were used for the induction of direct and indirect organogenesis and rootings. 91 % culture responded to shoot proliferation using stolon bud explants on MS having 6.0 mg L $^{-1}$BAP +1.0 mg L^{-1} NAA. Direct multiple shoot regeneration (83%) was also observed on MS supplementing 6.0 mg L $^{-1}$BAP +1.0 mg L^{-1} NAA. *In vitro* derived petiole callus induction (65 %) was found in *C. esculenta* L. Schott on MS media containing 1.0 mg L $^{-1}$ BAP + 2.0 mg L^{-1} 2, 4-D. I*n vitro* derived callus regeneration (60 %) was observed in media having 1.0 mg L $^{-1}$BAP + 1.5 mg L^{-1}NAA in *C. escuelnta* (stoloniferous). Profuse root development was found MS having 0.5 mg L^{-1}IAA. Regenerated plantlets were acclimatized with all agronomic practices in the plastic pots for normal growth and development in successfully.

Key words: Aqua aroid, callus, *colocasia esculenta, in vitro*, regeneration, MS.

1.Introduction

The under utilized Crops, aqua aroid locally called panikachu belongs to the genus *Colocasia*, within the subfamily colocasioideae of the monocotyledonous family Araceae, is the staple food in many countries eg. Hawaii, PNG, Fiji and important secondary food over the Africa and Asian countries. The Araceae includes near about 106 genera and more than 2000 species occur in the tropics and subtropics. Of the two botanical varieties *i) Colocasia esculenta* (L.) Schott var. esculenta *ii) Colocasia esculenta* (L.) Schott var.antiquorum (Schott) which is synonymous with *C. esculenta* var.globulifera [**1**]**.** The sterile appendage is a distinguishing taxonomic characteristic between dasheen and eddoe types of taro. In eddoe types, the sterile appendage is longer than the male section of the spadix; in dasheen types, the appendage is shorter than the male section. In Bangladesh, several types of aroid genera and species occur in different locations /districts, which can be categorized into edible, poisonous, medicinal and ornamental. The whole plant of panikachu including leaf, petiole, rhizome, corms, flowers, stolons are edible in Bangladesh for animal and human beings in different ways. The rhizome and stolons are the main edible part which are very popular and export vegetable in Bangladesh. They play a significant role for nutrient status, medicinal and industrial values. It constitutes moisture 63 - 85 %, carbohydrate (mostly starch) 13 - 29 %, protein 1.4 - 3 %, fat 0.16 - 0.36 %, crude fibre 0.60 - 1.18 %, ash 0.60 - 1.3 %, vitamin C 7 - 9 mg /100g, Thiamine 0.18mg /100g, riboflavin 0.04mg/100g, niacin 0.9mg/100g [**2, 3**]**.** It can helps Bangladesh to reduce food deficit in every year and maintain the substitute the present food habit. The diversity of taro is fast disappearing from many parts of the world, in part because of dietary changes and urban migration as well as pests and diseases. The wide genetic diversity must be exploited both directly by the evaluation of cultivars regarding their resistance to disease, yield and nutritional value and by genetic improvement. *In vitro* cultivation enables healthy and easily transportable propagation material to be obtained. Now the alomae/ bobone disease is

mainly confined to the Pacific, the dasheen mosaic virus disease occur world wide. Most taro producing countries in the Asia/ Pacific region have these diseases [4]. Severe cases of alomae can result in total crop loss, while bobone can cause up to 25 % yield loss. So the present investigation helps to improve large number better disease resistant high yielding genotypes rapidly of the whole year round using this protocols..

2.Materials and Methods

2.1.Materials

2.1.1.Plant materials

In this present study panikachu (aqua-aroid), the important edible aroid was selected for *in vitro* micropropagation evaluation. This was collected from Bangladesh Agricultural Research Institute (BARI), Joydevpur and different possible local cultivated areas of Bangladesh. Collected plant materials were maintained in the experiment farm of Institute of Biological Institute, Rajshai University, Rajshahi, Bangladesh. Different plant parts (leaf petiole, stolon buds) were used as explants for *in vitro* culture purpose.

2.1.2..Laboratory equipment and chemicals:

All recommended laboratory equipments, chemical reagents and MS basal media were used for this study.

2.2. Methods

The explants, stolon buds were collected from the field grown plants and taken to the laboratory. The excess unnecessary parts like roots, over mature leaves were removed and then these were cut into segments into convenient size, if necessary. Then the explants were transferred into savlon (powerful germicide eg.- chlorthexidine gluconate+cetrimide) water (1ml /l) for 15 minutes and washed thoroughly under running tap water for one hour to remove lose contaminants attached to explants and taken into a conical flask containing distilled water with a few drops of tween - 80 for about 7 minutes. This was followed by second washing with distilled water to remove all traces of above chemicals. Surface sterilization was carried by dipping in 0.1 % H_gCl_2 by gentle shaking for 18 minutes according to nature of explants. The materials were washed five times with sterile distilled water immediately to remove all traces of $HgCl_2$. MS [5] basal medium was used for the purpose of direct shoot regeneration, callus induction, callus regeneration. The pH of the medium was usually adjusted to 5.70 ± 0.1 with 0.1 N KOH. The culture nutrient media were gelled with 8 g. for 1 litter of agar medium. Then the whole mixture was gently heated on micro oven until the agar melted completely making the solution clear. The prepared melted medium was dispersed into culture vessels like test-tube or conical flask while medium still hot. The culture vessels were plugged with plastic cap. The culture vessels containing medium were then autoclaved at 1.1 kg /cm^2 pressure and at temperature of 121 ^oC for 20 minutes to ensure sterilization. Then the vessels with the medium were allowed to cool vertically and marked with the help of a glass marker to indicate specific hormonal supplements. The sterilization was done in front of the laminar air flow cabinet and it was equipped with all necessary devices. Sterilized explants were taken in a petri dish by sterilized forceps and incised at right angles by cold sterile scalpel at required size of explants depending on the nature of experiment. Prepared explants were carefully inoculated in culture vessels containing sterilized agar gelled

medium. After inoculation the culture vessels were sealed by or plastic cap and labeled by glass marker with inoculation date. The inoculated culture vessels were then incubated in a growth chamber containing special culture environment. The cultures were maintained at 27 ± 1 ^{o} C under the warm florescent light intensity varied from 2000 - 3000 lux. The photoperiod was maintained generally 16 hours light and 8 hours dark. Experiments for adventitious root formation on the shoots proliferated *in vitro* were conducted only after having sufficient amount of shoot cultures. After 10 -12 weeks, the usable shoot (> 2 cm) from proliferated multiple shoots were separated and individual shoots were placed in rooting media supplemented with different concentrations of auxins and without auxins. Data of different parameters from different treatments of shoot proliferation were recorded after four weeks of culture and computed in percentage using formula. Experiments were consisted of 10 explants and each of the experiment repeated thrice and means values were calculated separately for each replication.

3. Results and Discussion

3.1.Direct regeneration

Stolon buds explants were initially cultured on to MS medium containing different concentrations of BAP, Kn, NAA, IBA, IAA and 2, 4-D. Data on morphogenic responses were collected after four weeks inoculation and results were summarized and presented in **Table 1(a) and Fig.(a).** Stolon bud explants cultured on to medium with 2.0, 3.0, 4.0, 5.0 and 6.0 mg L^{-1} BAP did not show significantly response on morphogenic traits. Different concentrations of Kn and IBA do not show any response on morphogenic traits. Highest percentage of shoots was noticed on MS medium supplemented with 1.5 mg L^{-1} NAA and shoot lengths were highest (3.60 cm) in the media formulation.

In this experiment the effect of BAP with NAA on stolon bud culture in respect of shoot proliferation was studied. Results obtained on the percentage of cultures responded to shoot proliferation percentage, number of shoot per plant and shoot lengths are shown in **Table – 1(b)** and **Fig.a**. Highest percentage (91.00) of culture responded to shoot proliferation in 6.0 mg L^{-1} BAP +1.0 mg L^{-1} NAA. On the other hand lowest percentage (24.00 %) was recorded in 5.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA. The average number of shoots per explant was 3.60 and average length of shoot 3.60 cm was recorded after 25 days of culture. The highest percentage (22.00) was recorded in 5.0 mg L^{-1} BAP + 2.5 mg L^{-1} IBA. Average number of shoots was found and shoot length varies 0.30 cm to 2.10 cm (5.0 mg L^{-1} BAP + 2.5 mg L^{-1} IBA). Effect of different concentrations of BAP and IAA on shoot proliferation from stolon bud was also observed on percentage of shoot proliferation, the number of shoots per culture and average shoot length. The percentage of shoot forming explants was very low in combination with 5.0 mg L^{-1} BAP+1.5 mg L^{-1} IAA (6.20). On the other hand highest percentage (12.00) was recorded in 5.0 mg L^{-1} BAP + 2.0 mg L^{-1} IAA. Number of shoots per plant was same and shoot length varies from 1.50 cm to 1.90 cm. Effect of different concentrations of BAP and NAA on multiple shoot proliferation from stolon axillary bud.

3.1.1.Direct multiple shoot regeneration

In this experiment effect of BAP with NAA on stolon bud culture in respect of multiple shoot proliferation was studied. Results obtained on the percentage of cultures responded to multiple shooting, average number of shoot per culture and shoot lengths are shown in **Table –1(c) and Fig-b.** Lowest percentage of (24.00) culture responded to multiple shoots in 4.0 mg L^{-1} BAP + 2.5 mg L^{-1} NAA. On the other hand highest percentage (83.00) was recorded in 6.0 mg L^{-1} +1.0 mg L^{-1} NAA with aging. Highest number (7.80) of shoot and shoot length (3.80 cm) also observed in this concentrations. In case of different concentration of BAP

and 2, 4-D was also showed multiple shoot formation, number of shoot per plant and shoot length variations. Highest percentage of multiple shoot production from stolon bud explant was (58.00) in 1.0 mg L^{-1} BAP +1.0 mg L^{-1} 2, 4-D. Number of shoot length varies from 2.60 to 4.20 and shoot length varies from 2.60 cm to 3.80 cm in1.0 mg L^{-1} BAP + 1.0 mg L^{-1} 2, 4-D.

Table-1(a): Effect of different concentration of BAP, IAA and NAA on direct shoot proliferation singly from stolon bud explants of *C. esculenta* L.Schott.

Growth regulator mgL^{-1}	Days to shoot proliferation	Percentage of shoot formation	No. of shoot per Plant	Shoot length Cm
BAP			**Mean±S.E**	**Mean±S.E**
2.00	-	-	-	-
3.00	-	-	-	-
4.00	17	4.00	1.00±0.0000	.0.1800±0.0020
5.00	17	11.00	1.20±0.2000	0.600±0.00316
6.00	17	5.400	1.00±0.000	0.18±0.0020
NAA				
0.50	-	-	-	-
1.00	17	51.00	1.00±0.000	3.00±0.3161
1.50	17	45.00	1.00±0.000	3.60±0.5099
2.00	17	39.00	1.00±0.000	2.00±0.3162
2.50	17	28.00	1.60±0.2449	2.00±0.3162
IAA				
1.00	-	-	-	-
1.50	-	-	-	-
2.00	17	15.00	1.00±0.000	1.60±0.1871
2.50	17	33.00	1.00±0.000	1.80±0.1225
3.00	17	28.00	1.00±0.000	1.50±0.1581

- = no response

Table 1(b): Effect of different concentrations and combinations of BAP, IBA, NAA,IAA on direct shoot proliferation from stolon bud explants of *Colocasia esculenta* L. Schott.

Growth regulator mgL^{-1}	Days to shoot proliferation	Percentage of shoot formation	No. of shoot per Plant	Shoot length Cm
BAP+IBA			**Mean ±S.E**	**Mean ± S.E**
5.00+1.00	17	06.00	1.00±.000	0.300±0.005
5.00+1.50	17	12.00	1.00±0.00	0.860±0.009
5.00+2.00	17	12.00	1.00±0.00	1.04±0.1288
5.00+2.50	17	22.00	1.00±0.00	2.10±0.2449
5.00+3.00	17	9.00	1.00±0.00	1.10±0.0010
BAP+NAA				
5.00+1.00	17	24.00	1.00±0.00	0.82±0.1114
5.00+1.50	17	48.00	1.20±0.20	1.50±0.1581
5.00+2.00	17	64.00	1.40±0.24	1.90±0.2449
5.00+2.50	17	72.00	1.40±0.24	3.00±0.1581
5.00+3.00	17	55.00	1.60±0.24	2.60±0.2449
6.00+0.50	17	76.00	2.40±0.244	2.50±0.2236
6.00+1.00	17	**91.00**	**3.60±0.244**	**3.60±0.5099**
6.00+1.50	17	69.00	2.40±0.244	3.40±0.4000
6.00+2.00	17	64.00	2.00±0.316	2.20±0.2000
6.00+2.50	17	54.00	2.00±0.316	1.90±0.0010
BAP+IAA				
5.00+1.50	17	6.00	1.00±0.000	1.80±0.1225
5.00+2.00	17	12.00	1.00±0.000	1.50±0.2236
5.00+2.50	17	9.00	1.00±0.000	1.90±0.3317

Table 1(c): Effect of different concentrations and combinations of BAP, NAA, 2, 4-D on multiple shoot proliferation from stolon bud explants of *C. esculenta* L.Schott.

Growth regulator (mgL^{-1})	Days to shoot proliferation	% of shoot formation	No. of shoot per plant	Shoot length cm
BAP+2,4-D			**Mean±S.E**	**Mean±S.E**
1.00+0.50	30	**36.00**	2.60±0.244	2.60±0.244
1.00+1.00	30	58.00	4.20±0.583	3.80±0.200
1.00+1.50	30	52.00	3.00±0.316	3.10±0.244
1.00+2.00	30	34.00	2.80±0.200	3.20±0.200
1.00+2.50	30	14.00	2.80±0.200	3.10±0.244
BAP+NAA				
4.00+0.50	30	-	-	-
4.00+1.00	30	24.00	3.00±0.000	2.10±0.000
4.00+1.50	30	40.00	3.00±0.000	2.40±.244
4.00+2.00	30	60.00	2.80±0.200	2.70±0.200
4.00+2.50	30	24.00	2.80±0.200	2.50±0.223
5.00+0.50	30	29.00	1.60±0.244	1.10±0.290
5.00+1.00	30	52.00	2.90±0.001	1.80±0.200
5.00+1.50	30	52.00	3.00±.000	2.80±0.122
5.00+2.00	30	38.00	2.90±0.001	2.20±0.122
5.00+2.50	30	38.00	3.10±0.249	2.50±0.158
6.00+0.50	30	54.00	3.20±0.200	2.60±0.244
6.00+1.00	30	**83.00**	**7.80±0.663**	**3.80±0.339**
6.00+1.50	30	73.00	6.00±0.547	3.80±0.374
6.00+2.00	30	57.00	4.20±0.583	3.60±0.374
6.00+2.50	30	41.00	3.20±0.200	3.60±0.894

3.2.Callus induction

Different concentrations (0.5, 1.0, 1.5, 2.0, 2.5 mg L^{-1}) of auxin 2, 4-D and different concentrations (0.50, 1.0, 1.5, 2.0 mg L^{-1}) of BAP was used in combination to investigate the initiation of callus and its subsequent

regeneration. Petiole segments were used as explants for callus induction. Horizontally oriented explants produced callus throughout their entire surface more rapidly than those, which were placed vertically. Therefore petiole explants were always placed horizontally on the agar surface to induce callus in all experiments. It was further observed that IBA, IAA, NAA alone or in combinations with cytokinins failed to initiate callus. The effect of different concentrations BAP with different concentrations of 2, 4-D were studied and their results are presented in **Table-2 and Fig- (c&d)**. In present investigation petiole explants excised from *in vitro* grown plants (*Colocasia esculenta* (L) Schott) were used to investigate the effect of different hormonal concentrations and combinations. Explants were cultured on MS media supplemented with different concentrations and combinations of BAP and 2, 4-D. Callus proliferation was not noticed in all media formulation, days to callus initiation occurred within 50 days. Frequency of callus formation ranged from 5 % to 65 %. Highest percentage (65 %) of callus formation occurred in MS medium containing 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} 2, 4-D and followed by 55 % in MS medium containing 1.0 mg L^{-1} BAP + 2.5 mg L^{-1} 2, 4-D. In most cases colour of the callus was green, light green and dark green and texture of calli were compact and friable. Calli were dried gradually in a few days or they were alive in very short term. The optimum callus growth in terms of fresh weight was 2.00 ± 0.158 g. in 1.0 mg L^{-1} BAP +1.5 mg L^{-1} 2, 4-D followed by 1.80 ± 0.122 g. in 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} 2, 4-D. The minimum callus weight was 1.20 ± 0.003 g obtained in 2.0 mg L^{-1} BAP + 2.0 mg L^{-1} 2, 4-D.

Table-2: Effect of phytohormones on induction of calluses from petiole explants of (*c esculenta* L.Schott.).

Treatments mgL^{-1}	Days to callus initiation	% callus formation	Callus colour	Texture of callus	Fresh weight of callus g.	Organogenic Response		Intensity of callus growth
BAP+2,4-D					*Mean±S.E*	Root	Shoot	
1.0+0.5	50	30.00	G	F	1.18±0.008	-	-	
1.0+1.0	50	48.00	G	F	1.18±0.008	-	-	*
1.0+1.5	50	52.00	DG	F	2.00±0.158	-	+	**
1.0+2.0	50	**65.00**	**DG**	**C/F**	1.80±0.1225	-	+	***
1.0+2.5	50	55.00	G	F	1.10±0.004	-	+	*
2.0+0.5	50	30.00	DG	F	1.12±0.003	-	-	*
2.0+1.0	50	35.00	G	F	1.26±0.002	-	+	**
2.0+1.5	50	40.00	G	F	1.44±0.005	-	+	**

2.0+2.0	50	32.00	DG	F	1.20±0.003	-	-	*
2.0+2.5	50	30.00	G	F	1.06±0.004	-	-	*

" - ' no response , * poor growth ,** moderate growth , *** profuse growth.
" –"No. of root /shoot growth, "+" root/ shoot (1-3)/callus, LG =light Green, DG = Dark Green, C = Compact, F = Friable.

3.3.Callus regeneration

In this experiment petiole derived calli of aqua aroid, (Co*locasia esculenta* L. Schott) were used to investigate the effect of different hormonal concentrations and combinations. For shoot differentiation greenish, friable and soft small calli were transferred on MS medium supplemented with different concentrations and combinations of BAP and Kn alone and in combination with different concentration of NAA and IAA. Morphogenic potentialities of cultured calli varied with hormonal treatments and results are presented in **Table-3** and **Fig-(e&f).** Calli were transferred with different concentrations of IAA and NAA, Kn (0.50 -2.50 mg L^{-1}) alone and combined failed to differentiate any shoots. Highest (60 %) percentage of shoot regeneration was recorded in media having 1.0 mg L^{-1}BAP with 1.5 mg L^{-1} NAA followed by 45 % in media having 5.0 mg L^{-1}BAP with 1.0 mg L^{-1} NAA. The lowest 5 % of shoot regeneration was recorded in media having 2.0 mg L^{-1} BAP with 2.5 mg L^{-1} NAA, 3.0 mg L^{-1} BAP with 2.0 mg L^{-1}NAA and 4.0 mg L^{-1} BAP with 2.0 mg L^{-1}NAA. The highest number of shoots per callus was recorded 3.50 ± 0.158 in MS media having 5.0 mg L^{-1} BAP with 1.50 mg L^{-1} NAA. Highest length of shoot (5.00 ± 0.273) cm was recorded in media supplementing with 1.00 mg L^{-1}BAP with 1.5 mg L^{-1} NAA and followed by 1.0mg L^{-1} BAP with 1.0 mg L^{-1} NAA (4.00 ± 0.273) and the lowest length of shoot was 1.0 ± 0.00 cm in media having 3.0 mg L^{-1} BAP with 0.5 mg L^{-1}NAA formulation.

Table 3: Effect of phytohormones on regeneration of calluses from *in vitro* derived petiole explant of panikachu (*C. esculenta* L.Schott stoloniferous*)*.

Treatments mgL^{-1}	Days to callus regenera	%callus regeneratin	Fresh weight of callus with	Organogenic response		Intensity of callus	Average number of shoot per culture	Average length of shoot per culture
BAP+NAA			*Mean±S.E*	**Root**	**Shoot**		*Mean±S.E*	**Mean±S.E**
1.0+0.5	25	10.00	2.50±0.223	-	++	*	2.00±0.273	3.00±0.273
1.0+1.0	25	30.00	3.50±0.223	--	+++	**	2.20±0.316	4.00±0.273
1.00+1.5	25	60.00	4.30±0.223	-	+++	**	2.40±0.00	5.0±0.221
1.00+2.0	25	35.00	4.00±0.316	-	++	*	1.50±0.00	2.20±0.221

1.00+2.5	25	30.00	3.50±0.223	-	++	**	1.50± 0.222	3.00±0.221
5.0+0.5	25	40.00	2.00±0.160	-	+	*	3.50±0.158	4.00±0.374
5.0+1.0	25	45.00	3.00±0.158	--	+	*	3.40±0.158	3.50±0.158
5.0+1.5	25	30.00	3.50±0.158	-	+	*	3.50±0.158	3.0±0.158
5.0+2.0	25	25.00	2.50±0.160	-	+	*	2.25±0.160	3.0±.0.266

" - 'no response, * poor growth, ** moderate growth, *** profuse growth.

" –" No root / shoot growth , "+" root/ shoot (1-3)/ callus.

4. *Root induction*

Through direct or indirect organogenesis from stolon buds were grown on different concentrations of auxin (IBA, IAA, NAA and 2, 4-D) supplemented with MS media. Each hormone containing from 0.2 - 0.6 mg L^{-1} of which IAA 0.5 mg L^{-1} produced 91.30 percent of root induction (highest) but lowest (IAA 0.2 mg L^{-1}). Root induction percentage varies from 35.10 % - 91.30 %. Highest numbers of roots were observed at 0.5 mg L^{-1} IAA and root lengths were highest within 10 days. Different concentrations of IBA response slightly, range from 8.30 to 11.50 %. But NAA and 2, 4-D did not response with root induction at all (**Table- 4** and **Fig-(g&h)).** The adventitious roots were produced from the excised of micro shoots with in 2 - 3 weeks of culture on suitable medium. After washing in the tap water plantlets were then transplanted in the small plastic pots with soil which facilitated gradual acclimatization of the transplant in *ex vitro* stress environment. When the root system developed, the potted plantlets were transferred in medium type plastic pot with garden soil, compost and sand. Potted plantlets were successfully acclimatized with natural condition after 6 - 8 weeks.

Table-4: Effect of different concentration of auxin on root induction from the stolon bud explant derived plants of *Colocasia esculenta* L.Scott.

Growth regulators mgL^{-1}	Days to root proliferation	Percentage of root proliferation	Number of root per plant Mean ± S.E	Root length cm Mean ± S.E
IBA-0.2	-	-	-	-
IBA-0.3	-	-	-	-
IBA-0.4	10	10.80	1.80±0.2494	1.65± 0.1302
IBA-0.5	10	11.50	1.95±0.2167	1.60± 0.0010

IBA-0.6	10	8.30	1.67±0.1202	1.20± 0.1106
IAA-0.2	10	35.10	3.40±0.3055	2.30±0.2134
IAA-0.3	10	44.60	3.40±0.3055	3.10±0.2333
IAA-0..4	10	78.30	11.40±.4522	3.30±0.2134
IAA-0.5	**10**	**91.30**	**12.00±0.4472**	**5.00±0.3651**
IAA-0.6	10	81.90	10.40±.2667	4.00±0.1667

- = not response

There is no significant tissue cultural research work was not done in Bangladesh even in the world. Taro plantlets (*C. esculenta* var.antiquorum cv Bilashi) obtained by [**6**] from shoot tip derived calli cultured on MS medium supplemented with 1.0 mgL^{-1}NAA and 5.0 mg L^{-1} BA. Friable callus from two cultivars (Yatsugashira) or 2 mg L^{-1} 2, 4-D and 2ip (Malaysiano-4) in Taro, *Colocasia esculenta* Schott which proliferated on MS medium supplemented with 30g L^{-1}sucrose,1 mg L^{-1} 2, 4-D and 2ip obtained by [**7**]**.** Primary shoot apices axillary buds and protocorm like bodies cultured on two modification of Linsmaier and Skoog medium containing 5.5 mg L^{-1}naphthaleneacetic acid and 0.2 mg L^{-1}Kinetin.or 1.85 mg L^{-1}napthalene Acetic acid and 2 mg L^{-1}Kinetin and supplemented with 10^{-4} or 10^{-3} mol l^{-1} of polyamine putresetne-arginine and ornithine by [**8**]**.** for *in vitro* growth and multiplication of taro (*Colocasia esculenta* var.antiquorum cv. keladi). Single shoots and many roots developed by [**9**] on half strength Murashige and Skoog (HMS) medium supplemented with or without benzylamino purine (BAP)and they also investigated that most suitable plant growth regulators concentration was 1 mg L^{-1}BAP and 1 mg L^{-1} 2, 4-D for callus induction and proliferation in Japanese taro. Friable calli were induced in taro (*Colocasia esculenta* Schott cv.eguimo).by [**10**] culturing etiolated stem segments on MS medium supplemented with 30 g L^{-1}sucrose, 2 mg L^{-1}2, 4 D and 2 mg L^{-1}2ip.calli. The shoots initiated roots after being transferred to MS medium without phytohormone. Callus formation and multiple plantlets production by [**11**] through culturing axillary bud explants from south pacific (Solomon Islands) taro, *Colocasia esculenta* var.esculenta cv. Akalomamale (Araceae) on a modified Murashige Skoog medium containing 1mg L^{-1} NAA and TE. Introduction of taro callus from shoot tip explants on modified Nitsch medium supplemented with 2 4 dichlorophenoxyacetic acid (2,4-D) and 6 Benzyle adenine (BA) at 1 mg L^{-1}each and shoot differentiation was observed on the medium supplemented with 1 mg L^{-1}BA as early as 10 weeks of culture by [**12**]**.** A rapid method for direct regeneration of over 80 shoots per corm in 8 weeks was achieved by [**13**] through thin section culture of tissue cultures taro corm on MS medium containing 50μM BA. Regenerated shoots developed roots readily when transferred to hormone free medium. High frequency (0.62) and number (3.4) of plantlets produced per explant media containing 1.0 mg L^{-1}each of kinetin and NAA by [**14**] .

5.Acclimatization

Plantlets with rootings were removed from the culture vessels and then washed with running distilled water and then transferred to wet fertile loamy soil contained plastic pots for hardening. Within one month or before the plantlets showed best performance with acclimatization.

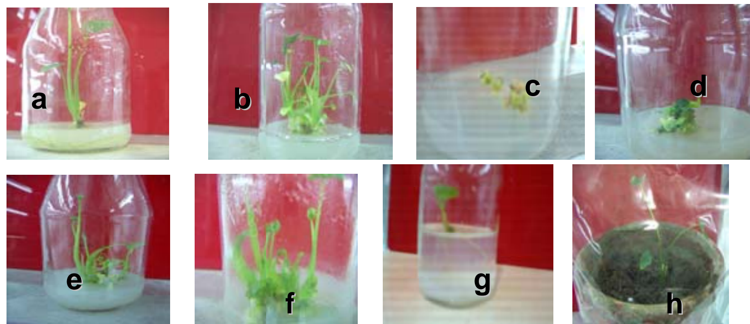

Fig-: Showing different stages of direct regeneration using stolon bud explant (**a**)**:** on MS medium containing 6.0 mg L^{-1}BAP +1.0 mg L^{-1}NAA after two weeks. (**b**)**:** direct multiple shoot regeneration . **(c&d):** different stages callus induction using petiole explants containing MS medium substitute with 1.0 mg L^{-1}BAP +2.0 mg L^{-1}2, 4-D.after 30 & 50 days. **Fig.(e&f)**:different stages callus regeneration on MS medium supplemented with 1.0 mg L^{-1}BAP + 1.5 mg L^{-1}NAA using petiole explant callus after 15 days and 30 days observation (**g&h**) rootings and acclimatization of regenerated shoot of aqua aroid or panikachu.

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