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Full Length Research Paper

Studies on the intiation of callusing and regeneration of plantlets in three different basal media with varied plant growth regulators for the micropropagation of *Anthurium scherzeriaum* using leaf and spathe as explants

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Anthurium scherzeriaum **is one of the most common ornamentals used in floriculture industry. However, there is shortage of planting material supplied to the cultivators. To overcome this problem, many breeders adopted micropropagation techniques. The usage of high dosage of plant growth regulators in the media resulted in mutation and loss of potentiality in the regeneration. An attempt was made to derive a suitable medium and selection of proper explants to avoid undesirable results. Explants like young old leaf segments and spathe were used. Three different basal medias such as Murashige and Skoog (MS), Nitsch and White' media were used with varying concentration of growth regulators. The obtained results indicated marked improvement in the growth pattern and morphological features of the plants obtained by culturing spathe segments in Nitsch' medium.**

Key words: *Anthurium scherzeriaum*, tissue culture, floriculture, ornamental plants.

INTRODUCTION

Anthurium scherzeriaum is one of the most common ornamentals extensively used as cut flowers in floriculture industry, because of sparkling texture of its spathe. It is a hybrid with great genetic diversity. Propagation of this plant by conventional method is very difficult. The clonal propagation using suckers is adopted by the cultivators and breeders as well.

Only a limited amount of plants can be produced in a year by this method, which is time consuming and laborious. Keeping this in mind, many breeders ventured into micropropagation techniques using shoot apex and leaf segments as explants. They were able to produce a large number of planting material by this method.

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However, the disadvantage of this method is the cell lines under going mutation and loosing the potentiality of regeneration. This may be mainly due to the use of large amount of growth regulators in the media for the rapid multiplication and also may be due to the kind of explants they selected for the induction of the callus. The present investigation was made to minimize the usage of plant growth regulators in the medium. Also spathe of the inflorescence was used as explants.

MATERIALS AND METHODS

A. scherzeriaum of different stages was collected from the nurseries and growers and maintained in a Polyhouse PG Centre, St Josephs College, Bangalore, here are various parts of a plant which can be used as an explant. The large explant consisting of parenchyma, vascular tissue and cambium have greater regenerative ability than the smaller explant. Small groups of homogenous tissue taken from the epidermal and sub epidermal layer could directly give rise to complex organs such as flowers or buds or roots. The tissue pieces

Table 1. Growth of culture of *A. scherzeriaum* in full strength of MS medium with 50 ml/L CW.

S/N	Type of explant	Time taken for callusing (day)	Hormone supplement (mg/L)		
			6 - BAP	2, 4 - D	NAA
1	Young leaf segment	40-42	2.0	0.5	-
		35 - 40	2.0	-	2.0
2	Old leaf segment	No callusing even after 40 days	2.0	0.5	-
			2.0	-	2.0
3	Spathe	30-35	2.0	0.5	-
		28 - 30	2.0	-	2.0

Table 2. Growth of culture of *A. scherzeriaum* on half strength MS medium with 50 ml/L CW.

S/N	Type of explant	Time taken for callusing (day)	Hormone supplement (mg/L)		
			6 - BAP	2, 4 - D	NAA
1	Young leaf segment	28-30	2.0	0.5	-
		22 - 25	2.0	-	2.0
2	Old leaf segment	45-50	2.0	0.5	-
		45 - 50	2.0	-	2.0
3	Spathe	22-25	2.0	0.5	-
		22 - 25	2.0	-	2.0

Table 3. Growth of callus of *A. scherzeriaum* in Nitsch's medium with 50 ml/L CW.

S/N	Type of explant	Time taken for callusing (day)	Hormone supplement (mg/L)		
			6 - BAP	2,4 - D	NAA
1	Young leaf segment	30-32	2.0	0.5	-
		28 - 30	2.0	-	2.0
2	Old leaf segment	No callusing even after 40 days	2.0	0.5	-
			2.0	-	2.0
3	Spathe	22-25	2.0	0.5	-
		22 - 25	2.0	-	

obtained by aseptically homogenizing the plants in a blender produce numerous new plants. In this present investigation, five samples, each consists of young, old leaf segments and spathe were used as explants.

RESULTS

The cultures were regularly monitored at regular intervals of the time and observations made were tabulated (Tables). The respective hormones were supplemented in different concentrations with the different stages of the explant to understand the better growth of the callus. Table 1 represents the culture of *A. scherzeriaum* in the full concentration of MS medium with 50 ml/L CW (coconut water). In this process, the spathe showed significant production of callus for 28 to 30 days and in old explants, the callus formation did not take place, while the young leaf segment responded within 35 to 42 days.

Table 2 represents the culture of *A. scherzeriaum* in the half strength of MS medium with 50 ml/L CW. In this process, the spathe and the young leaf segment with 6-BAP (2.0 mg/L) and NAA (2.0 mg/L) hormone showed significant production of callus in 22 to 25 days and in the older leaf segments, the callus formation took place after 45 to 50 days.

Table 3 represents the culture of *A. scherzeriaum* in the full concentration of Nitsch's medium with 50 ml/L CW. In this process, the spathe showed significant production of

Table 4. Growth of culture of *A. scherzeriaum* in whites medium supplemented with 50 ml/L CW.

S/N	Type of explant	Time taken for callusing (day)	Hormone supplement (mg/L)		
			6 - BAP	2,4 - D	NAA
1	Young leaf segment	25-28	2.0	0.5	-
		28 - 30	2.0	-	2.0
2	Old leaf segment	No callusing even after 40 days	2.0	0.5	-
			2.0	-	2.0
3	Spathe	22-25	2.0	0.5	-
		22 - 25	2.0	-	2.0

Table 5. Growth of culture of *A. scherzeriaum* in full strength MS medium supplemented with various concentrations of hormones.

S/N	Time taken for redifferentiation (day)	Hormone concentration (mg/L)		
		BAP	2,4 - D	NAA
1	18 - 20	0.5	-	0.5
2	13 - 15	1.0	-	-
3	20 - 22	1.0	0.5	-
4	24 - 26	2.0	-	1.0
5	28 - 30	2.0	-	-
6	33 - 35	2.0	1.0	-
7	33 - 35	2.0	-	1.0
8	38 - 40	2.0	0.5	-
9	No response	2.0	1.0	1.0

callus for 22 to 25 days and in the older explants, the callus formation did not take place. However, the young leaf segments showed response within 28 to 30 days with 6-BAP (2.0 mg/L) and NAA (2.0 mg/L).

Table 4 represents the culture of *A. scherzeriaum* in the full concentration of whites medium with 50 ml/L CW. In this study, the spathe showed significant production of callus in 22 to 25 days and in the older explants, the callus formation did not take place, while the young leaf segment responded within 28 to 30 days with 6-BAP (2.0 mg/L) and NAA (2.0 mg/L).

The redifferentiation growth of culture in full strength MS medium supplemented with various concentrations of hormones has been studied and represented in Table 5. In lower concentration of BAP and NAA (0.5 mg/L) the faster growth of culture was noticed. The normal growth of the plant was also observed in the same concentration (up to 5, 10, 20 and 25 cycles, Tables 6 to 10). 1.0 mg/L of 6-BAP and 0.5 mg/L of NAA has prompted highest shoot length and number of roots production respectively.

DISCUSSION

In the present investigation, an attempt was made to propagate *A. scherzeriaum in vitro* using different basal media. Earlier workers like Vargas et al. (2004) made an attempt to culture *A. scherzeriaum* seeds collected from the spadix in a medium containing 2.2 mg/L B.A. Later, the seedlings were subcultured using micro-cuttings on a medium containing 4.4 mg/L BA. and 0.05 mm NAA. The type of explant plays an important role in the regeneration of plants from the callus obtained. Hence, explants like young and old, leaf and spathe segments showed excellent callusing and regeneration capacity. Different media like M.S. and Nitsch were tried for the initiation of callus. There was good response in the callusing of leaf segments in MS medium supplemented with 2 mg/L of 2,4-D and 50 ml/L of CW. In the initial stage, the callus tissue formed was looking healthy and green, but later started yellowing. This was mainly due to excess of macro and micro salts concentration in the MS medium. To avoid this, half concentration MS medium was tried with the same hormone concentration. The cultures were incubated in dark for few days in the beginning (for about 25 days). Healthy callusing was noticed in this medium after 25 days of incubation at 24 ± 2°C temperature (Figure 1). The callus so obtained was shifted to bright light (1500 to 2000 Lux). The regeneration of callus cells into plantlets started in 15 days (Figure 2). Similar result

Table 6. Growth of culture of *Anthurium scherzeriaum* in full strength MS medium supplemented with various concentrations of hormones (up to 5 cycles).

S/N	Growth and multiplication of plantlet	Hormone concentration (mg/L)		
		6 BAP	2,4-D	NAA
1	Normal	0.5	-	0.5
2	Normal	0.5	0.5	-
3	Normal	1.0	-	1.0
4	Abnormal	1.0	1.0	-
5	Normal	1.5	-	0.5
6	Normal	2.0	-	1.0
7	Abnormal	2.0	1.0	-
8	Abnormal	3.0	-	2.0
9	Abnormal	4.0	-	2.0
10	Abnormal	5.0	-	2.0

Table 7. Growth of culture of *A. scherzeriaum* in full concentration MS medium supplemented with various concentrations of hormones (up to 10 cycles).

S/N	Growth and multiplication plantlet	Hormone concentration (mg/L)		
		6 BAP	2,4- D	NAA
1	Normal	0.5	-	0.5
2	Normal	0.5	0.5	-
3	Normal	1.0	-	1.0
4	Abnormal	1.0	1.0	-
5	Normal	1.5	-	0.5
6	Normal	2.0	-	1.0
7	Abnormal	2.0	1.0	-
8	Abnormal	3.0	-	2.0
9	Abnormal	4.0	-	2.0
10	Abnormal	5.0	-	2.0

was also noticed in the spathe culture (Figure 3).

In case of spathe, the young and old spathes showed turned poor callusing rather than the intermediate ones. The callus was slightly creamish white in colour in the beginning and became green (Figure 4). The rate of callusing was found to be much faster in spathes than in leaves. The regeneration of plantlets from the callus was much slower when compared to other plants. Earlier workers have shown that the vegetative propagation of several *Anthurium* species is a very difficult task (Kuehnle and Sugii, 1991), the obtained embryogenic callus from plant spadix and organogenic callus from leaf explants of several *Anthurium* hybrids. They maintained the callus culture for a very long time and found its capacity of regeneration.

Puchooa and Sookun (2003) studied the tissue culture response of *Anthurium andraeanum* and induced mutation through gamma ray irradiation to obtain new varieties. They used two different media for tissue culture studies (Murashige and Skoog, 1962; Nitsch, 1969). They found that callus induction in *A. scherzeriaum* was rapid and prolific in Nitsch's medium supplemented with BA at 1 mg/L and 2,4-D at 0.1 mg/L with the reduced concentration ammonium nitrate (200 mg/L). Shoot formation occurred when BA concentration was reduced to 0.5 mg/L and the ammonium nitrate level increased to 720 mg/L. Regenerated shoots started rooting on Nitsch medium containing IBA (1.0 mg/L).

In the present investigation, the Nitsch's medium supplemented with 1 mg/L of BAP and 0.5 mg/L of 2,4-D and 100 ml/L of CW formed prolific callusing (Figure 5). The concentration of ammonium nitrate was found with little effect on the shoot generation as indicated by the earlier research of Puchooa and Sookun (2003). However, the reduction of all other macro nutrients in the Nitsch's medium had considerable effect on the organogenesis. The callus showed reorganization within 20 days of transflasking in reduced macro-contents of Nitsch medium supplemented with just 1 mg/L BAP and 1 mg/L thymine HCl. The plantlets showed significant rooting when transferred to Nitsch medium containing 2 mg/L NAA and 2 g/L activated charcoal (Figure 6).

Table 8. Growth of culture of *A. scherzeriaum* in full strength MS medium supplemented with various concentrations of hormones (up to 20 cycles).

S/N	Growth and multiplications plantlet	Hormone concentration (mg/L)		
		6 BAP	2,4-D	NAA
1	Normal	0.5	-	0.5
2	Normal	0.5	0.5	-
3	Normal	1.0	-	1.0
4	Abnormal	0.5	-	0.5
5	Abnormal	0.5	0.5	-
6	Abnormal	1.0	-	1.0
7	Abnormal	2.0	1.0	-
8	Abnormal	3.0	-	2.0
9	Abnormal	4.0	-	2.0
10	Abnormal	5.0	-	2.0

Table 9. Morphological features of plantlets cultured in different concentration of hormones using full concentration of MS medium for *A. scherzeriaum* (up to 25 cycles).

S/N	Shoot length (cm)	Number of leaves	Number of root	Hormone concentration (mg/L)		
				6 BAP	2,4, - D	NAA
1	2 - 2.5	4 - 5	3 - 4	0.5	-	0.5
2	2 - 3	5 - 6	4 - 6	0.5	0.5	-
3	4 - 5	6 - 8	6 - 8	1.0	-	1.0
4	3 - 3.5	3 - 4	5 - 6	1.0	1.0	-
5	2.5 - 3	3 - 4	5 - 6	1.0	-	0.5
6	2 - 2.5	2 - 3	3 - 4	2.0	-	1.0
7	2 - 2.5	2 - 3	3 - 4	2.0	1.0	-
8	2 - 2.5	2 - 3	3 - 4	3.0	-	2.0
9	1.5 - 2	2 - 3	3 - 4	4.0	-	2.0
10	1.5 - 2	2 - 3	3 - 4	5.0	-	2.0

The cultures were further subjected to higher concentration of hormones ranging from 2 mg/L to 10 mg/L of NAA, 2 mg/L to 5 mg/L of BAP and 100 ml/L CW. The multiplication of plantlets was found to be rapid in all the above media combinations. However, the plantlets started producing enormous callus and rhizogenous roots at the base (Figure 7). Later, the plantlets started growing vigorously in height with very long internodes which were slender. The leaf size also showed considerable morphological changes such as narrowing and decrease in the area of leaf lamina etc. However, all these observations varied depending upon the concentration of auxins and cytokinins in the media (Figure 8). The variations observed in the plants regenerated from cultured cells are derived from two sources *viz*., 1) some of the variations could be revelation of the inherent cellular heterogeneneity of the plant and 2) culture conditions may bring about new genetic changes (Bhojwani and Razdan, 2004).

Vargas et al. (2004) also made an attempt of callus culture in *A. andraeanum* using MS as basal medium supplemented with 8.9 mg/l BA and 2.7 mg/L NAA. However, they incubated the culture under continuous fluorescent light at 25°C. In the present investigation, it is noticed that the callus formation occur better in the half concentration MS medium supplemented with minimum quantity of plant regulators such as 2,4-D and BAP with CW and incubated under dark. Such callus tissue obtained also indicated the presence of more number of healthy somatic embryoids than higher quantity of growth regulators. The callusing was also noticed faster and the regeneration process was much better. The explants which were exposed to light also formed callus. The callus cells so formed indicated less number of healthy somatic embryoids and were slow in the regeneration process.

Puchoa and Sookum (2003) also made observations such as the number of callus forming shoots or number of shoots per callus etc. in their studies. In the present investigation also similar attempt was made to count the

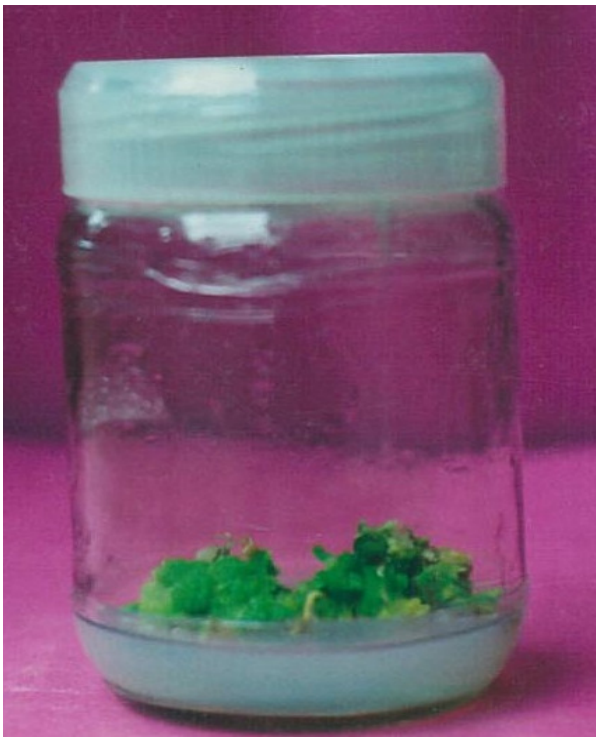

Figure 1. Healthy callusing after 25 days of incubation in dark from spathe explants.

Figure 3. Regeneration of callus into plantlets from spathe explants.

Figure 2. Regeneration of callus into plantlets from spathe explants.

Figure 4. Callus of older spathe tissue.

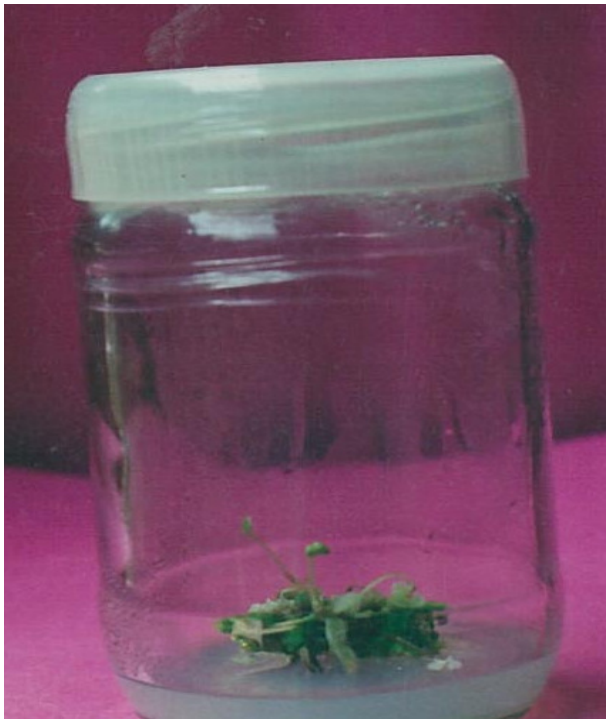

Figure 5. Callus from the spathe cultured in Nitsch's medium.

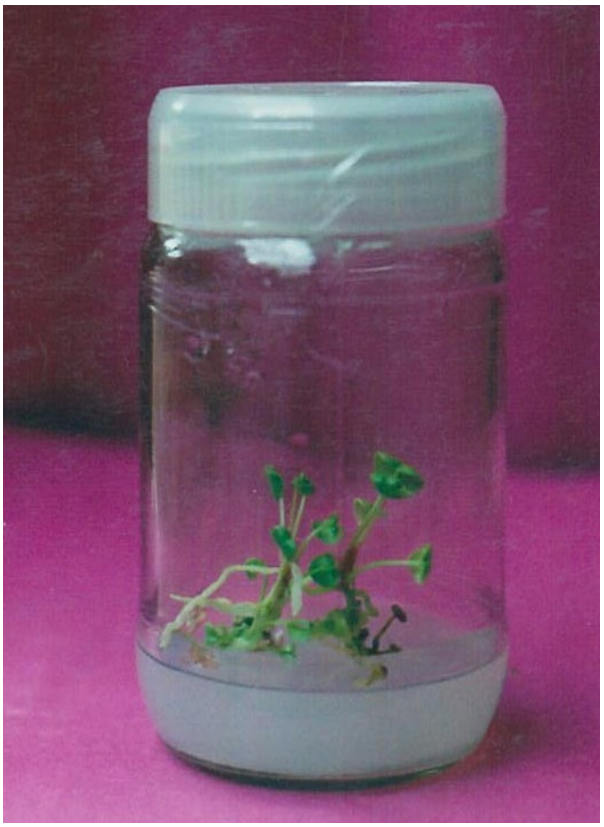

Figure 6. The plantlets in Nitsch'medium with activated charcoal as supplements.

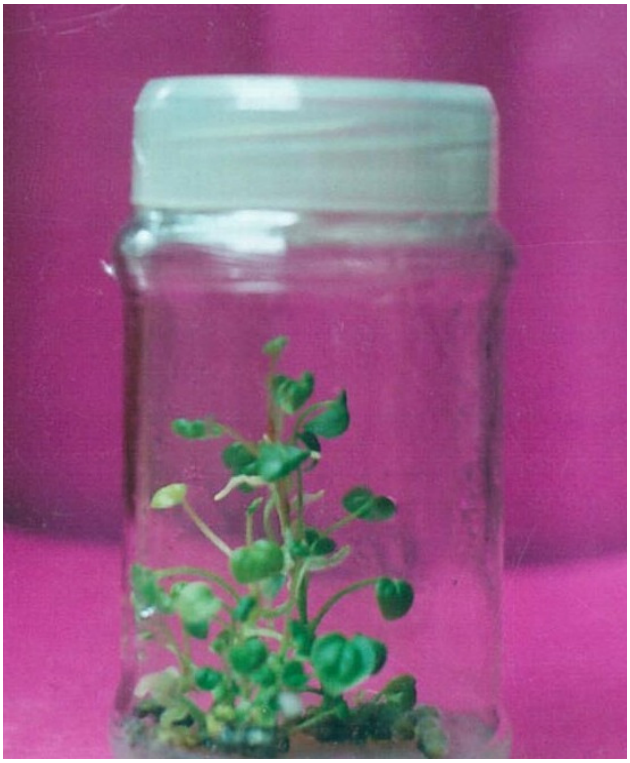

Figure 7. The plantlets with abnormal callusing and rhizogenous roots at base.

Figure 8. The variations of leaf size and leaf lamina in increased concentration (2 mg/l to 10 mg/l of NAA, 2 mg/l to 5 mg/l BAP and 100 ml/l CW).

number of plantlets formed from callus cells. In the initial stages of culture the formation of shoots was found more which gradually decreased as the number of cycles repeated. The callus cells started loosing their regeneration capacity and started developing into benign mass of cells without somatic embryoides. This clearly

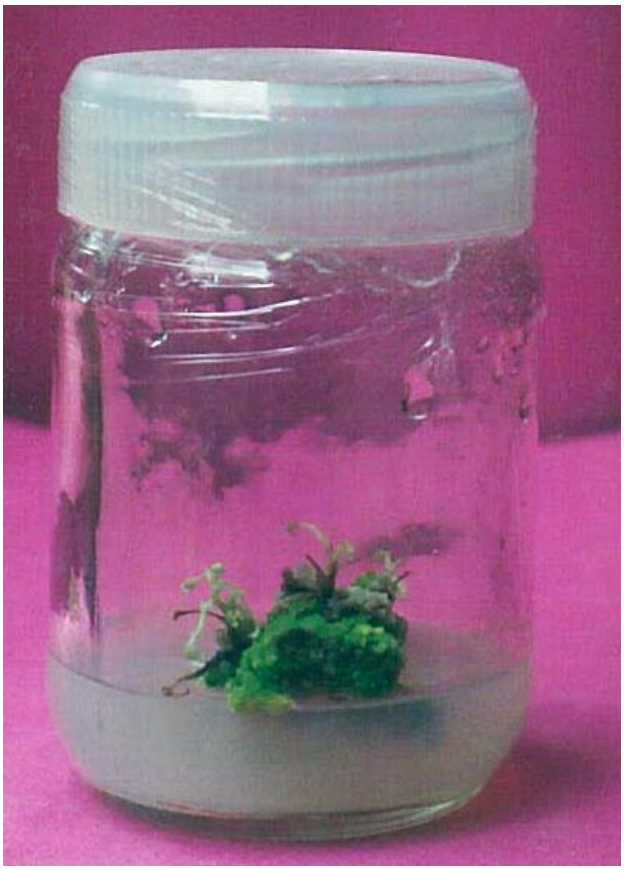

Figure 9. Rapid multiplication of callus cells and shoots in the increased concentration of 2 to 10 mg/L of NAA, 2 to 5mg/L of BAP and 100 ml/L of CW

Table 10. Morphological features of plantlets cultured up to 20 cycles in different concentration of hormones using full strength MS medium for *Anthurium scherzeriaum* (After 20 cycles).

S/N	Shoot length (cm)	Number of leaves	Number of root	Hormone concentration (mg/L)		
				6 BAP	2,4 –D	NAA
1	2 - 2.5	4 - 5	3 - 4	0.5	-	0.5
2	2 - 3	5 - 6	4 - 6	0.5	0.5	-
3	4 - 7	3 - 4	6 - 8	1.0	-	1.0
4	4 - 8	3 - 4	6 - 10	1.0	1.0	-
5	5 - 10	2 - 3	6 - 10	1.0	-	0.5
6	6 - 8	2 - 3	6 - 10	2.0	-	1.0
7	6 - 8	2 - 3	6 - 12	2.0	1.0	-
8	4 - 5	2 - 3	6 - 12	3.0	-	2.0
9	3 - 4	2 - 3	6 - 12	4.0	-	2.0
10	2 - 3	2 - 3	6 - 12	5.0	-	2.0

indicate that the hormone in the media influence the cells to loose their capacity to form healthy embryoides. Increase in the concentration of hormones produce vigorous callusing at the base and multiplication of shoots (Figure 9). Figures 1 to 9 represents the different stages of *A. scherzeriaum in vitro*. However, the regeneration capacity of the tissue will drastically come down within 10 cycles of culturing.

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