Pak. J. Bot., 41(5): 2347-2355, 2009.

AN EFFICIENT PROTOCOL FOR RAPID MULTIPLICATION OF *BRYOPHYLLUM PINNATUM* AND *BRYOPHYLLUM DAIGREMONTIANUM*

SHAGUFTA NAZ[*], SUMERA JAVAD[*], SAIQA ILYAS[*] AND AAMIR ALI[**]

[*]*Department of Botany, Lahore College for Women University, Lahore, Pakistan*
[**]*Department of biological Sciences, University of Sargodha, Sargodha, Pakistan*

Abstract

A rapid multiplication protocol for two species of *Bryophyllum pinnatum* and *Bryophyllum daigremontianum* has been optimized in the present study. Leaf sections of 1x1cm^2 were used as explants. The effect of various concentrations of thidiazuron TDZ (2.5, 5.0, 10.0, 15.0, 20.0 µM) and BAP alone and their combinations on MS medium were examined. TDZ proved more potent as its lower concentrations produced multiple shoots in *B. daigremontianum* i.e. 13.4^b±1.22 in 5 µM TDZ and 20.2^a±0.33 in 10 µM TDZ along with roots. The regeneration frequency and number of shoots per explant were also enhanced on these concentrations. Supplementation of media with different concentrations of BAP (1, 2, & 3 µM) and combinations of BAP and NAA did not improve shoot regeneration. Only shoots were produced from leaf sections in lower concentrations of BAP (1 µM) while the higher concentration of BAP did not show the optimum response. These micro shoots were then transferred to selected combinations of TDZ for further multiplication. TDZ 10 µM gave better results for shoot proliferation and elongation in both varieties. Plantlets produced by this method were shifted in green house for acclimatization. The best survival rate was 91% in 75% sand with 25% coco peat for *B.pinnatum* and 94% was noticed in 33% sand with 33% farmyard and leaf mould in *B. daigremontianum*.

Introduction

Genus *Bryophyllum* of the family *Crassulaceae* is a valuable medicinal as well as ornamental plant. It is a genus of about 125 species of tropical region that grows 3-5 feet high. These plants are cultivated as ornamental house plants and rock or succulent garden plants (Kulka, 2006). It has a definite ornamental value (Souza-Brito *et al*., 1993) because of its beautiful inflorescence. *Bryophyllum blossfeldiana* is a popular flowering potted indoor and garden plant around the globe and represents one of the economically most important potted plants in Europe. *B. pinnatum* is the air plant, miracle leaf or life plant is a native to Madagascar. It is a popular houseplant and has become naturalized in temperate regions of Asia, the Pacific and Caribbean, Australia, New Zealand, West Indies, Macaronesia, Mascarenes, Galapagos, Melanesia, Polynesia and Hawaii (Zamora *et al.*, 1998).

B. daigremontianum is called the Devils' backbone, Mexican hat plant or Mother of millions. It is native of western Indian Ocean of Africa and naturalized elsewhere in the world**.** It is an annual succulent, up to 3 feet tall, having triangular leaves up to 6 inches long with beautiful inflorescence (Kulka, 2006).

Both species are used as ornamental potted plant around the globe. Along with its ornamental values these species also contain a lot of medicinal values (Ofokansi *et al*., 2005; Nahar *et al*., 2008). In traditional medicine, these species have been used to treat ailments such as infections, rheumatism and inflammation. Kalanchoe extracts also have immunosuppressive effects. *Kalanchoe pinnatum* has been recorded in Trinidad and

Tobago as being used as a traditional treatment for hypertension and for the treatment of kidney stones (Tedge *et al*., 2005). It is useful to prevent from alcoholic liver damage, viral liver damage and toxic liver damage. Aqueous extract of its leaves shows anti-inflammatory, anti-diabetic and anti-tumor activity (Sidhartha & Chandhuri, 1990; Supratman *et al*., 2001; Torres-Santos *et al*., 2003). Alcoholic extracts of *Bryophyllum pinnatum* showed antimicrobial activity against a number of Gram positive and Gram-negative bacterial strains (Akinsulire *et al*., 2007). Three main compounds are present in *Bryophyllum* which has their unique medicinal value. One of them is Bryophillin A which shows strong anti-tumor activity. Others are Bersaldegenin-3-acetate and Bryophillin C which shows insecticidal properties (Supratman *et al*., 2001).

As these species produce valuable secondary metabolites having different types of medicinal importance, so it is necessary to develop protocols for rapid multiplication through micropropagation, callogenesis and organogenesis, because efficient regeneration system is prerequisite for all biotechnological tools (Philip & Gamborg, 2005). Regeneration of various species of Kalanchoe was obtained from leaf explants by direct organogenesis. Medium containing 0.5 mg/l TDZ and 0.04 mg/l IAA showed good shoot–generating capacity with all four species. Shoot elongation proved to be problem in some species (Sanikhani *et al*., 2006). Some species of *Bryophyllum* are grown ornamentals like *K. blossfeldiana* has being by far the most important and it has been regenerated *In vitro* and transformed. Gabryszewska & Hemple (1985) reported regeneration of roots in Kalanchoe first time. A few other species of *Bryophyllum* have been regenerated e.g., *K. daigremontianum* and *K. laciniata* (Frello *et al*., 2002) for the purpose of finding an effective protocol. Another effort was also reported on another species of *K. tomentosa* from shoot tips. Multiplication and growth response were obtained on a hormone free MS basal medium suggesting that there is a little role of plant growth hormones in the *In vitro* development, multiplication and organogenesis of Kalanchoe (Khan *et al*., 2006). In this context, *In vitro* propagation of other species is an interesting topic. Thus *In vitro* propagation could be a valuable alternative to propagation by seeds or cuttings, especially when breeders want to work with species hybrids. Single valuable hybrids can be propagated in this way, when other means are inaccessible. As the development of a regeneration protocol is often time consuming, it would be an advantage to have a protocol for more species.

There is therefore a need to develop tissue culture protocols for the rapid propagation of selected elite varieties of *Bryophyllum*. The present research report is directed towards the rapid propagation of *Bryophyllum* by using different types and concentration of phytohormones.

Materials and Methods

Leaves from *B. pinnatum* and *B. daigremontianum* were taken and washed with tap water to remove sand and dust particles and then thoroughly washed with household detergent i.e., ultra surf for about 10 minutes, then leaves were rinsed with tap water to make it free from detergent. It was followed by dipping of the leaves in 50% Sodium hypochlorite solution for 40 minutes. The leaves were then washed with autoclaved water for at least three times to remove the smell of bleaching solution.

Sections of equal sizes (1x1 cm^2) were cut from these leaves and were grown on MS (Murashige & Skoog, 1962) media supplemented with TDZ (at concentrations of 2.5, 5,10, 15 and 20 µM), BAP (at concentrations of 0.5, 1, 2 & 3 µM) at establishment stage

for initiation of multiple shoots. After optimization of the best media these microplants were further sub cultured for multiplication. The multiplication media contain selected concentrations of TDZ (5, 10, 15 µM) and combinations of BAP and NAA (1, 2 +10 µM) All media were based on MS basic salts, vitamins, sucrose 30g/l. The pH of media was adjusted at 5.57. For solidification of media phytagel with conc., of 1.5 g/l was used before autoclaving.

The cultures of *Bryophyllum* were grown in culture room with temperature 21± 2^{o}C and light intensity of 2000-3000 lux, while the cultures were maintained at photoperiod of 8 hours light and 16 hours dark. Days of initiation for shoots and roots were recorded for both the varieties of *Bryophyllum*. Then observations based on days to shoot/root initiation, frequency of shoot/root formation, number of shoots/roots per explant and shoot/root height (cm), were recorded after 40 days after establishing the culture. After establishing the plantlets, these were shifted in green house for acclimatization. Different types of soil media having different ratio of coco peat, biofert, farmyard manure and leaf mould were used. The data about survival rate was noticed in different soil mixtures and the best one was selected.

A completely randomized design with 5 replicates was used for the experiment. The data for each parameter were subjected to analysis of variance (ANOVA) using the COSTAT V.63: statistical software (Cohort software, Berkely, California). The mean values were compared with the least significant difference test following Duncan' new multiple range test at 5% level.

Results and Discussions

The disinfection treatment used was efficient for the *In vitro* establishment of both *Bryophyllum* species, with approximately 90% of explants remaining aseptic and showing new growth after 20 days of culture. Around 80% of the disinfected explants produced at least one shoot, demonstrating a successful establishment of shoot cultures (Fig. 1a).

To induce shoot regeneration, individual leaves of at least 1x1cm^2 of both *Bryophyllum* species were transferred to MS medium supplemented with different concentrations of TDZ (Tables 1 & 2). New shoots first appeared as small, green protuberances at the leaf bases and developed into shoots. (Fig. 1b). Initiation of shoots in both the species in most of the treatments ranged within 7-22 days in TDZ (Tables 1 & 2). Rapid and early shoot initiation for both these species was observed in 5 and 10 µM TDZ (Fig. 1b) with 8.4^{a}±0.61 and 7.00^{b}±0.4 days of initiation of shoot respectively for *B. pinnatu*m. The best medium was M.S + 10 µM of TDZ for shoot initiation as it yielded shoots from 100% of leaf sections of *B. pinnatum* and *B. daigremontianum*. In MS medium + 10µM TDZ 100% and 80% of leaf sections produced roots for *B. pinnatum* and *B. daigremontianum*. Again MS medium + 5µM TDZ also showed better results as compared to other concentrations i.e., 92% of leaf sections produced shoots while 82% produced roots in *B. pinnatum*. In case of *B. daigremontianum*, 92% of leaf sections gave rise to roots.

The effect of thidiazuron (TDZ) on the micropropagation response of *Bryophyllum* is well documented. The beneficial effect of TDZ on shoot regeneration and proliferation (Zamora *et al*., 1998; Sanikhani *et al*., 2006) and induction of multiple shoots (Frello, *et al*., 2002) was reported in different species of *Crassulaceae*. The medium containing the 10 µM TDZ can be used as basis for constructing media for regeneration of other *Bryophyllum* species as well as other species from the *Crassulaceae*, using leaf segment as explants.

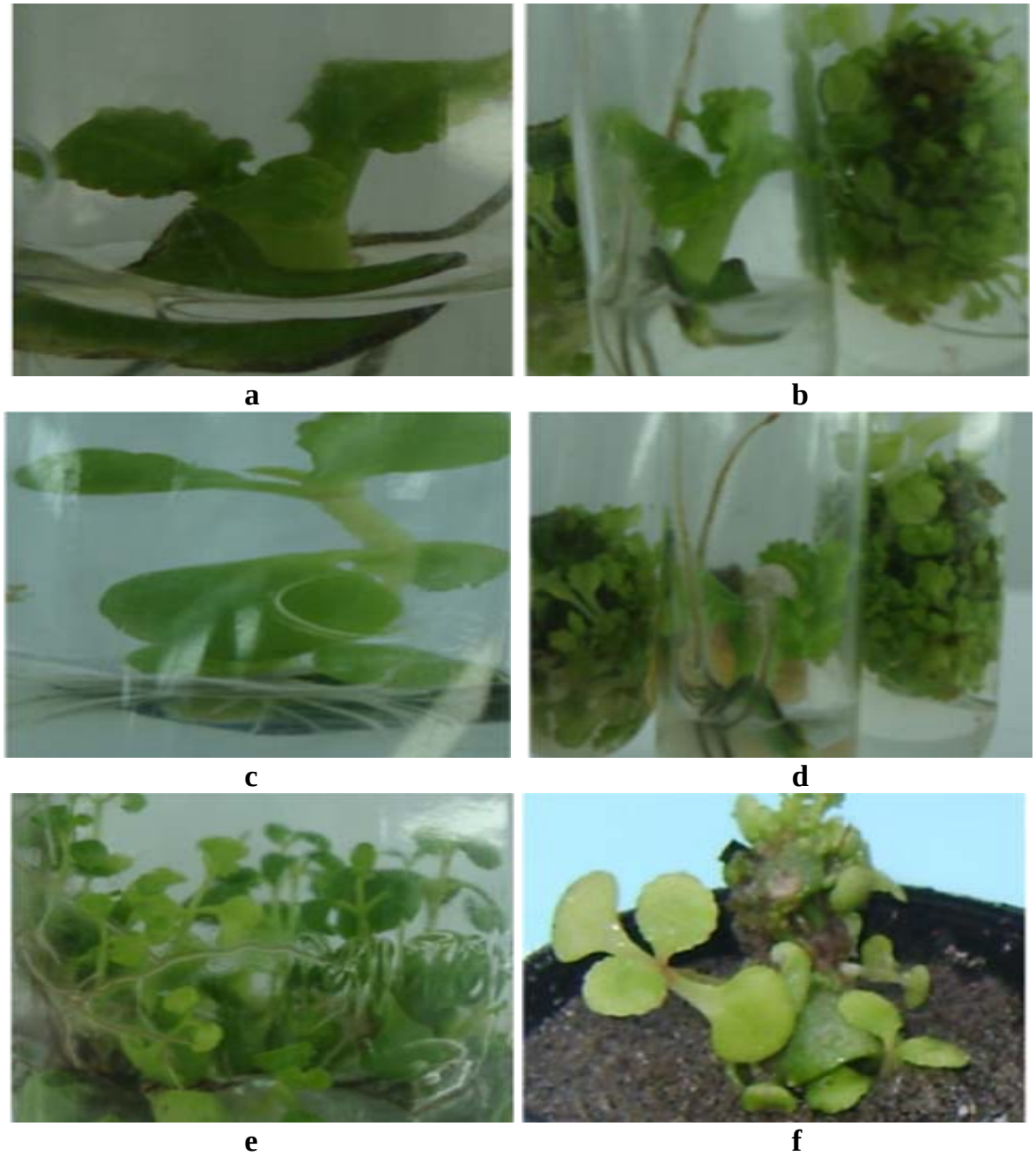

Fig. 1. a) Emergence of shoot in MS+TDZ 5µM in *B. pinnatum.* b) Initiation of multiple shoots in MS+TDZ 20µM in *B. pinnatum*. c) Initiation of single shoot in MS+TDZ 10µM in *B. daigremontianum*. d) Multiple shoot formationin MS+TDZ 10µM in *B. daigremontianum*. e) Mass multiplication of micro shoots to TDZ 10µM in *B.pinnatum* from BAP 2µM+10NAA. f) Acclimatization in autoclaved sand in green house.

In *B. pinnatum*, maximum number of shoots per explants was observed in MS medium + 20µM TDZ with an average value of 2.8^{a}±0.44 shoots. So it showed that lower concentrations of TDZ were not able to produce multiple shoots in *B. pinnatum*. Maximum number of roots per explants was seen in MS media+15µM TDZ i.e., 3.54^{a}±0.13 roots. In case of *B. daigremontianum*, MS medium+10µM TDZ produced 20.2^{a}±0.33 shoots per explant and MS medium+5µM TDZ produced 13.4^{b}±1.22 shoots per explants which are pretty good number for micro propagation (Fig. 1d). Maximum number of roots per explant was observed in MS medium+2.5µM TDZ. So TDZ showed very positive effect on the number of shoots per explants of *B. daigremontianum*. Root length was maximum in MS medium+15µM TDZ for *B. pinnatum* (4.28^{a}±0.19cm) and *B. daigremontianum* showing maximum average root length of 2.22^{a}±0.07cm in MS medium+5µM & MS medium +10µM TDZ.

Table 1. Effect of different concentrations of TDZ on initiation of shoots and roots of *Bryophyllum pinnatum*.

Conc. of TDZ µM	No. of Explants	Days to initiation of		Frequency of initiation of (%)		Per explant no. of		Length (cm)	
		Shoot	Root	Shoots	Roots	Shoots	Roots	Shoots	Roots
2.5	10	15.2^{b} ±2.44	14.0^{c}±0.28	62	42	1.4^{b}±0.36	2.00^{b}±0.49	0.32^{c}±0.04	1.50^{d}±0.23
5.0	10	8.4c±0.61	10^{d}±0.03	92	82	1.0^{b}±00	1.42^{b}±0.11	0.32^{c}±0.004	1.40^{d}±0.1
10	10	7.00c±0.4	10.9^{d}±0.22	100	100	1.0^{b}±00	3.16^{a}±0.07	0.92^{a}±0.08	3.50^{b}±0.2
15	10	22.2^{a}±0.77	18.8^{a}±0.44	69	78	1.0^{b}±00	3.54^{a}±0.13	0.80^{ab}±0.04	4.28^{a}±0.19
20	10	16.8^{b}±0.72	17.2^{b}±0.66	72	78	2.8^{a}±0.44	1.80^{b}±0.18	0.70^{b}±0.07	2.20^{c}±0.18
LSD		1.82	1.27			0.83	0.81	0.18	0.69

Table 2. Effect of different concentrations of TDZ on initiation of shoots and roots of *Bryophyllum daigremontianum*.

Conc. of TDZ µM	No. of Explants	Days to initiation of		Frequency of initiation of (%)		Per explant no. of		Length (cm)	
		Shoot	Root	Shoots	Roots	Shoots	Roots	Shoots	Roots
2.5	10	15.8^{b}±0.33	14.4^{b}±0.22	98	82	1.00^{c}±00	7.4^{a}±0.23	1.44^{b}±0.07	1.54^{b}±0.02
5.0	10	9.80^{d}±0.18	12.0^{c}±0.00	92	90	13.4^{b}±1.22	1.0^{d}±0.03	1.06^{c}±0.01	2.22^{a}±0.07
10	10	10.0^{d}±00	12.0^{c}±0.03	100	80	20.2^{a}±0.33	1.0^{d} ±00	1.72^{a}±0.09	2.22^{a}±0.07
15	10	14.0^{c}±0.49	13.8^{b}±0.44	70	70	1.00^{c}±00	2.8^{c}±0.18	1.22^{bc}±0.10	1.20^{c}±0.11
20	10	22.6a±0.22	15.8^{b}±0.18	70	72	2.60^{c}±0.36	5.4^{b}±0.36	0.76^{d}±0.09	0.80^{d}±0.07
SD		0.97	0.77			1.94	0.67	0.27	0.19

*: Data was collected after 40 days of culture. Each value is mean of three replicate with standard error (Mean ± SD) a,b,c: Mean with same superscript are not significantly different from each other at 5% level by Duncan' new multiple range test.

Table 3. Effect of different concentrations of BAP and its combination with NAA on Shoots and Roots of *Bryophyllum pinnatum*.

Conc. of TDZ µM	No. of Explants	Days to initiation of		Frequency of initiation of (%)		Per explant no. of		Length (cm)	
		Shoot	Root	Shoots	Roots	Shoots	Roots	Shoots	Roots
0.5	10	15.0^{c}±0.57	14.4^{c}±0.22	61	41	1^{a}±0	2.6^{b}±0.36	0.36^{c}±0.04	1.64^{c}±0.12
1.0	10	14.8^{c}±0.18	0.00^{d}±0.00	82	00	1^{a}±0	0.0^{c}±0.00	0.80^{b}±0.00	0.00^{d}±0.00
2.0	10	18.0^{b}±0.49	0.00^{d}±0.00	100	00	1^{a}±0	0.0^{c}±0.00	1.00^{a}±0.06	0.00^{d}±0.00
3.0	10	11.0^{d}±0.28	0.00^{d}±0.00	77	00	1^{a}±0	0.0^{c}±0.00	0.28^{c}±0.04	0.00^{d}±0.00
1+10µM NAA	10	18.8^{ab}±0.44	17.2^{a}±0.33	82	80	1^{a}±0	4.0^{a}±0.28	0.38^{c}±0.04	2.10^{b}±0.09
2+10µM NAA	10	19.4^{a}±0.21	16.2^{b}±0.18	79	80	1^{a}±0	4.2^{a}±0.33	0.96^{a}±0.05	3.04^{a}±0.11
LSD		1.27	0.58			0	0.75	0.15	0.25

Effectiveness of TDZ as compared with other plant growth hormones was also reported by Jaiswal (2006) in *B. pinnatum*. Frello *et al*., 2002 reported that regeneration was more effective with TDZ than with BAP in *K. blossfeldiana*. The shoot regeneration frequency and number of shoots per explants were also enhanced by increasing concentration of TDZ in seven cultivars of *Kalanchoe blossfeldiana* (Sanikhani *et al*., 2006).

Leaf explants were also cultured on MS basal media supplemented with different concentrations of BAP and its combinations with NAA. Initiation of shoots in both species for most of the treatments ranged within 11-19 days (Tables 3 & 4). For *B. pinnatum* minimum days for shoot initiation were observed in MS medium + 3µM BAP while 100% of leaf sections produced shoots in MS medium + 3µM B while in *B. daigremontianum*, there was no particular effect of BAP concentrations on days of initiation as all the explant initiated shoots within the range of 16-18 days. While minimum days of initiation of shoots were observed in MS medium + BAP 2 µM + NAA 10 µM, but all these concentrations of BAP failed to produce roots except 0.5µM of BAP. This indicates that BAP has inhibitory effects on root initiation in *B. daigremontianum.* It is evident from Tables 3 & 4 that 100% shoots frequency was observed in MS medium + 2µM BAP in *B. pinnatum* and in MS medium + 2µMBAP + 10µM NAA in *B. daigremontianum.* In both the *Bryophyllum* species different BAP concentrations did not affect the number of shoots per explant as in all the concentrations only one shoot per explant was produced. In *B. pinnatum* and *B. daigremontianum*, maximum shoot length was produced in MS medium + BAP 2µM + NAA10µM. As simple BAP failed to produce roots in *Bryophyllum* species, so combination of BAP with NAA proved to be excellent for root growth. Because *B. pinnatum* produced $4.2^{a}\pm0.33$ roots per explant and *B. daigremontianum* produced $7.2^{a}\pm0.44$ roots per explant. So explants were then grown on the combinations of BAP and NAA. These explants yielded roots within 12-17 days and shoots within 13-19 days (Fig. 1c). Growth regulators added to the growth media during the shoot proliferation stage played a definitive role in growth enhancement of *Bryophyllum* cultured *In vitro*. This is consistent with results of Duane & Riserberg (1983) who found that the *B.pinnatum* produced 2.8 times more shoots in 10^{-6}M BAP as compared to water. Karapoff (1982) reported that bud initiation was stimulated by BAP and he obtained average of 1.18 ± 0.82 roots in 10^{-6} mol BAP. Heide (1965) investigated that budding on intact leaves are promoted by BAP. To fulfil the increasing requirement of nutrients and space the directly regenerated shoots from both varieties were transferred in fresh medium containing selected combinations of BAP, TDZ and NAA for further multiplication (Fig. 1d) in culture flasks. Proliferation of shoots started and during this secondary proliferation stage, lateral shoots developed from axils of lower leaves of newly initiated shoots. As a result a dense mass of shoots (15-50) was formed in each culture flask (Fig. 1e). The results were excellent for both of the *Bryophyllum* species with a shoot length of $2.20^{a}\pm0.13$ with 10µmol of TDZ (Table 5 & Fig. 1e). These proliferated shoots were cut into segments containing 2-4 shoots and transferred to fresh medium in jars. These segments gave rise to fresh clumps of 4-9 shoots within 4-5 weeks of transfer. In this way shoot proliferation was maintained for several passages by monthly transfer to fresh medium. So by reculturing the clumps about 120-150 shoots were produced from single explants with 4-5 passages and maintained for one year.

Table 4. Effect of different concentrations of BAP and its combination with NAA on Shoots and Roots of *Bryophyllum daigrimontianum*.

Conc. of TDZ µM	No. of Explants	Days to initiation of		Frequency of initiation of (%)		Per explant no. of		Length (cm)	
		Shoot	Root	Shoots	Roots	Shoots	Roots	Shoots	Roots
0.5	10	$16.0^{c}\pm0.49$	$14.2^{a}\pm0.44$	98	80	$1.00^{a}\pm0.0$	$4.0^{c}\pm0.28$	$1.60^{b}\pm0.07$	$1.46^{a}\pm0.31$
1.0	10	$17.0^{bc}\pm0.28$	$0.00^{c}\pm0.00$	84	00	$1.00^{a}\pm0.0$	$0.0^{d}\pm0.00$	$1.10^{c}\pm0.02$	$0.00^{d}\pm0.00$
2.0	10	$18.0^{b}\pm0.28$	$0.00^{c}\pm0.00$	80	00	$1.00^{a}\pm0.0$	$0.0^{d}\pm0.00$	$1.86^{b}\pm0.05$	$0.00^{d}\pm0.00$
3.0	10	$16.2^{c}\pm0.44$	$0.00^{c}\pm0.00$	79	00	$1.00^{a}\pm0.0$	$0.0^{d}\pm0.00$	$1.16^{c}\pm0.08$	$0.00^{d}\pm0.00$
1+10µM NAA	10	$13.4^{d}\pm0.22$	$12.2^{b}\pm0.18$	81	78	$1.00^{a}\pm0.0$	$7.2^{a}\pm0.44$	$1.26^{c}\pm0.12$	$1.14^{b}\pm0.11$
2+10µM NAA	10	$19.2^{a}\pm0.33$	$14.4^{a}\pm0.22$	100	100	$1.00^{a}\pm0.0$	$6.0^{b}\pm0.28$	$2.20^{a}\pm0.13$	$0.56^{c}\pm0.15$
LSD		1.16	0.69			00.0	0.79	0.29	0.31

*: Data was collected after 40 days of culture. Each value is mean of three replicate with standard error (Mean ± SD) a,b,c: Mean with same superscript re not significantly different from each other at 5% level by Duncan' new multiple range test.

Table 5. Micro propagation of *B. pinnatum* and *B. daigremontianum* in different concentrations of TDZ.

Conc. of TDZ µM	No. of Explants	*Bryophyllum pinnatum*				*Bryophyllum daigremontianum*			
		Days to initiation of		Frequency of initiation of (%)		Per explant no. of		Length (cm)	
		Shoot	Root	Shoots	Roots	Shoots	Roots	Shoots	Roots
5	10	$11.0^{c}\pm0.01$	$2.0^{c}\pm0.00$	$1.16^{c}\pm0.08$	$0.33^{c}\pm0.00$	$14.0^{c}\pm0.02$	$1.0^{b}\pm0.00$	$2.20^{c}\pm0.03$	$1.53^{c}\pm0.22$
10	10	$18.0^{a}\pm0.01$	$6.0^{a}\pm0.28$	$1.26^{b}\pm0.12$	$1.14^{a}\pm0.11$	$22.0^{a}\pm0.06$	$3.0^{a}\pm0.00$	$2.89^{a}\pm0.11$	$1.99^{a}\pm0.34$
15	10	$17.0^{b}\pm0.03$	$4.0^{b}\pm0.28$	$2.20^{a}\pm0.13$	$0.56^{b}\pm0.15$	$20.0^{b}\pm0.10$	$3.0^{a}\pm0.00$	$2.60^{b}\pm0.26$	$1.72^{b}\pm0.04$
LSD		0.12	0.79	0.29	0.31	0.09	0.00	0.38	0.41

*: Data was collected after 40 days of culture. Each value is mean of three replicate with standard error (Mean ± SD) a,b,c: Mean with same superscript are not significantly different from each other at 5% level by Duncan' new multiple range test.

Then these all plantlets were transferred to green house for acclimatization in 100% humidity. Different types of soil media having different ratio of sand, coco peat, biofert, farmyard manure and leaf mould were used. Six different types of soil combinations were used like 100% sand, 33% sand with 33% of farmyard and leaf mould, 50% sand with 50% coco peat, 50% sand with 50% biofert, 25% sand with 25% of coco peat, biofert and farmyard. Survival percentage was noticed in all the combinations. The success of any process of plant cloning can be evaluated by the number of regenerated plants that can survive in field conditions, following acclimatization and hardening. Our results showed that plant survival rates was 91% observed in 75% sand with 25% cocopeat *for B.pinnatum* and 94% was noticed in 33% sand with 33% farmyard and leaf mould in *B.daigerimontinum* (Fig. 1f). Assays of acclimatization carried out by other authors working with different species of *Bryophyllum* showed that plantlets obtained *in vitro* are easily acclimatized (Khan, *et al*., 2006; Sanikhani *et al*., 2006).

The procedure reported in this paper suggests that multiplication *via* tissue culture could be a commercially feasible method for *Bryophyllum* propagation. Micropropagation is more rapid, continuous and efficient than propagation *via* conventional cutting because it can supply uniform and consistent plant material for investigations of important secondary metabolites produced by this species, as well as its use as an ornamental plant.

References

Akinsulire, R. Odunayo, Aibinu, E. Ibukun, Adenipekun, Tayo, Adelowotan, Toyin and Odugbemi. 2007. *In Vitro* antimicrobial activity of crude extract from plants *Bryophyllum pinnatum* and Kalanchoe. *African journal of traditional of Traditional Complementary and Alternative Medicines*, 4(3): 338-334.

Duane, F.H. and L.H. Riserberg. 1983. Hormonal regulation of epiphyllous buds release in development in *Bryophyllum calycinum*. *American Journal of Botany*, 70(6): 912-915.

Frello, S., E. Venerus and M. Serek. 2002. Regeneration of various species of Crassulaceae with special reference to Kalanchoe. *Journal of Horticulture Science & Biotechnology*, 77(2): 204-208.

Gabryszewska, E. and M. Hemple. 1985. Rooting of Kalanchoe *In vitro* and *In vivo*. *Symposium "In Vitro Problems Related to Mass Propagation of Horticulture Plants"*. 16-20 Sept. 1985. Gembloux, Belgium.

Heide, O.M. 1965. Effects of 6-Benzyleamino-Purine and 1-Naphthalene acetic acid on the epiphyllous bud formation in *Bryophyllum. Planta (Berl.)*. 67: 281-296.

Jaiswal, S. and S. Sawhney. 2005. Correlation of epiphyllous bud differenciation with foliar senescence in crassulacean succulent *Kalanchoe pinnatum* as revealed by thidiazuron and ethrel application. *J. Plant physiology*, 163(7): 717-722.

Karapoff, J.A. 1982. Harmones and early *In vitro* development of epiphyllous propagules on *Bryophyllum calycinium*. *American Journal of Botany*. 69(3): 348-355.

Khan, S., S. Naz, K. Ali and S. Zaidi. 2006. Direct organogenesis of *Kalanchoe tomentosa* (Crassulaceae) from shoot tips. *Pak. J. Bot*., 38(4): 977-981.

Kulka, R.G. 2006. Cytokinin inhibit epiphyllous plantlet development on leaves of *Bryophyllum marnierianum*. *Exp. Bot*., 17(77): 122-125.

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

Nahar, K., G.U.K. Mohammad and R.S. Mohammad. 2008. Antimicrobial and Cytotoxic activities of *Bryophyllum daigremontianum*. *J. Pharm. Sci*., 7(1): 99-101.

Ofokansi, K.C., C.O. Esimone and C.R. Anele. 2005. Evaluation of the *in vitro* combined anti bacterial effect of the leaf extracts of *Bryophyllum pinnatum* and *Ocimum gratissimum (Labiatae). Plant product Res. J*., 9: 6-10.

Philips, G.C. and O.L. Gamborg. 2005. Plant cell tissue and organ culture. (Eds.): N.K. Mehra. Narosa publishing house 6, community centre. New Delhi, India. pp. 91-93.

Sanikhani, M., F. Stefan and S. Margrethe. 2006. TDZ induces shoot regeneration in various *Kalanchoe blossfeldiana* pollen cultivars in the absence of auxin. *Plant cell, Tissue and Organ Culture*, 85(1): 75-82.

Sidhartha, P. and A.K.W. Chandhuri. 1990. Anti-inflammatory action of *Bryophyllum pinnatum* leaf extract. *Fitoterapia*, 41(6): 527-533.

Souza-Brito, A.R.M. and A.A. Souza-Brito. 1993. Forty years of Brazilian medicinal plant research. *Journal of Ethnophormocology*, 39(1): 53-67.

Supratman, U., T. Fujita, K. Akiyama, H. Hayashi, A. Murakami, H. Sakai, Koshimizu and H. Ohigashi. 2001. Anti tumor promoting activity of Bufadienolides from *Kalanchoe pinnata* and *Kalanchoe daigremontiana x tubiflora*. *Bioscience, Biotechnology and Biochemistry*, 65(4): 947-949.

Tedge, H., E. Mohammad, K. Asres and G. Mariam. 2005. Antimicrobial activities of some selected traditional Ethopian medicinal plants used in the treatment of skin disorders. *Journal of Ethnophormacology*, 100(1/2): 168-175.

Torres-Santos, E.C., S.A.G. Da Silva, S.S. Costa, A.P.P. Santos, A.P. Almeida and B. Rossi-Bergmann. 2003. Toxicological analysis and effectiveness of oral *Kalanchoe pinnata* on human case of cutaneous leishmaniasis. *Phytotherapy research*, 17:801-803.

Zamora, A.B., S.S. Gruezo and S.V. Siar. 1998. Somaclonal variation: Breeder's tool for recovering potencial cultivars in ornamentals (Kalanchoe). 14. *Annual Scientific Conference of the federation of Crop Science Societies of the Philippines, Cebu City (Philippines*)., 19-24 April.

(Received for publication 3 May 2009)