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In vitro regeneration and rapid multiplication of *Kalanchoe blossfeldiana*: An important ornamental plant

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Abstract
In the present study, the effect of different auxins and cytokinins alone and in combinations were studied for *in vitro* regeneration of whole plant of Kalanchoe. At initiation stage, longest shoot (1.5 cm) was obtained on MS + 0.4 mg/l BAP while at multiplication, maximum number of shoots (8) were obtained on MS + 2.5 mg/l BAP using nodal explant. The greenish and higher mass of callus (56.6 mg per explants) was obtained on MS + 2 mg/l 2,4-D + 2 mg/l BAP. The maximum number of shoots (7) was observed when callus was grown on MS + BAP (0.2 mg/l) and NAA (0.1 mg/l). The best rooting response (7 roots) was observed on MS + 1mg/l IBA. The micro propagated plantlets established successfully in acclimatization medium containing soil:cocopeat (1:1).

Keywords: Kalanchoe**,** 2, 4-D, BAP, *in vitro*, explants

Introduction
Kalanchoe (*Kalanchoe blossfeldiana*, 2n=34) is an ornamental plant, commonly known as Panda plant, belongs to family Crassulaceae, potted around the world (Ofokansi *et al*., 2005; Nahar *et al*., 2008) [14, 13]. The genus Kalanchoe was first described by Michel Adanson in 1763 which consists of about 130 species of annual and perennial shrubs, climbers and small trees. The species of Kalanchoe mostly found in south-eastern Asia and China (Allorge-Boiteau *et al*., 1996) [1]. Kalanchoe is a beautiful and succulent plant with dense white hairs-like covering. Usually, it is cultivated as garden ornamental in rock and sand gardens or as novelty gifts with a medium humidity (Brito and Brito, 1993) [4]. The species is characterized by a high level of cardiac glycosides. Therefore, it is gaining importance in pharmacy and medicine. By recent studies, Kalanchoe could be valuable in anticancer therapy due to the high content of metabolites of antimitotic activity (Garces *et al*., 2009) [6]. Moreover, it is used to treat wounds, allergies, and skin diseases (Hsieh *et al*., 2013) [7].
Kalanchoe is a slow growing plant therefore it is extremely necessary to develop a tissue culture system for rapid production for commercial and medicinal purposes. Micropropagation through tissue culture permits the regeneration of large numbers of disease free plants from small pieces (explants) of stock plants in a relatively short period without seasonal restrictions (Preil *et al*., 1988) [15]. Because of its medicinal importance and potential to produce value added secondary metabolites in tissue culture, it is of great interest to develop biotechnological methods to improve the production of this plant *in-vitro* (Khan *et al*., 2006) [9]. Although Kalanchoe is readily propagated by leaf and stem cuttings, this procedure is rather slow and insufficient, frequently resulting in low-quality plants. Bhuiyan *et al*. (2005) reported regeneration of *Kalanchoe blossfeldiana* for the first time achieving fast propagation of high quality plants. For rapid production of superior quality plants, *in-vitro* propagation is essential (Ioannou *et al*., 1992; Frello *et al*., 2002; Khan *et al*., 2006; Sanikhani *et al*., 2006) [8, 5, 9, 16].

Materials and Methods
The present investigation was conducted at Plant Tissue Culture Laboratory of Yash Biotechnology, Nashik, Maharashtra, India. Two explants namely apical node and leaf from healthy *Kalanchoe blossfeldiana* was used for regeneration and rapid multiplication. The explants was surface sterilized as per protocol described by Kordi *et al*. (2013) [10]. For *in-vitro* plant regeneration, MS medium supplemented with different hormones like cytokinins [6-benzyl amino purine (BAP), kinetin] and auxins [indole-3-acetic acid (IAA), 1-napthelene

acetic acid (NAA), indole 3-butyric acid (IBA)] at different concentrations was used. The inoculated culture bottles were incubated at 24 ± 2 ^{0}C for 16 hr light (2000-3000 Lux) at 60-65% relative humidity. Callus culture was maintained at 24 ± 2^0 in dark condition at 60-65% relative humidity for 15-21 days. For acclimatization, microshoots having well developed roots was put in soil: FYM: cocopeat (1:1:1) and in soil: cocopeat (1:1).

Results and Discussion

The effort was made to develop an efficient protocol for *in vitro* regeneration and multiplication of Kalanchoe. The experiment was conducted using nodal and leaf explants of Kalanchoe. The nodal explant was used for direct organogenesis while both nodal and leaf explants were used for indirect organogenesis.

Establishment of *Kalanchoe blossfeldiana in vitro*

After four weeks of inoculation, nodal segments of *Kalanchoe blossfeldiana* showed initiation of culture. The longest shoot (1.5 cm) was obtained on MS medium supplemented with 0.4 mg/l BAP (Table 1 and fig. 1a). In comparison with our result, Khan *et al.* (2006) [9] reported less number of shoots (1.66) but greater length of shoot on MS medium with devoid of hormones.

Shoot multiplication

Generally, *in vitro* shoot from various explants is known to be largely determined by the balance between different concentrations of auxins and cytokinins in the growth medium. The shoot initiation obtained from the *in vitro* established cultures were subjected for multiplication. In the present investigation, shoot length varied under various BAP concentrations. The maximum number of shoots (8) per nodal explant was obtained on MS medium supplemented with 2.5 mg/l BAP (Table 2 and fig. 1b). This has been supported by Bhuiyan *et al.* (2006) [3] who revealed that MS medium supplemented with 0.4 mg/l IAA and 1.0 mg/l TDZ, proved as the best medium for shoot regeneration of *K. daigremontiana*.

Callus induction

For getting multiple plants within short time, callus culture is the best *in vitro* culture. Considering this, leaf and nodal explants were used for callus induction. Total seven treatment of different concentrations of BAP and 2, 4-D was used for callus induction. Out of seven treatments, five treatments showed greenish callus in both leaf and nodal explants while other two treatments (T_1 and T_2) could not produce callus (Table 3 and Fig. 1c). The higher mass of callus was observed from nodal explant supplemented with 2.0 mg/l BAP + 2.0 mg/l 2, 4-D with MS medium. Kumlay and Ercisli (2015) [12] reported higher callus weight (2.04 g) on MS medium containing 3.0 mg/l BAP + 2.0 mg/l NAA in *Solanum tuberosum* which was four times more compared to present study. In other studies, green compact callus of *Celosia argentea* was recorded on MS medium supplemented with 0.5 mg/l NAA and 1.0 mg/l BA (Bakar *et al*., 2014) [2].

Shooting of callus tissue

The multiple shoot lets were developed from callus when MS medium supplemented with BAP and NAA. The maximum number of shoots (7) were observed when MS medium supplemented with NAA (0.1 mg/l) and BAP (0.2 mg/l) (Table 4 and fig. 1d). Similarly in other study, the best results for shoot regeneration were obtained in 0.5 mg/l NAA and 1.0 mg/l BA **(**Bakar *et al*., 2014**)** [2].

Rooting

In plant tissue culture, high concentration of auxin and low concentration of cytokinin showed rooting response to shootlets. In present study, we were utilized different concentrations of auxins like, IBA, NAA and IAA with MS medium for rooting purpose. The best rooting response (7 roots) was observed on MS medium containing 1mg/l IBA compared to MS medium supplemented with 1mg/l NAA and IAA (Table 5 and fig. 1e). However, in other studies of root induction, MS medium supplemented with 1mg/l NAA showed highest number of roots (9.71) (Kordi *et al*., 2013) [10]. Khan *et al*. (2006) [9] revealed that *in vitro* roots could be induced with devoid of any plant growth regulator in MS medium. This indicates that rooting depends on type of cultivar because some cultivar may have endogeneous auxins for rooting.

Hardening

Total six shootlets having fully expanded leaves and well grown roots putforth for hardening. Out of six, three microshoots were put in soil: FYM: cocopeat (1:1:1) while other three kept in soil: cocopeat (1:1) for hardening. One plantlet from soil: FYM: cocopeat (1:1:1) and two plantlets from soil: cocopeat (1:1) was successfully acclimatized (fig. 1f). Thus, total 50% plants successfully survived after hardening. The probable reason of dying of 50% plants may be due to low humidity, high temperatures and intense light irradiation in summer season (Kulus, 2015) [11]. Also, kind of hardening medium effects on acclimatization. Khan *et al*., (2006) [9] reported acclimatization of the *in vitro* grown plantlets with the survival rate of 96% using sand: charcoal (1:1) as a hardening medium.

The tissue culture of Kalanchoe are very important and useful for several reason including ornamental and medicinal purpose. Tissue culture could provide large scale multiplication of *Kalanchoe blossfeldiana* within short period due to market demand.

Table 1: Establishment nodal explant using different combination of BAP after four weeks of culture

Treatment	MS + BAP (mg/l)	Length of shoot (cm)
T1	0.0	0.5
T2	0.1	0.5
T3	0.2	0.7
T4	0.3	1.0
T5	0.4	1.5

Table 2: Shoot multiplication at different concentration of BAP

Treatment	MS + BAP (mg/l)	Length of shoot (cm)	Number of shoots
T1	1.0	1.8	2.0
T2	1.5	2.3	2.0
T3	2.0	2.8	3.0
T4	2.5	3.2	8.0
T5	3.0	2.6	5.0

Table 3: Effect of BAP and 2, 4-D on callus induction

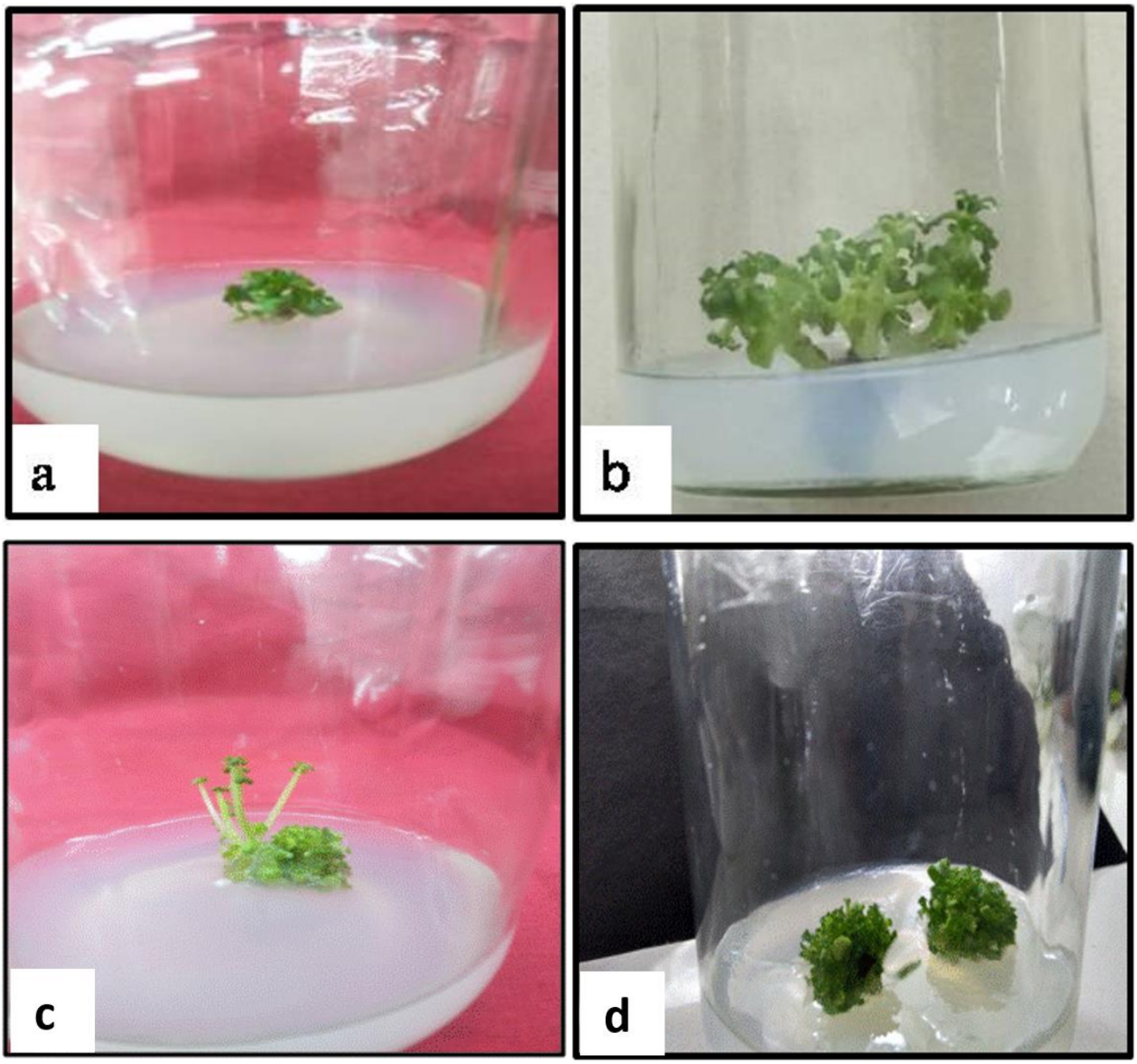
Treatment	Supplement (mg/l) with MS medium		Type of explant inoculated	Weight of callus (mg)	Colour of callus
	BAP	2, 4-D			
T1	0.5	0.5	Leaf disc	-	-
T2	0.5	1.0	Leaf disc	-	-
T3	1.0	1.0	Leaf disc	35.0	Greenish
T4	1.5	1.0	Node	38.0	Greenish
T5	1.5	1.5	Node	41.0	Greenish
T6	2.0	2.0	Node	56.6	Greenish
T7	2.5	2.5	Node	50.0	Greenish

Table 4: Effect of BAP and NAA on shoot induction from callus

Treatment	MS + BAP (mg/l)	MS + NAA (mg/l)	No. of shoots per callus	Length of shoot/s (cm) per callus
T1	0.1	0.1	5.0	1.8
T2	0.2	0.1	7.0	2.3
T3	0.3	0.1	6.0	2.2
T4	0.4	0.1	6.5	2.1
T5	0.5	0.1	5.5	2.0

Table 5: Effect of IBA, IAA and NAA on root induction

Treatment	MS + Hormone (mg/l)	No. of root/s per explants	No of shoot/s per explant (cm)
T1	IBA 1mg/l	7.0	2.5
T2	IAA 1mg/l	5.0	1.8
T3	NAA 1mg/l	3.0	2.0



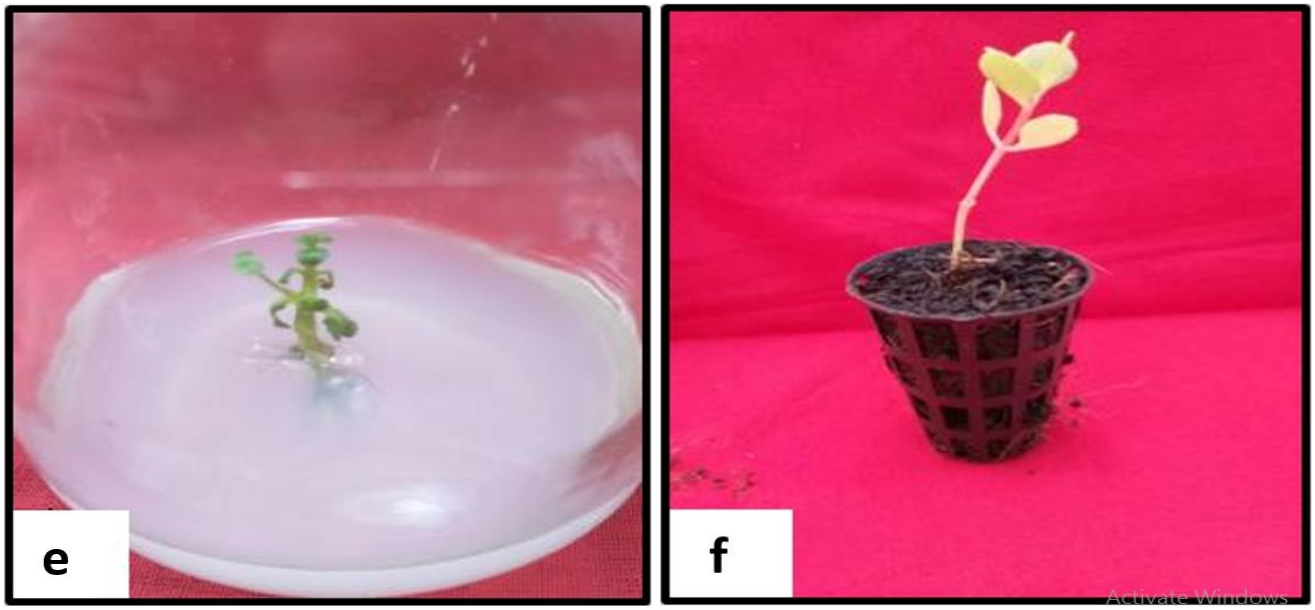


Fig 1: *In vitro* regeneration of Kalanchoe a)shoot initiation using MS + BAP (0.4 mg/l) b) Multiple shoot regeneration using MS + BAP (2.5 mg/l) c) Callus induction on MS + 2,4-D (2.0 mg/l) and BAP (2.0 mg/l) d) Multiplication of shoots from callus using MS + BAP (0.2 mg/l) and NAA (0.1mg/l) e) Induction of root on MS + IBA (1 mg/l),IAA (1mg/l) and NAA (1mg/l) f) Primary hardening of regenerated plants on soil: cocopeat (1:1).

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