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In Vitro Propagation of Valuable Succulents: A Review

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ABSTRACT

Succulents, popular group of terrestrial plants known for their unique appearance and ability to thrive in tropical arid environments. They can be vegetatively propagated through cuttings and seeds, also through *in vitro* propagation mass plantlets can be produced. Tissue culture raised plantlets have a number of advantages over traditional propagation methods, such as ability to produce large numbers of plants from a small amount of starting material, plants with free of pests, diseases and the ability to produce plants that are resistant to adverse environmental conditions. In this review, various results and discussion regarding different concentrations of plant growth regulators for multiplication, rooting of succulents with effective growth condition are described.

Keywords: Succulents, *In vitro* propogation, Growth regulators, Regeneration

INTRODUCTION

Succulent plants are thickened, fleshy and engorged, usually to retain water in arid climates or soil conditions. They store water in various structures, such as leaves and stems. The water content of some succulent organs can get up to 90–95%. The habitats of these water-preserving plants are often in areas with high temperatures and low rainfall, such as deserts, but succulents may be found even in alpine ecosystems growing in rocky soil. Succulents are characterized by their ability to thrive on limited water sources, such as mist and dew, which makes them equipped to survive in an ecosystem that contains scarce water sources. Succulents come in a wide variety of shapes, sizes and colors, making them popular houseplants and garden additions (Anonymous, 2023a).

Succulents plant parts like leaf sap, resinous solid known as aloe lump or cape aloe which is used as a laxative. Leaves or roots that are boiled in water are taken as a laxative, arthritis, eczema, conjunctivitis, hypertension and stress. Succulents can contribute to reducing flu-like symptoms, headaches, fever and coughs. Leaves can remove many volatile organic compounds (VOCs) from the air. Plants emit water vapor, and that in turn generates a pumping action that pulls contaminated air down to the roots of the plant. As adaptogens, succulents target inflammatory, circulatory, oxidant, and ultraviolet aggressors, as well as improve the texture of skin, when regularly used topically (Anonymous, 2023).

Succulents are popular ornamental plants for both indoor and outdoor use. They are relatively easy to care for and can add a touch of beauty to any space. Some succulents are edible, including aloe vera,

prickly pear and dragon fruit. These succulents can be eaten raw, cooked or used in processed foods. Succulents have been used for centuries to treat a variety of medical conditions. Aloe vera is known for its ability to soothe and heal burns. Other succulents have been used to treat digestive problems, skin conditions and respiratory infections. Succulents can help to purify the air by removing pollutants and toxins. This is especially beneficial in indoor environments. Succulents are very drought-tolerant plants, which makes them ideal for planting in dry areas. They can also be used to help prevent erosion and desertification. (Anonymous, 2023b).

Succulents can be propagated by different means. The most common is vegetative propagation; this includes cuttings where several inches of stem with leaves are cut and after healing, produce a callus. After a week or so, roots may grow. A second method is division consisting of uprooting an overgrown clump and pulling the stems and roots apart. A third method is propagation by leaf by allowing the formation of a callus. During this method, a bottom leaf is fully removed from the plant often by twisting or cutting. The leaf then dries out and a callus forms preventing the leaf from absorbing too much moisture and thus rotting. This method typically takes up to a few weeks to produce healthy roots that would eventually create new plants (Anonymous, 2023a).

Succulents are susceptible to rots caused by fungi, bacteria and this makes it difficult to maintain a large number of succulents. Tissue culture can be used to propagate succulents much faster than traditional methods (Anonymous, 2023b). To overcome various problems, tissue culture is a proven way to culture succulents under *in vitro* conditions (Anonymous, 2023c). *In vitro* propagation has the potential to revolutionize the succulents industry. By making it possible to produce large numbers of high-quality plants quickly and efficiently, *in vitro* propagation can help to the growing demand for succulents.

In addition to its commercial applications, *in vitro* propagation can also be used to propagate rare or endangered succulents. This can help to preserve biodiversity and ensure the survival of these unique plants (Anonymous, 2023b)

Some of the most types of succulents include:

1 **Cacti:** Cacti are a type of succulent that is native to the Americas. They are known for their thick, fleshy stems and spines. Some popular cacti include the *saguaro cactus*, the *prickly pear cactus* and the *barrel cactus.*

2 **Agaves:** Agaves are another type of succulent that is native to the Americas. They have large, thick leaves that grow in a rosette pattern. Some popular agaves include the century plant, the blue agave and the queen *Victoria agave.*

3 **Aloes:** Aloes are type of succulent that is native to Africa. They have long, fleshy leaves that are often used in medicinal products. Some popular aloes include the aloe vera plant and the coral aloe plant.

4 **Echeverias:** Echeverias are a type of succulent that is native Central and South America. They have rosettes of thick, fleshy leaves that come in a variety of colors. Some popular echeverias include the hens and chicks plant, the black prince echeveria and the rainbow echeveria. (Anonymous, 2023b).

***In vitro* Establishment of Succulents: Problems and solutions**

In vitro establishment of succulents can present various challenges for horticulturists and plant enthusiasts. There is continuous challenge to obtain quality planting materials and reaches a bottleneck when producing in large quantities. One common problem is contamination by microorganisms, such as bacteria and fungi, which can hinder the growth of succulent plantlets. To lower-down these

contaminants and to improve efficiency of sterilants, newly emerged lateral branches were selected before 6 days of culture initiation and sprayed with solution of bavistin (0.1%) and streptomycin (100mg/l). Also, to increase efficiency of procedures and to overcome various problems during culture initiation, following strategies were employed:

1. ***Size of explant:***

Size of explants matters in ascetic culture initiation. If size of explants is bigger then surface of explants also lager which contains greatest microbial load on it. Small size of explant gives more beneficial results than the larger explants. But if size of explants is small it will show poor response

2. ***Position and size of nodal segments:***

Surface sterilization treatment plays an important role in establishment of *in vitro* cultures. Sterilants not only removes micro-organisms but also causes phyto-toxic effect in plant tissue. After sterilization, immature and small size explants turn to brown and simultaneously dead. Large size explants, contains more microbial count, tolerate harsh sterilants treatment but hinder bud sprouting. Many researchers, used buds from newly emerging sprouts for culture establishment. Hence, to select proper size and nodal position of explants, newly emerged sprouts are selected.

3. ***Per-treatment of nodal explants:***

To remove excess microbial load present on surface of explants pre-treatment of selected explants employed. Without removal of leaf sheaths (covering buds), nodal segments were swabbed with 70% ethanol using absorbent cotton and then leaf sheaths removed without any injury to bud and kept under running tap-water for 30 minutes. Explants were pre-treated with non-ionic, polysorbate surfactant 1% Tween-20 for 15 minutes and then with antiseptic solution of 1% Savlon (Chlorhexidine Gluconate I.P. 1.5% v/v & Cetrimide Solution Cetrimide I.P. 3.0% w/v) for 15 minutes followed by 2-3 wash of sterilized distilled water. These explants were then treated with fungicide Bavistin (*Carbendazim*-50% (W.P.) for 60 minutes and rinse 3 times with sterilized distilled water.

4. ***Surface sterilization and in vitro establishment***

After pre-treatments, these explants taken in laminar air flow cabinet and transferred to sterilized bottle jar. These explants were treated with different sterilants at different concentration and time**Error! Reference source not found.**. Also, a stable, broad spectrum antibiotic 'Plantomycin' (Aries agro limited) with active content of Streptomycin sulphate = 9%, Tetracycline Hydrochloride = 1% used. Use of sterilizing chemicals is beneficial, like, $HgCl_2$, H_2O_2, NaOCl, Bromine water, etc. Regular subculturing and the use of anti-microbial agents can also help control contamination.

5. ***Manipulation of in vitro growth:***

Another issue in succulent *in vitro* culture is the formation of callus tissue instead of shoot regeneration. This can occur due to excessive levels of plant growth regulators, particularly auxins. Adjusting the hormone concentrations and exploring different combinations can promote the formation of shoots instead of callus. Inadequate rooting is a common problem during succulents. To facilitate root development, the addition of auxins like indole-3-butyric acid (IBA) to the culture medium can be beneficial. It important to strike a balance between the auxin concentration and the succulents species requirements to achieve successful rooting.

Furthermore, proper acclimatization is crucial for transferring *in vitro* grown succulent plantlets to *ex-vitro* conditions. Gradually exposing them to ambient humidity and light levels helps prevent stress and

enhances their survival rates when moved to soil. These challenges and solutions underscore the importance of meticulous care and attention to detail in the *in vitro* establishment of succulents, ultimately

Table 1: *In vitro* reviews of important species of succulents

Sr. no.	Varieties	Medium combinations			Culture condition	Results	Reference
		Medium	Cytokinin	Auxin			
	Haworthia turgida Haw.	(Murashige & Skoog 1962) MS Medium	BA 1.0 mg L^{-1}	NAA 0.1 mg L^{-1}	Temp. 25±2°C & 70% relative humidity	Multiplication: MS + BA 1.0 mg L^{-1} Rooting: MS + NAA 0.1 mg L^{-1} TDZ 2.5 mg L^{-1}	Liu *et.al*, 2017
	Cotyledon orbiculata L. (Crassulaceae)	MS medium	BA 2.0 µM	IBA 10 µM	–	Multiplication: MS + BA 2.0µM Rooting: MS + IBA 10 µM	Kumari *et. al*, 2016
	Portulaca grandiflora.	MS medium	BAP 4.0mgLG	NAA 0.75mgLG	Temp. 25± 2°C and 70-80% relative humidity	Multiplication: MS + BAP 4mgLG Rooting: MS+ NAA 0.75mgLG	Jain *et.al*, 2010
	Tinospora cordifolia (Willd.)	MS medium	BA (0.44–13.31 µM) Kinetin (0.46–13.94 µM)	IBA 6.43 µM	Temp. 25 °C and 55% relative	Multiplication: MS + Kinetin 4.36 µM	Sivakumar *et.al*, 2014

					humidity 16hrs. photoperiod	Rooting: MS + IBA 6.43µM	
	Caralluma sarkariae.	MS medium	BAP (0.1,0.5,1.0,2.0 mg/L) Kinetin (0.1,0.5 mg/L)	IAA, NAA, IBA (0.10,0.50,1.00,2.00,3.00 mg/L)	Temp. 25±2°C 16hrs. photoperiod	Multiplication: MS + BAP 2.0 mg/L Kinetin 0.5 mg/L Rooting: MS+ IAA 2.00 mg/L	Rani *et.al,* 2008
6	*Pilosoceres robinii* (Lemaire)	MS medium	BAP 13.32µM	-	Solar light 48.1-62.5 µE.m^{-2} s^{-1} at 28±2ºC.	Multiplication: MS + 13.32µM 6-BAP	Mendoza. *et.al,* 2009
7	*Pelecyphora aselliformis ehrenberg and p. Strobiliformis werdermann* (Cactaceae)	MS medium	BA 8.8 µM	IAA 2.85 µM IBA 2.46µM	Temp. 25 ± 2°C 16/8-hrs. light/dark photoperiod	Multiplication: MS + BA 8.8µM Rooting: MS + IBA 4.90µM	Balch. *et. al,* 2002
8	*Copiapoa tenuissima Ritt. forma monstruoa*	MS medium	-	2,4-D 9.05µM	Temp. 24 ± 2°C 16hrs. photoperiod	Rooting: MS+ 2,4-D 9.05µM	Rumins ka *et. al,* 2011

9	*Agave parrasana*	MS medium	BA 0-53.2 µM	2,4-D 0-0.18 µM	Temp. 27±2 ◦C. 16hrs. photoperiod	Multiplication: MS + BA 53.2 µM Rooting: MS+0.11 µM 2,4-D	Fernando *et.al*, 1999
10	*Aloe vera L.*	MS medium	-	IBA 1mg/L	Temp. 22±1 °C and 17±1 °C.	Rooting: MS+ IBA 1mg/L	Hamdeni *et.al*, 2021
11	*Aloe barbadensis Miller*	MS medium	BAP 10-40 µg/L	IBA+NAA (1.5+2.0 µg/L)	Temp. 24±2ºC and 16hrs. photo period	Multiplication: MS + BAP 2mg/L Rooting: MS+ IBA 50 µg/L	Jayakrishna *et.al*, 2011
12	*Mammillaria genus* (Cactaceae)	MS medium	BA, KIN, Metatopolin, TDZ (0.4-8.9µM)	-	Temp. 25 ± 1°C with a 16hrs. photoperiod	Multiplication: MS+ BA, KIN, Metatopolin, TDZ (0.4-8.9µM)	Castellanos *et.al*, 2018
13	*Cotyledon orbiculata*	MS medium	5µM TDZ and 2.0µ M BA.	10µM IBA	Temp. 25 ± 2°C with a 16hrs. photoperiod	Multiplication: MS + 5 µM TDZ & 2.0µM BA Rooting: MS+0.5 µM NAA	Staden *et.al*, 2016
14	*Coryphantha minima*	MS medium	BA 4.40µM 46.0µM	NAA 2.27µM	Temp. 25-27°C	Multiplication: MS + BA 1mg L^{-1}	Malda *et.al*, 1999

						Rooting: MS+ NAA 0.05mg L^{-1}	
15	*Obregonia denegrii Fric. and Coryphana minima*	MS medium	BA 0.5 mg L^{-1}	NAA 0.1mg L^{-1}	Temp. 25 - 27°C	Multiplication: MS + BA 1mg L^{-1} Rooting: MS+ NAA 0.1mg L^{-1}	Malda *et.al*, 1999
16	*Copiapoa tenuissima Ritt. forma monstruosa*	MS medium	-	9.05 µM 2,4-D	Temp. 24 ± 2°C. 16hrs. photoperiod	Rooting: MS+9.05 µM 2,4-D	Rumins ka , 2011
17	*Notocactus magnificus*	MS medium	BAP (0, 2.2, 8.9 or 22.2 µM) kinetin (0, 4.6, 9.3 or 23.2 µM)	NAA (0, 2.7, 10.7 or 26.9 µM) IAA (0, 2.9,5.7µM)	Temp. 25±2 °C with 14hrs. photoperiod	Multiplication: MS + BAP 22.2 µM Rooting: MS+IAA 2.9 µM	Medeiros *et.al*, 2005
18	*Opuntia ficus-indica*	MS medium	BA (0.5, 1.0, 1.5, 3.0 and 5.0 mg L^{-1}) Kinetin (0.5, 1.0, 1.5, 3.0 and 5.0 mg L^{-1})	NAA 0.5mg L^{-1} IAA (0.25, 0.5 or 1.0 mg/L)	Temp. 25±°C with 16hrs. photoperiod	Multiplication: MS + BA 5.0 mg /L Rooting: MS+ IAA 0.5mg/L	Khalafalla *et.al*, 2007
19	*Purple pitahaya (Hylocereus costaricensis)*	MS medium	BAP (0, 5, 15, 30, 45, or 60µM)	NAA 0.5µ*M*	Temp. 24-25°C 12hrs. Photoperiod	Multiplication: MS + BAP 30µM	Vinas *et.al*, 2012

						Multiplication: MS + NAA 0.5µ*M*	
20	*Obregonia denegrii FrIC.* (Cactaceae)	MS medium	BAP 4.4µM	NAA 10.7µM	Temp. 25 ± 1°C 16hrs. photoperiod	Multiplication: MS + BAP 4.4µM Rooting: MS+ NAA 10.7µM	Cardarelli *et.al,* 2010
21	*Turbinicarpus laui Glass et Foster* (Cactaceae)	MS medium	BA (0, 2.2, 4.4, 8.8, 13.32 µM)	NAA (0, 0.54, 2.68 µM)	Temp. 27±2°C 16hrs. photoperiod	Multiplication: MS + BA 13.32µM Rooting: MS+ NAA 2.68µM	Rosas *et.al,* 2001
22	*Pilosocereus robinii*		BA (0.0, 0.5, 1.0, 2.0 and 4.0 mg/L)	IAA, IBA or NAA (0.0, 0.1, 0.2 and 0.4 mg/L)	Temp 22 ± 2 °C	Multiplication: MS + KN (2.0 mg/L) Rooting: MS+ NAA 0.4 mg/L	Khattab *et.al,* 2013
23	*Salicornia brachiata*	MS medium	BA (4.44,8.9,13.3,22 .2,44.4 µM)	NAA (5.37 µM) 2,4-D (4.52 µM)	Temp. 25±1°C 16hrs. photoperiod	Multiplication: MS + BA 8.9µM Rooting: MS+ NAA 5.37 µM	Joshi *et.al,* 2011

24	*Escobaria minima* (Baird), *M. pectinifera, P. aselliformis*	MS medium	BA (22.18-44.4µM) & KN (23.23 or 4.65 mM)	NAA 0.05 µM	Temp. 23 ±1°C and 16hrs. photoperiod	Multiplication: MS + BA 22.20 µM Rooting: MS+2,4-D 0–9 µM	Giusti *et. al*, 2002
25	*Opuntia*	MS medium	BA 0.5 µM	IBA 5.5 µM	Temp. 27 ± 2 °C 16hrs. photoperiod	Multiplication: MS + BA 0.5 µM Rooting: MS+ IBA 5.5 µM	Saucedo *et.al*, 2005
26	*Nopalxochia ackermannii*	MS medium	BA 0.5 mg·L^{-1}	IBA 0.25 mg·L^{-1}	Temp. 25±2°C 14hrs. photoperiod	Multiplication: MS + KT 1.0 mg·L^{-1} Rooting: MS+ NAA 0.2 mg·L^{-1}	Deng *et.al*, 2018
27	*Coryphantha macromeris*	MS medium	BA 44 µM	2,4-D 0.5 µM	Temp. 26±3°C & 16hrs. photoperiod	Multiplication: MS + BA 44 µM Rooting: MS+2,4-D 0.5 µM	Smith *et.al*, 1991
28	*Tuberaria major*	MS medium	BA, Kinetin, Zeatin 0.1, 0.2, or 0.5 mg/L^{-1}	IBA 0.2 or 0.5 mg/L^{-1}	Temp. 25±2°C 16hrs. photoperiod	Multiplication: MS + BA 0.2 mg/L^{-1} Rooting: MS+ IBA 0.5 mg/L^{-1}	Goncalves *et. al*, 2010

29	Cactus *(Cereus peruvianus l.)*	MS medium	BAP 5 mg/l	NAA 0.1 mg/l	Temp. 24±1°C	Multiplication: MS + BAP 5 mg/l Rooting: MS+ NAA 0.1 mg/l	Sawsan *et.al,* 2004
30	*Melocactus salvadorensis*	MS medium	BAP 4 mg L^{-1}	NAA 0.1 mg L^{-1}	Temp. 25±2°C	Multiplication: MS + BAP 5 mg L^{-1} Rooting: MS+ NAA 1 mg L^{-1}	Monostori *et.al,* 2012
31	*Pachyveria pachytoides and Sedum morganianum*	MS medium	BAP 6.0 mg l^{-1}	NAA 0.1 mg l^{-1}	Temp. 20 -25 °C	Multiplication: MS + BAP 6.0 mg l^{-1} Rooting: MS+NAA 0.1 mg l^{-1}	Xiaodan Xu *et.al,* 2017
32	*Rauvolfia serpentina*	MS medium	TDZ (0.1, 0.3, 0.5, 0.8, 1.0, 2.5 µmol/L)	IBA (0.1, 0.5, 1.0, 1.5, 2.0 µmol/L)	Temp. 24±2°C 16hrs. photoperiod	Multiplication: MS + TDZ 0.8 µmol/L Rooting: MS+ IBA 1.0 µmol/L	Alatar et.al, 2015
33	*Brahmi Bacopa monniera (L.) Pennell*	MS medium	BAP (0.5, 1.0, 1.5, 2.0, 2.5mg/L)	NAA (0.5–1.0mg/L)	Temp. 25±2°C 8hrs.	Multiplication: MS + BAP 0.5mg/L	Tanveer *et.al,* 2010

					photoperiod	Rooting: MS+ NAA 0.5mg/L	
34	*Rhodiola imbricata*	MS medium	BAP (0.25–10 µM), KN (0.25–10 µM), TDZ (0.1–5 µM)	NAA (1–10 µM), IBA (2.5–10 µM), IAA (2.5–10 µM)	Temp. 22±2°C 16hrs. photoperiod	Multiplication: MS + TDZ 1.0µM BAP 5µM KN 5µM Rooting: MS+ IBA 5µM IAA 5µM	Bhardwaj *et.al*, 2018
35	*Aloe pruinosa*	MS medium	BA (5,10 µM)	IAA (2,4 µM)	Temp. 25±2°C 16hrs. photoperiod	Multiplication: MS + BA 5 µM Rooting: MS+ IAA 4 µM	Baskaran *et.al*, 2015
36	Pistachio *(Pistacia vera L.)*	MS medium	Metatopolin 2 mg l^{-1}	IBA (0.5-2.5mg l^{-1}) NAA (0.5-2.5mg l^{-1})	–	Multiplication: MS + Metatopolin 2 mg l^{-1} Rooting: MS+ IBA 2.5 mg l-1 NAA 1 mg l^{-1}	Benmahioul *et.al*, 2017
37	Cactus *(Opuntia Ficus indica)*	MS medium	BAP (0, 2, 2.5, 3, 3.5, 4 mg/l)	IBA 0.5 mg/l NAA 0.5 mg/l	Temp. 25±1°C 16hrs. photoperiod	Multiplication: MS + BAP 3 mg/l Rooting: MS+IBA 0.5 mg/l	Fatima *et.al*, 2022

						NAA 0.5 mg/l	
38	*Mexican cacti*	MS medium	BA 8.85 µM	NAA 0.01mg IBA 4.90 µM	Temp. 25 ± 2°C 16hrs. photoperiod	Multiplication: MS + BA 0.44 µM Rooting: MS+NAA 0.01 mg IBA 2.46 µM	Balch *et.al*, 1998.
39	*Cactus and Agave*	MS medium	KIN 1,2 and 3 mg/l	NAA 1, 2 and 3 mg/l	Temp. 27 ±2°C	Multiplication: MS + KIN 2 mg/l Rooting: MS+NAA 1mg/l	Ruvalcaba *et.al*, 1998
40	*Echinocereus cinerascens*	MS medium	BAP (0.5, 1.0, 1.5, 2.0 mg l^{-1})	NAA (0.5, 1.0, 1.5, 2.0 mg l^{-1})	Temp. 27 ±2°C	Multiplication: MS + BAP 1.5 mg l^{-1} Rooting: MS+ NAA 2.0 mg l^{-1}	Elias *et.al*, 2014
41	*Sansevieria Trifasciata Var. Laurentii* (Prain)	MS medium	-	IBA (1.00–10.00 mgl $^{-1}$)	Temp. 22±2°C 16hrs. photoperiod	Rooting: MS+ IBA 10 mgl^{-1}	Kaur *et.al*, 2021
42	*Pyrus communis*	MS medium	BA 1mg/L	IBA 0.01mg/L	Temp. 25°C	Multiplication: MS + BA 1mg/L	Anno *et.al*, 1989

					16hrs. photoperiod	Rooting: MS+ IBA 0.01mg/L	
43	*Micranthocereus Backeb. (Cactaceae)*	MS medium	-	NAA 1.34µM L^{-1}	Temp. 25±3°C 16hrs. photoperiod	Rooting: MS+ NAA 1.34µM L^{-1}	Civatti *et.al* 2017
44	*Dactylopius opuntiae (Hemiptera : Dactylopiidae)*	MS medium	BA 1.0mg/L^{-1}	IAA 0.1mg/L^{-1}	Temp. 27±2°C 16hrs. photoperiod	Multiplication: MS + BA 1.0mg/L^{-1} Rooting: MS+ IAA 0.1mg/L^{-1}	Houllou *et.al*, 2009
45	*Tinospora cordifolia (wild.)*	MS medium, Nitsch and Nitsch medium	Kinetin 0.5 mg/L	2,4-D (3ppm, 5ppm, 7ppm) IBA, IAA, NAA (0.2, 0.4, 0.6,0.8 mg/L)	Temp. 25°C 16hrs. photoperiod	Multiplication: MS + Kinetin 0.5 mg/L Rooting: MS+ IAA 0.5 mg/L NAA 0.2,0.4 mg/L IBA 0.4,0.6 mg/L 2,4-D 3ppm	Paul *et.al*, 2012
46	*Pitaya*	MS medium	ZT 13.68 µM, TDZ 0.11 µM, BA 17.76 µM	2,4-D 0.23 µM	Temp. 25 ± 2 °C	Multiplication: MS + ZT 13.68 µM	Hua *et.al*, 2014

					16hrs. photoperiod	Rooting: MS+ IBA 2.46 µM	
47	*Plectranthus bourneae*	MS medium	BA (0.7 mg/l) TDZ (1.0 mg/l)	IBA (1.5 mg/l)	Temp 26 ± 2°C 16hrs. photoperiod	Multiplication: MS + BA 0.7 mg/l Rooting: MS+ IBA 1.5 mg/l	Thaniarasu *et.al*, 2015
48	*Pilosocereus robinii*	MS medium	BA (0.0, 0.5, 1.0, 2.0 and 4.0 mg/L)	IAA, IBA or NAA (0.0, 0.1, 0.2 and 0.4 mg/L)	Temp 22 ± 2 °C	Multiplication: MS + KN (2.0 mg/L) Rooting: MS+ NAA 0.4 mg/L	Khattab *et.al*, 2013

leading to the successful propagation of these unique and often sought-after plants.

Conclusion

In conclusion, the outcome of the various researchers indicates that, endogenous level and exogenously supplied plant growth regulator plays an important role during regeneration of plantlets and these are species specific. Although there is contamination problem during establishment of nodal explants, multiplication rate can be increases by manipulating PGR concentrations. During establishment of culture, all culture induced on 0.25 BAP. When a huge mother culture induced on 0.25 mg/L BAP, then PGR combination employed. For multiplication combinations ranging from 0.25 mg/L BAP to 5 mg/l BAP. Another problematic step in micropropagation of *succulents* is rooting, as increased level of endogenous cytokinin in previous step of shoot multiplication. To increase rooting response it is important to lower down endogenous level of cytokinin, for this one passage carry out on lower concentration of auxins or on growth regulator free medium. For rooting induction combination ranging from 0.25 mg/l IBA /IAA /NAA to 2.0 mg/l may use. During hardening temperature, light, humidity with respect to species maintained. The most effective culture condition is 25°C temperature and 16/8 light/Dark photoperiod.

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