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In vitro micropropagation of insulin (*Costus pictus*)

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Abstract
The present investigation has been conducted on "*In vitro* micropropagation of Insulin *(Costus pictus)* from nodal bud was undertaken to identify suitable media combinations for micropropagation. The mother plant of *Costus pictus* was collected from Sai Nursery, Chinchpur. Tal: Rahata, Dist: Ahmednagar, Maharashtra. The explants were initiated on MS media with BAP (0.5 mg/l BAP, 1 mg/l BAP, 1.5 mg/l BAP, 2 mg/l BAP, 2.5 mg/l BAP) and IAA (0.5 mg/l IAA). The best results of explants initiation were obtained on MS media containing 1.5 mg/l BAP and 0.5 mg/l IAA, after 2 weeks of culture. Multiplication was observed after 6 weeks of growth cycle. The best result of multiplication was observed for 1.5 mg/l BAP AND 1.5 mg/l IBA hormonal combination. Best result of rooting was observed for MS media supplemented with 1.5 mg/l IBA and 1 mg/l IAA. After one week the culture were transferred to hardening in control condition at polyhouse.

Keywords: *Costus pictus, in vitro* propagation, Micropropagation, BAP, IBA, IAA

1. Introduction

The *Costus* is genus of perennial tropical herbaceous flowering plants belongs to the family *Costaceae*. It is often characterized and distinguished from relatives such as Zingiber (true ginger) by their spiraling stems. It is originated in South and Central America. It can be identified by particular characteristic broad, dark green leaves and beautiful solid red stems. It grows with an outstanding spiral effect along with the green inflorescences, with their yellow-with-red-striped flowers. It is widely cultivated in south India and also grows wild in many places.

It can be identified by particular characteristic broad, dark green leaves and beautiful solid red stems *C. pictus* is also well known for its medicinal value mainly antiseptic, tonic, aphrodisiac, carminative, stomachic, and vermifuge. It is able to prevent the hair turning grey and its root is anodyne, antibacterial properties (Devi VD, Urooj A, 2008). It is widely used as a remedy for diabetes. Powdered leaves of *C. pictus* known to possess therapeutic effect, when supplemented to streptozotocin induced diabetic rats, is found to reduce blood glucose level by 21% after 15 days of supplementation. The Methanolic leaf extract of *C. pictus* used to lower blood glucose level in alloxan induced diabetic rats.

2. Materials and Methods

The research work was carried out in the Department of Plant Biotechnology, College of Agricultural Biotechnology, Loni, and Affiliated by MPKV, Rahuri.

1. Collection of Plant Material

Explants were collected from the healthy and virus free mother plants, from the Sai Nursery,

Surface Sterilization of explants

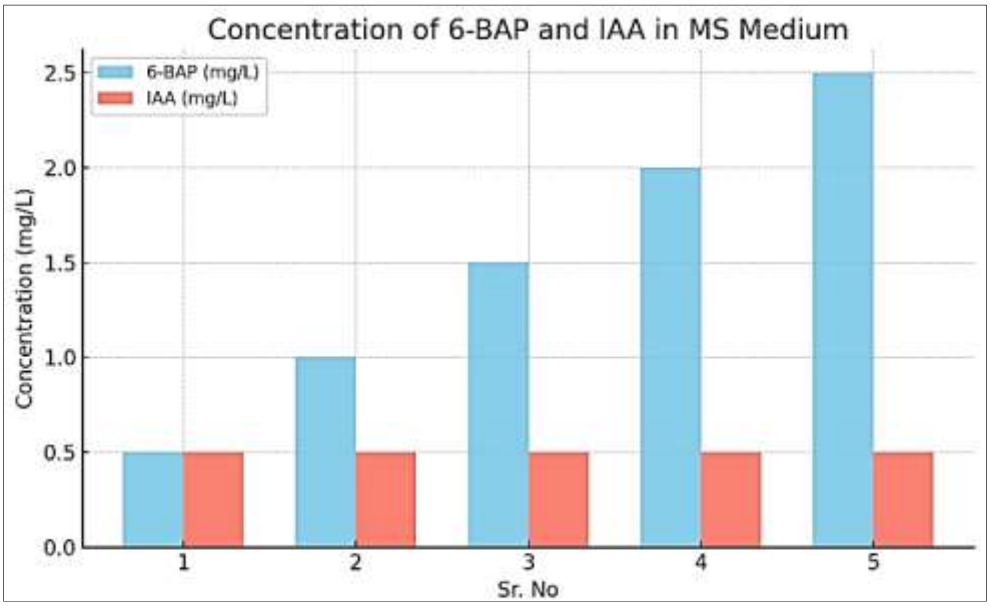
The young shoot tips (1-2 cm) were washed thoroughly under running tap water for up to 10 minutes to remove the traces of dust etc. Followed by treatment with 0.5% Bavistin for 3-5 min. Then treatment followed by successive three washing with distilled water to make the explants free from detergent. Again, the explants were washed with 70% ethanol for 30 seconds & washed with distilled water for 3-4 times. After that, the explants were transferred to laminar air flow chamber and finally explants sterilized with 0.1% $Hgcl_2$ for 1 minute in LAF. Then explants washed with sterile distilled water for 3-4 times.

2. Inoculation of Culture

A piece of plant tissue explants was excised from the plant

Table 1: Different Media combinations for Initiation

Sr. No	MS Media+ 6- BAP+IAA(Mg/L)	
	6-BAP	IAA
1.	0.5	0.5
2.	1.0	0.5
3.	1.5	0.5
4.	2.0	0.5
5.	2.5	0.5



Graph 1: Different Media combinations for Initiation

4. Maintenance of Culture Room

All the inoculated culture tubes were incubated at 25±2 °C temperature for 16 hours light and 8 hours dark per day in 2000-3000 lux light intensity under cool fluorescent white light in the culture room.

5. Initiation of Culture: The inoculated culture bottles are observed for 2-3 weeks. The cultured explants started to initiate shoot formation within 1 week. After 2-3 weeks, well grown shoots were observed

6. Shoot Multiplication

Shoot multiplication was carried out using the 4-5 cm long and most vigorous shoots from the initiation stage. Newly formed shoots were cut in 1.5-2 cm size and cultured on shoot multiplication media containing hormones concentration of BAP (0.5, 1.0, 1.5, 2.0 mg/l) and IBM 1.5 mg/l. Initiated cultures were transferred to autoclaved medium within bottles in laminar air flow hood only. Every possible care has been taken to prevent any further contamination. Shoot proliferation was determined after six weeks of culture. Data were recorded in terms of mean number of shoots per explants and survival percentage.

7. Rooting

After 4 weeks of newly formed shoots on multiplication medium measuring length 2-4 cm were excised individually from the cluster and transferred to rooting media under aseptic conditions in laminar air flow hood. Four types of media used for rooting are given below,

Table 2: Media used for rooting

Sr. No.	MS medium	IBA(Mg/L)	NAA(Mg/L)
1.	MS medium	0.5	0.0
2.	MS medium	1.0	0.5
3.	MS medium	1.5	1.0
4.	MS medium	2.0	1.5

8. Hardening

After one week of rooting culture were transferred to hardening in controlled condition at polyhouse.

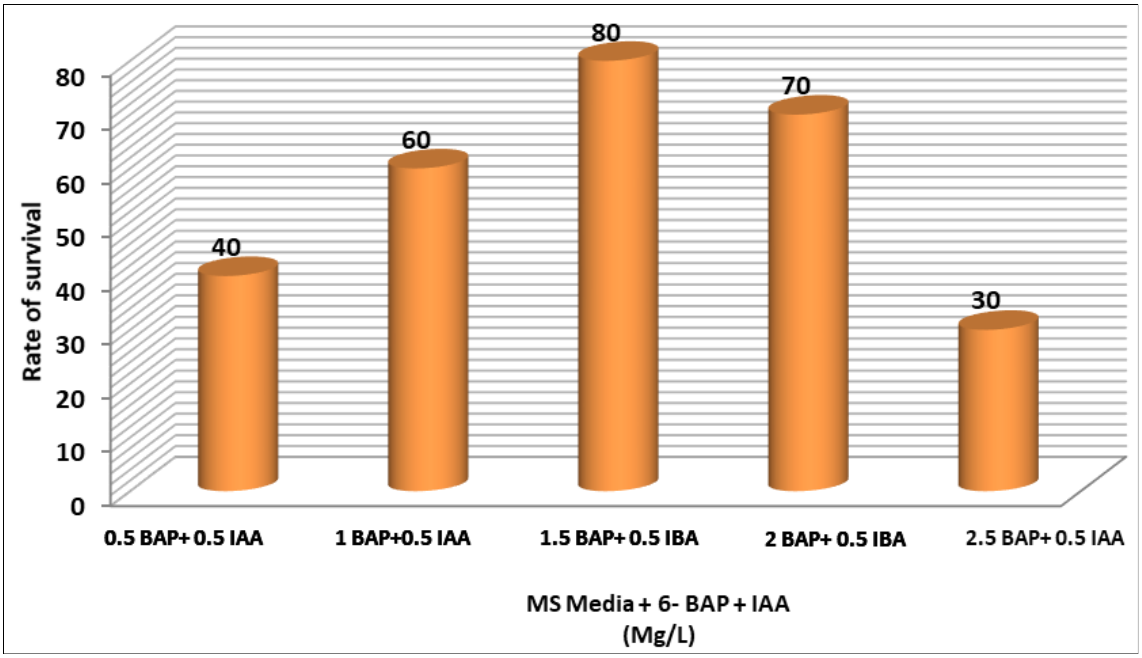
3. Result

A. Shoot Initiation Result

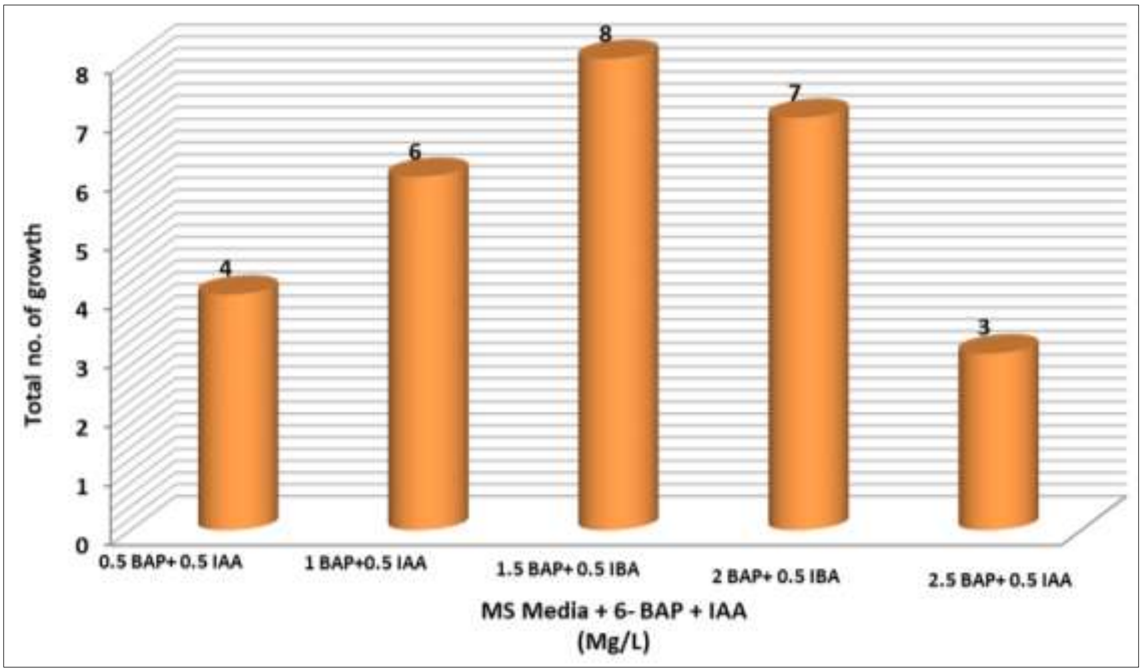
Shoot initiation and establishment from *Costus pictus* nodal buds explant were cultured on MS medium (Control) MS Media + 0.5 mg/l BAP + 0.5 mg/l IAA, MS Media + 1.0 mg/l BAP + 0.5 mg/l IAA, MS Media + 1.5 mg/l BAP + 0.5 mg/l IAA, MS Media + 2.0 mg/l BAP + 0.5 mg/l IAA, MS Media + 2.5 mg/l BAP + 0.5mg/l IAA was used

Table 3: Result of Initiation and Rate of Survival

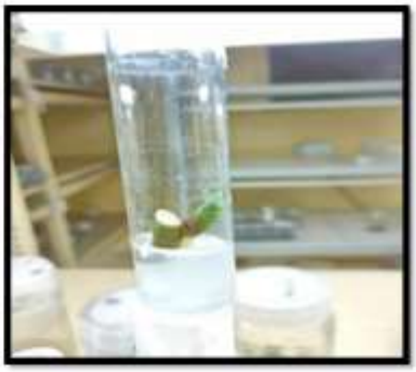
Sr. No	MS Media + 6- BAP + IAA (Mg/L)		Total no. of inoculations	Total no. of contaminated bottles	Total no. of growth	Rate of survival
	6-BAP	IAA				
1.	0.5	0.5	10	6	4	40
2.	1.0	0.5	10	4	6	60
3.	1.5	0.5	10	2	8	80
4.	2.0	0.5	10	3	7	70
5.	2.5	0.5	10	7	3	30



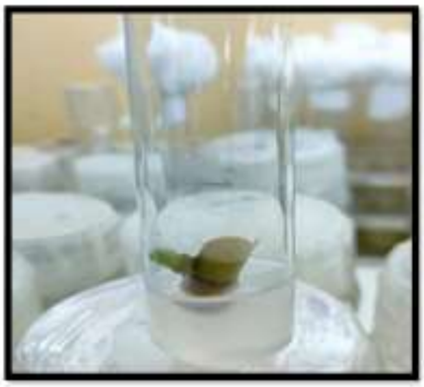
Graph 2: Rate of Survival after initiation



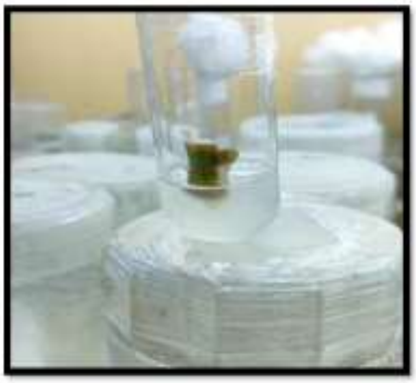
Graph 3: Total no. of growth



MS Media + 0.5 mg/l BAP + 0.5 mg/l IBA



MS Media + 1.0 mg/l BAP + 0.5mg/l IBA



MS Media + 1.5 mg/l BAP + 0.5mg/l IBA

MS Media + 2.0 mg/l BAP + 0.5mg/lIBA

MS Media + 2.5 mg/l BAP + 0.5mg/l IBA

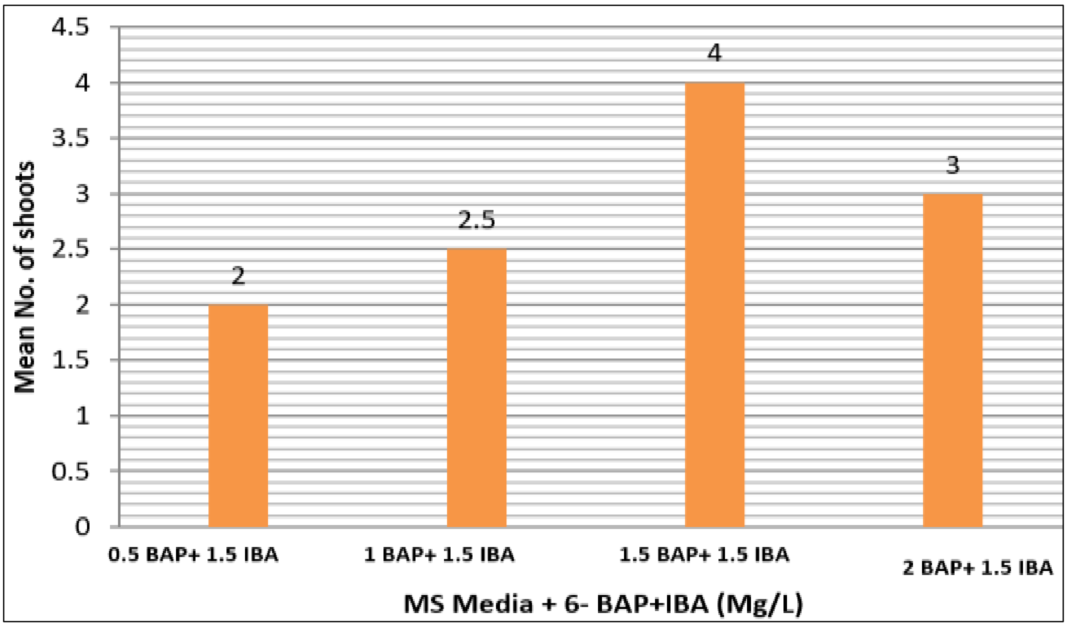
Plate 1: Initiation Result of *Costus pictus* With Different Concentration

B. Multiplication

After 6 weeks of the growth cycle, established cultures were subjected to different media concentrations for multiplication. The shoot initials obtained from the *in vitro* established cultures were subjected for multiplication.

Table 4: Result of Multiplication and mean number of shoots

Sr. No.	MS Media + 6- BAP+IBA (Mg/L)		Mean no of shoots
	6-BAP	IBA	
1.	0.5	1.5	2
2.	1.0	1.5	2.5
3.	1.5	1.5	4
4.	2.0	1.5	3



Graph 4: Result of Multiplication and mean number of shoots

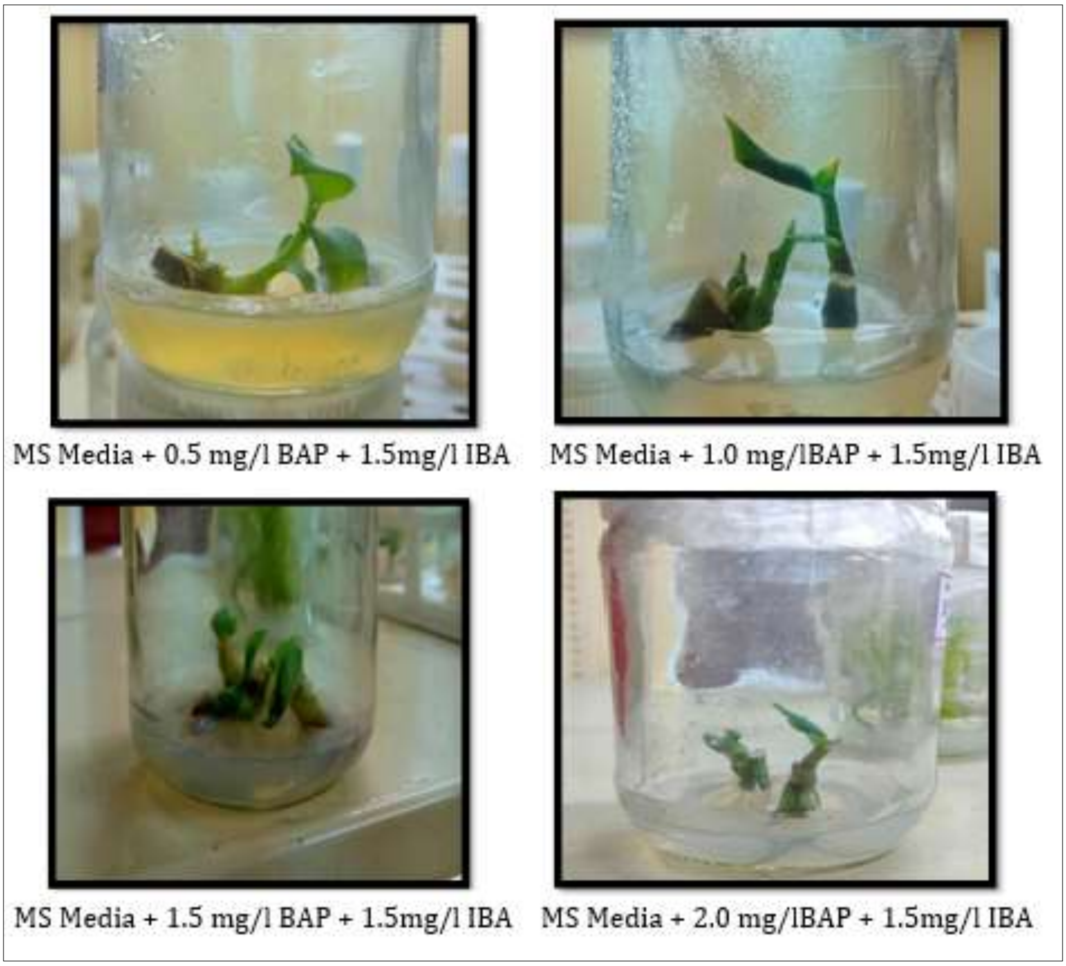


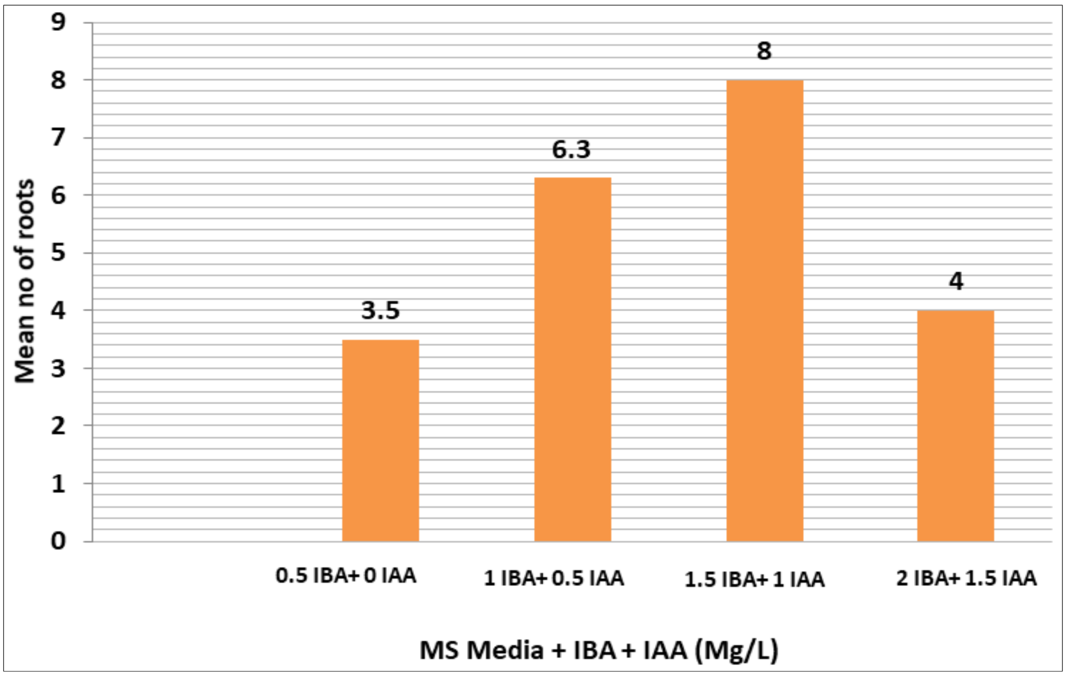
Plate 2: Multiplication Result of *Costus pictus* With Different Concentration

C. Rooting

During multiplication rooting has been occur after that culture were transferred to rooting media concentration was MS Media + 1.5 mg/l IBA + 1.0 mg/l IAA after one week the culture were transferred to hardening in control condition at polyhouse.

Table 5: Result of Rooting and mean number of roots

MS Media + IBA + IAA (Mg/L)		Mean no of roots	Longest root length
IBA	IAA		
0.5	0.0	3.5	2.7
1.0	0.5	6.3	3.3
1.5	1.0	8	4.9
2.0	1.5	4	3.8



Graph 5: Result of Rooting and mean number of roots

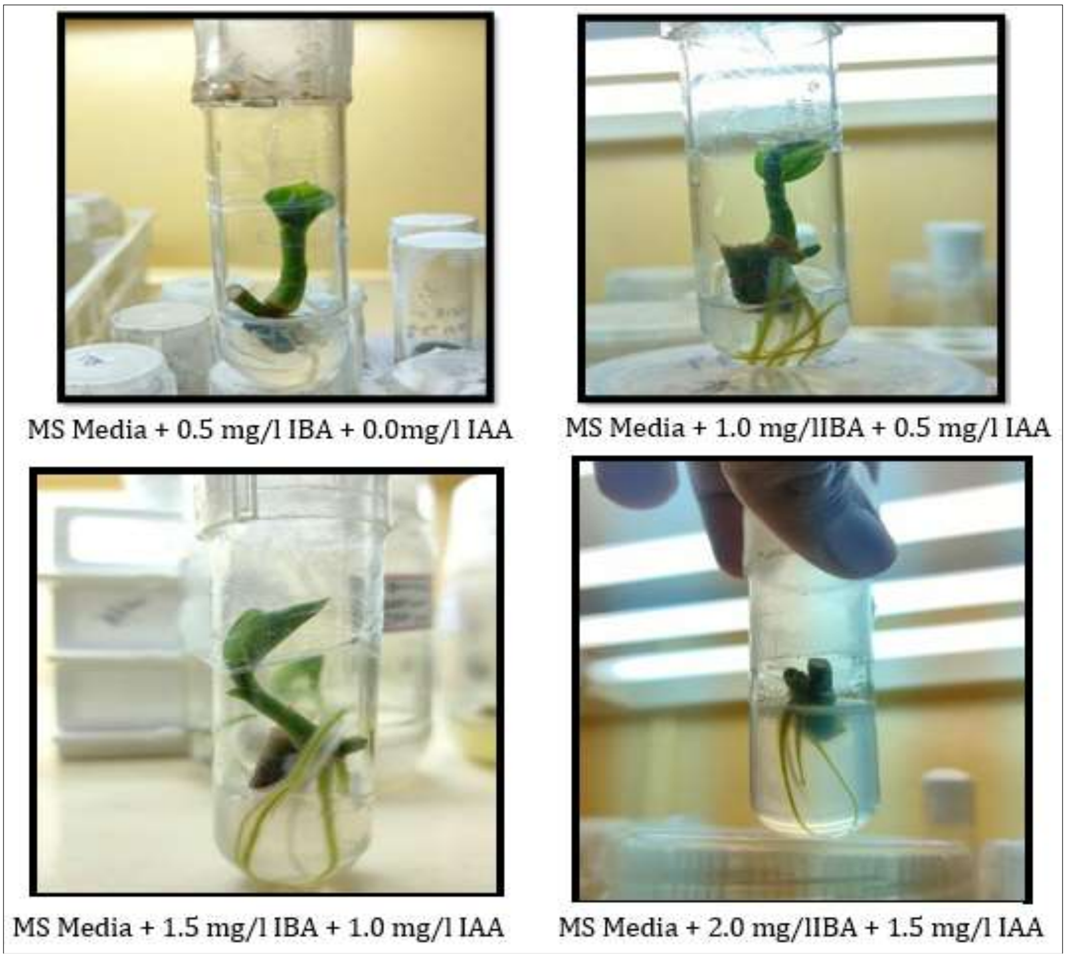


Plate 3: Result Rooting with Different Concentration

D. Hardening: Plantlets was transplanted in to pots as - paper cup, containing mixture of autoclaved red soil, farm yard manure and sand in ratio 2:1:1.

4. Conclusion

The nodal segment of Insulin plant can be effectively used for the micropropagation. The *Costus pictus* is an important medicinal herb with various pharmacological actions. The productions of large numbers of Insulin plants were possible through *in vitro* propagation technique.

5. References

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