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Induction of multiple shoots in *Zamioculcas zamiifolia* Engl. under *in vivo* condition

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Abstract
Zamioculcas zamiifolia Engl. is an ornamental potted foliage plant and a relatively new introduction to the world of interior plants. However, its slow rate of growth and lower rate of multiplication making it very expensive and propagules such as leaflets or leaflet sections or rachis give rise to only one shoot per propagule under *in vivo* conditions. In the present study that aimed at developing multiple shoots, two leaflets with rachis were used as propagule for *in vivo* experiments and treated with 1.0 - 3.0 g L^{-1} BA and kinetin, 0.1 - 0.3 g L^{-1} NAA, IBA and their combinations. The number of rhizomes formed per propagule was highest in 1.0 g L^{-1} kinetin + 0.2 g L^{-1} NAA at 90 days. More roots were formed when the propagule was treated with 1.0 g L^{-1} kinetin+0.1 g L^{-1} NAA at 120 days and on an average 2.2 multiple shoots had formed per propagule at180 days, when it was treated with 1.0 g L^{-1} kinetin + 0.2 g L^{-1}NAA.

Keywords: ZZ. Growth regulators, two leaflets with rachis, multiple shoots

Introduction
Zamioculcas zamiifolia Engl. (ZZ), is a suberect, stemless, herbaceous monocotyledonous perennial plant belonging to Araceae family (Feng *et al.*, 2006; Wong, 2009 and Harrison, 2009) [7, 16, 8]. It is a native of Eastern Africa, growing in tropical moist forest floor or stony ground with large pinnate leaves and a thick succulent horizontal rhizome (with tuber like formations) that is underground (Lopez *et al*., 2009) [10] and is a diploid species (2n = 34). This is a wonder plant commonly known by several names such as "Zanzibar Gem'', "Fat Boy", "ZZ Plant", "Eternity Plant", "African coontie", "Aroid palm", "Arum fern", "Cardboard palm", "Emerald frond" and "Golden tree" *etc.*

In ZZ plants, the compound leaves arise from the underground stem. Above the ground each compound leaf consists of 4-8 pairs of oblong-elliptic, glabrous, coriaceous, slightly succulent leaflets borne on an elongate rachis with a succulent petiole (Mayo *et al.*, 1997) [11]. The petiole of the leaflet is very short and attached to the central rachis. The inflorescence consists of a spadix enclosed by a modified bract called a spathe. The spathe is greenish in color, ovate, 7 cm long, and 3 cm wide. The spadix is a fleshy cylindrical spike, 6 cm long, covered with many small unisexual flowers. Spadices contain male flowers on the upper portion and female flowers on the lower portion, with a short-constricted zone in between bearing sterile flowers (Chen and Henny, 2003) [3]. Underground rhizomes vary in size depending on the age of the plant (Mayo *et al.*, 1997) [11]. The plant has no apparent limiting insects or disease problems under interior conditions and is extreme tolerant to low light and drought (Chen *et al*., 2002) [4]. The ability of the plant to withstand water stress and low light has elevated its (ZZ) horticultural importance at international level (Chen and Henny, 2003) [3].

ZZ plant can be propagated vegetatively using leaflets. The bottle neck in its large-scale production is its slow rate of growth and lower rate of multiplication making it very expensive and propagules such as leaflets or leaflet sections or rachis give rise to only one shoot per propagule under *in vivo* conditions (Nirmala, 2016) [12]. The present study was carried out to develop a methodology to enhance the production of multiple shoots under *in vivo* conditions for accelerated production of potted plants.

Material and methods
The stock plants of ZZ for the present study were obtained from a Private Nursery and maintained under the shade net in the Farm Section of UAS, GKVK, Bengaluru.

The propagule for *in vivo* studies were collected from the actively growing shoots of stock plants. The plant is botanically depicted in Plate 1. 'Leaf cuttings' (botanically two leaflets with rachis) (Plate 2) were selected from well matured shoots. Third, fourth and fifth pair of leaflets from basal end were taken and leaf cuttings were prepared before two days of planting.

Plate 1: Stock plant of *Zamioculcas zamiifolia*

Leaf cuttings were dipped in growth regulator solutions like BA (1-3 g L^{-1}), kinetin (1-3 g L^{-1}) NAA (0.1-0.3 g L^{-1}), IBA (0.1-0.3 g L^{-1}) and its combinations for 30 seconds and planted in a potting media containing vermiculite: coir pith (1:1) (Plate 3)

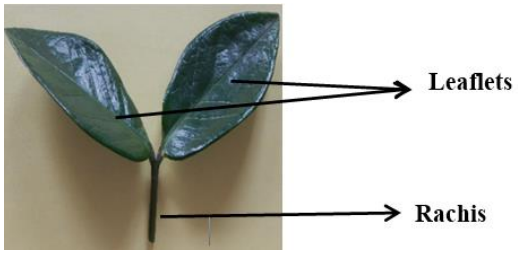

Plate 2: Two leaflets with rachis

Plate 3: General view of *in vivo* experiment

Results and discussion

ZZ plants follow a definite pathway during regeneration of new plants from leaf cuttings. (Plate 4). Initiation of rhizome was noticed at the distal end of the rachis prior to which the inner white or creamish white tissue emerged out from the cut end at the base of the rachis that gradually developed into rhizome. Followed by the development of rhizomes, emergence of roots was noticed from the upper part of these newly formed rhizomes. These rhizomes are formed by the downward-movement of food material and its accumulation in the distal end. Cutter, 1962 reports that growth hormones may be involved in rhizome formation *i.e.,* biologically active substances of hormones, present in the leaflet of *Z. zamiifolia*, are responsible for the development of rhizomes. With the development of new plantlet, there was decay of the original plant material. After complete formation of the rhizomes, adventitious shoot buds appeared on the terminal portion of the rhizome. These buds developed into cotyledonary leaf like structures. Further, there was emergence of new leaf from the base of this cotyledonary leaf which unfurled, and the leaflets expanded (Plate 4i).

In the present study, the initial response of swelling at the cut end of leaves (Plate 4b) was noticed in eight days after planting followed by the formation of rhizomes. Rhizome initiation occurred after 20.40 days followed by formation of roots at 35.69 days in the presence of 0.2 g L^{-1} NAA. (Table 1.). Early root initiation was noticed in the presence of 0.2 g L^{-1} NAA and other treatments at 30.59 days and delayed root initiation was recorded at 50.99 days when no growth regulator was used (Table 1.). Singh and Chettri (2013) [15], in their study reported that the leaf cuttings of chrysanthemum treated with 100 ppm IBA + 25 ppm BA showed root initiation in 16.42 days. In ZZ, however, time required for root initiation appears to be more as they emerge only after the formation of rhizome. Seneviratne *et al*. (2013) opinion that root number (1.30) was higher in the basal leaflet without rachis, middle leaflet without rachis and two terminal leaflets with rachis than in the other cutting types and root length (0.8cm) was higher in the basal leaflet without rachis than the two basal leaflets with rachis, terminal leaflet without rachis and two terminal leaflets with rachis. To increase the rooting percentage of cuttings in *Rosa centifolia* Akhtar *et al*. (2015) [2] conducted a study by applying different concentrations of IBA, NAA, IAA and BAP at 450 ppm, 700 ppm and 950 ppm to the cuttings. The highest shoot length (10.67 cm), shoot dry weight (3.02 g), number of roots (14.00), root length (11.90 cm) and root dry weight (0.50 g) was recorded in IBA at 450 ppm as compared to other treatments. However, in the present study IBA did not have any significant influence on number of roots.

Early development of new shoots was noticed at 91.78 days when the leaf cuttings were treated with 3.0 g L^{-1} BA before planting which was delayed upto 152.96 days when no growth regulators were used (Table 1). As per Cutter (1962) [5], absolute concentration of cytokinins and auxins are effective in formation of shoots and hence the present result. Up to four rhizomes formed per leaf cutting when treated with 1.0 g L^{-1} kinetin + 0.2 g L^{-1}NAA at 90 days as against one rhizome in control (Table 1 and Figure 1). The higher level of cytokinin to auxin ratio may be responsible for enhanced formation of rhizome, which is otherwise normally only one. Cytokinins are known to induce multiple shoots in many ornamental plants and this is commercially exploited in chrysanthemums. In ZZ the rhizomes are nothing but underground stem (Wong, 2008) and cytokinin and its concentration has induced formation of more rhizomes, further resulting in formation of multiple shoots following the specific pathway (Plate 4) as described earlier.

The size of the underground rhizome is known to vary, diameter ranging from 0.4 cm for those newly formed from leaf cuttings to 10 cm or larger after two years of growth (Mayo *et al.*, 1997) [11]. We observed that the diameter of rhizome was highest when leaf cuttings were treated with 0.3 g L^{-1} NAA (23.74mm) and lowest was recorded in 3.0 g L^{-1} kinetin+ 0.3 g L^{-1}NAA (4.81mm) at 90 days (Table 1 and

Figure 2). Similar results were reported by Nirmala (2017) [12] while studying on the effect of potting media and growth regulators on initiation of rhizomes in different ZZ propagules. Though kinetin has induced more number of rhizomes as reported in the earlier experiment, NAA alone is found to be responsible for increased rhizome size, as noticed in the present experiment.

Development of roots and shoots from rhizomes that form at the base of propagules is equally important for the formation of complete plantlet.

Roots formation was best when the propagule was treated with 1.0 g L^{-1} kinetin+0.1 g L^{-1} NAA and as many as 10.20 roots formed per rhizomes while only 4.82 roots were recorded in the absences of growth regulator treatment at 120 days (Table 1). Auxins are responsible for induction of tuber and roots. NAA has a stimulatory effect on root formation and NAA alone seems to suppress shoot formation. However, absolute concentration of cytokinins and auxins are effective in formation of shoots. Anatomical studies of regeneration show that the initiation of shoots and roots occurs in or close to the vascular tissue, but tuber formation takes place in the parenchymatous tissue of the midrib (Cutter, 1962) [5]. However, in the present study more number of roots are found to form in the presence of higher kinetin and lower NAA concentrations. This is because the number of roots is related to the number of rhizomes formed and in the present experiments more number of rhizomes have been recorded when the propagule was treated with higher kinetin and lower NAA concentrations.

It was observed that the root length also followed similar trend with maximum of 8.50 cm recorded in 1.0 g L^{-1} kinetin + 0.2 g L^{-1}NAA at 120 days and lowest of 5.4 cm was recorded in control (Table 1). The reason for increased root length in the presence of kinetin is not known, though NAA is known to influence rooting of cuttings. One of the reasons may be accumulated food in the already developed rhizomes. An average of 2.20 shoots formed per rhizome when the propagule was treated with 1.0 g L^{-1} kinetin + 0.2 g L^{-1} NAA at 180 days and lowest of 1.32 was recorded in 0.1 g L^{-1} IBA as against one per rhizome when growth regulators were not used (Table 1 and Figure 3). According to Cutter (1962), it seems likely that the applied growth substance has mobilized carbohydrates towards the site of application; possibly the meristematic activity induced acts as a sink of utilization of such materials. To increase the rooting percentage of cuttings in *Rosa centifolia* Akhtar *et al*. (2015) [2] conducted a study by applying different concentrations of IBA, NAA, IAA and BAP at 450 ppm, 700 ppm and 950 ppm to the cuttings. The highest shoot length (10.67 cm), shoot dry weight (3.02 g), number of roots (14.00), root length (11.90 cm) and root dry weight (0.50 g) was recorded in IBA at 450 ppm as compared to other treatments. However, in the present study IBA did not have any significant influence on number of roots. Shimada *et al*. (2005) [14] has carried studies on adventitious bud formation from leaf cuttings in *Begonia*. Small leaf pieces without petiole did not form adventitious bud, however, addition of BA at more than 0.25 ppm resulted in adventitious bud formation up to 80 per cent. Higaki and Rasmussen (1979) reported that anthurium plants of 'Ozaki Red' when sprayed with PBA, BA or ethephon at a concentration of 100, 500, 1000 or 1500 mg L^{-1} induced adventitious bud formation. Maximum number of shoots (3.6 shoots/plant) was formed in BA at 1000 mg L^{-1}. Similarly influence of another cytokinin kinetin, in the presence of very low concentrations of NAA was also noticed in our study.

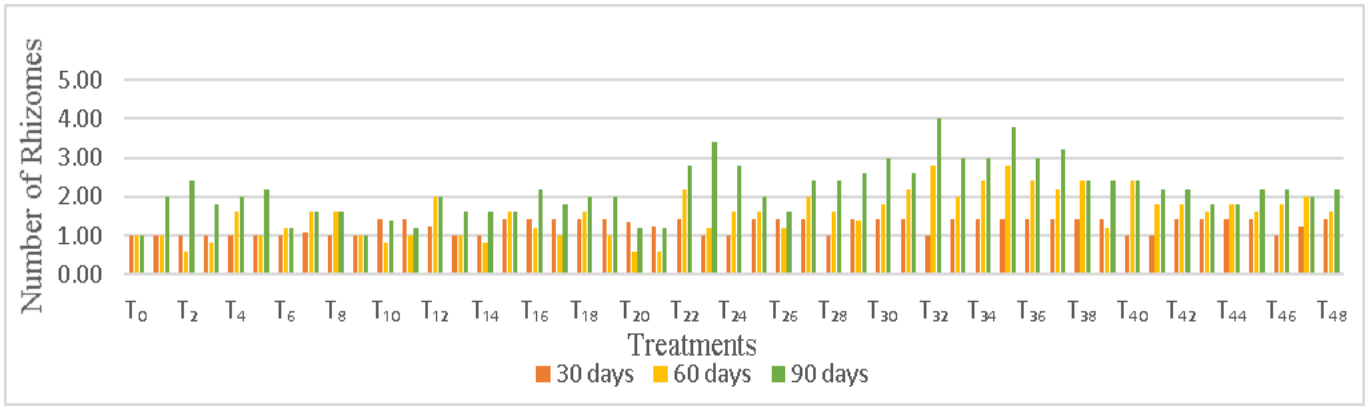

Fig 1: Influence of growth regulators on number of rhizomes formed from two leaflets with rachis of ZZ under *in vivo* condition

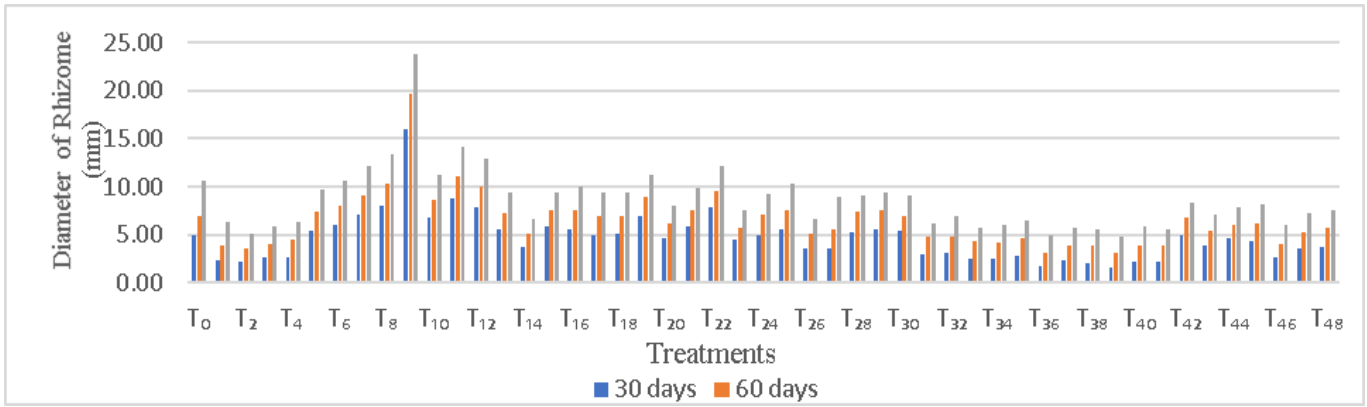

Fig 2: Influence of growth regulators on size of rhizomes formed from two leaflets with rachis of ZZ under *in vivo* condition (in mm)

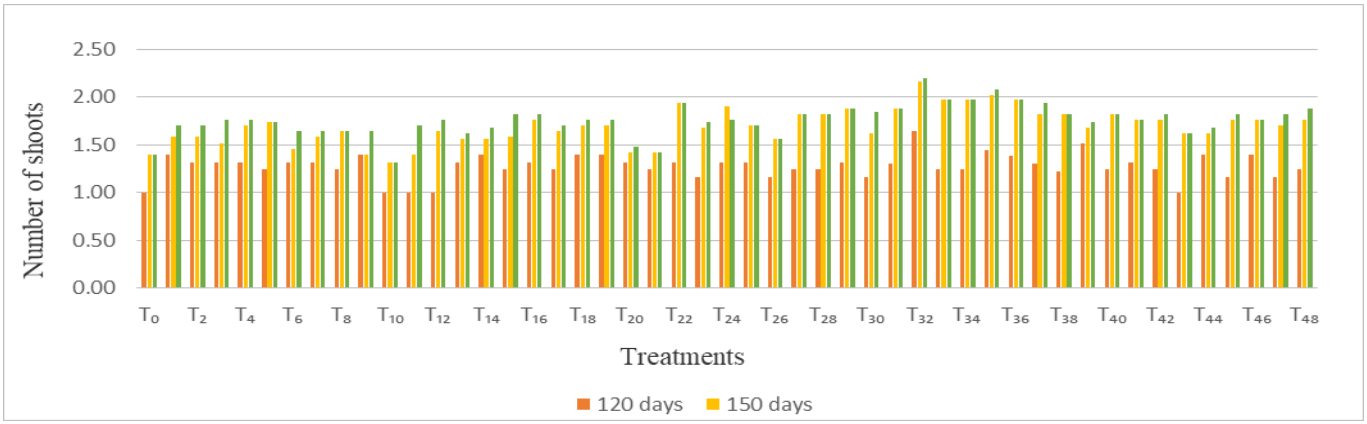

Fig 3: Influence of growth regulators on number of shoots produced from rhizomes of ZZ under *in vivo* condition

Treatments	Time taken for rhizome initiation (in days)	Number of rhizomes formed at 90 days (in numbers)	Diameter of the rhizome at 90 days (mm)	Time taken for root initiation (in days)	Number of roots formed at 120 days (in numbers)	Root length at 120 days (in cm)	Time taken for shoot initiation (in days)	Number of shoots formed at 180 days (In numbers)
T_0: Control	35.69	1.00	10.63	50.99	4.82	5.64	152.96	1.40
T_1: 1 g L^{-1}BA	30.59	2.00	6.36	40.79	6.00	6.60	122.37	1.70
T_2: 2 g L^{-1}BA	30.59	2.40	5.19	38.75	6.60	6.50	122.37	1.70
T_3: 3 g L^{-1}BA	28.55	1.80	5.94	38.75	6.00	6.70	91.78	1.76
T_4: 1 g L^{-1} Kinetin	28.55	2.00	6.31	40.79	6.80	7.13	122.37	1.76
T_5: 2 g L^{-1} Kinetin	29.57	2.20	9.64	38.75	9.00	7.60	122.37	1.74
T_6: 3 g L^{-1} Kinetin	30.59	1.20	10.64	40.79	6.40	7.10	93.82	1.64
T_7: 0.1 g L^{-1} NAA	22.43	1.60	12.18	36.71	8.00	7.17	122.37	1.64
T_8: 0.2 g L^{-1} NAA	20.40	1.60	13.33	30.59	7.40	7.10	122.37	1.64
T_9: 0.3 gL^{-1} NAA	20.40	1.00	23.74	30.59	8.00	6.80	122.37	1.64
T_{10}: 0.1 g L^{-1} IBA	28.55	1.40	11.23	40.79	6.00	6.30	128.49	1.32
T_{11}: 0.2 g L^{-1} IBA	28.55	1.20	14.14	40.79	6.40	6.60	122.37	1.70
T_{12}: 0.3 g L^{-1} IBA	30.59	2.00	12.91	38.75	6.60	7.00	122.37	1.76
T_{13}: 1 g L^{-1} BA + 0.1 g L^{-1} NAA	25.49	1.60	9.42	36.71	7.00	6.30	128.49	1.62
T_{14}: 1 g L^{-1} BA + 0.2 g L^{-1} NAA	26.51	1.60	6.72	40.79	8.00	6.70	122.37	1.68
T_{15}: 1 g L^{-1} BA + 0.3 g L^{-1} NAA	20.40	1.60	9.37	32.63	6.20	6.00	122.37	1.82
T_{16}: 2 g L^{-1} BA + 0.1 g L^{-1} NAA	20.40	2.20	9.96	32.63	6.40	6.00	128.49	1.82
T_{17}: 2 g L^{-1} BA + 0.2 g L^{-1} NAA	26.51	1.80	9.47	40.79	6.00	6.00	122.37	1.70
T_{18}: 2 g L^{-1} BA + 0.3 g L^{-1} NAA	22..42	2.00	9.44	32.63	6.60	6.00	122.37	1.76
T_{19}: 3 g L^{-1} BA + 0.1 g L^{-1} NAA	26.51	2.00	11.22	40.79	6.00	6.00	128.49	1.76
T_{20}: 3 g L^{-1} BA + 0.2 g L^{-1} NAA	20.40	1.20	8.05	32.63	6.00	6.00	122.37	1.48
T_{21}: 3 g L^{-1} BA + 0.3 g L^{-1} NAA	20.40	1.20	9.80	32.63	6.00	6.00	122.37	1.42
T_{22}: 1 g L^{-1} BA + 0.1 g L^{-1} IBA	28.55	2.80	12.12	40.79	6.20	6.30	128.49	1.94
T_{23}: 1 g L^{-1} BA + 0.2 g L^{-1} IBA	30.59	3.40	7.64	40.79	6.60	6.90	122.37	1.74
T_{24}: 1 g L^{-1} BA + 0.3 g L^{-1} IBA	28.55	2.80	9.22	40.79	6.00	6.00	122.37	1.76
T_{25}: 2 g L^{-1} BA + 0.1 g L^{-1} IBA	28.55	2.00	10.27	40.79	6.00	6.00	128.49	1.70
T_{26}: 2 g L^{-1} BA + 0.2 g L^{-1} IBA	30.59	1.60	6.62	40.79	6.20	6.60	122.37	1.56
T_{27}: 2 g L^{-1} BA + 0.3 g L^{-1} IBA	28.55	2.40	8.90	40.79	6.00	6.00	128.49	1.82
T_{28}: 3 g L^{-1} BA + 0.1 g L^{-1} IBA	28.55	2.40	9.05	40.79	6.00	6.70	122.37	1.82
T_{29}: 3 g L^{-1} BA + 0.2 g L^{-1} IBA	28.55	2.60	9.41	40.79	6.00	6.00	122.37	1.88
T_{30}: 3 g L^{-1} BA + 0.3 g L^{-1} IBA	30.59	3.00	9.06	40.79	6.00	6.00	128.49	1.84
T_{31}: 1 g L^{-1} Kinetin + 0.1 g L^{-1} NAA	26.51	2.60	6.21	40.79	10.20	7.50	93.82	1.88
T_{32}: 1 g L^{-1} Kinetin + 0.2 g L^{-1} NAA	20.40	4.00	6.90	30.59	10.00	8.50	91.78	2.20
T_{33}: 1 g L^{-1} Kinetin + 0.3 g L^{-1} NAA	22.43	3.00	5.79	32.63	7.40	7.50	91.78	1.98
T_{34}: 2 g L^{-1} Kinetin + 0.1 g L^{-1} NAA	20.40	3.00	6.01	32.63	6.60	7.50	95.86	1.98
T_{35}: 2 g L^{-1} Kinetin + 0.2 g L^{-1} NAA	22.43	3.80	6.55	30.59	6.60	8.00	91.78	2.08
T_{36}: 2 g L^{-1} Kinetin + 0.3 g L^{-1} NAA	26.51	3.00	4.93	38.75	8.60	7.40	91.78	1.98
T_{37}: 3 g L^{-1} Kinetin + 0.1 g L^{-1} NAA	26.51	3.20	5.70	38.75	6.80	7.50	93.82	1.94
T_{38}: 3 g L^{-1} Kinetin + 0.2 g L^{-1} NAA	20.40	2.40	5.64	30.59	7.40	7.60	91.78	1.82
T_{39}: 3 g L^{-1} Kinetin + 0.3 g L^{-1} NAA	20.40	2.40	4.81	32.63	6.80	7.10	91.78	1.74
T_{40}: 1 g L^{-1} Kinetin + 0.1 g L^{-1} IBA	28.55	2.40	5.89	38.75	6.40	7.10	128.49	1.82
T_{41}: 1 g L^{-1} Kinetin + 0.2 g L^{-1} IBA	28.55	2.20	5.58	38.75	6.80	7.60	122.37	1.76
T_{42}: 1 g L^{-1} kinetin + 0.3 g L^{-1} IBA	30.59	2.20	8.37	40.79	7.60	7.80	122.37	1.82
T_{43}: 2 g L^{-1} Kinetin + 0.1 g L^{-1} IBA	28.55	1.80	7.10	40.79	7.40	7.75	128.49	1.62
T_{44}: 2 g L^{-1} Kinetin + 0.2 g L^{-1} IBA	28.55	1.80	7.82	40.79	6.80	7.00	122.37	1.68
T_{45}: 2 g L^{-1} Kinetin +0.3 g L^{-1} IBA	28.55	2.20	8.20	40.79	8.00	7.50	122.37	1.82
T_{46}: 3 g L^{-1} Kinetin + 0.1 g L^{-1} IBA	28.55	2.20	6.06	40.79	6.00	6.50	128.49	1.76

T_{47}: 3 g L^{-1} Kinetin + 0.2 g L^{-1} IBA	32.63	2.00	7.19	40.79	6.40	6.50	122.37	1.82
T_{48}: 3 g L^{-1} Kinetin + 0.3 g L^{-1} IBA	32.63	2.20	7.59	40.79	6.60	7.30	122.37	1.88
p=0.05	*	*	*	*	*	*	*	*
S.Em±	0.44	0.33	0.85	0.63	0.46	0.30	1.95	0.07
CD	1.23	0.90	2.36	1.75	1.27	0.81	5.41	0.20

BA - 6-Benzylaminopurine NAA- Naphthaleneacetic acid IBA- Indole-3-butyric acid S.Em± - Standard error mean CD- Critical difference * Significant at 5%

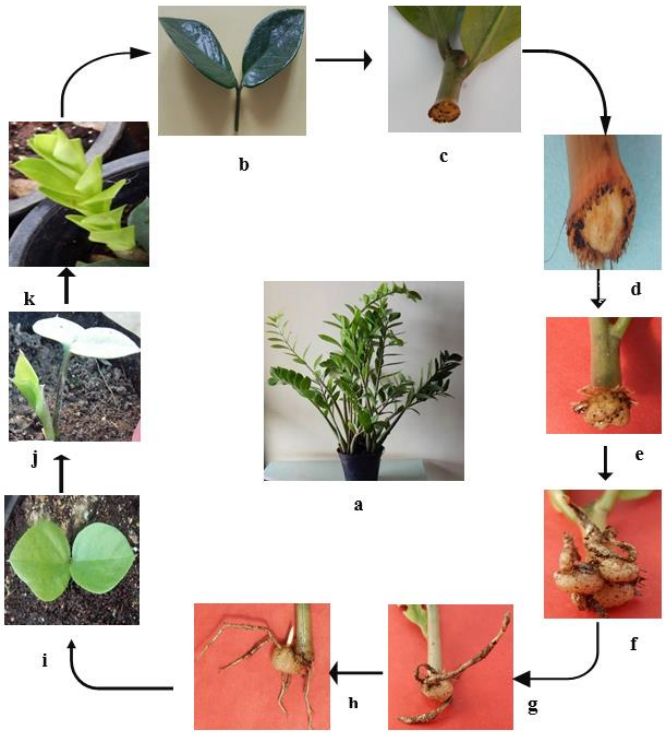

Plate 4: Pathway of formation of new plants from propagule – two leaflets with rachis (a)Stock plant (b) Two leaflets with rachis (c) Swelling of cut end of the propagule with necrotic tissue (d) Emergence of inner creamish white tissue out of the necrotic cell layer in the cut end (e)Formation of rhizomes (f) Whole rhizome with roots (g) Elongation of roots on the rhizomes (h) Emergence of adventitious shoot buds from rhizomes (i) Emergence of leaves resembling cotyledons (j) Emergence of new leaf from the base of cotyledonary leaves (k) Unfurling of leaflets forming shoot like structure

Conclusion

The study on the induction of multiple shoots in *Zamioculcas zamiifolia* Engl. confirmed that the number of rhizomes formed per propagule was highest in 1.0 g L^{-1} kinetin + 0.2 g L^{-1} NAA at 90 days. More roots were formed when the propagule was treated with 1.0 g L^{-1} kinetin+0.1 g L^{-1} NAA at 120 days and on an average 2.2 multiple shoots had formed per propagule at 180 days, when it was treated with 1.0 g L^{-1} kinetin + 0.2 g L^{-1}NAA under *in vivo* conditions

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