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Characterization of mother palms and novel techniques to produce elite seedlings of coconut var. Chowghat Orange Dwarf

S. Rohith[1], S. Kavibalan[1], K. Thangaraj[2], J. Suresh[4], M. Ananthan[3] and R. Renuka[1*]

[1]Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore -641 003
[2]Centre of Innovation, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai – 625 104
[3]Department of Horticulture, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai – 625 104
[4]Coconut Research Station, Aliyar Nagar, Coimbatore – 642 101
***E-Mail**: renukaraman@tnau.ac.in

Abstract
Coconut is one of the major plantation crops of India and the demand for elite seedlings is exorbitant. In this study, superior mother palms of Chowghat Orange Dwarf (COD) which is not completely homozygous were identified based on Phenotypic Co-efficient of Variation (PCV), Genotypic Co-efficient of Variation (GCV), heritability and genetic advance of morphological traits for applications in crop improvement. Explants from superior palms *viz*., mature embryos (whole/sliced) and shoot tips (whole/sliced), were used for direct organogenesis. Among these, sliced shoot tips (4 slices) in Y3 basal + 200 µM Thidiazuran (TDZ), recorded highest shoot induction (350 %) and regeneration frequency (325%) at 30 Days After Inoculation (DAI) and 60 DAI respectively than sliced embryos (4 slices) which recorded lesser shoot induction (170 %) and regeneration frequency (125%). This is the first study that demonstrates shoot tips as one of the potential explants for *in-vitro* production of quality coconut seedlings.

Keywords: Coconut, genetic variability, direct organogenesis, mature embryo culture and shoot tip culture

INTRODUCTION

Coconut (*Cocos nucifera* L.) is one of the principal plantation crops in India. Coconut cultivation is diversified across various regions of India, predominantly in the southern states. In the global context, India stands third in terms of area of cultivation and leads in the coconut production with the annual coconut production of 20.73 billion nuts from 2.19 million hectares (ha) with an average productivity of 9430 nuts/ha (ICC Statistical Year Book, 2021). However, the area of cultivation, production and productivity of coconut is decreasing in India (https://coconutboard.gov.in/Statistics.aspx) due to aged coconut gardens, senile palms, infestation of pest and diseases and lack of quality seedlings. The traditional method of planting using seeds does not generate an adequate number of elite seedlings leading to increased demand for top-notch planting material. To meet out the greater demands for elite seedlings, mother palms with superior characteristics are essential for breeding programs.

Cocos nucifera, a monotypic species with no wild relatives, displays substantial diversity and heterozygosity broadly classifying them into talls and dwarfs. Dwarfs are gaining

commercial significance due to their short stature, early bearing and tender nut qualities. The Chowghat Orange Dwarf (COD) is a tender nut variety recommended for cultivation in India. The tender nut of COD at seven months has the highest sugar content of 7.0 g/100 ml and potassium of 2000 ppm (Niral *et al.*, 2019). This variety has served as a parental palm for developing coconut hybrids like Kera Sankara and Chandra Sankara. Niral and Jerard (2018) reported that the dwarf palms are more homozygous than talls, due to a high degree of self-pollination. However, in spite of overlapping male and female phase which promotes self pollination, natural crosses have been reported in COD (Satyabalan, 1956). Whitehead (1976), observed some degrees of cross pollination in dwarf coconut varieties when present in the vicinity of tall coconut varieties. Kurian and Peter (2007) established the occurrence of natural cross dwarfs in open pollinated progenies of dwarf to an extent of 20%. Ninan and Satyabalan (1964) documented 41.3 per cent off-types in COD that showed greater vigor than pure dwarf seedlings. The study further declared that the dwarfs are not homozygous to the extent it is conceived. Thomas *et al*. (2015) recorded slightly higher heterozygosity at molecular level in Malayan Green Dwarf (MGD) which was attributed to its breeding behaviour where only 60% of the MGD palms showed complete overlapping of male and female phases and pointed out the possibilities of out crossing in 40% MGD palms. Therefore, identification of superior mother palms of COD which is not strictly self pollinated and lacks complete homozygosity will assist in breeding programs and also in propagation through seeds. However, both traditional seed propagation and conventional breeding methods does not fulfil the demand of quality seedling materials as significant proportion of coconut plantations necessitates replanting. Thereby, in addition to seed propagation and conventional breeding methods, novel techniques like plant tissue culture can provide a solution to keep up with the swiftly increasing demand of elite planting material of coconut.

Over the past 65 years, *in-vitro* culture studies for the propagation of coconut have been explored (Chan *et al.,* 1998; Cutter and Wilson, 1954; Nguyen *et al*., 2015; Renuka *et al*., 2018; Rillo and Paloma 1991; Wilms *et al*., 2021). Various explants *viz*., immature leaves (Karunaratne *et al*., 1991), immature inflorescence (Verdeil *et al*., 1994), anthers and unfertilized ovary (Perera *et al*., 2007), slices of immature and mature embryos (Adkins *et al*., 1999; Samosir *et al*., 1999) rachilla from immature inflorescence (Hornung and Verdeil, 1999), apical meristem (Verdeil and Buffard-Morel, 1995) and plumule from zygotic embryos (Saenz *et al*., 2006) were used for *in-vitro* studies. Among these, plumule explants proved to be the most responsive explants for embryogenic callus, somatic embryo formation (Hornung, 1995) and production of complete plantlets (Perez-Nunez *et al*., 2006). However, use of plumules from cross pollinated zygotic embryos would result in plantlets with unpredictable agronomic traits. Therefore, it is necessary to identify most responsive explants other than plumules for the production of elite coconut seedlings with known agronomic traits.

Till date, SE has been generally considered as the promising biotechnological method to produce clonal coconut palms (Hornung and Verdeil, 1999; Perez-Nunez *et al*., 2006). SE has been proposed as a viable supplementary tool to seed propagation and conventional breeding for generating a significant quantity of coconut plantlets through tissue culture. However, an alternative approach to achieve clonal coconut propagation exists in the form of direct organogenesis, a lesser-explored avenue for plant regeneration in coconut. This process entails the direct formation of shoots from tissues without an intervening callus phase (Bhatia and Sharma *et al.,* 2015).

Sushmitha *et al*. (2019) explored direct organogenesis using different treatments on zygotic embryos from the COD variety. Chandrakala *et al*. (2019) documented regeneration of shoots through direct organogenesis in coconut var. East Coast Tall (ECT). Wilms *et al*. (2021) produced plantlets by culturing apical meristem which led to auxiliary shoot proliferation and regeneration of plantlets through direct organogenesis. In a perennial crop like coconut, regeneration through direct shoot organogenesis is found to be a secure pathway than somatic embryogenesis as the risk of somaclonal variation is lower (Abahmane, 2011). A repeatable and viable direct organogenesis protocol for commercial production of quality coconut seedlings is a prerequisite. In this study, characterisation of COD mother palms which is not strictly self pollinating has been attempted to identify best parental palms for breeding programs and *in-vitro* studies. Under *in-vitro* studies different types of explants and slicing techniques has been explored to assess its impact on shoot induction and shoot regeneration through direct organogenesis for its applications in coconut micropropagation.

MATERIALS AND METHODS

Characterization of coconut mother palms: Morphological characterisation was carried out at Coconut Research Station, Aliyar Nagar, Coimbatore, Tamil Nadu. The experimental material (25 palms) was selected among the 19 years old 50 palms of COD during 2021-2023 based on annual yield/palm. The palms with the spacing of 25 feet X 25 feet were maintained under well irrigated conditions with a standard package of practices. Morphological observations which include vegetative, reproductive and fruit traits were recorded for the selected 25 palms in three replicates. The vegetative and reproductive traits comprised of stem girth (cm), number of leaves on crown, length of inflorescence (cm), number of spikes/ inflorescence, length of spikes (cm), number of female flowers per inflorescence and nuts per palm per year

while the fruit component traits comprised of fruit weight (g), fruit length (cm), fruit breath (cm), weight of husked fruit (g), weight of broken nut (g), weight of coconut water (g) and weight of kernel (g). For the traits stem girth and number of leaves on crown, observations was recorded once in every six months and for the trait nuts/palm/year, observations were recorded annually for three years (2021 – 2023). For all other traits, the observations were documented in three replicates using randomly selected three matured nuts / fully developed inflorescence per palm during 2023. To select the superior mother palms for breeding programs and micropropagation studies, the data was subjected to statistical analysis. The phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) (Burton, 1952), heritability (h^2) (Robinson *et al*., 1949) and genetic advance as percentage of mean (GAM) (Johnson *et al*., 1955) were used to assess the variation among the mother palms and to identify the traits that contribute significantly to productivity. Based on this study, superior mother palms for *in-vitro* studies were selected.

In-vitro studies for propagation of coconut: The tissue culture studies were carried out at the Department of Biotechnology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai. From the superior mother palms, nuts were collected for *in-vitro* studies. These nuts were used for explants collection. The explants *viz*., mature embryos were isolated from 12-months old nuts. The fibrous mesocarp of coconuts was removed, and the nuts were cut horizontally. A section of the broken nuts, which consisted of three eyes, were used for isolation of mature embryos. For isolation of shoot tips 13 months old sprouted nuts were used. The fibrous mesocarp was removed carefully and the sprouts were cut from the base of the nut and dissected further to obtain shoot tips of 5 – 10 mm.

Mature embryo culture: The largest eye of the broken nuts was identified and endosperm cylinders were isolated using tender coconut opener. These endosperm cylinders were sterilized in 5% sodium hypochlorite solution to facilitate transportation to the laboratory. The endosperm cylinders were then triple rinsed with distilled water containing tween 20 solution, followed by sterilization with 0.1% mercuric chloride solution for 20 minutes and washes with sterile distilled water. Further the endosperm cylinders were sterilized with 70% ethanol for one minute and rinsed thrice with sterile distilled water. Endosperm cylinders were dissected and mature embryos were isolated. Mature embryos were sterilized with 0.1% mercuric chloride for three minutes followed by sterile distilled water washes and inoculated as whole embryos for the treatments T1 – T5 in sterilized modified Y3 medium, supplemented with sucrose (6 %), activated charcoal (1%), agar (0.8%) and growth regulator *viz*., TDZ at various concentrations (0 µM, 50 µM, 100 µM, 150 µM and 200 µM). For the treatments T6-T10, the whole embryos were sliced vertically into two halves and each slice was inoculated separately in modified Y3 media containing TDZ of varied concentrations *viz*., 0 µM, 50 µM, 100 µM, 150 µM and 200 µM. For the treatments T11-T15, the whole embryos were sliced vertically into four slices and each slice was inoculated in modified Y3 media supplemented with TDZ (0 µM, 50 µM, 100 µM, 150 µM and 200 µM) (**Table 1**). For each treatment 20 whole embryos were used. The experiments were replicated sufficiently to meet the standards of statistical analysis. The cultures were incubated at 26±2 ℃ at dark for four weeks and later transferred to 16/8 hrs photoperiod. Subculturing was carried out at 45 days interval. Shoot induction and regeneration in whole/sliced embryos were recorded at 45 DAI and 90 DAI respectively. The frequency of shoot induction and regeneration was calculated at 45 DAI and 90 DAI respectively as furnished below:

$$\text{Shoot induction/regeneration frequency of whole embryos} = \frac{\text{Number of whole embryos responded}}{\text{Total number of whole embryos inoculated}} \times 100$$

$$\text{Shoot induction/regeneration frequency of sliced embryos} = \frac{\text{Number of embryo slices responded}}{\text{Total number of whole embryos inoculated}} \times 100$$

Shoot tip culture: The broken nuts with sprouted embryos (**Table 2** and **Fig. 1**) were used for shoot tip isolation. The sprouts were cut from the base of the largest eyes and stored in 5% sodium hypochlorite solution until transportation to the laboratory. The sprouts were then triple rinsed with distilled water containing tween 20 solution, followed by sterilization with 0.1% mercuric chloride solution for 20 minutes and three washes with sterile distilled water. The sprouts were dissected and shoot tips of 5 mm – 10 mm were isolated. For the treatments T1-T5 whole shoot tips were inoculated in sterilized modified Y3 medium supplemented with sucrose (6 %), activated charcoal

Table 1. Treatments for direct organogenesis using mature embryo

Treatments	Whole embryo	Sliced embryo (2 slices)	Sliced embryo (4 slices)
Y_3 basal	T1	T6	T11
Y_3 basal + 50 µM	T2	T7	T12
Y_3 basal + 100 µM	T3	T8	T13
Y_3 basal + 150 µM	T4	T9	T14
Y_3 basal + 200 µM	T5	T10	T15

A. Sprouted nut (13 month old) **B. Sterilization of sprouts with 5% NaOCl**

Fig. 1. Sprouted embryos and sterilization of sprouts

(1%), agar (0.8%) combined with growth regulator *viz*., TDZ at various concentrations (0 µM, 50 µM, 100 µM, 150 µM and 200 µM). For the treatments T6-T10, shoot tips were sliced vertically into two halves and each slice was inoculated separately in modified Y3 medium containing TDZ at different concentrations (0 µM, 50 µM, 100 µM, 150 µM and 200 µM). For the treatments T11- T15, the shoot tips were sliced vertically into four slices and each was inoculated in modified Y3 medium with TDZ at varied concentrations (0 µM, 50 µM, 100 µM, 150 µM and 200 µM). For each treatment 20 whole shoot tips were used. The experiments were replicated sufficiently to satisfy statistical requirements. The cultures were incubated at 26±2 ℃ at dark for four weeks and later transferred to 16/8 hrs photoperiod. Subculturing was carried out at 30 days interval. Shoot induction and regeneration in whole/sliced shoot tips were recorded at 30 DAI and 60 DAI respectively. The frequency of shoot induction and regeneration was calculated at 30 DAI and 60 DAI respectively as furnished below:

$$\text{Shoot induction/regeneration frequency of whole shoot tips} = \frac{\text{Number of whole shoot tips responded}}{\text{Total number of whole shoot tips inoculated}} \times 100$$

$$\text{Shoot induction/regeneration frequency of sliced shoot tips} = \frac{\text{Number of shoot tip slices responded}}{\text{Total number of whole shoot tips inoculated}} \times 100$$

Statistical analysis: The data recorded on morphological characterisation of coconut were subjected to statistical analysis using MS Excel spreadsheet, while for the *in-vitro* studies Completely Randomized Design was adopted as per the standard procedure of Panse and Sukhatme (1985). The analysis was carried out with WASP software (https://ccari.icar.gov.in/wasp2.0/index.php).

RESULTS AND DISCUSSION

Superior mother palms determine the success of crop improvement programs and tissue culture studies. COD is not completely homozygous due to certain extent of cross pollination which resulted in natural cross dwarfs and off-type progenies (Kurian and Peter, 2007; Ninan and Sathyabalan (1964); Whitehead (1976)). Morphological characterisation and genetic variability analysis in coconut mother palms var. COD is essential to identify superior mother palms suitable for breeding new varieties and production of true to type seedlings through *in-vitro* studies.

Genetic variability analysis: Assessment of variability parameters *viz*., PCV, GCV, heritability and genetic advance will categorize the key characters that influence the productivity. In the present study, all the characters exhibited marginally high PCV than GCV which indicated marginal influence of environment factors on its expression. Low PCV and low GCV (<10 %) were recorded for all the vegetative and reproductive traits and fruit component traits except for number of female flowers per inflorescence which recorded moderate PCV and fruit length which recorded moderate PCV and GCV (**Table 3**). Low PCV and GCV indicated the low range of genetic variability and narrow genetic base in COD for these characters and therefore direct selection based on these traits should be avoided. However, Sudha *et al*. (2019) has documented high PCV and high GCV for number of female flowers per inflorescence, nuts per palm per year and fruit length in West Coast Tall population. Sivakumar *et al*. (2020) recoded high PCV and GCV for tender nut water content, whole nut weight, dehusked nut weight and nut yield. The analysis on heritability showed that number of female flowers per inflorescence and nuts per palm per year had low heritability and low genetic

Table 2. Treatments for direct organogenesis using shoot tip

Treatments	Whole shoot tip	Sliced shoot tip (2 slices)	Sliced shoot tip (4 slices)
Y_3 basal	T1	T6	T11
Y_3 basal + 50 µM	T2	T7	T12
Y_3 basal + 100 µM	T3	T8	T13
Y_3 basal + 150 µM	T4	T9	T14
Y_3 basal + 200 µM	T5	T10	T15

Table 3. Vegetative and reproductive traits for genetic variability analysis in COD

Traits	Grand Mean	Range		PCV (%)	GCV (%)	Heritability (%)	GAM (%)
		Min	Max				
SG	68.97	64.00	75.33	4.64	3.80	67.01	6.41
NLC	22.36	18.33	26.00	9.31	5.59	36.09	6.92
LOI	64.20	62.33	66.00	1.54	1.42	84.72	2.69
NOS	26.97	23.67	32.67	8.53	7.71	81.62	14.34
LOS	24.49	22.00	28.33	6.43	5.66	77.44	10.26
NFF/I	15.61	09.00	18.67	14.26	7.79	29.84	8.77
NPY	131.72	125.33	155.67	4.02	3.81	89.63	7.43

Stem girth (SG), number of leaves on crown (NLC), length of inflorescence (LOI), number of spikes (NOS), length of spikes (LOS), number of female flower per inflorescence (NFF/I) and nuts per year (NPY)

advance while number of spikes per inflorescence, length of the spike, fruit weight, fruit length, weight of broken nut and weight of coconut water recorded high heritability and moderate genetic advance (**Table 3 and 4)**. Suchitra and Paramaguru (2018) found that spadix length, number of inflorescence per palm and kernel thickness has high heritability with moderate genetic advance. In this study six superior mother palms of COD were selected out of 25 palms for micropropagation studies based on fruit component traits viz., fruit weight, fruit length, weight of broken nut and weight of coconut water. These traits can also be used to select superior mother palms for breeding programs as COD is not completely homozygous.

Direct organogenesis through mature embryo culture: Kong *et al*. (2021) opined direct shoot organogenesis to be a better solution for clonal propagation of coconuts. In this study, mature embryos were used to induce direct shoot organogenesis. The results revealed that Y3 + 200 µM TDZ has favoured maximum shoot induction and shoot regeneration frequency in all the treatments. Similar results have been reported in coconut var. COD and ECT, where TDZ has promoted shoot induction and regeneration Chandrakala *et al*. (2019); Sushmitha *et al*. (2019). TDZ has been found to be effective at very low concentrations (0.0091 to 3.99 µM) for micropropagation of many plant species (Lu, 1993). However, it has been used at higher concentrations (2.27 to 145.41 µM) for propagation of tree species including *Zanthoxylum rhetsa* (Augustine and D'Souza, 1997). Among various treatments under mature embryo culture, T15 (Y3 basal + 200 µM TDZ, sliced embryo (4 slices)) recorded highest shoot induction frequency of 170 % and highest shoot regeneration frequency of 125 %, which is highly significant than other treatments (**Table 4; Fig. 2**). Generally, in traditional method of seed propagation in coconut 1:1 ratio of seed to seedling is obtained. In sliced embryo culture (4 slices), 1: 1.7 and 1.25 ratio of shoot induction and shoot regeneration are reported in this study. Sisunandar *et al*. (2015) produced 56 shoots from 30 zygotic embryos (1:1.8) by adopting embryo incision technique. Among the different slicing methods, sliced embryo (4 slices) recorded significantly higher shoot induction and shoot regeneration than T10 (Y3 basal + 200 µM TDZ, sliced embryo (2 slices)) (**Table 5**) which implies the importance of slicing techniques in coconut micropropagation. This study reveals that the sliced embryo (2/4 slices) are able to regenerate shoots through the production of new meristem and primordial leaves as reported during regeneration of halved embryos (Sisunandar *et al*., 2015).

Direct organogenesis through shoot tip culture: In this study, shoot tip isolated from sprouted nuts were used as explants as whole shoot tip and as sliced shoot tips (2/4slices). The results revealed that T15 under shoot tip culture (Y3 basal + 200 µM TDZ, sliced shoot tip (4 slices)) recorded highest shoot induction frequency of 350 % and highest shoot regeneration frequency of 325 %, which is highly significant than other treatments (**Table 5; Fig 3**). In sliced shoot tip culture (4 slices), 1: 3.5 and 3.25 ratios of shoot induction and shoot

Table 4. Fruit component traits for genetic variability analysis in COD

Traits	Grand Mean	Range		PCV (%)	GCV (%)	Heritability (%)	GAM (%)
		Min	Max				
FW	900.17	731.67	1025.33	7.61	7.61	99.93	15.67
FL	20.44	16.00	23.70	11.00	10.12	84.65	19.17
FB	13.73	12.07	15.13	6.13	5.04	67.57	8.54
WHF	507.56	465.00	562.67	4.83	4.83	99.99	9.95
WBN	465.41	371.67	527.33	7.53	7.53	99.99	15.52
WOW	100.27	88.00	120.00	6.79	6.55	93.08	13.02
WOK	191.79	178.00	204.33	3.07	2.93	91.17	5.77

Fruit weight (FW), fruit length (FL), fruit breath (FB), weight of husked fruits (WHF), weight of broken nuts (WBN), weight of water (WOW), weight of kernel (WOK)

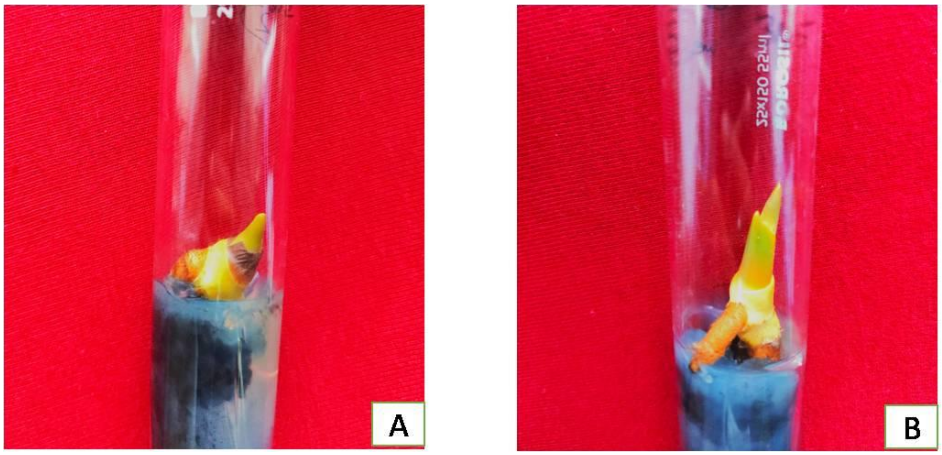
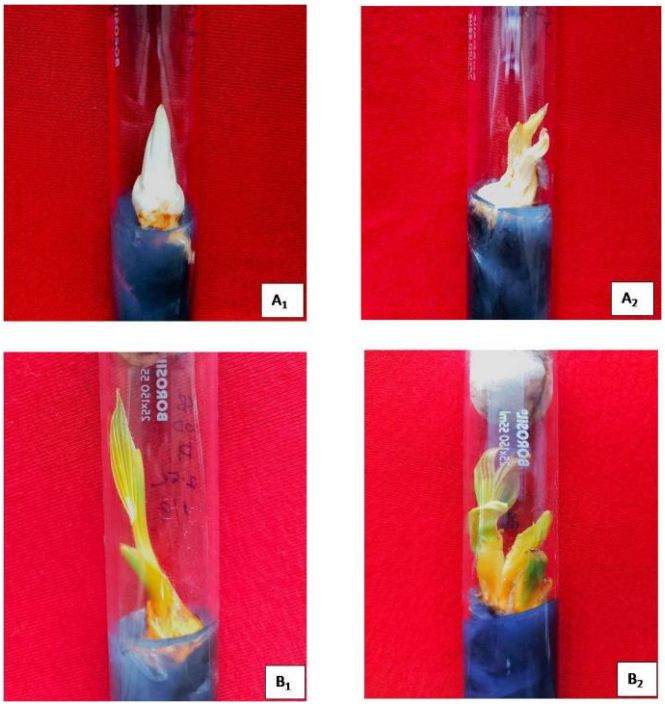


Fig. 2. Mature embryo culture (whole embryos), A: 60 DAI, B: 90 DAI

Table 5. Shoot induction and shoot regeneration through mature embryo culture

Treatments	Shoot induction frequency (%) 45 DAI			Shoot regeneration frequency (%) 90 DAI		
	Whole embryo	Sliced embryo (2 slices)	Sliced embryo (4 slices)	Whole embryo	Sliced embryo (2 slices)	Sliced embryo (4 slices)
Y_3 basal	52.6^{d}	60.0^{d}	50.0^{d}	50.0^{d}	50.0^{e}	40.0^{e}
Y_3 basal + 50 µM TDZ	57.3^{c}	62.0^{d}	75.0^{e}	55.0^{c}	58.3^{d}	65.0^{d}
Y_3 basal + 100 µM TDZ	57.3^{c}	90.0^{c}	110.0^{c}	56.0^{c}	80.0^{c}	100.0^{c}
Y_3 basal + 150 µM TDZ	65.0^{b}	110.0^{b}	140.0^{b}	62.0^{b}	90.0^{b}	109.6^{b}
Y_3 basal + 200 µM TDZ	78.0^{a}	120.0^{a}	170.0^{a}	73.6^{a}	100.0^{a}	125.0^{a}
CD (0.01)	4.341	7.521	7.800	3.013	3.241	7.173

regeneration is reported in this investigation. Sliced shoot tips (4 slices) in Y3 basal + 200 µM TDZ (T15), recorded significantly higher shoot induction and shoot regeneration than T10 (Y3 basal + 200 µM TDZ, sliced shoot tip (2 slices)) (**Table 6**). This study shows that sliced shoot tips (2/4 slices) are able to regenerate shoots by overcoming the apical dominance. The damage caused to the shoot apex during slicing would have promoted shoot induction. This is one of the first studies where shoot tips isolated from sprouts of 13 month old nuts were used as explants for micropropagation which has resulted in shoot regeneration in the ratio of 1:3.25.



A1 and A2: Shoot induction of sliced meristem at 30 DAI; B1 and B2: Shoot regeneration of sliced meristem at 60 DAI

Fig. 3. Shoot induction and regeneration through shoot tip culture

Table 6. Shoot induction and shoot regeneration through shoot tip culture

Treatments	Shoot induction frequency (%) 30 DAI			Shoot regeneration frequency (%) 60 DAI		
	Whole Shoot tip	Sliced shoot tip (2 Slices)	Sliced shoot tip (4 Slices)	Whole shoot tip	Sliced shoot tip (2 Slices)	Sliced shoot tip (4 Slices)
Y_3 basal	65.0^{d}	98.0^{e}	169.0^{e}	61.0^{e}	70.0^{d}	155.0^{e}
Y_3 basal + 50 µM TDZ	71.6^{c}	127.6^{d}	193.0^{d}	65.0^{d}	120.0^{c}	185.0^{d}
Y_3 basal + 100 µM TDZ	80.0^{b}	136.6^{c}	300.0^{c}	68.0^{c}	125.0^{c}	275.0^{c}
Y_3 basal + 150 µM TDZ	80.0^{b}	150.0^{b}	320.0^{b}	73.0^{b}	135.0^{b}	310.0^{b}
Y_3 basal + 200 µM TDZ	90.0^{a}	180.0^{a}	350.0^{a}	84.0^{a}	164.0^{a}	325.0^{a}
CD (0.01)	4.569	5.621	19.931	2.350	9.915	16.910

Wilms *et al*. (2021) used apical mersitems of 4 months old *in-vitro* coconut seedlings produced from culturing of matured embryos of 10-11 months old nuts and recorded meristem proliferation after 45 days which produced plantlets after 6-8 months. From the present investigation, it is inferred that *in-vitro* culturing of shoot tips isolated from sprouts will hasten the process of elite coconut seedling production and will also reduce the cost of production as the duration of culturing under laboratory is decreased. The response of shoot tips to *in-vitro* culturing was faster than the matured embryos either whole /sliced embryos. Shoot tips (whole / sliced) under *in-vitro* conditions exhibited shoot induction and shoot regeneration at 30 and 60 DAI respectively while in mature embryos shoot induction and shoot regeneration was recorded at 60 and 90 DAI.

Among various explants used for coconut micropropagation, plumules have been identified as promising explants for coconut micropropagation. However, it does not guarantee plants with known agronomic traits (Perez-Nunez *et al*., 2006). The present investigation is the first study that demonstrates shoot tips as potential explants for the production of elite coconut seedlings. Fine tuning of the culture conditions, culture methods and slicing techniques for direct organogenesis

through shoot tip culture will improve the multiplication ratio thereby leading to production of elite coconut seedlings.

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