Induction of somatic embryogenesis and plantlet regeneration in Areca concinna, an endangered palm species

Aparna Veluru (aparna.cpcri@gmail.com)
Central Plantation Crops Research Institute
Neema Scientist Mohammed
Central Plantation Crops Research Institute
Sandip Senior Scientist Shil
CPCRI: Central Plantation Crops Research Institute
Krishna Prakash
Central Plantation Crops Research Institute
Kavya Kotian
Central Plantation Crops Research Institute
Muralikrishna KS
Central Plantation Crops Research Institute
Anitha Karun
Central Plantation Crops Research Institute

Research Article

Keywords: Areca concinna, Zygotic embryo, Callogenesis, Somatic embryogenesis, In-vitro multiplication

Posted Date: September 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2012044/v1

Abstract

Areca concinna (Arecaceae) is an endangered plant species endemic to south western Sri Lanka. The palm was listed as endangered plant species by IUCN due to habitat loss; hence a multiplication and conservation strategy needs to be accorded. Here at ICAR CPCRI, a protocol was developed for clonal propagation of *A. concinna.* The explants, immature inflorescence, immature and mature zygotic embryos were tested for callogenesis and subsequent somatic embryogenesis in M72 basal media supplemented with the auxins 2, 4-D and Picloram. Of the three types of explants inoculated, somatic embryogenesis was observed only from mature zygotic embryos. The auxin 2, 4-D enhanced callus multiplication and somatic embryogenesis when compared to picloram. Somatic embryos grown in Y3 or 1/2 Y3 basal medium supplemented with BAP (1 mg/L), NAA (0.5 mg/L), and IBA (0.25 mg/L) developed into plantlets. This protocol has its application in the rapid multiplication of *A. concinna* palms thereby enhancing the possibility of conservation of the endangered palm.

Key Message

Areca concinna, wild relatives of cultivated areca nut, *Areca catechu* L. is an endangered species. Indirect somatic embryogenesis protocol was developed from mature embryo explants of the palm. This study will provide a valuable reference for the propagation and conservation of A. *concinna*.

Introduction

Areca concinna Thwaites or 'Squirrel catechu' is a medium-sized palm with a low clustering habit bearing attractive lush green leaves, white fragrant flowers and bright red coloured fruits. The palm belongs to the genus Areca L. and the family Arecaceae which comprises 45 species (Plant List 2013). The plant is most suitable for use as an ornamental palm due to its attractive plant characteristics. It is considered a wild relative of the only cultivated palm from the Areca genus i.e. *Areca catechu* L. (beetle nut). Similar to beetle nuts, A. concinna nuts are also used as masticatory (Murthy and Pillai 1982), especially in Sri Lanka, where the nuts are occasionally chewed (Nair, 2021). The extracts from *Areca concinna* peel were used as a natural dye for dyeing cotton fabric (Ranathunga et al. 2021). The plant feed additive Peptasan® containing *Acacia concinna* can be effectively used for preventing coccidiosis in broiler chickens. (Sánchez et al. 2019). Matsumoto and Yamaguchi (2007) reported that *A. concinna* could effectively remove the indoor air contaminant toluene under lighting conditions. Moreover, the species was utilized in hybridization programmes for the production of interspecific hybrids with beetle nuts. These interspecific hybrids were resistant to Phytophthora meadii infections as A. concinna exhibited resistance to P. meadii (Prathibha et al. 2015). The seeds of *A. concinna* exhibit anti microbial and antioxidant properties (Rathnapriya et al. 2019) but are orthodox (Gama-Arachchige and Alahakoon, 2017) with low germination percentage when compared with same genus members like *A. triandra* (Muralikrishna et al. 2017).

However, because of habitat loss in endemic regions, the palm was included in the IUCN Red list of endangered species in the year 1998 (Johnson 1998). So, in addition to conventional propagation methods, it is essential to take up *in-vitro* propagation and conservation strategies for preserving these species. Even though *in vitro* propagation of many palm species had been successful (Arecanut- Karun et al. 2004; Coconut - Shareefa et al. 2019; Date palm - Al-Khayri and Naik 2022; Cane palm- Nijabat et al. 2022; Oil palm – Karim 2022), the same in *A. concinna* was not reported till date. In the present study, we report the successful *in vitro* propagation technique of *A. concinna* via somatic embryogenesis.

Materials And Methods

Plant materials and explants preparation

The study was conducted at the Indian Council of Agricultural Research- Central Plantation Crops Research Institute (ICAR – CPCRI), Kasaragod, Kerala (12° 31' 02.64" N, 74° 59' 26.38" E) from November 2018 to June 2021.

The explants used were immature inflorescence, and immature and mature zygotic embryos, which were collected from *Areca concinna* palms between November 2018 and February 2019 and maintained in the research field of ICAR-CPCRI. The inflorescence collected was of the − 3 stage (just opened inflorescence was considered as 0). These were about 12–15 cm in length. The unopened inflorescences were wiped with 80% alcohol and subjected to flame sterilization (Neema et al. 2022). The spathe was removed aseptically by giving a vertical cut from tip to base that joins with the horizontal cut at the base and pulling it off outward. The middle portions of the exposed rachilla (4 cm from the base and 3 cm from the tip) were chopped into 2–4 mm pieces and were inoculated into the callus initiation media. The immature zygotic embryos were extracted from 4 months old green nuts while mature zygotic embryos were obtained from 6-month-old red-coloured nuts. The nuts collected were washed under running tap water for 15 min, followed by the application of a few drops of detergent Tween 20 for 10 min and again rinsing with running tap water. The calyx was removed from the nuts and then treated with 0.01% Mercuric chloride for 3 minutes and then rinsed three times with sterile distilled water to remove the traces. Subsequently, they were transferred to a laminar airflow chamber where the husk was separated from the nuts to obtain the kernels. The kernels were sterilized using 20% sodium hypochlorite for 10 minutes followed by rinsing in autoclaved sterile water 5 to 6 times. Embryos were carefully excised from the kernels and inoculated in the callus induction media.

Medium and cultural conditions for callus induction

Four different media combinations (M1 – M4) were tested (Table 1) for callus induction. The basal media used was M72 (Karunaratne and Periyapperuma 1989). All the media combinations were uniformly supplemented with 3% sucrose, 0.1% activated charcoal and 0.8% agar. The pH of the medium was adjusted to 5.7 ± 0.2 with 0.1 N NaOH or 0.1 HCl before autoclaving the media at 10 K Pa for 15 min at 121°C. Inoculated plates were incubated at 27°C under dark conditions continuously for three months. The chopped inflorescence was inoculated to each media combination. Ten ZE each from both immature

and mature nuts was inoculated and the experiments were replicated thrice. The cultures were monitored regularly for fungal or bacterial contamination.

Table 1
Inoculation media types and their hormonal compositions

Media	Plant growth chemicals				
	Basal media	2,4-D (mg/L)	Picloram (mg/L)	2-iP (mg/L)	BAP (mg/L)
M1	M72	-	48.29	-	-
M2	M72	25	-	-	-
M3	M72	-	36.21	-	3
M4	M72	15	-	3	-

[M72 basal media (Karunaratne S, Periyapperuma K, 1989); 2, 4-D: 2, 4-dichlorophenoxyacetic acid; 2-ip: 2-isopentenyl adenine; BAP: 6- benzylaminopurine]

Medium and culture conditions for somatic embryogenesis

Three months after the initial inoculation, the callus obtained from the explants was transferred to the same media with 1/10th of the initial auxin concentration of the media that induced callus. The cultures were maintained in dark at 27°C and 80% RH. Monthly subculture was done to the same media until the development of somatic embryos.

Medium and culture conditions for somatic embryo germination and plantlet regeneration

The somatic embryos formed were transferred to hormone-free Eeuwen's Y3 basal media for germination. Germinated cultures were maintained under white LED lights (100 µmol m^{-2} s^{-1}) for a photoperiod of 16 h light and 8 h dark. The temperature and RH were maintained at 27 ± 1°C and 80% respectively. For the growth and development of the somatic embryos, two media combinations were tried; full-strength Eeuwen's Y3 basal medium supplemented with 1 mg/L of BAP, 0.5 mg/L NAA, and 0.25 mg/L of IBA (T1) and half-strength Eeuwen's Y3 basal medium supplemented with 1 mg/L of BAP, 0.5 mg/L NAA), and 0.25 mg/L of IBA (T2) Subculture is carried out at monthly intervals for two months in the same media. In the third month, subculture was carried out to liquid media with the same media composition.

Acclimatization and hardening of plantlets

After one month of culturing in liquid plantlet development media, plants with 2–4 leaves and a sufficient number of roots were potted into a potting mixture containing sand, soil, and coir pith in the ratio 3:1:1 for initial acclimatization. The potted plants are covered tightly with polythene bags and placed under the

LEDs to maintain a very high humid condition. After one week, small perforations are made on the polythene cover. The polythene covers are removed after one month and the plantlets are transferred to the net house for hardening.

Statistical analysis

The explants' response and effect of different media for callus induction were determined after 90 days of culture. The growth and development of *A. concinna* somatic embryos on germination media were determined 60 days after germination. Analysis of variance was performed over the completely randomized design trials using R software Version 4.1.3 (R Core Team 2022) by applying rules of arc sine data transformation (Parsad 2005) since the response was measured in percentage. The significance of differences between treatment means (Fisher-LSD test) was determined by using the "Agricola" package in R (Mendiburu 2020).

Results And Discussion

Explant response and media standardization for embryogenic callus

Of the three explants viz. immature inflorescence, immature and mature ZE (zygotic embryos) inoculated; callus induction was observed only in mature ZE of *A. Concinna*. Immature ZE and inflorescence cultures did not respond to the callus initiation media M1 – M4 (Table 2). Even though callus initiation was observed from mature ZE explants in M2 and M4 media (Table 3), SE from the initiated calli was observed only in M2 media. A somatic embryo from the ZE callus was observed after 3 months when the callusing explants were subcultured to the responsive media with 1/10th of the initial 2, 4-D concentration. The mature ZE bulged after 1–2 weeks of dark incubation. Even though white translucent calli were induced from the enlarged mature ZE after two months of initial inoculation (Fig. 1B), some of the embryos germinated giving rise to the shoot initials on the same media. Germination of the mature ZE (8.6–30%) was observed in all the tested media (Table 3).

Table 2
Explant-response for callusing in callus initiation media

Explant types	**Media combinations**			
	M1	M2	M3	M4
Mature ZE	-	++++	-	++
Immature ZE	-	-	-	-
Immature inflorescence	-	-	-	-
(+: low response for callusing; ++: medium response for callusing; ++++: high response for callusing; -: no response for callusing)				

Table 3
Response of mature ZE explants to inoculation media for callusing and germination

Media	Explant Browning (%)	ZE germination (%)	Callusing (%)	Embryogenic callus (%)
M1	8.33(2.85a)	11.66(3.39a)	0.00(0.00b)	0.00(0.00b)
M2	10.00 (3.09a)	8.66(2.92a)	23.33(4.80a)	8.33(2.85a)
M3	11.66(3.29a)	10.00(3.09a)	0.00(0.00b)	0.00(0.00b)
M4	6.66(2.54a)	30.0(16.64a)	3.33(1.05b)	1.66(0.74b)
Mean	9.16(2.94)	15.08(6.51)	6.66(1.46)	2.49(0.89)
CV (%)	54.54(28.63)	39.03(156.88)	61.23(68.44)	81.65(72.94)
CD at 0.05	N/A(1.68)	11.25(20.42)	7.80(2.00)	3.90(1.31)
S.Em.	2.88(0.71)	3.39(104.4)	2.35(1.00)	1.17(0.43)

(Figures in parentheses square root transformed values; CV is the coefficient of variation and CD is the critical difference)

Maximum response for callusing i.e. 23.33% was observed in M2 than M4 (3.3%) (Table 3; Fig. 1B &C); ie enhanced callusing was observed in basal media with 2, 4-D alone when compared to the 2, 4-D + 2-iP media. 2, 4-D has been used successfully in tissue culture of diverse plant species, especially for the induction of embryogenic calli and somatic embryos in palms (Silva-Cardoso et al. 2022; Ferreira et al. 2022). Embryogenic callus could be obtained in different plants by manipulating 2, 4-D concentration and the extent of its presence in induction media (Zheng and Konzak 1999). For example, 1 mg/L of 2, 4-D for a week duration is effective for induction of embryogenic calli and subsequent regeneration in rice seed explants (Carsono et al. 2021), whereas the same chemical works only if it's used at 66 mg/L for eight months to obtain the embryogenic calli from immature inflorescence explants of coconut (Verdeil et al. 1994). Palms are comparatively difficult species to grow in *vitro*. The recalcitrant nature of the palms was explained due to lower explant response, slower growth rate, excessive browning, and lack of repeatability (Fernando et al. 2010; Shareefa et al. 2019).

In the current study, we used 2, 4-D and picloram to induce the embryogenic calli. The effectiveness of 2, 4-D was tested in different palms to induce organogenesis and somatic embryogenesis (Sáenz et al. 2018; Zayed et al. 2017). Similarly, several researchers demonstrated the positive role of the synthetic auxin picloram in the clonal multiplication of palms like coconut (*Cocos nucifera L.*), areca nut (*Areca catechu L.*), pejibaye palm (*Bactris gasipaes* H.B.K), rattan palm (*Calamus manan* Miquel.) and Canary Island date palm (*Phoenix canariensis*) (Raju 2006; Karun et al. 2004; Valverde et al. 1987; Goh et al. 1999; Huong et al. 1999). Though both the auxins are found to have a role in callogenesis and clonal

multiplication of palms, the response of callusing in *A. concinna* was seen only on induction media supplemented with 2,4-D.

When explant sources (mature, immature ZEs and immature inflorescence tissues) of *A. concinna* were compared for the response, only mature ZE survived and gave rise to callus (3.3–23.33%) on callus induction media. There was no response for callusing from the other two explants i.e., immature ZE and inflorescence explants (Table 2). Even though browning was observed in all three explant tissues, intense browning was observed in inflorescence explants rather than in embryos. Eventually, these browned tissues lost viability by the end of the 60th day after inoculation. Oxidative browning is one of the common problems faced in plant tissue culture resulting in lower rates of regeneration (Laukkanen et al. 2000; Aliyu 2005) or sometimes even leading to the death of explant tissue (Panaia et al. 2000; Tabiyeh et al. 2006). Explant browning and its deleterious effects on palm tissue culture were reported in many studies (EL-Gioushy et al. 2020; Al-Mayahi et al. 2018; Karun et al. 2004). Even though oxidative browning of mature ZE in all four media combinations ranged from 6.66–11.66% significant differences were not found among the media combinations. However, the browning of mature ZE didn't lead to the death of explant tissue in *A. concinna*.

Somatic embryogenesis and plantlet regeneration

The calli obtained from responsive induction media (Table 2, Fig. 1B) were subcultured to Eeuwen's Y3 media containing 2.5 mg/L of 2, 4-D for callus multiplication and somatic embryogenesis. Only 35 to 49% of the calli obtained from inoculation media showed regenerative capacity (Table 3; Fig. 1C). Somatic embryo formation was observed; when embryogenic callus was transferred to hormone-free Eeuwen's Y3 media supplemented with 3% sucrose, 0.1% activated charcoal and 0.8% agar for maturation and germination (Fig. 1E). The germinated somatic embryos were transferred to full and half strength Y3 media supplemented with 1 mg/L of BAP, 0.5 mg/L NAA) and 0.25 mg/L of IBA for proper growth and development of shoot and roots (Table 3; Fig. 1F).

Relatively equal growth and development of somatic embryos were observed in both media compositions (Table 4). Around 78% of the somatic embryos developed exhibited proper shoot and root initials. Fully developed plantlets with 2–4 expanded leaves were obtained after 16 weeks of culture in the same medium. Approximately 67% of developed plantlets showed healthy growth in tubes. These healthy plantlets with proper roots were subjected to *in vitro* acclimatization/hardening (Fig. 1G). Plants are transferred to plastic pots containing sand: soil: coir pith (3:1:1) mixture and were covered initially with polyethylene bags and incubated in a culture room under white LEDs (200 µmol m^{-2} s^{-1}) for a photoperiod of 16 h light and 8 h dark. The temperature and RH were 27 ± 1°C and 80% respectively. During *in vitro* acclimatization plants were watered with diluted (1:2) MS macro solution once a week. After *in vitro* hardening only 35% plantlet recovery was obtained due to poor root development and contamination issues during *in vitro* hardening. After two months of *in vitro* acclimatization, these plantlets were transferred to a shade net- house to carry *ex vitro* hardening process

Table 4
Growth and development of *A. concinna* somatic embryos on germination media

Treatment	Shoot initiation (%)	Rooting (%)	Plantlet survival before hardening (%)	Plantlet survival after hardening (%)
T1	80.00(8.93a)	80.00(8.93a)	71.66(25.86a)	33.33(16.98a)
T2	76.66 (8.75a)	66.66(25.57a)	63.33(42.78a)	38.33(30.15a)
Mean	78.33 (8.84)	73.33(17.25)	67.49(34.32)	35.83(23.57)
CV (%)	10.42 (6.9)	11.13 (122.14)	12.46(61.4)	23.48(81.78)
CD 0.05	NA (2.14)	NA (74.04)	NA(74.05)	NA(67.73)
S.Em.	4.71 (0.37)	4.71 (444.1)	4.85(444.2)	4.85(371.6)

(Figures in parentheses square root transformed values; Compositions of T1 and T2 (somatic embryo germination media); T1:Y3 media + 1 mg/L BAP + 0.5 mg/L NAA + 0.25 mg/L IBA, T2:1/2 Y3 media + 1 mg/L BAP + 0.5 mg/LNAA + 0.25 mg/L IBA; CV is the coefficient of variation and CD is critical difference)

Conclusions

In the current study, mature zygotic embryos were used for plantlet regeneration in the endangered palm species, *Areca concinna* through *in vitro* culture. The explants selection, culture medium and hormonal concentrations played an essential role in callogenesis and somatic embryogenesis of the palm. The auxin 2,4-D influences callogenesis and somatic embryo formation in A. *Concinna.* This protocol could be used for mass multiplication of the palms, thereby acting as a means to safeguard the germplasm of the endangered plant species *A. concinna*.

Abbreviations

2, 4-D 2, 4-dichloro phenoxy acetic acid

BAP N6-benzylaminopurine

IBA Indole butyric acid

LED Light emitting diodes

NAA Naphthalene acetic acid

PGRs Plant growth regulators

Picloram 4-Amino-3, 5, 6-trichloropyridine-2-carboxylic acid

SE Somatic embryogenesis

Y3 Eeuwen's Y3 basal medium

ZE Zygotic embryo

Declarations

Acknowledgements

The authors acknowledge the Indian Council of Agricultural Research, for providing the necessary funds to carry out the research.

Author contributions

AV planned and conducted the study, MKS collected the explant, NM, KP, and AK supervised the experiment. AV wrote the manuscript, SS analysed the experimental data; NM revised the manuscript. All authors read and approved the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

1. Aliyu OM (2005) Application of tissue culture to cashew (*Anacardium occidentale*. p. L.) breeding: An appraisal. Afr J Biotechnol 4:1485–1489. https://www.ajol.info/ index.php/ ajb/ article/view/116551
2. Al-Khayri JM, Naik PM (2022) Influence of 2iP and 2, 4-D Concentrations on the accumulation of biomass, phenolics, flavonoids and radical scavenging activity in date palm (*phoenix dactylifera* l.) cell suspension culture. Horticulturae 8: 683 https://www.mdpi.com/2311-7524/8/8/683
3. Al-Mayahi AMW, Ali AH, Shareef HJ (2018) Influence of cold pretreatment on shoot regeneration from callus in date palm (*Phoenix dactylifera* L.) cv. 'Barhee'. J Genet Eng Biotechnol 16:607–612. https://doi.org/10.1016/j.jgeb.2018.07.002
4. Carsono O, Juwendah E, Liberty, Sari S, Damayanti F, Rachmadi M (2021) Optimize 2, 4-D concentration and callus induction time enhance callus proliferation and plant regeneration of three rice genotypes. Biodiversitas 22:2555–2560. https://doi.org/10.13057/biodiv/d220702
5. Eeuwens CJ (1976) Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut palms (*Cocos nucifera*) and cultured *in vitro*. Physiol Plant 36:23–28. https://doi.org/10.1111/j.1399-3054.1976.tb05022.x

6. EL-Gioushy SF, Liu R, Fan HK (2020) A complete protocol to reduce browning during coconut (*Cocos nucifera* L.) tissue culture through shoot tips and inflorescence explants. Plant Arch 20:2196–2220
7. Fernando SC, Vidhanaarachchi VRM, Weerakoon LK, Santha ES (2010) What makes clonal propagation of coconut difficult? Asia-Pac J Mol Biol Biotechnol 18:163–165
8. Ferreira JCB, de Araújo Silva-Cardoso IM, de Oliveira Meira R, Scherwinski-Pereira JE (2022) Somatic embryogenesis and plant regeneration from zygotic embryos of the palm tree *Euterpe precatoria* Mart. Plant Cell Tiss Organ Cult 148:667–686
9. Gama-Arachchige NS, Alahakoon AACB (2017) Seed Dormancy and Germination Requirements of Two Endemic Palm Species of Sri Lanka. *In Proceedings of International Forestry and Environment Symposium* (Vol. 22).) DOI:10.31357/fesympo.v22i0.3246
10. Goh DKS, Michaux FN, Monteuuis O, Bon MC (1999) Evidence of somatic embryogenesis from root tip explants of rattan *Calamus manan.* In Vitro. Cell Dev Biol - Plant 35:424–427
11. Huong LTL, Baicco M, Hug BP, Burno M, Santilachi R, Rosati P (1999) Somatic embryogenesis of Canary Island date palm. Plant Cell Tiss Organ Cult 56:1–7
12. Johnson D (1998) *Areca concinna*, The IUCN Red List of Threatened Species 1998: e.T38186A10099202. https://dx.doi.org/1
13. Karim SKA (2022) An overview of oil palm cultivation via tissue culture. Elaeis guineensis p. 191. DOI:10.5772/intechopen.99198
14. Karun A, Siril EA, Radha E, Parthasarathy VA(2004) Somatic embryogenesis and plantlet regeneration from leaf and inflorescence explants of areca nut (*Areca catechu* L.).Cur Scipp.1623–1628
15. Karunaratne S, Periyapperuma K (1989) Culture of immature embryos of coconut (*Cocos nucifera* L.): callus proliferation and somatic embryogenesis. Plant Sci 62:247–253
16. Laukkanen H, Rautiainen L, Taulavuori E, Hohtola A (2000) Changes in cellular structures and enzymatic activities during browning of Scots pine callus derived from mature buds. Tree Physiol 20:467–475
17. Matsumoto H, Yamaguchi M (2007) Experimental study on the effect of foliage plants on removing indoor air contaminants. *CLIMA 2007 Well Being Indoors*, p.A03
18. Mendiburu FD (2020) agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-3. https://CRAN.R-project.org/package=agricolae
19. Muralikrishna KS, Rajesh MK, Sajini KK, Nagaraja NR, Ananda KS, Karun A (2017) Immature embryo culture in wild Areca spp
20. Murthy KN, Pillai RNS (1982) Botany. In: Bavappa KVA, Nair MK, Kumar PT (eds) The Arecanut palm. Central Plantation Crops Research Institute, Kasaragod, pp 11–49
21. Nair KP (2021) Arecanut (*Areca catechu* L.). Tree Crops. Springer, Cham.), pp 1–25
22. Neema M, Aparna V, Chandran KP(2022) Contrast analysis recommends flame sterilization for surface depuration in coconut (*Cocos nucifera*) meristem culture. Cur Hort p.41

23. Nijabat A, Naveed NH, Faiz S, Yasin NA, Ali A (2022) Combinatorial effects of thidiazuron and gibberellic acid on in vitro propagation of an endangered tree. Cane Palm (Dypsis lutescens)
24. Panaia M, Senaratna T, Bunn E, Dixon KW, Sivasithamparam K (2000) Micropropagation of the critically endangered Western Australian species, *Symonanthus bancroftii* (F Muell.) L. Haegi (Solanaceae). Plant Cell Tiss Organ Cult 63:23–29
25. Parsad R (2005) Transformation of data. Design, Analysis Agricultural Experiments Indian Agricultural Statistics Research Institute, New Delhi, India, 637–647
26. Prathibha VH, Hegde V, Sharadraj KM, Nidhina K, Nagaraja NR, Chaithra M (2015) Identification of sources of resistance against *Phytophthora* in areca nut. In Proceedings of 3rd International Symposium on Phytophthora held at Bengaluru, India, 9-12th September 2015, P. 40
27. Raju CR (2006) Direct *in vitro* shoot development from immature rachilla explants of coconut (*Cocos nucifera* L.). Cord 22:51–58
28. Ranathunga RGSM, Wanigasekara KV, Udayakumara SV (2021) Dying of cotton fabric with a natural dye extracted from. Areca concinna peel
29. Rathnapriya RHDL, Senevirathne SA, Edirisinghe EMRKB (2019) Investigation of biochemical properties of *Oncosperma fasciculatum* and *Areca concinna* seeds
30. Sáenz L, Chan JL, Narvaez M, Oropeza C (2018) Protocol for the micropropagation of coconut from plumule explants. In: Loyola-Vargas VM, Ochoa-Alejo N (eds) Plant Cell Culture Protocols. Humana Press, New York, NY, USA, pp 161–170
31. Sánchez-Hernández C, Trejo-Castro JA, Mendoza-Martínez L, Gloria-Trujillo GD (2019) A Evaluation of a feed plant additive for coccidiosis control in broilers herbals for coccidiosis control. Brazil J Poultry Sci 21
32. Shareefa M, Thomas RJ, Sreelekshmi JS, Rajesh MK, Anitha K (2019) *In vitro* regeneration of coconut plantlets from the immature inflorescence. Curr Sci 117:813–820
33. Silva-Cardoso IMDA, Meira FS, Scherwinski-Pereira JE (2022) The maturity level of the explant plays a key role in somatic embryogenesis of the palm tree *Syagrus oleracea* [Mart.] Becc. Acta Physiol Plant 44:1–17
34. Tabiyeh DT, Bernard F, Shacker H (2006) Investigation of glutathione, salicylic acid, and GA_3 effects on browning in *Pistacia vera* shoot tips culture. Acta Hortic 726:201
35. The Plant List (2013) *Version 1.1.* Published on the internet http://www.theplantlist.org/ (accessed 3rd December 2018)
36. Valverde R, Arias O, Thorpe TA (1987) Picloram-induced somatic embryogenesis in pejibaye palm (*Bactris gasipacs* H.B.K.). Plant Cell Tiss Organ Cult 10:149–156
37. Verdeil JL, Huet C, Grosdemange F, Buffard-Morel J (1994) Plant regeneration from cultured immature inflorescences of coconut (*Cocos nucifera* L.) evidence for somatic embryogenesis. Plant Cell Rep 13:218–221

38. Zayed EMM (2017) Direct organogenesis and indirect somatic embryogenesis by *in vitro* reversion of mature female floral buds to a vegetative state. In: Al-Khayri JM, Jain SM, Johnson DV (eds) Date Palm Biotechnology Protocols Volume I: Tissue Culture Applications, Methods in Molecular Biology. Humana Press, New York, pp 47–59
39. Zheng MY, Konzak CF (1999) Effect of 2, 4-D on callus induction and plant regeneration in anther culture of wheat. Plant Cell Rep 19:69–73

Figures

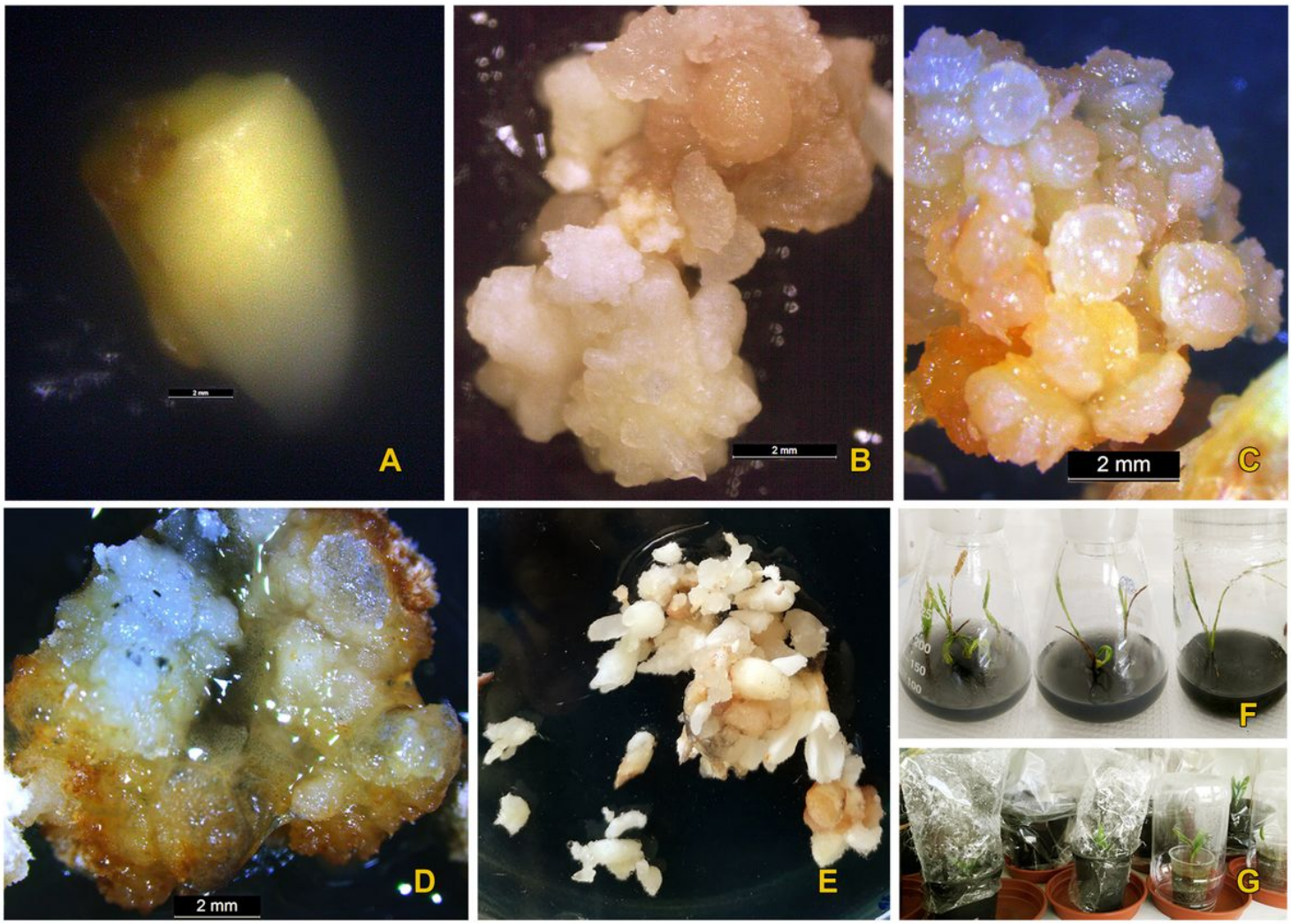

Figure 1

Micropropagation of *Areca concinna* via somatic embryogenesis from mature zygotic embryos; (A).Inoculation of mature zygotic embryos of *A.concinna* in callusing media, (B). Callus initiation from zygotic embryos of *A.concinna*, (C). Embryonic calli of *A.concinna* (D). Non-Embryogenic calli, (E). Somatic embryogenesis from the calli, (F). Germination and development of *A.concinna* Somatic embryos, (G). *In vitro* hardening of somatic embryo-derived plants from *A.concinna*