ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021

Exploring Somatic Embryogenesis and Plantlet Regeneration in Various Oil Palm (Elaeis guineensis Jacq.) Genotypes: A Comparative Study

D. S. Sparjanbabu[1*], M. S. R. Krishna[1]

1 Koneru Lakshmaiah Educational Foundation Deemed to be University, Guntur, Andhra Pradesh, India.

Abstract

In this study, we evaluated the capacity for somatic embryogenesis and plantlet regeneration in four elite genotypes: P-1 (240D X 281D), P-2 (80D X 281D), C-1 (98C X 254D), and C-2 (98C X 208D). To initiate callus formation and proliferation, zygotic embryos (ZEs) were cultured on N6 media supplemented with 2 mgL-1 Dicamba for a duration of 90 days. Following induction, embryogenic calli were cultured for 120 days in N6 media containing 0.1 mgL-1 2,4-D, 0.16 mgL-1 putrescine, 0.5 mgL-1 casein, and 2.0 g/L activated charcoal to promote somatic embryogenesis and maturation. The differentiated polyembryoids were then transferred to regeneration media consisting of N6 with 0.5 mgL-1 NAA, 1.0 mgL-1 BAP, and 0.5 mgL-1 activated charcoal. Among the studied genotypes, P-2 and P-1 exhibited the highest rates of callus induction, embryogenic calli, differentiated polyembryoids, and a greater number of plantlets per somatic embryo cluster. Overall, P-2 and P-1 genotypes displayed more promising results throughout the entire process of somatic embryogenesis and regeneration from matured ZEs of the dura variety.

Keywords: Dura, Callus induction, Dicamba, Elite genotype

1. INTRODUCTION:

Oil palm, being a highly versatile crop with numerous applications in various industries, holds a prominent position in the global vegetable oil market [1]. Consequently, its cultivation has significantly expanded over the last three decades. However, India faces a substantial shortage of vegetable oils [2], leading to the import of around two-thirds of its total edible oil, incurring significant foreign exchange expenditure. To address this issue, extensive breeding using diverse genetic resources is essential [3]. Unfortunately, oil palm has a limited genetic diversity [4], making the availability of genetic variability a prerequisite for genetic improvement [5]. Therefore, conserving germplasm accessions has become imperative [6]. However,

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021

maintaining perennial oil palm germplasm ex situ is resource-intensive, and due to its single growing apex, vegetative multiplication is not feasible [7]. The conventional propagation method for oil palm is solely through seeds, but long-term storage of seeds is challenging due to their intermediate storage behavior [8]. Thankfully, cryopreservation offers a viable solution for conserving valuable germplasm, as both zygotic embryos (ZEs) and somatic embryos can be stored for extended periods through this technique [9]. Therefore, it is necessary to develop an in vitro regeneration method for the isolated Zygotic Embryos (ZEs). While there have been various studies on in vitro regeneration using direct and indirect somatic embryogenesis with ZEs [10], a consistent and dependable protocol is lacking due to the crop's genetic variability.

2. PLANT MATERIAL AND EXPLANT:

The mature fresh fruit bunches of four elite genotypes, namely P-1, P-2, C-1, and C-2, [11] were collected from the seed garden of ICAR-Indian Institute of Oil Palm Research in Pedavegi [12], Andhra Pradesh, India, located at coordinates 16°48'41.6" N and 81°07'51.0" E[13].

3.PREPARATION OF EXPLANT:

Following the harvest, the fruitlets were depericarped to extract the seeds, and then the seeds were cracked to obtain the kernels [14]. The kernels underwent surface sterilization using a mixture of Tween-20 solution (0.5 mL/100 mL) and a fungicide solution (1% Carbendazim and 1% Mancozeb) for 20 minutes within a sterile environment [15]. Subsequently, the kernels were rinsed three times with sterile water [16]. After halving the kernels, the embryos were carefully removed and subjected to sterilization with a 20% (v/v) sodium hypochlorite solution for 20 minutes [17], followed by three washes with sterile deionized water. Finally, the sterilized embryos were inoculated onto the culture medium [18].

2. SOMATIC EMBRYOGENESIS AND MATURATION:

To induce somatic embryo differentiation and maturation, the embryogenic calli obtained from the callus induction medium were transferred to N6 medium[19] supplemented with 2,4-D (0.1 mgL-1), putrescine (0.16 mgL-1), casein (0.5 mgL-1), and activated charcoal (2.0 mgL-1) [Table 1][20]. The cultures were then kept in darkness at a temperature of 27°C for a period of 120 days[21].

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021

Table 1: Components of the culture media in different stages of somatic embryogenesis and planet regeneration from oil palm zygotic embryo.

Components	Stage I	Stage II	Stage III
	Callus induction and proliferation (90 days)	Somatic embryogenesis and maturation (120 days)	Plantlet regeneration from polyembryoids (60 days)
Culture Media	N6	N6	N6
Dicamba (mg L^{-1})	2.0	-	-
2,4-D (mg L^{-1})	-	0.1	-
NAA (mg L^{-1})	-	-	0.5
BAP (mg L^{-1})	-	-	1.0
Putrescine (g L^{-1})	-	0.16	-
Casein (g L^{-1})	-	0.5	-
Activated charcoal (g L^{-1})	-	2.0	0.5
Sucrose (g L^{-1})	30.0	30.0	30.0
Agar (g L^{-1})	8.0	8.0	8.0

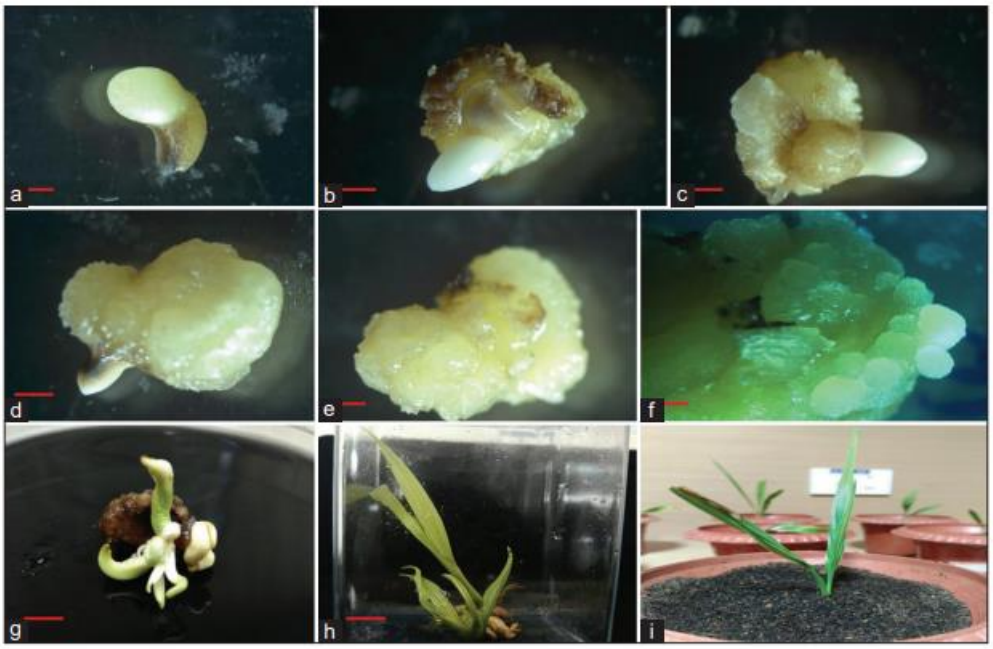

Figure 1: Morphological stages of somatic embryogenesis from mature zygotic embryo of dura oil palm. (a) Swollen zygotic embryo before formation of callus. (b) 25% explant surface covered by callus. (c) 50% explant surface covered by callus. (d) 75% explant surface covered by callus. (e) 100% explant surface covered by callus. (f) Somatic embryos with globular forms. (g) Germinating polyembryoids. (h) Regenerated plantlets. (a-e) calli on callus induction and proliferation medium. (f-g) calli on somatic embryogenesis and maturation medium. (h) plantlet regeneration medium. (i) Hardening in soil less media. *Bars*, 0.2 cm (a-e); 0.1 cm (f); 1.0 cm (g); 2.0 cm (h).

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021

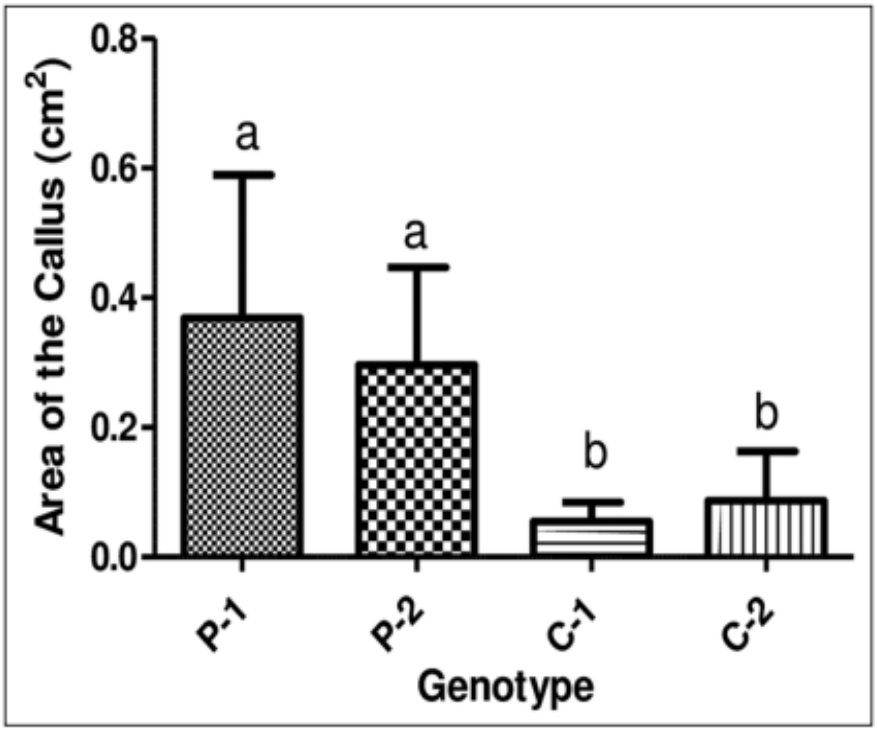

Figure 2: Effect of genotype on callus size-area (cm^2).

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021

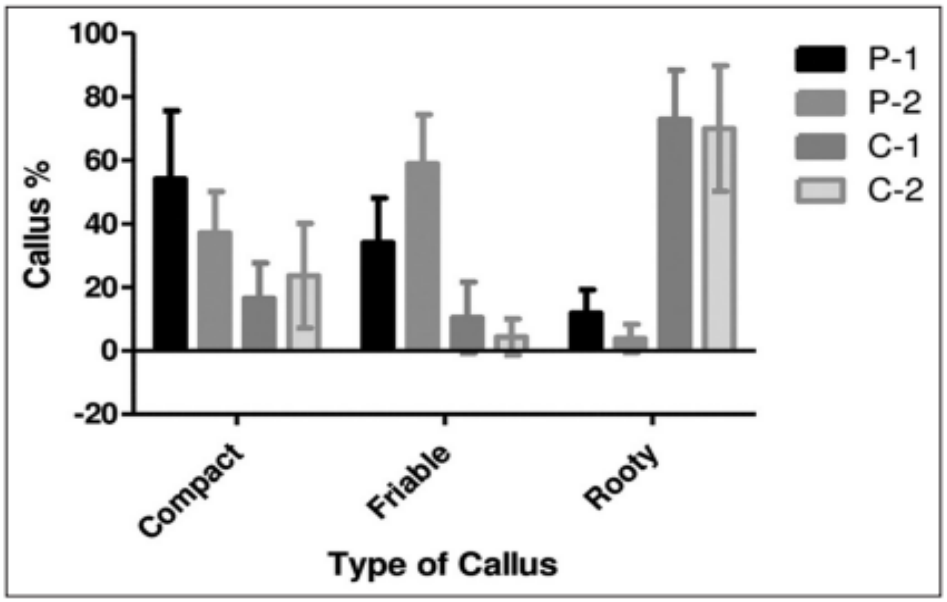

Figure 3: Effect of genotype on type of callus; values are mean ± SE.

Table 2: Effect of genotypes on callus induction and color of the callus from the zygotic embryos of oil palm.

Genotype	Callus induction (%)	Color of the callus (%)		
		Yellow	White	Translucent
P-1 (240D X 281D)	88.618^{ab}	76.293^{a}	16.467^{b}	7.240^{bc}
P-2 (80D X 281D)	94.998^{a}	86.953^{a}	9.663^{b}	2.273^{c}
C-1 (98C X 254D)	83.796^{ab}	10.515^{b}	48.710^{a}	40.775^{a}
C-2 (98C X 208D)	76.630^{b}	11.443^{b}	61.243^{a}	24.933^{ab}

*Letters indicate significant differences between treatments (callus induction significant at 5% level of Significance CD (0.05) whereas colour of the callus significant at 1% CD (0.01) and 5% CD (0.05).

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021

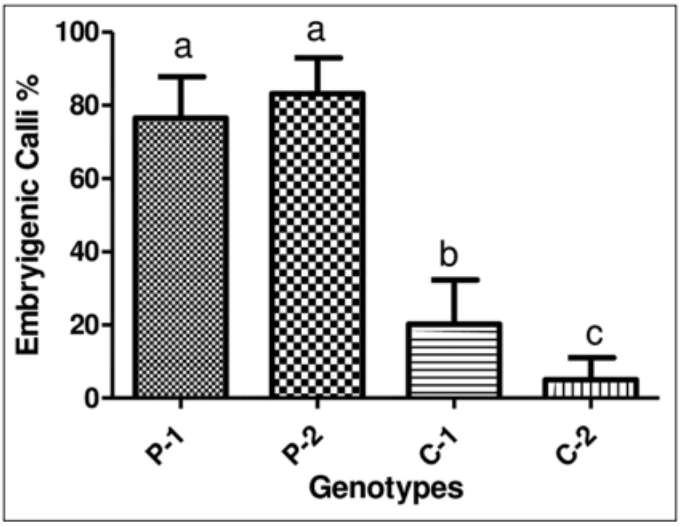

Figure 4: Effect of genotype on embryogenic calli percent.

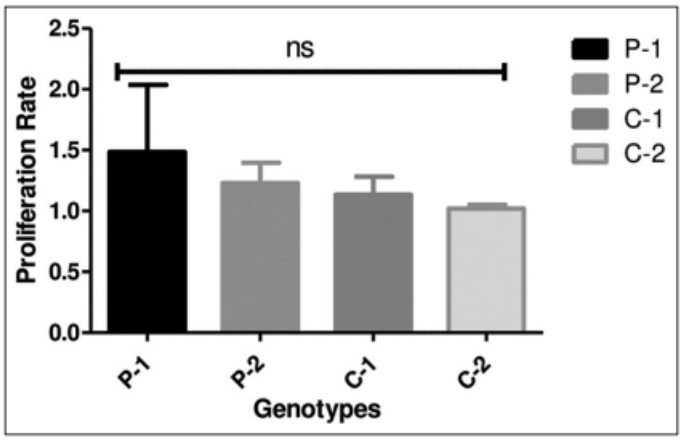

Figure 5: Effect of genotypes on callus proliferation rate; ns-not significant

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021

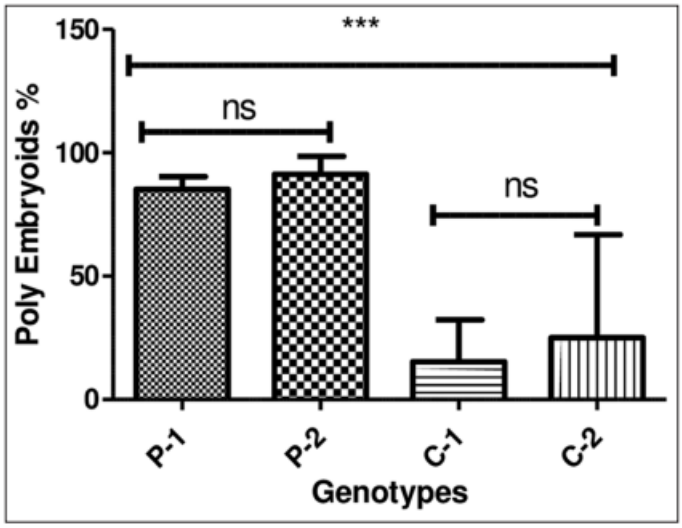

Figure 6: Effect of genotypes on polyembryoids from the pre somatic embryos ns-not significant within same group; *** cumulatively highly significant.

Table 3: Effect of genotype on explant surface covered by the callus.

Genotype	Explant surface covered by the Callus (%)				
	0	25	50	75	100
P-1 (240D X 281D)	11.382^{ab}	0.000^{b}	0.000^{c}	27.147^{a}	63.901^{b}
P-2 (80D X 281D)	5.002^{b}	0.000^{b}	8.352^{bc}	4.556^{b}	82.090^{a}
C-1 (98C X 254D)	16.204^{ab}	7.778^{ab}	32.778^{a}	10.880^{b}	32.361^{c}
C-2 (98C X 208D)	23.370^{a}	10.135^{a}	23.204^{ab}	10.735^{b}	32.556^{c}

*Letters indicate significant differences between treatments (0, 25 and 75 significant at 5% level of Significance CD (0.05) whereas 50 and 100 significant at 1% CD (0.01) and 5% CD (0.05).

CONCLUSION:

Our evaluation of four elite Indian oil palm genotypes for the differential response to somatic embryogenesis results in significant variation among genotypes in terms of all characters studied. Genotypes P-2 (80D X 281D) and P-1 (240D X 281D) showed impeccable potential for somatic embryogenesis and plantlet regeneration among genotypes.

REFERENCES:

1. Corley RH. How much palm oil do we need? Environ Sci Policy 2009;12:134-9.

2. Jackson TA, Crawford JW, Traeholt C, Sanders JA. Learning to love the world's most hated crop. J Oil Palm Res 2019;31:331-47.

3. Shigetomi Y, Ishimura Y, Yamamoto Y. Trends in global dependency on the Indonesian palm oil and resultant environmental impacts. Sci Rep 2020;10:20624.

4. Hardon J. Breeding and selection of the oil palm in Malaysia. Oléagineux 1968;23:85-90.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021

5. Ooi SC, Rajanaidu N. Establishment of oil palm genetic resources theoretical and practical considerations. Malaysian Appl Biol 1979;8:15-28.

6. Kumar PN, Sparjanbabu DS, Ravichandran G, Anitha M, Satyanarayana G, Mandal G, et al. Effect of low temperature storage on oil palm (Elaeis guineensis Jacq.) seed viability. Inter J Trop Agri 2015;33:155-9.

7. Ellis RH, Hong TD, Roberts RH, Tao KL. Low moisture content limits to relations between seed longevity and moisture. Ann Bot 1990;65:493-504.

8. Ellis RH. The longevity of seeds. Hortscience 1991;26:1119-25.

9. Chu CC, Wang CC, Sun CS, Hsu C, Yin KC, Chu CY, et al. Establishment of an efficient medium for another culture of rice through comparative experiments on the nitrogen sources. Scientia Sinica 1975;18:659-68.

10. Jayanthi M, Susanthi B, Mohan NM, Mandal PK. In vitro somatic embryogenesis and plantlet regeneration from immature male inflorescence of adult dura and tenera palms of Elaeis guineensis Jacq. Springerplus 2015;4:256.

11. de Silva RC, Luis ZG, Scheswinski-Pereira JE. The histodifferentation events involved during the acquisition and development of somatic embryogenesis in oil palm (Elaeis guineensis Jacq.) Plant Growth Regul 2014;72:67-80.

12. Thawaro S, Te-chato S. Effect of genotypes and auxins on callus formation from mature zygotic embryos of hybrid oil palm. J Agri Technol 2009;5:167-77.

13. Williams EG, Maheswaran G. Somatic embryogenesis: Factors influencing coordinated behaviour of cells as an embryogenic group. Ann Bot 1986;57:443-62.

14. Pádua MS, Santos RS, Labory CR, Stein VC, Mendonça EG, Alves E, et al. Histodifferentiation of oil palm somatic embryo development at low auxin concentration. Protoplasma 2017;255:285-95.

15. Balzon TA, Luis ZG, Scherwinski-Pereira JE. New approaches to improve the efficiency of somatic embryogenesis in oil palm (Elaeis guineensis Jacq.) from mature zygotic embryos. In vitro Cell Dev Biol Plant 2013;49:41-50.

16. Fukuda H. Programmed cell death of tracheary elements as a paradigm in plants. Plant Mol Biol 2000;44:245-53.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021

17. Besse I, Verdeil JL, Duval Y, Sotta B, Maldiney R, Miginia E. Oil palm (Elaeis guineensis Jacq.) clonal fidelity: Endogenous cytokinins and indoleacetic acid in embryogenic callus cultures. J Exp Bot 1992;43:983-9.

18. Durand-Gasselin T, Guen VL, Konan E, Duval Y. Oil palm (Elaeis guineensis Jacq.) plantations in Côte d'Ivoire obtained through in vitro culture. First results. Oléagineux 1990;45:1-11.

19. Hanower J, Hanower P. Inhibition et stimulation, en culture in vitro, de l'embryogenèse des souches issues d'explants foliaires de palmiers à huile. C R Acad Sci Paris Ser 1984;298:45-8.

20. Abdullah R, Zainal A, Heng WY, Li LC, Beng YC, Phing LM, et al. Immature embryo: A useful tool for oil palm (Elaeis guineensis Jacq.) genetic transformation studies. Electron J Biotechnol 2005;8:0717-3458.

21. Eeuwens CJ, Lord S, Donough CR, Rao V, Vallejo G, Nelson S. Effects of tissue culture conditions during embryoid multiplication on the incidence of "mantled" flowering in clonally propagated oil palm. Plant Cell Tissue Organ Cult 2002;70:311-23.