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# Differential response of oil palm (*Elaeis guineensis* Jacq.) genotypes on somatic embryogenesis and plantlet regeneration from zygotic embryo

D. S. Sparjanbabu[1]*, Naveen Kumar Prathapani[2], M. S. R. Krishna[1], D. Ramajayam[3], B. Susanthi[4]

[1]Koneru Lakshmaiah Educational Foundation Deemed to be University, Guntur, Andhra Pradesh, India.
[2]ICAR-Directorate of Floricultural Research, Pune, Maharashtra, India.
[3]ICAR-National Research Centre for Banana, Tamil Nadu, India.
[4]ICAR-Indian Institute of Oil Palm Research, Pedavegi, Andhra Pradesh, India.

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**ABSTRACT**

Oil palm's enormous economic potential necessitates the genotype evaluation and conservation for its accelerated breeding programs. Hence, in this study, we have evaluated the somatic embryogenesis and regeneration potential of four elite genotypes P-1 (240D X 281D), P-2 (80D X 281D), C-1 (98C X 254D), and C-2 (98C X 208D). For callus induction and proliferation, zygotic embryos (ZEs) were cultured on N6 media supplemented with 2 $mgL^{-1}$ Dicamba for 90 days. After induction, for somatic embryogenesis and maturation, embryogenic calli were cultured for 120 days in the N6 media with 0.1 $mgL^{-1}$ 2,4-D, 0.16 $mgL^{-1}$ putrescine, 0.5 $mgL^{-1}$ casein, and 2.0 g/L activated charcoal. Differentiated polyembryoids were, further, transferred into the 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 genotypes studied, P-2 and P-1 have shown the highest callus induction rate, embryogenic calli, differentiated polyembryoids, and more plantlets per somatic embryo cluster. Overall, P-2 and P-1 genotypes have shown conspicuous results over the C-1 and C-2 genotypes in the whole somatic embryogenesis and regeneration process from the matured ZEs of the dura.

## 1. INTRODUCTION

Oil palm is the most traded oil crop in the global vegetable oil market due to its innumerable uses in various industries [1,2]. Hence, in the past three decades, the expansion of the crop increased enormously. Due to the ever-increasing shortage of vegetable oils in the country, India imports nearly two-thirds of its total edible oil by spending valuable foreign exchange. Thus, it necessitates extensive breeding with variable genetic resources. Whereas, oil palm has a narrow genetic base [4,5], for any genetic improvement availability of the genetic variability is a pre-requisite. Hence, the conservation of germplasm accessions has become mandatory, but maintaining perennial oil palm germplasm in *ex situ* is high resource-demanding, and due to its single growing apex, it cannot be multiplied vegetatively also. In oil palm, conventional propagation is only through seed, whereas long-term storage of seed is also impossible by its intermediate storage behavior [6-8]. However, zygotic embryos (ZEs) and somatic embryos can be cryopreserved for the long term to conserve valuable germplasm. Thus, it entails establishing *in vitro* regeneration protocol for the excised ZEs. Although there are several reports on *in vitro* regeneration through direct and indirect somatic embryogenesis using ZEs, still there is a lack of reliable repetitive protocol due to crop heterogeneity. Hence, in this study, we have evaluated the four elite Indian genotypes for their differential response to somatic embryogenesis and plantlet regeneration capacity.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material

Mature fresh fruit bunches of four elite genotypes P-1, P-2, C-1, and C-2 were harvested from the ICAR-Indian Institute of Oil Palm Research seed garden (16°.48′ 41.6″ N 81°07′ 51.0″ E) Pedavegi, Andhra Pradesh, India.

### 2.2. Preparation of Explant

After harvesting, fruit lets were depericarped to acquire the seeds and seeds were cracked to obtain the kernels. Then kernels were surface sterilized by Tween-20 solution (0.5 mL/100 mL), and fungicide solution (1% Carbendazim and 1% Mancozeb) for 20 min in the clean chamber and were rinsed with sterile water 3 times. Then, the kernels were halved, and embryos were excised and sterilized with 20% (v/v)

*Corresponding Author:
*D. S. Sparjanbabu,*
*Koneru Lakshmaiah Educational Foundation*
*Deemed to be University, Guntur, Andhra Pradesh, India.*
*E-mail: samuelsparjan @ gmail.com*

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sodium hypochlorite solution for 20 min and washed thrice with sterile deionized water and inoculated on culture medium.

### 2.3. Callus Induction and Proliferation

For callus induction and proliferation, surface-sterilized ZEs were cultured on autoclave sterilized N6 [9] media, supplemented with 2 $mgL^{-1}$ Dicamba in 25 mm test tubes. The treatments consisted of six replicates, with 20 explants per replicate. All the cultures were incubated in the dark chamber at 27 ± 2°C and sub-cultured every 30 days on the same media, for three passages. Callus induction percentage was determined by counting the number of ZEs forming callus out of the total number of embryos cultured and multiplied by 100. The size of each callus/area ($cm^2$) was measured, and the percentage of explant surface covered by the callus was also measured. To further characterize the color of the callus, the type of the callus and callus proliferation rate was evaluated during the proliferation phase.

### 2.4. Somatic Embryogenesis and Maturation

For somatic embryo differentiation and maturation, embryogenic calli induced on callus induction medium were transferred onto N6 medium containing 2,4-D (0.1 $mgL^{-1}$), putrescine (0.16 $mgL^{-1}$), and casein (0.5 $mgL^{-1}$) with activated charcoal (2.0 $mgL^{-1}$) [Table 1]. The cultures were maintained in the dark at 27°C for up to 120 days.

### 2.5. Plantlet Regeneration

For plantlet development, somatic embryos and differentiating polyembryoids were transferred onto the regeneration media consisting of the N6 media with NAA (0.5 $mgL^{-1}$), BAP (1.0 $mgL^{-1}$), and activated charcoal (0.5 $mgL^{-1}$) [Table 1]. These cultures were maintained in Magenta boxes under a white fluorescent light chamber (227 µmol $m^{-2}$ $s^{-1}$) with a 16 h photoperiod at 27 ± 2°C for nearly 60 days. For further hardening, well-grown *in vitro* plantlets with a balanced shoot and root were transferred to pots in soilless media comprising vermiculite, cocopeat, and soilrite (1:1:1) for nearly 2 months.

### 2.6. Statistical Analysis

The whole experiment was set up in Randomized Block Design; values were based on six replications consisting of 20 explants per replicate as a mean. Data were subjected to ANOVA ($P < 0.05$) using WASP 2.0, a web-based software developed by ICAR-Central Coastal Agricultural Research Institute, Goa, India.

## 3. RESULTS AND DISCUSSION

### 3.1. Callus Induction and Proliferation

Irrespective of the genotypes, all explants (ZEs) showed swelling [Figure 1a] after 1 week of culture. However, Jayanthi *et al*. [10], de Silva *et al*. [11] and Thawaro and Te-chato [12] observed the differential response of genotypes over callus type, color, and size only after 4 to 9 weeks of culture. Among genotypes, P-2 (80D X 281D) induced highest callus per cent (94.998) which was on par with genotypes P-1 (240D X 281D) and C-1 (98C X 254D), whereas genotype C-2 (98C X 208D) showed 76.63% of callus induction, as mentioned in Table 2. After 4 weeks of culture during the multiplication of the calli repetitive, cellular division was observed, where ZEs embryonic cells started dedifferentiation into callus cells [13] resulting from the variation in the color of the distal region of the meristematic cells. During this phase, genotypes showed differential responses on callus size and significantly varied by genotype, where genotypes P-1 and P-2 induced large calli compared to C-1 and C-2 genotypes [Figure 2]. Similarly, the differential response was observed on callus color and type, where white, yellow, and translucent colored calli were marked with compact, friable, and rooty calli. According to Pádua *et al*, [14], Balzon *et al*. [15] and Thawaro and Techato [12] yellow colored friable calli were due to their intense cell division which forms embryogenic clusters. Whereas translucent calli formed by elongated cells with broken cell walls and vacuoles [14] may be due to their apoptotic nature [16], these cannot form embryogenic calli. Similarly, due to a lack of intense cell division and the formation of abnormal large elongated cells, rooty calli were non-embryogenic. Among the genotypes tested, P-2 and P-1 genotypes induced the highest per cent yellow coloured calli, whereas C-1 and C-2 genotypes induced the highest percentage of white and translucent calli [Table 2].

In terms of callus type, P-1 and P-2 genotypes induced the highest embryogenic compact and friable calli, whereas C-1 and C-2 genotypes generated the highest per cent of non-embryogenic rooty calli [Figure 3]. As stated by de Silva *et al*. [11], Besse *et al.* [17], Durand-Gasselin *et al*. [18], and Hanower and Hanower [19], both compact and friable calli possess the tendency for embryogenesis. Thus, among genotypes, P-2, and P-1 genotypes induced the highest per cent embryogenic calli (83.217 and 76.550), whereas genotypes C-1 and C-2 produced the highest non-embryogenic calli [Figure 4]. However, there was no significant difference among genotypes on callus proliferation rate [Figure 5]. Whereas genotypes P-1 and P-2

**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 |

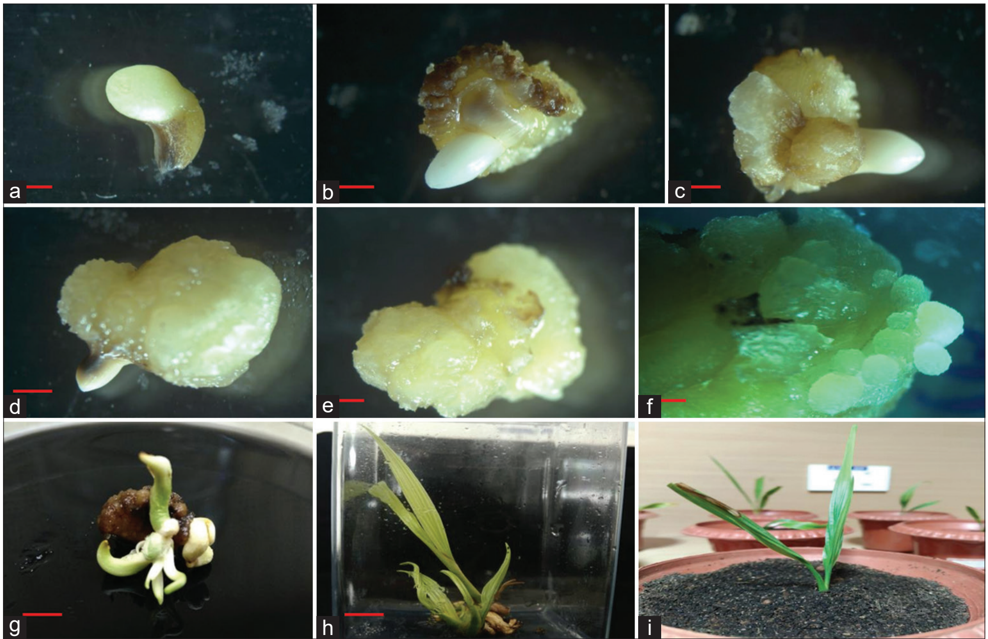


**Figure 1:** Morphological stages of somatic embryogenesis from mature zygotic embryo of dura oil palm. (a) Swollen zygotic embryo before formation of callus. (b) 25% explant surface covered by callus. (c) 50% explant surface covered by callus. (d) 75% explant surface covered by callus. (e) 100% explant 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).

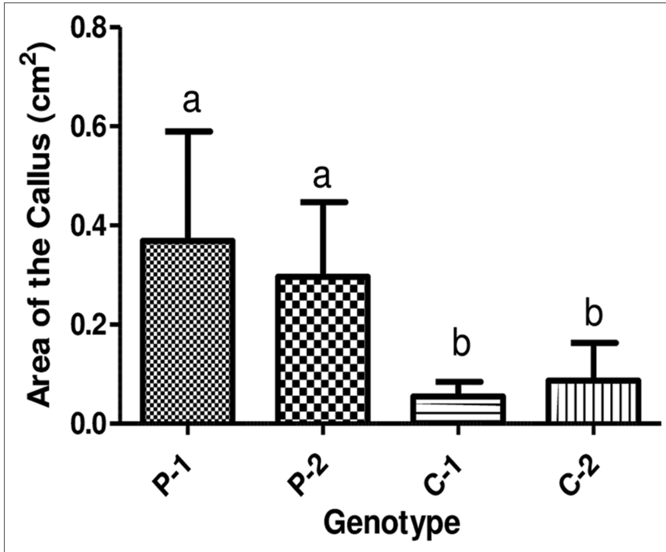


**Figure 2:** Effect of genotype on callus size-area (cm$^2$).

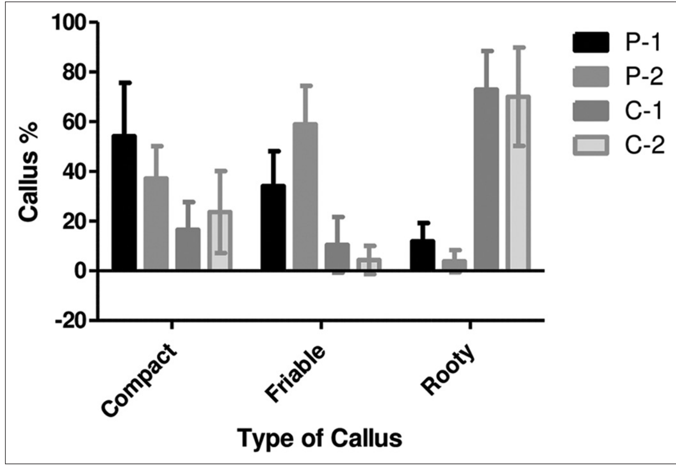


**Figure 3:** Effect of genotype on type of callus; values are mean ± SE.

have shown the highest percentage of the callus area and explant covered by the callus [Table 3 and Figure 1a-e] over genotypes C-1 and C-2, similar results were reported by Balzon *et al.* [15] and Abdullah *et. al* [20], where increased coverage of explant by the callus aids further embryogenesis.

### 3.2. Somatic Embryogenesis and Maturation

After 90 days of callus induction and proliferation, embryogenic calli were transferred to somatic embryogenesis and maturation media [Table 1]. During this phase, several morphological changes were observed by forming the globular-shaped pre-embryonic structures [Figure 1f] further differentiated into polyembryoids [Figure 1g]. During the maturation of these polyembryoids, the addition of vigorous

**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).

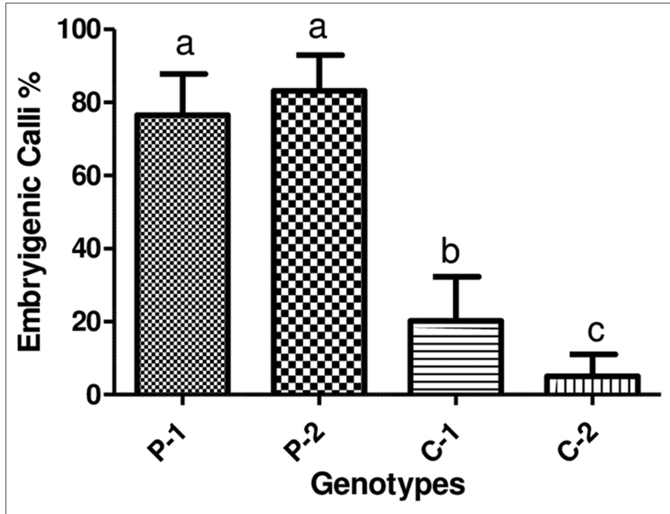


**Figure 4:** Effect of genotype on embryogenic calli percent.

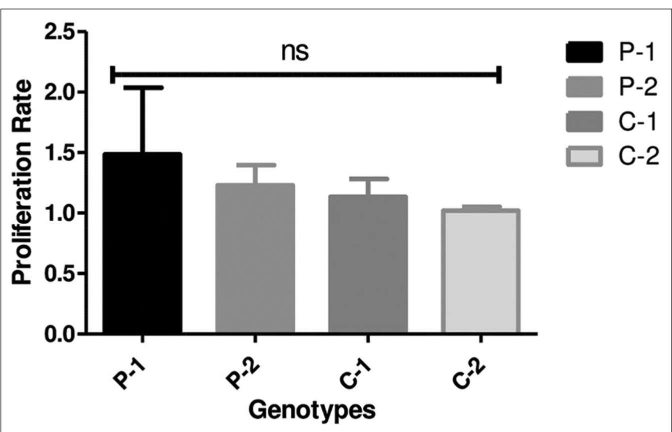


**Figure 5:** Effect of genotypes on callus proliferation rate; ns-not significant

Auxin 2,4-D in the somatic embryogenesis media additionally induces the embryogenic competence of the calli [21-23]. The addition of casein also plays a significant impact on somatic embryogenesis [24], similar results were also observed by Thuzar *et al.,* [24] and Feher *et al.,* [25]. However, this somatic embryogenesis and maturation process are not only affected by the growth regulator and composition of the medium but also by genotype [26,27]. Thus, among the genotypes tested, genotype P-2 produced more differentiated polyembryoids (91.341%) which was on par with the genotype P-1 (85.220%), and the least polyembryoids were produced by the genotype C-1 (15.278%) followed by genotype C-2 [Figure 6], similar results also obtained by Sparjanbabu *et al.,* [27] in *in vitro* germination and growth of oil palm ZEs.

### 3.3. Plantlet Regeneration

Somatic embryo clusters from the differentiated polyembryoids [Figure 1g] were transferred into plantlet regeneration media [Table 1]. After 120 days of culture on somatic embryogenesis and maturation media, among genotypes, the maximum number of plantlets per somatic embryo cluster was acquired from the P-2 (4.167) followed by P-1 (3.167), which was significantly higher than that of other genotypes C-1 (1.0) and C-2 (1.1). Furthermore, P-2 and P-1 genotypes were found superior for the formation of shoots and roots simultaneously; similar results were reported by Sparjanbabu *et al*. [27,28]. For obtaining the well-grown plantlets [Figure 1h] with shoots and roots, cultures were grown in the regeneration media for up to 60 days, and further, hardening plantlets [Figure 1i] were transplanted to soilless media containing vermiculite, cocopeat, and soilrite (1:1:1) for nearly 2 months.

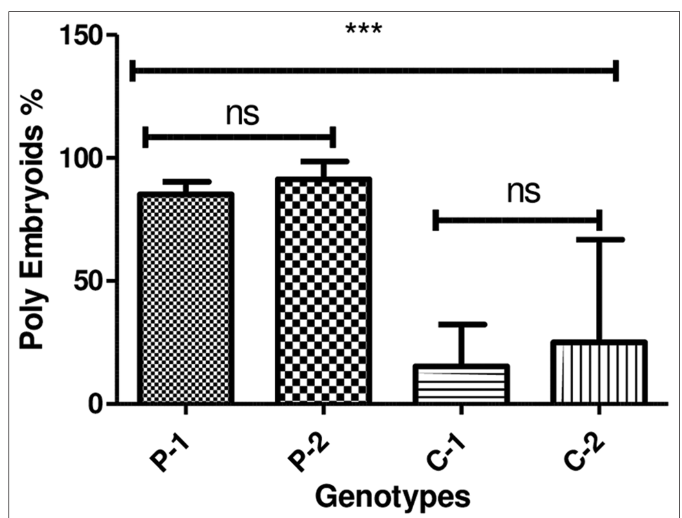


**Figure 6:** Effect of genotypes on polyembryoids from the pre somatic embryos ns-not significant within same group; *** cumulatively highly significant.

**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).

## 4. 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. Selection and conservation of such genotypes would be a promising stride for the Indian oil palm genetic improvement and breeding programs.

## 5. ACKNOWLEDGMENT

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## 6. AUTHORS' CONTRIBUTIONS

DSS: Executed the experiment, collected the data, interpreted the data, and wrote the manuscript; PN: Concept and design of the investigation and supervision; MSR: Interpreted the data, revision of the manuscript;

DR: Concept and design of the investigation and analyzed the data; BS: Executed the experiment and collected the data.

## 7. FUNDING

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## 8. CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## 9. COMPLIANCE WITH ETHICAL STANDARDS

This study does not involve experiments on animals or human subjects.

## 10. DATA AVAILABILITY

All data generated or analysed during this study are included in this manuscript. Apart from these, there are no datasets that were generated or analyzed during the study.

## 11. PUBLISHER'S NOTE

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