PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.10708

eISSN 2348-1900

RESEARCH ARTICLE

Ultrastructure of scutellum-induced callus deciphers stages of somatic embryogenesis and shoot regeneration in *indica* rice

Piyali Das[1], Anu Kalia[2], Ekta Patel[1], Somak Hazra[1], Ajinder Kaur[1] & Jagdeep Singh Sandhu[1*]

[1]School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana 141 004, Punjab, India
[2]Electron Microscopy and Nanoscience Laboratory, Punjab Agricultural University, Ludhiana 141 004, Punjab, India

*Correspondence email - js_sandhu@pau.edu

Received: 17 July 2025; Accepted: 06 December 2025; Available online: Version 1.0: 04 February 2026

Cite this article: Piyali D, Anu K, Ekta P, Somak H, Ajinder K, Jagdeep SS. Ultrastructure of scutellum-induced callus deciphers stages of somatic embryogenesis and shoot regeneration in *indica* rice. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.10708

Abstract

This study describes somatic embryogenesis from scutellar epithelial tissue-induced callus in *indica* rice cultivar PR126 followed by shoot regeneration. The highlighting feature was histological identification of distinct embryogenic regions revealing origin of embryogenesis from upper epidermal layer of callus cells. This layer comprised of clusters of small, compact and isodiametric pro-embryogenic cells with dense cytoplasm. These cells enlarged and differentiated, leading to the development of pro-embryos having distinctive protodermis that produced globular somatic embryos with an average frequency of 5.36 ± 2.89 % in 25 days old callus. The embryos formed discrete leaf primordium-like structures from the epidermal layer after 14 days of callus transfer on regeneration. The leaf primordia developed into shoot apices, followed by normal shoot formation at 77.06 ± 10.82 % frequency within 45 days. The study demonstrates that the embryogenic regions in calli are fewer; their early detection and promotion can lead to effective shoot regeneration. This approach can be used for trait improvement in recalcitrant commercial rice cultivars through genome editing.

Keywords: leaf primordium; *Oryza sativa* L.; pro-embryos; shoot apices; somatic embryos

Introduction

Rice (*Oryza sativa* L.) is amenable to *in vitro* callus induction and shoot regeneration. The prolific induction of callus, followed by high frequency regeneration is pre-requisite for genetic transformation and genome editing experiments (1). The shoot regeneration is influenced by several factors such as, type of explant, plant growth regulators, culture conditions etc. (2). Amongst these, the plant growth regulators have a role in determining the type of cells (e.g. embryogenic or non-embryogenic) that develop from the explant(s) by controlling the degree of de-differentiation and re-differentiation (3). The accurate identification of embryogenic cells can be achieved by examining spatial and temporal aspects of *in vitro* induced callus through histology (4).

The actively dividing meristematic cells in embryogenic callus give rise to pro-embryos. Pro-embryo is the earliest morphologically distinguishable stage in somatic embryogenesis. Subsequently, somatic embryos are formed following 4 distinct stages viz. globular, heart-shaped, torpedo-shaped and cotyledonary (5, 6). The cell structure analyses of rice calli have provided insights into somatic embryogenesis and shoot regeneration (7). These analyses have mainly focussed on specific developmental stage rather than addressing the entire shoot regeneration process. However, a comprehensive histological and morphological analysis of the entire developmental process involved in shoot regeneration from rice callus is required to identify abnormal developments and enable application of optimized conditions for enhancing regeneration efficiency (8). The histology of callus cultures can facilitate early differentiation of embryogenic calli from non-embryogenic calli and furnish ample information on the regeneration capacity of the callus cultures (9).

In the present study, histological and morphological analysis of shoot regeneration from scutellum epithelial tissue-derived rice callus was carried out. The microscopic examination of callus sections revealed distinct embryogenic and non-embryogenic regions. The embryogenic regions developed into pro-embryos and somatic embryos. These differentiated and developed to form leaf primordia-like structures. The presence of these structures in large numbers was indicative of higher shoot induction frequency. The study provides systematic examination of the ultrastructure of calli, thus aiding in the early selection of embryogenic regions suitable for subsequent shoot regeneration.

Materials and Methods

Plant material

Mature seeds of *indica* rice cultivar PR 126 were used as explants for establishing callus cultures. The seeds were obtained from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India.

Callus induction

The dehusked healthy seeds were rinsed with mild detergent and washed under running tap water for 3 min. The cleansed seeds were surface sterilised using Carbendazim solution (0.1 % w/v) for 2 hr,

followed by 3 washes with sterile distilled water. Subsequently, these were sterilised with mercuric chloride (0.1 % w/v) for 4 min under sterile conditions and blot-dried on sterile filter paper (Whatman, USA). The seeds were aseptically cultured on MS (Murashige and Skoog) medium (10) supplemented with 2, 4-D (2, 4-Dichlorophenoxyacetic acid (1.5 mg/L), kinetin (0.5 mg/L), proline (600 mg/L), sucrose (3 % w/v) and agar (0.8 % w/v) having a pH of 5.8 (11). The seeds were placed with scutellum part just above the medium and endospermic portion embedded inside. The cultures were incubated in dark at 25 ± 2 ℃ for callus induction. Seven independent sets of experiments/biological replicates were performed. All media components were obtained from HiMedia (Mumbai, India).

Shoot regeneration

The calli were transferred on shoot regeneration medium containing MS salts supplemented with BAP (6-Benzylaminopurine) (1.5 mg/L), kinetin (1 mg/L), NAA (Naphthaleneacetic acid) (1 mg/L), myoinositol (100 mg/L), sucrose (3 % w/v) and agar (0.8 % w/v) with pH of 5.8 (12). The cultures were incubated under 16 hr photoperiod using fluorescent lamps at 25 ± 2 ℃.

Root induction and hardening

In vitro regenerated shoots were excised and placed on half-strength basal MS medium without growth regulators for root induction. The plantlets were hardened (by transferring on water-moistened cotton) in jam jars under 16:8 hr light/dark period at 25 ± 2 ℃ for 3 days. The jars carrying plantlets were covered with polythene sheets to maintain humidity. Subsequently, the plantlets were transferred to pots containing a mixture of soil and cocopeat. The potted plantlets were maintained at 28–30 ℃ in a glasshouse and watered twice daily.

Histological analysis

Histological analysis of callus was carried out at various developmental stages using standard optical microscopy according to the standard procedures (13). The samples included 10, 15, 20 and 25 days old callus, as well as embryogenic callus after 7 and 14 days of placement on shoot regeneration medium. The standard free-hand sectioning of calli was carried out with steady strokes to make thin slices (14). The sections were moved from blade to a drop of water on a microscope slide. The excess water was gently removed using tissue paper and 2 distinct staining solutions containing 1 % Congo red and 1 % methylene blue were applied individually to the sections (15, 16). Fixation and microtomy were avoided since rice calli are soft, friable and highly hydrated, making them susceptible to distortion and shrinkage during fixation, dehydration and embedding. Free-hand sectioning enabled preservation of native cellular integrity and morphology for direct microscopic observation. The images were captured using Leica application suite (LAS 6.0, Leica Microsystems, Mannheim, Germany) on an optical research microscope (Leica DM 5000) at 100 X magnification.

Statistical analysis

The callus induction frequency (%) was analyzed as: Number of calli induced/Total number of seeds inoculated × 100. The regeneration frequency (%) was calculated as: Number of calli regenerated/Total number of calli induced × 100. The frequency of plantlet survival (%) was calculated as: Number of surviving plantlets/Total number of plantlets developed× 100. The experimental data were analysed as mean ± SD (standard deviation) to study variation among 7 independent sets of biological replicates.

Results

Callus induction and shoot regeneration

The scutellar region of seeds from recalcitrant *indica* rice cultivar PR 126 showed callus initiation on induction medium with a mean frequency of 69.41 ± 10.55 % after 10–15 days of culturing (Table 1). The calli became compact, nodular and creamish-white at an average frequency of 5.36 ± 2.89 % after 10 days of induction (Fig. 1A). Upon transfer to regeneration medium, green islands appeared on calli after 14 days (Fig. 1B). Multiple shoots (8–10) regenerated from the green islands at an average frequency of 77.06 ± 10.82 % in next 21 days (Fig. 1C). The shoots displayed root formation upon transfer to root induction medium in 14 days (Fig. 1D). The plantlets were hardened and transferred to soil (Fig. 1E & 1F). The plants were morphologically uniform with normal leaves and growth patterns. The number of plantlets developed is shown in Table 1.

Ultrastructure of rice callus for analysing somatic embryogenesis

The cell organization in 10 days old calli induced from scutellum epithelial tissue was determined through histological analysis (Fig. 2). The results revealed presence of 2 different types of cells in the same callus i.e., i) small, compact, isodiametric cells with starch-

Table 1. Callus induction and plantlet development from *indica* rice cultivar PR 126

Experiment (No.)	Number of seeds inoculated	Number of callus induced	Number of calli showing embryogenic regions	Number of embryogenic regions showing shoot regeneration	Regeneration efficiency (%)	Number of plantlets developed	Number of hardened plantlets	Number of surviving plantlets
1	140	107 (76.42)	4 (3.74)	3 (75.00)	2.80	3	1	1 (33.33)
2	150	129 (86.0)	5 (3.87)	3 (60.00)	2.32	3	2	1 (33.33)
3	190	130 (68.42)	3 (2.31)	2 (66.66)	1.54	2	1	1 (50.00)
4	210	155 (73.80)	6 (3.87)	5 (83.33)	3.22	5	3	2 (40.00)
5	260	174 (66.92)	9 (5.17)	7 (77.77)	4.02	7	5	5 (71.43)
6	310	186 (60.00)	15 (8.06)	13 (86.66)	6.99	13	11	9 (69.23)
7	350	190 (54.28)	20 (10.53)	18 (90.00)	9.47	18	17	17 (94.44)
Mean ± SD		69.41 ± 10.55	5.36 ± 2.89	77.06 ± 10.82				55.97 ± 23.13

Figures in parenthesis represent per cent values and these were used to calculate Mean ± SD

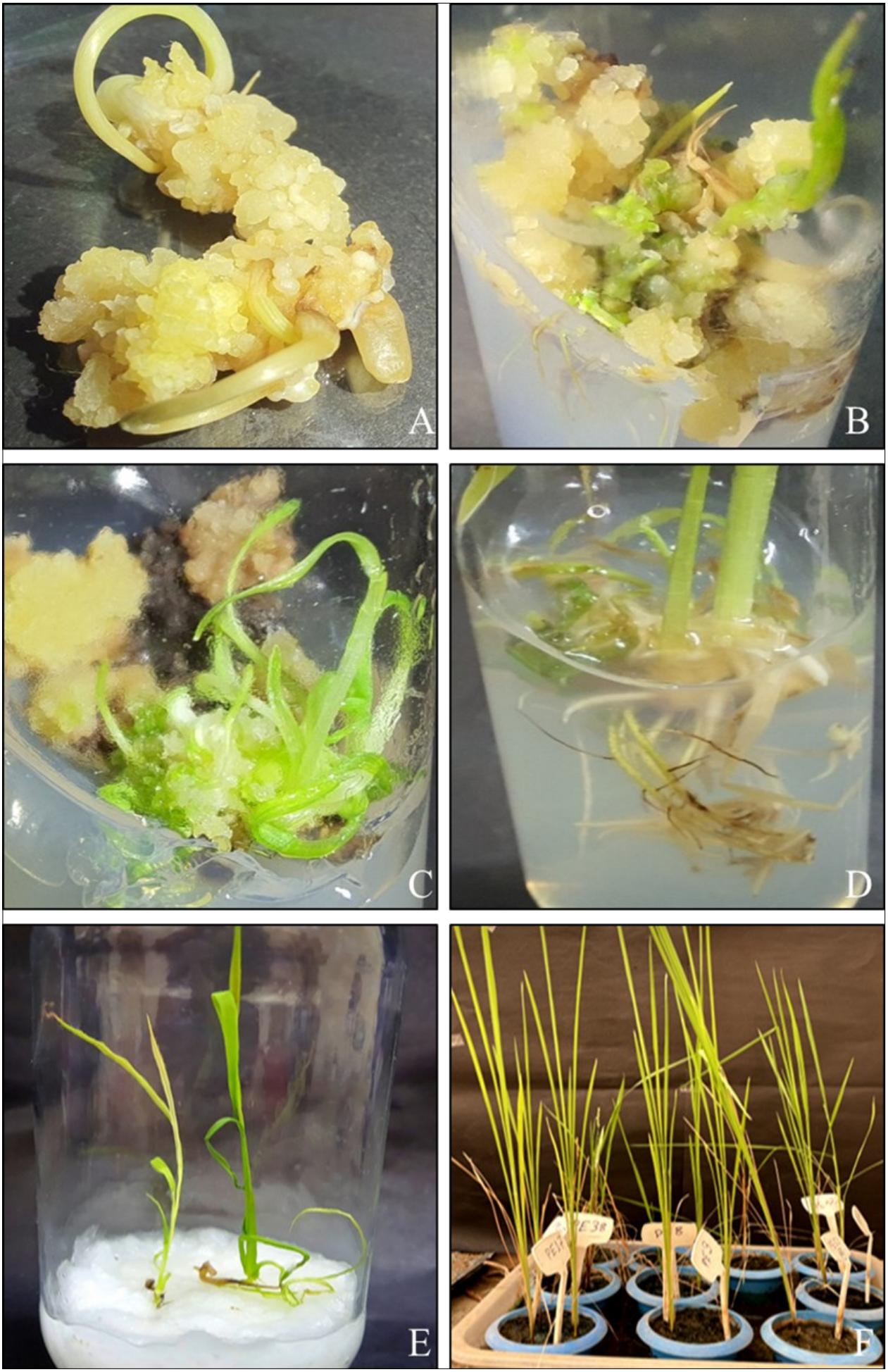

Fig. 1. Callus induction and shoot regeneration in *indica* rice. (A) Callus induction from scutellar region of the seed, (B Appearance of green islands and shoot regeneration, (C) Multiple shoot regeneration, (D) Root induction, (E) Hardening of plantlets, (F) Plants in soil.

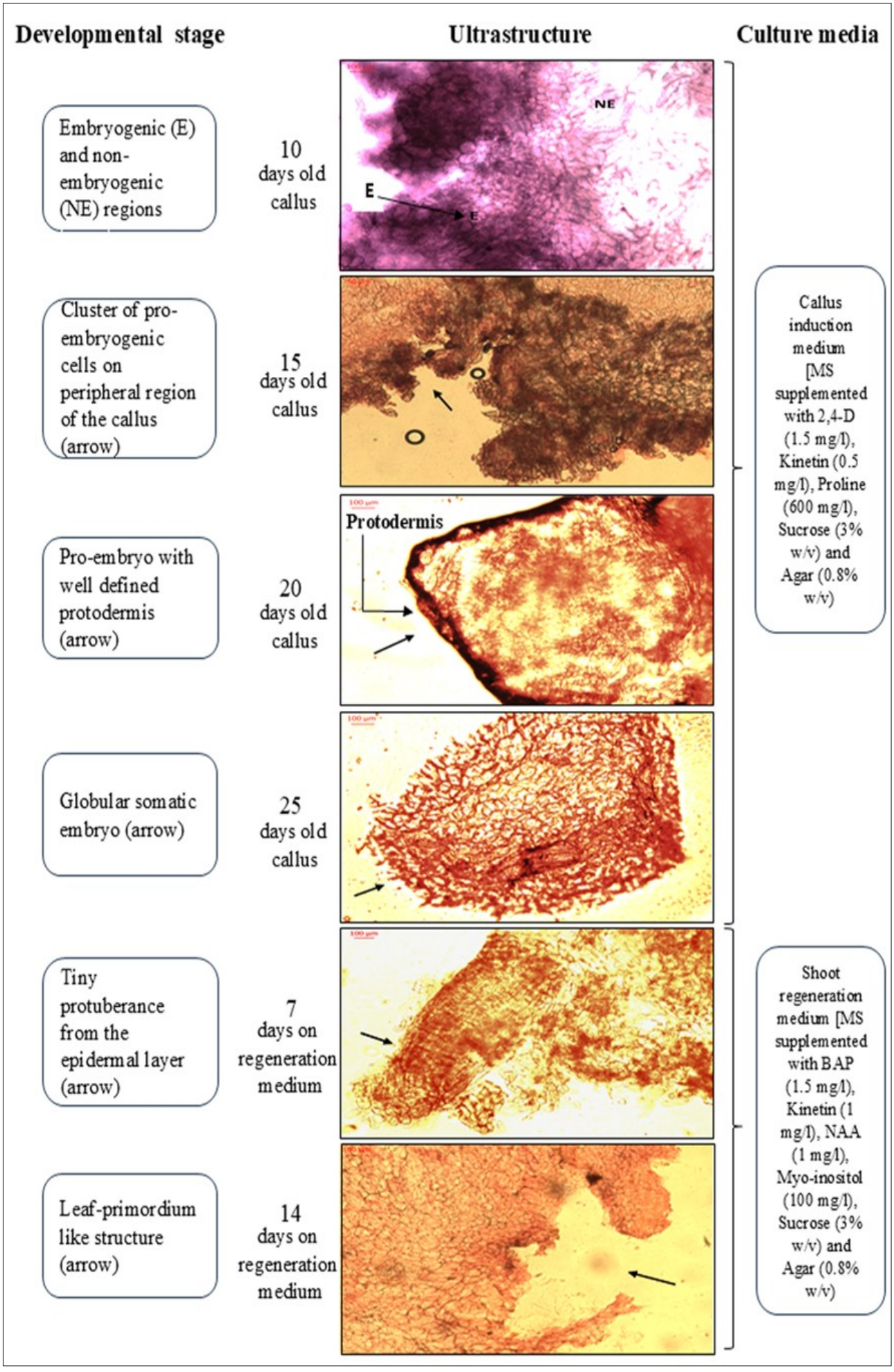

Fig. 2. Histology of developmental stages in rice from callus to shoot regeneration. The scale is presented in each image.

rich dense cytoplasm and dark stained nuclei and ii) large, highly vacuolated cells. The former were classified as embryogenic, while the latter as non-embryogenic. Further, the morphological changes on the callus surface were examined to understand the process of shoot formation. Clusters of pro-embryogenic cells were observed on the peripheral region (upper epidermal layer) of 15 days old calli on callus induction medium. The pro-embryogenic cells increased in size leading to formation of somatic pro-embryos with a clearly defined protodermis in 20 days old calli. The pro-embryos developed into globular somatic embryos in 25 days old calli on induction medium.

The development of shoot formation was analysed after 7 and 14 days of transfer on shoot regeneration medium. The club-shaped outgrowths with tiny protuberances composed of randomly arranged cells were observed on callus surface after 7 days. The number of protuberances increased after 14 days and resembled leaf primordia-like structures that were present on the upper epidermal layer. Following these sequential events, rice callus cultures displayed formation of shoot apices and shoots within a total period of 45 days.

Discussion

This study describes somatic embryogenesis from scutellar epithelial tissue-derived callus in *indica* rice cultivar PR 126 followed by shoot regeneration. The distinction was early identification of discrete embryogenic regions that originated from peripheral cells of callus (upper epidermal layer). Histological analysis of calli at various developmental stages revealed presence of pro-embryogenic cells in the peripheral region forming somatic embryos that subsequently differentiated into leaf primordia-like structures. The organisation of developmental features provides insight into the precise structure and positioning of callus cells within their native environment, which is crucial for determining their fate and behaviour. The *in-situ* localisation of callus cells is crucial for comprehending their interactions over time and potential for regeneration either through somatic embryogenesis or organogenesis (17).

The examination of callus in the present study demonstrated cells in specific regions were small, compact, isodiametric with dense cytoplasm and numerous starch granules that were classified as embryogenic. Similar characteristics i.e. small and isodiametric cells, dense cytoplasm with large nucleus have been recorded for embryogenic cells in a number of crops (18–21). Such cells are enriched with organelles and display active metabolism along with high mitotic activity (22). In contrast, non-embryogenic callus cells are loosely arranged, elongated and vacuolated with sparse cytoplasm with low metabolic activity. In rice, the embryogenic callus had compact clusters of meristematic cells with dense cytoplasm and distinct nuclei, whereas non-embryogenic callus was irregular containing highly vacuolated cells (23). Likewise, the embryogenic callus in rice was characterised by small cells with high nucleus-to-cytoplasm ratio and compact organization resembling early embryonic structures, whereas non-embryogenic callus contained enlarged, vacuolated cells with reduced cytoplasmic density and limited regenerative potential (6). Additionally, dense cytoplasm containing starch granules is typical of embryogenic cells and indicative of their high metabolic rate and dedifferentiation potential (6). High starch density is a crucial requirement for dedifferentiation (24). Several studies have depicted that starch granules not only serve as a morphological feature but also a marker of cellular totipotency. The accumulation of starch reflects a high metabolic state of cells by providing energy for rapid cell division and differentiation during somatic embryo formation (6, 25). Similarly, during organogenesis, starch granules within plastids of meristemoidal cells were frequently observed surrounding the nucleus; such arrangement represents a metabolically active environment, a pre-requisite for organogenesis (26).

The histological analysis in our study demonstrated that somatic embryos developed from peripheral cells of calli through different developmental stages. The peripheral layer of calli has been reported to be a meristematic layer consisting of diploid and totipotent cells that give rise to genetically stable embryoids and shoots (27, 28). The features, such as clusters of pro-embryogenic cells, pro-embryoids, globular embryos, small protuberances and leaf-primordia like structures appeared to be associated with shoot development. The embryogenic calli are destined to display these stages and produce fully developed shoots. The developmental stages identified in the present study are consistent with previous reports on rice somatic embryogenesis and organogenesis. Histological studies have shown that embryogenic callus contains actively dividing meristematic cells that undergo physiological and molecular reprogramming leading to autonomous differentiation into compact embryogenic cell clusters, subsequently giving rise to pro-embryos (6). The pro-embryo formation is considered as the earliest morphologically distinguishable stage in the process of somatic embryogenesis. Pro-embryos often arise either at the peripheral or internal zones of callus depending on its physiological state and culture media composition (7). As the culture advances, pro-embryos progressively develop into somatic embryos, following 4 distinct stages viz. globular, heart-shaped, torpedo-shaped and cotyledonary (29, 30). The transition from pro-embryo to mature cotyledonary somatic embryo is governed by a complex interplay of genotypic, physiological and biochemical factors such as, ratio of auxins to cytokinins, stress signals and expression of embryogenesis-related genes (20).

The occurrence of small protuberances and leaf-primordia like structures from mature embryos in the present study are associated with shoot development and the observation is consistent with early works where shortly after the formation of leaf primordia-like structure, several primary shoot initiations and leafy regions became visible on the surfaces of *Oryza glaberrima* calli (17). Likewise, the stratified peripheral cells, small protuberances and leaf-primordia like structures are connected to the organisation of rice shoots (31).

Comparative analysis of shoot regeneration in *japonica* and *indica* subspecies demonstrated significant phenotypic differences in formation of green spots, an indicator of regeneration (32, 33). Most of *japonica* calli exhibited greening by 9th day post-light treatment, whereas *indica* calli showed considerably less greening. Further, *japonica* cultivars exhibited a higher frequency of compact, creamy-white embryogenic calli with strong regeneration potential (34). In contrast, *indica* cultivars mainly form loose, watery or non-embryogenic calli with low regeneration efficiency often resulting in fewer green shoots. Our study also demonstrates that embryogenic regions in *indica* calli were fewer, however their early detection and selection led to effective shoot regeneration. The variations in rice

subspecies are largely attributed to underlying genetic and physiological differences, including hormonal sensitivity, metabolic activity and gene regulation pathways associated with somatic embryogenesis.

BABY BOOM1 (*OsBBM1*) transcription factor acts as a key developmental regulator by activating *OsYUC* genes responsible for embryogenic reprogramming and embryo development (35). In addition, other transcription factors, such as *LEC1*, *LEC2*, *FUS3*, *WUS* and *SERK* enhance embryogenic potential through activation of embryo identity genes and coordination of hormone-mediated signaling. The interplay between auxin, cytokinin and abscisic acid pathways, along with epigenetic modifications like DNA methylation and histone remodeling, further fine-tunes gene expression during embryogenic transition (36, 37). Collectively, these interconnected networks establish molecular foundation for both zygotic and somatic embryogenesis in rice.

To summarise, the present study demonstrates formation of shoots from rice callus involving a series of sequential processes. The development began with (1) induction of pro-embryogenic cell clusters in the peripheral region; (2) occurrence of various callus regions with one or more characteristics related to shoot organisation; (3) formation of tiny protuberances composed of cells arranged randomly on upper epidermal layer; and (4) formation of leaf primordia-like structures. Following this sequence of processes, normal shoots eventually developed. Moreover, the presence of structures resembling somatic embryos and leaf primordia indicated the occurrence of somatic embryogenesis. The histological analysis provides insights into the ultrastructure of calli demonstrating fewer embryogenic regions. The selection of such regions at early stage is essential to establish shoot regeneration in recalcitrant commercial rice cultivars for trait improvement through genome editing.

Conclusion

This study provides a comparative analysis of rice callus tissues developed *in vitro* and *in vivo*, offering insights into structural differences crucial for accurate identification of culture-induced changes. Through detailed morphological and histological examination, it enhances our understanding of the rice plantlet regeneration process. The findings are applicable across rice cultivars, highlighting common developmental patterns. This knowledge could pave the way for improved strategies in manipulating rice callus, contributing to the refinement of efficient regeneration protocols. While the present study successfully identifies key embryogenic regions within calli, their contribution to regeneration remains to be validated. Future work will focus on substantiating this relationship to refine and optimize shoot regeneration.

Acknowledgements

The authors thank School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana for providing infrastructural and in-house funding support to carry out this research.

Authors' contributions

PD, EP, SH executed lab experiments and collected data. AK[1] carried out microscopy. AK[2] analysed the data and edited the manuscript. JSS conceptualized, designed research work and prepared manuscript. All authors read and approved the final manuscript [AK[1] - Anu Kalia; AK[2] - Ajinder Kaur].

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Altpeter F, Springer NM, Bartley LE, Blechl AE, Brutnell TP, Citovsky V, et al. Advancing crop transformation in the era of genome editing. Plant Cell. 2016;28:1510–20. https://doi.org/10.1105/tpc.16.00196
2. Deo PC, Tyagi AP, Taylor M, Harding R, Becker D. Factors affecting somatic embryogenesis and transformation in modern plant breeding. South Pac J Nat Appl Sci. 2010;28(1):27–40. https://doi.org/10.1071/SP10002
3. Subban P, Kutsher Y, Evenor D, Belausov E, Zemach H, Faigenboim A, et al. Shoot regeneration is not a single cell event. Plants. 2020;10 (1):58. https://doi.org/10.3390/plants10010058
4. Narciso JO, Hattori K. Genotypic differences in morphology and ultrastructures of callus derived from selected rice varieties. Philipp Sci Lett. 2010;3:59–65.
5. Abe T, Futsuhara Y. Efficient plant regeneration by somatic embryogenesis from root callus tissues of rice. J Plant Physiol. 1985;121(2):111–18. https://doi.org/10.1016/S0176-1617(85)80035-3
6. Vega R, Vásquez N, Espinoza AM, Gatica AM, Valdez-Melara M. Histology of somatic embryogenesis in rice (*Oryza sativa* cv. 5272). Rev Biol Trop. 2009;57:141–50. https://doi.org/10.15517/RBT.V57I0.21291
7. Mendoza AB, Futsuhara Y. Histological observations on plant regeneration in rice (*Oryza sativa* L.) calli. Jpn J Breed. 1992;42(1):33 –41. https://doi.org/10.1270/jsbbs1951.42.33
8. Kruglova N, Zinatullina A, Yegorova N. Histological approach to the study of morphogenesis in callus cultures *in vitro*: a review. Int J Plant Biol. 2023;14(2):533–45. https://doi.org/10.3390/ijpb14020042
9. Ijaz B, Sudiro C, Hyder MZ, Malik SI, Farrukh S, Schiavo FL, et al. Histo-morphological analysis of rice callus cultures reveals differential regeneration response with varying media combinations. *In vitro* Cell Dev Biol Plant. 2019;55:569–80. https://doi.org/10.1007/s11627-019-09974-6
10. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 1962;15 (3):473–97. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
11. Sharma M. Genetic transformation of indica rice for sheath blight resistance using chitinase gene. PhD Thesis. Ludhiana: Punjab Agricultural University; 2021.
12. Pathania S, Lore JS, Kalia A, Kaur A, Sharma M, Mangat GS, et al. Conversion of sheath blight susceptible indica and japonica rice cultivars into moderately resistant through expression of antifungal β-1,3-glucanase transgene from *Trichoderma* spp. Transgenic Res. 2022;31(4):537–51. https://doi.org/10.1007/s11248-022-00318-6
13. Johansen DA. Plant microtechnique. New York: McGraw-Hill Book Co. Ltd.; 1940.
14. Ruzin SE. Plant microtechnique and microscopy. New York: Oxford University Press; 1999.

15. Verbelen JP, Kerstens S. Polarization confocal microscopy and Congo red fluorescence: a simple and rapid method to determine the mean cellulose fibril orientation in plants. J Microsc. 2000;198 (2):101–07. https://doi.org/10.1046/j.1365-2818.2000.00691.x

16. Humphrey CD, Pittman FE. A simple methylene blue–azure II–basic fuchsin stain for epoxy-embedded tissue sections. Stain Technol. 1974;49(1):9–14. https://doi.org/10.3109/10520297409116929

17. Brisibe EA, Miyake H, Taniguchi T, Maeda E. Callus formation and scanning electron microscopy of plantlet regeneration in African rice (*Oryza glaberrima* Steud.). Plant Sci. 1992;83(2):217–24. https://doi.org/10.1016/0168-9452(92)90081-V

18. Falco MC, Mendes BMJ, Neto AT, Glória BA. Histological characterization of *in vitro* regeneration of *Saccharum* sp. R Bras Fisiol Veg. 1996;8(2):93–97.

19. Fransz PF, Schel J. Cytodifferentiation during the development of friable embryogenic callus of maize (*Zea mays*). Can J Bot. 1991;69 (1):26–33. https://doi.org/10.1139/b91-005

20. Mostafz SB, Wagiran A. Efficient callus induction and regeneration in selected indica rice. Agronomy. 2018;8(5):77. https://doi.org/10.3390/agronomy8050077

21. Taylor MG, Vasil IK. The ultrastructure of somatic embryo development in pearl millet (*Pennisetum glaucum*; Poaceae). Am J Bot. 1996;83(1):28–44. https://doi.org/10.1002/j.1537-2197.1996.tb13871.x

22. Fransz PF, Schel JHN. Ultrastructural studies on callus development and somatic embryogenesis in *Zea mays* L. Berlin, Heidelberg: Springer; 1994.

23. Sangduen N, Klamsomboon P. Histological and scanning electron observations on embryogenic and non-embryogenic calli of aromatic Thai rice (*Oryza sativa* L. cv. Khao Daw Mali 105). Kasetsart J (Nat Sci). 2001;35:427–32.

24. Mangat BS, Pelekis MK, Cassells AC. Changes in the starch content during organogenesis in *in vitro* cultured *Begonia rex* stem explants. Physiol Plant. 2006;79(2):267–74. https://doi.org/10.1111/j.1399-3054.1990.tb06741.x

25. Appezzato-da-Glória B, Machado SR. Ultrastructural analysis of in vitro direct and indirect organogenesis. Rev Bras Bot. 2004;27:429–37.

26. Quiroz-Figueroa FR, Rojas-Herrera R, Galaz-Avalos RM, Loyola-Vargas VM. Embryo production through somatic embryogenesis can be used to study cell differentiation in plants. Plant Cell Tiss Org Cult. 2006;86(3):285–301. https://doi.org/10.1007/s11240-006-9139-6

27. Mantell SH, Matthews JA, McKee RA. Principles of biotechnology. Oxford: Blackwell Scientific Publications; 1985.

28. Razdan MK. Introduction to plant tissue culture. New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.; 2019.

29. Abe T, Futsuhara Y. Genotypic variability for callus formation and plant regeneration in rice (*Oryza sativa* L.). Theor Appl Genet. 1986;72(1):3–10. https://doi.org/10.1007/BF00261446

30. Islam MM, Haque ME, Alam S, Islam MA, Khalekuzzaman M, Sikdar B. Morphological and histological observation of embryogenic calli derived from immature embryo of BRRI dhan28 (*Oryza sativa* L.) variety. Res Plant Biol. 2013;3:21–27.

31. Nakano H, Maeda E. Shoot differentiation in callus of *Oryza sativa* L. Z Pflanzenphysiol. 1979;93(5):449–58. https://doi.org/10.1016/S0044-328X(79)80179-8

32. Indoliya Y, Tiwari P, Chauhan AS, Goel R, Shri M, Bag SK, et al. Decoding regulatory landscape of somatic embryogenesis reveals differential regulatory networks between *japonica* and *indica* rice subspecies. Sci Rep. 2016;6:1–15. https://doi.org/10.1038/srep23050

33. Kalhori N, Nulit R, Go R, Zulkifly S, Azizi P, Abiri R. Selection, characterization and somatic embryogenesis of Malaysian salt-tolerant rice (*Oryza sativa* cv. MR219) through callogenesis. Int J Agric Biol. 2017;19:157–63.

34. Yan LN, Xia LI, Dan WU. The comparison in tissue culture ability of mature embryo in different cultivars of rice. Agric Sci China. 2010;9:840–46. https://doi.org/10.1016/S1671-2927(09)60162-0

35. Khanday I, Santos-Medellín C, Sundaresan V. Rice embryogenic trigger BABY BOOM1 promotes somatic embryogenesis by upregulation of auxin biosynthesis genes. bioRxiv. 2020:2020.08.24.265025. https://doi.org/10.1101/2020.08.24.265025

36. Elhiti M, Stasolla C, Wang A. Molecular regulation of plant somatic embryogenesis. *In vitro* Cell Dev Biol Plant. 2013;49(6):631–42. https://doi.org/10.1007/s11627-013-9547-3

37. Sivanesan I, Nayeem S, Venkidasamy B, Kuppuraj SP, Rn C, Samynathan R. Genetic and epigenetic modes of the regulation of somatic embryogenesis: a review. Biol Futur. 2022;73:259–77. https://doi.org/10.1007/s42977-022-00126-3

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.