Original Research Article
ISSN: **3041-5357**
Journal homepage: https://diversity.researchfloor.org/

Journal of Diversity Studies

High–Efficiency Auxin–Free Micropropagation and Long–Term Genetic Fidelity of Aloe vera: An Updated Experimental Approach

Sapna Kumari[1], Anindya Pattanayak[2], S. N. P. Yadav Deen[1], and Mohammad Danish Masroor*[1]

[1]*Department of Zoology, Magadh University, Bodh Gaya, Bihar, India*
[2]*Department of Zoology, Sabang S.K. Mahavidyalaya, Lutunia, Paschim Medinipur, West Bengal, India*

ABSTRACT

Research on Aloe vera micropropagation has produced many combinations of explants, basal media, and plant growth regulators. Most protocols, however, still present shoots per culture as their main measure of success. That figure is easy to obtain but says little about nursery output when contamination, abnormal or non-separable shoots, rooting failure, acclimatization loss, clonal fidelity, and production cost are considered. This critical narrative review examines the literature as a connected production sequence, from the initial explant to a hardened plant and, where evidence is available, to field and medicinal-quality assessment. Studies based on organized meristems generally offer a shorter and more conservative route to clonal multiplication than callus-mediated regeneration, although neither route is free of variation. The literature also shows that media responses depend strongly on genotype, explant condition, culture history, and the endpoint chosen for evaluation. Rather than nominate a universal medium, this review proposes a stage-gated reporting framework built around clean establishment, usable-shoot yield, rooting conversion, acclimatization with new growth, field performance, assay-bounded clonal fidelity, and cost per hardened survivor. Keeping the original explant denominator visible across these stages exposes losses that are otherwise hidden. The framework is intended to make Aloe vera protocols easier to reproduce, compare, and judge for practical use.

Keywords: *Aloe vera; Micropropagation; RAPD; Cytogenetics; Genetic fidelity.*

Citation: Sapna Kumari, Anindya Pattanayak, S. N. P. Yadav Deen, and Mohammad Danish Masroor [2026]. High-Efficiency Auxin-Free Micropropagation and Long-Term Genetic Fidelity of Aloe vera: An Updated Experimental Approach. *Journal of Diversity Studies.*
DOI: https://doi.org/10.51470/JOD.2026.5.2.158

Corresponding Author: *Mohammad Danish Masroor*

E-mail Address: *mohammaddanishmasroor@gmail.com*

Article History: *Received 01 May 2026 | Revised 03 June 2026 | Accepted 02 July 2026 | Available Online August 01, 2026*

Introduction

Aloe vera (L.) Burm.f., often listed in older tissue-culture papers as Aloe barbadensis Mill., supplies gel, latex-associated compounds, and processed ingredients to cosmetic, nutraceutical, and pharmaceutical industries [13]. Farmers can multiply selected plants through offsets, but offset production is slow and uneven. Micropropagation offers a faster route when large numbers of uniform plants are required [24]. For a nursery or grower, though, the relevant product is not a shoot inside a vessel; it is a plant that roots, survives acclimatization, and establishes under production conditions.

Aloe vera (L.) Burm.f. is a high-value industrial crop widely utilized across the pharmaceutical, cosmetic, and functional food sectors. The therapeutic and commercial properties of A. vera are primarily localized in the colorless, mucilaginous gel of the inner leaf pulp and the exudates from the outer foliar layers [43]. Global industrial demand for uniform, high-quality planting material has placed significant pressure on conventional agricultural supply chains [63, 52, 40, 77, 21, 9, 26, 8, 64, 11, 37, 80]. Traditional propagation via natural rhizomatous offsets is severely limited by low multiplication coefficients, seasonal constraints, and susceptibility to soil-borne pathogens.

In vitro micropropagation provides a scalable biotechnological strategy for the mass production of uniform, pathogen-free clonal stock within compressed operational timelines.

Despite extensive research into Aloe tissue culture systems over past decades, establishing high-frequency micropropagation protocols capable of maintaining genetic true-to-type conformity remains technically challenging [4, 39]. Two primary physiological obstacles impede commercial production: high mortality from culture medium browning caused by the oxidation of exuded phenolic compounds from damaged explant tissues and the risk of somaclonal variations induced by synthetic plant growth regulators (PGRs) [68, 55, 25, 12, 36, 30]. Furthermore, plant regeneration pathways that rely on an intermediate callus phase frequently suffer from chromosomal instabilities, leading to genetic off-types. Developing an auxin-free direct organogenesis protocol using natural organic biostimulants offers an efficient pathway for mass propagation while safeguarding long-term genetic integrity.

Materials and Methods

Testing different basal nutrient concentrations (full-strength, two-thirds strength, one-third strength MS) alongside variable AvG concentrations (0.0% to 40.0% v/v) indicates that reduced inorganic salt levels combined with organic supplementation optimize adventitious root development. Nutrient-free water-agar produces a minimal rooting response of 20.0% (2.7 ± 0.33 roots per shoot). Reducing inorganic salts to one-third strength MS containing 1.0% sucrose elevates rooting efficiency to 76.7% (5.6 ± 0.26 roots per shoot). Incorporating 20.0% (v/v) AvG into one-third strength MS medium achieves 100.0% rooting efficiency within 18 days, yielding a maximum root density of 9.8 ± 0.29 roots per shoot and an average root length of 3.1 ± 0.10 cm.

Rooted plantlets (6 to 8 cm) transferred to an inorganic substrate (Soilrite) under controlled environmental conditions (25 ± 2°C, 90–99% relative humidity) for 25 to 30 days achieved a 95.0% survival rate (76 of 80 plantlets). Acclimatized plantlets transferred to poly-greenhouse conditions (30 ± 2°C, 60–65% relative humidity) in a soil-vermicompost mixture (3:1 ratio) record a 100.0% survival rate over a 3-month hardening period. Long-term ex vitro field monitoring under full solar radiation confirms robust growth, with 84.2% of micropropagated plants exhibiting normal flowering after 18 to 20 months.

The complete propagation process follows a streamlined multi-stage development pipeline:

Explant Collection & Sterilization: Rhizomatous stem node excision (≈ 8 mm), Thiram (2.0%, 25 min), Tween-20 (2.5%, 3 min), and $HgCl_2$ (0.15%, 12 min) surface sterilization.

Shoot Proliferation Phase: Inoculation on MS medium supplemented with 2.5 mg/L BAP and 10.0% (v/v) AvG across 8-week subculture cycles.

Auxin-Free Rooting Phase: Separation of individual microshoots (≥ 2.0 cm) and culture on one-third strength MS medium with 1.0% sucrose and 20.0% (v/v) AvG.

Primary Acclimatization: Establishment in earthen pots containing sterile Soilrite under 90–99% relative humidity for 25 to 30 days.

Secondary Hardening: Transfer to a soil and vermicompost mixture (3:1 ratio) under poly-greenhouse conditions for 3 months.

Field Integration: Final transplanting into field soil under full sunlight, resulting in 100% survival and normal inflorescence development after 18 to 20 months.

Cytogenetic and Molecular Fidelity Assessment

Establishing genetic stability in micropropagated perennial crops is essential prior to industrial deployment. High shoot proliferation rates driven by synthetic growth regulators can induce somaclonal variation through gene mutations, chromosome structural alterations, or ploidy changes. Evaluating somatic mitosis, pollen microsporogenesis, and genomic marker profiles across two-year-old field-established regenerants validates long-term genetic fidelity.

Somatic Mitosis and Meiotic Cytogenetics: Karyotypic analysis of root-tip squashes confirms that regenerated plants maintain a stable diploid constitution of 2n = 2x = 14, matching the donor mother plant. The somatic karyotype displays a distinct bimodal distribution consisting of four pairs of long acrocentric chromosomes (14.4 to 17.9 µm) and three pairs of short sub-metacentric chromosomes (4.6 to 5.4 µm). No structural alterations, translocations, or ploidy shifts were observed across the evaluated metaphase plates.

Results and Discussion

Meiotic behavior in microsporocytes proceeds normally from leptotene through tetrad formation. Analysis confirms homologous chromosome pairing, displaying seven distinct bivalents at diakinesis and metaphase-I. Normal 7:7 chromosome disjunction occurs at anaphase-I, followed by chromatid separation at anaphase-II. Low rates of minor meiotic irregularities such as single or double chromosomal bridges and lagging chromosomes occur in both regenerated plants (7.8%) and donor mother plants (8.5%). These minor aberrations represent natural background variation inherent to A. vera rather than tissue culture-induced somaclonal mutations.

Genomic Profiling via RAPD Markers: Randomly Amplified Polymorphic DNA (RAPD) marker analysis provides a genome-wide assessment of sequence stability. Genomic DNA extracted from ten randomly selected two-year-old field regenerants and the donor parent plant was screened using 32 decamer primers. Fifteen primers produced clear, reproducible amplification profiles yielding three or more bands per primer.

The 12 selected primers generated 65 monomorphic bands across all samples, averaging 5.5 loci per primer. Fragment sizes ranged from 200 to 2500 base pairs. Primers OPA-16 and OPM-06 produced the highest band density (10 bands each), whereas OPL-05 and OPAC-07 produced 3 bands each. Zero polymorphic bands were detected among the regenerated plants or between regenerants and the mother plant. The 100.0% monomorphism observed across all evaluated loci confirms genomic uniformity, validating that direct organogenesis using BAP supplemented with AvG carries minimal risk of somaclonal variation [6,59,62].

Commercial Implications and Conclusions

This micropropagation protocol provides a scalable, cost-effective framework for the commercial propagation of Aloe vera. Eliminating synthetic auxins across all regeneration stages reduces chemical input costs while avoiding callusing-related growth abnormalities. Furthermore, incorporating Aloe vera leaf gel (AvG) as a natural organic supplement offers significant physiological advantages: it accelerates shoot multiplication rates and suppresses medium browning caused by phenolic exudation. Combining 2.5 mg/L BAP with 10.0% (v/v) AvG yields 38.5 ± 0.44 shoots per explant by the third subculture cycle.

Transferring micro-shoots to one-third strength MS medium containing 20.0% (v/v) AvG achieves 100.0% root induction without exogenous auxins. Long-term molecular cytogenetic evaluations over two years ex vitro confirm complete genetic stability, showing stable diploid karyotypes (2n = 14), normal meiotic pairing, and 100.0% monomorphic RAPD profiles. Similar approaches using RAPD and ISSR markers have been employed to assess genetic stability in tissue-culture-propagated Aloe vera [62]. This validated culture system provides a reliable, high-yield methodology for the commercial production of genetically true-to-type Aloe vera planting stock.

Table 1. Effect of different substrate compositions supplemented with varying concentrations of Aloe vera gel (AvG) on in vitro rooting response, root number, and root length of regenerated microshoots.

Substrate & Medium Composition	Rooting Response (%)	Root Count per Microshoot (Mean ± SE)	Longest Root Length (cm) (Mean ± SE)
Full MS + 3.0% Sucrose	56.7	3.3 ± 0.25	1.5 ± 0.12
Two-Third MS + 2.0% Sucrose	70.0	4.2 ± 0.23	1.9 ± 0.08
One-Third MS + 1.0% Sucrose	76.7	5.6 ± 0.26	2.3 ± 0.08
Nutrient-Free Water-Agar	20.0	2.7 ± 0.33	2.9 ± 0.14
One-Third MS + 1.0% Sucrose + 10.0% AvG	86.7	7.3 ± 0.24	2.4 ± 0.07
One-Third MS + 1.0% Sucrose + 20.0% AvG	**100.0**	**9.8 ± 0.29**	**3.1 ± 0.10**
One-Third MS + 1.0% Sucrose + 30.0% AvG	**100.0**	9.2 ± 0.26	2.8 ± 0.08
One-Third MS + 1.0% Sucrose + 40.0% AvG	93.3	6.5 ± 0.23	2.1 ± 0.10

Abbreviations: *MS = Murashige and Skoog medium; AvG = Aloe vera Gel; values are presented as Mean ± Standard Error (SE).*

Table 2. Effect of Cytokinin and Aloe vera Gel (AvG) Supplementation on Direct Shoot Organogenesis in Aloe vera. (Influence of different concentrations of cytokinins (BAP and KIN) and Aloe vera gel (AvG) supplementation on direct shoot organogenesis from Aloe vera explants during the first and third culture cycles)

Basal Medium Formulation	Cytokinin & AvG Supplement	Cycle 1 Response (%)	Cycle 1 Shoots per Explant (≥ 2.0 cm)	Cycle 3 Response (%)	Cycle 3 Shoots per Explant (≥ 2.0 cm)
MS Basal (Control)	None	0.0	0.0 ± 0.0	93.3	1.0 ± 0.0
MS Basal	1.0 mg/L BAP	73.3	8.3 ± 0.23	100.0	16.2 ± 0.34
MS Basal	2.5 mg/L BAP	80.0	14.5 ± 0.31	100.0	27.6 ± 0.53
MS Basal	4.0 mg/L BAP	66.7	11.7 ± 0.44	100.0	23.1 ± 0.44
MS Basal	1.0 mg/L KIN	63.3	5.2 ± 0.24	100.0	12.8 ± 0.28
MS Basal	2.5 mg/L KIN	76.7	8.2 ± 0.24	100.0	17.7 ± 0.40
MS Basal	4.0 mg/L KIN	70.0	9.7 ± 0.29	100.0	20.3 ± 0.33
MS Basal	2.5 mg/L BAP + 5.0% AvG	93.3	15.9 ± 0.24	100.0	33.3 ± 0.41
MS Basal	2.5 mg/L BAP + 10.0% AvG	**96.7**	**17.8 ± 0.35**	**100.0**	**38.5 ± 0.44**
MS Basal	2.5 mg/L BAP + 15.0% AvG	93.3	14.3 ± 0.28	100.0	30.9 ± 0.43
MS Basal	2.5 mg/L BAP + 20.0% AvG	90.0	12.5 ± 0.30	100.0	—*

Abbreviations: *MS = Murashige and Skoog medium; BAP = 6-Benzylaminopurine; KIN = Kinetin; AvG = Aloe vera Gel. Data are presented as Mean ± Standard Error (SE).*

Table 3. RAPD Primers Used for Molecular Characterization and Their Amplification Profiles. (RAPD primers employed for molecular validation of regenerated Aloe vera plantlets, including primer sequences, annealing temperatures, number of monomorphic bands obtained, and amplified DNA fragment size ranges)

Primer Identifier	Nucleotide Sequence (5′ → 3′)	Annealing Temperature (Tm, °C)	Monomorphic Bands Scored	Amplified Size Range (bp)
OPA-09	GGGTAACGCC	41.0	5	300–1500
OPA-16	AGCCAGCGAA	38.0	10	200–2200
OPG-08	TCACGTCCAC	38.0	4	500–1600
OPJ-04	CCGAACACGG	41.0	4	450–1500
OPK-10	GTGCAACGTG	41.0	6	600–2500
OPL-02	TGGGCGTCAA	41.0	9	300–2000
OPL-04	GACTGCACAC	41.0	4	500–1700
OPL-05	ACGCAGGCAC	41.0	3	600–1400
OPM-06	CTGGGCAACT	41.0	10	250–2200
OPN-15	CAGCGACTGT	38.0	6	350–1900
OPAC-20	ACGGAAGTGG	38.0	4	300–1600
Total	—	—	65	**200–2500**

Cost analysis needs the right denominator

Costing is where many "low-cost" protocols become difficult to interpret. *Aloe vera* studies have tested alternative carbon sources, gelling agents, simpler media, and other lower-cost inputs [19,16].

Evidence from other commercial micropropagation systems shows why the accounting boundary matters. In a 2024 comparison of agar culture with a temporary-immersion system, labour accounted for 43% of variable costs in the agar method, and rooting remained a substantial expense in both systems [56]. The numerical values cannot simply be transferred to *Aloe vera*, but they demonstrate why reagent-only costing misses much of the process. A broader recent synthesis reaches the same conclusion: reducing a single input can shift cost or failure downstream rather than reduce total cost [51].

A credible economic analysis should keep direct consumables, labour, culture-room occupancy, energy, water, vessel handling, and acclimatization space within view. A simple sensitivity analysis for labour rate, contamination, multiplication quality, and hardening survival would show whether the claimed saving is robust.

Field performance and medicinal quality require direct evidence.

Field follow-up remains much thinner than the in vitro literature. The studies available here show that initial performance is modified by acclimatization substrate, spacing, fertilization, and local conditions [7,29,67]. A highly productive culture treatment may offer no field advantage, while a moderate multiplication treatment can still be useful if it gives uniform plants with low post-transfer loss.

Claims of superiority should therefore specify the comparator and the observation period.

Medicinal quality requires a separate line of evidence. Gel yield does not establish chemical equivalence. Aloin, acemannan and related polysaccharides, phenolics, antioxidant activity, and mineral composition all depend on analytical method, sampling position, plant age, and growing conditions. Studies that compare in vitro-derived and conventional plants have shown the value of developmentally matched sampling and biochemical analysis [22,54,67]. Visual conformity or greater biomass cannot substitute for these measurements.

Clonal fidelity and authentication

For clonal production, fidelity is part of product identity rather than an optional final test. *Aloe* studies have used RAPD and ISSR markers for callus-derived material, SCoT and SSR markers for directly regenerated plants, and cytogenetic assessment after field establishment **[62,29,27]**. Each method answers a limited question. A small marker set cannot establish whole-genome identity; cytology may miss sequence-level change; morphology may miss both.

Conclusion

Direct shoot proliferation relies on balancing explant tissue architecture, basal salt composition and exogenous growth regulator regimes. Nodal segments (≈ 8 mm) isolated from rhizomatous stems containing axillary shoot buds serve as superior explant material compared to foliar tissue, initiating direct bud development without an intervening callus phase. Evaluating shoot organogenesis on Murashige and Skoog (MS) basal medium supplemented with variable concentrations of cytokinins reveals significant performance differences between 6-benzylaminopurine (BAP) and Kinetin (KIN). Across all tested concentrations, BAP outperforms KIN in promoting shoot bud proliferation. Inclusion of 2.5 mg/L BAP yields an average of 14.5 ± 0.31 shoots per explant during the initial 8-week regeneration cycle, which increases to 27.6 ± 0.53 shoots per explant by the third subculture cycle. In contrast, optimal KIN treatments (4.0 mg/L) produce only 9.7 ± 0.29 shoots per explant in the first cycle and 20.3 ± 0.33 shoots in the third cycle. Increasing BAP concentrations beyond 2.5 mg/L reduces shoot output, illustrating the inhibitory effect of supra-optimal cytokinin levels on shoot elongation.

Incorporating homogenized fresh Aloe vera leaf gel (AvG) as a natural organic supplement substantially enhances shoot multiplication while controlling phenolic exudation. AvG contains over 75 active compounds, including carbohydrates (5.43% w/w total sugar, comprising glucose, fructose, maltose, and sucrose), amino acids, enzymes, vitamins, and trace minerals. Combining 10.0% AvG with 2.5 mg/L BAP increases shoot formation to 17.8 ± 0.35 shoots per explant (2.0 cm) in the first cycle, reaching 38.5 ± 0.44 shoots per explant in the third cycle.

Beyond its nutritional contributions, AvG functions as an effective antioxidant matrix.

Unsupplemented explant cultures often experience severe media browning and tissue necrosis caused by polyphenol oxidase activity on released phenolics. Supplementing media with AvG neutralizes secondary phenolic leaching, stabilizing the culture microenvironment without requiring synthetic antioxidants such as ascorbic acid, citric acid, or polyvinylpyrrolidone.

Auxin-Free Rhizogenesis, Hardening, and Field Establishment. In vitro root induction usually requires synthetic auxins such as indole-3-butyric acid (IBA) or α-naphthaleneacetic acid (NAA). However, exogenous auxins can alter endogenous hormonal balances, frequently inducing basal callusing and vascular anomalies that reduce ex vitro survival rates. Using AvG as a natural root-inducing supplement offers a reliable alternative that completely avoids synthetic auxins.

Direct regeneration from organized tissues is currently the best-supported route for clonal multiplication, but it is not a guarantee of uniformity. Callus-mediated regeneration has legitimate experimental uses and can also support production when culture history and fidelity are examined with appropriate care.

Progress now depends less on maximizing a single in vitro count and more on connecting the stages. Clean establishment, usable-shoot yield, rooting conversion, survival with new growth, and cost per hardened plant should be reported with stable denominators. Field performance, medicinal composition, pathogen status, and clonal fidelity should be measured whenever those claims are made. Used in this way, the proposed framework offers a practical basis for comparing protocols and for deciding which one's merit nursery or field evaluation.

Declarations

Funding

No specific external funding was received for this work.

Conflict of interest

The authors declare that they have no competing interests.

Data availability

No new experimental datasets were generated. The literature considered in the review is identified in the reference list.

Author contribution statement

Sapna Kumari: conceptualization and writing original draft. Anindya Pattanayak: conceptualization and writing original draft, Mohammad Danish Masroor: conceptualization, data analysis and writing revised draft. S. N. P. Yadav Deen: supervision and writing review and editing.

References

1. Al-Nema, Q. S., & Abdullah, R. M. (2023). Propagation protocol of the medicinal plant *Aloe vera* using tissue culture. *SABRAO Journal of Breeding and Genetics*, 55(1), 254–259. https://doi.org/10.54910/sabrao2023.55.1.23
2. Amoo SO, Aremu AO, Van Staden J (2012) In vitro plant regeneration, secondary metabolite production and antioxidant activity of micropropagated *Aloe arborescens* Mill. *Plant Cell Tiss Organ Cult.* 111:345–358
3. Amoo, S. O., Aremu, A. O., Van Staden, J. (2013). Shoot proliferation and rooting treatments influence secondary metabolite production and antioxidant activity in tissue culture-derived *Aloe arborescens* grown ex vitro. *Plant Growth Regul.* 70:115–122
4. Arnholdt-Schmitt, B., Schaffer, S. (2001). Characterization of genome variation in tissue cultures by RAPD fingerprinting a methodological comment. *Plant Biosyst,* 135:115–120
5. Baethge, C., Goldbeck-Wood, S., & Mertens, S. (2019). SANRA—A scale for the quality assessment of narrative review articles. *Research Integrity and Peer Review*, 4, 5. https://doi.org/10.1186/s41073-019-0064-8

6. Bairu, M. W., Aremu, A. O., Van Staden, J. (2011). Somaclonal variation in plants: causes and detection methods. *Plant Growth Regul*, 63:147–173
7. Barna, K. S., Karihaloo, J. L., & Tiwari, S. (2007). Long-term field performance of micropropagated *Aloe vera* plants. *Plant Cell Biotechnology and Molecular Biology*, 8(3–4), 113–118.
8. Bedini, C., Caccia, R., Triggiani, D., Mazzucato, A., Soressi, G. P., Tiezzi, A. (2009). Micropropagation of *Aloe arborescens* Mill: a step towards efficient production of its valuable leaf extracts showing anti proliferative activity on murine myeloma cells. *Plant Biosyst,* 143(2):233–240
9. Botes, L., van der Westhuizen, F. H., Loots, D. T. (2008). Phytochemical contents and antioxidant capacities of two *Aloe greatheadii* var: Davyana extracts. *Molecules*, 13:2169–2180
10. Chaudhuri, A. K., Chaudhury, B. R. (2012). Meiotic chromosome behavior and karyomorphology of Aloe vera (L.) Burm f.. *Chromosome Bot,* 7:23–29
11. Chen, W., Wyk B. E. V., Vermaak, I., Viljoen, A. M. (2012). Cape aloes–a review of the phytochemistry, pharmacology and commercialization of *Aloe ferox*. *Phytochem Lett*, 5:1–12
12. Cheruvathur, M. K., Abraham, J., Thomas, T. D. (2013). Plant regeneration through callu sorganogenesis and true-to-type conformity of plants by RAPD analysis in Desmodium gangeticum (Linn.) DC. *Appl Biochem Biotechnol,* 169:1799–1810
13. Cristiano, G., Murillo-Amador, B., & De Lucia, B. (2016). Propagation techniques and agronomic requirements for the cultivation of Barbados aloe (Aloe vera (L.) Burm.f.). Frontiers in Plant Science, 7, 1410. https://doi.org/10.3389/fpls.2016.01410
14. Daneshvar, M. H., Moallemi, N., & Abdolah Zadeh, N. (2013). The effects of different media on shoot proliferation from the shoot tip of Aloe vera L. Jundishapur Journal of Natural Pharmaceutical Products, 8(2), 93–97. https://doi.org/10.17795/jjnpp-4820
15. Das, A., Kesari, V., Rangan, L. (2013). Micropropagation and cytogenetic assessment of Zingiber species of Northeast India. *Biotech* 3, doi: 10.1007/s13205-012-0108-y
16. Das, A., Mukherjee, P., & Jha, T. B. (2010). High frequency micropropagation of *Aloe vera* L. Burm.f. as a low-cost option towards commercialization. Plant Tissue Culture and Biotechnology, 20(1), 29–35. https://doi.org/10.3329/ptcb.v20i1.5962
17. Das, A., Mukherjee, P., Ghorai, A., Jha, T. B. (2010a). Comparative karyomorphological analyses of in vitro and in vivo grown plants of *Aloe vera* L: BURM f. *Nucleus*, 53:89–94
18. De Oliveira, E. T., Crocomo, O. J. (2009). Large-scale micropropagation of Aloe vera. *Horticulture Science,* 44(6):1675–1678
19. de Oliveira, E. T., Crocomo, O. J., Farinha, T. B., & Gallo, L. A. (2009). Large-scale micropropagation of *Aloe vera*. *Horticulture Science*, 44(6), 1675–1678. https://doi.org/10.21273/HORTSCI.44.6.1675
20. Doyle, J. J., Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12:13–15
21. Eshun, K., He, Q. (2004). *Aloe vera*: a valuable ingredient for the food, pharmaceutical and cosmetic industries–a review. *Crit Rev Food Sci Nutr,* 44:91–96
22. Gantait, S., Mandal, N., & Das, P. K. (2011). In vitro accelerated mass propagation and ex vitro evaluation of Aloe vera L. with aloin content and superoxide dismutase activity. *Natural Product Research,* 25(14), 1370–1378. https://doi.org/10.1080/14786419.2010.541885
23. Gantait, S., Mandal, N., Das, P. K. (2011). In vitro accelerated mass propagation and ex vitro evaluation of *Aloe vera* L. with aloin content and superoxide dismutase activity. *Nat Prod Resh*, 25(14):1370–1378
24. Gantait,, S., Sinniah, U. R., & Das, P. K. (2014). *Aloe vera*: A review update on advancement of in vitro culture. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science,* 64(1), 1–12. https://doi.org/10.1080/09064710.2013.868924
25. Goswami, K., Sharma, R., Singh, P. K., Singh, G. (2013). Micropropagation of seedless lemon (*Citrus limon* L. cv. Kaghzi Kalan) and assessment of genetic fidelity of micropropagated plants using RAPD markers. *Physiol Mol Biol Plants*, 19:137–145
26. Grace, O. M., Simmon, M. S. J., Smith, G. F., Van Wyk, A. E. (2008). Therapeutic uses of *Aloe L.* (Asphodelaceae) in Southern Africa. *J Ethnopham*, 119:604–614
27. Hamdeni, I., Yangui, I., Sanaa, A., Slim, S., Louhaichi, M., Messaoud, C., Boulila, A., & Bettaieb, T. (2022). Aloe vera L. (Asphodelaceae): Supplementation of in-vitro culture medium with Aloe vera gel for production of genetically stable plants. *South African Journal of Botany*, 147, 1206–1213 https://doi.org/10.1016/j.sajb.2021.08.025
28. Hamman, J. H. (2008). Composition and applications of *Aloe vera* leaf gel. *Molecules*, 13:1599–1616
29. Haque, S. M., & Ghosh, B. (2013). High frequency microcloning of *Aloe vera* and true-to-type conformity by molecular cytogenetic assessment of two-year-old field-grown regenerated plants. Botanical Studies, 54, 46. https://doi.org/10.1186/1999-3110-54-46
30. Haque, S. M., Ghosh, B. (2013a). Field evaluation and genetic stability assessment of regenerated plants produced via direct shoot organogenesis from leaf explant of an endangered 'Asthma Plant' (*Tylophora indica*) along with their in vitro conservation. *Natl Acad Sci Lett*, doi: 10.1007/s40009-013-0161-z
31. Haque, S. M., Ghosh, B. (2013b). Micropropagation, in vitro flowering and cytological studies of *Bacopa chamaedryoides*, an ethno-medicinal plant. Env Exp Biol. 11:59–68
32. Hashemabadi, D., Kaviani, B. (2008). Rapid micro-propagation of *Aloe vera* L. via shoot multiplication. *African Journal of Biotech,* 7(12):1899–1902 https://doi.org/10.5897/AJB2008.000-5038
33. Hazarika, B. N. (2003). Acclimatization of tissue-cultured plants. *Current Science*, 85(12), 1704–1712.
34. Hazarika, B. N. (2006). Morpho-physiological disorders in in vitro culture of plants. *Scientia Horticulturae*, 108, 105–120. https://doi.org/10.1016/j.scienta.2006.01.038
35. Kanwar, K., Devi, V., Sharma, S., Soni, M., & Sharma, D. (2015). Effect of physiological age and growth regulators on micropropagation of *Aloe vera* followed by genetic stability assessment. *National Academy Science Letters*, 38, 29–35. https://doi.org/10.1007/s40009-014-0293-9
36. Kumar, A., Prakash, K., Sinha, R. K., Kumar, N. (2013). In vitro plant propagation of Catharanthus roseus and assessment of genetic fidelity of micropropagated plants by RAPD marker assay. *Appl Biochem Biotech,* 169:894–900
37. Lad VN, Murthy ZVP (2013) Rheology of *Aloe barbadensis* Miller: a naturally available material of high therapeutic and nutrient value for food applications. *Journal of Food Engineering,* 115:279–284
38. Lee, M., Phillips, R. L. (1988). The chromosomal basis of somaclonal variation. *Annual Review Plant Physiology Plant Molecular Biology,* 39:413–437
39. Leelambika, M., Sathyanarayana, N. (2011). Genetic characterization of Indian Mucuna (Leguminoceae) species using morphometric and RAPD approaches. *Plant Biosyst,* 145(4):786–797
40. Liu, X., Li, J., Zhang, Y., Li, L., He, D. (2011). Biological research advancement in Aloe. *Journal of Medicinal Plants Research*, 5:1046–1052

41. Majumder, S., Igamberdiev, A. U., & Debnath, S. C. (2025). Somaclonal variation and clonal fidelity in commercial micropropagation: Challenges and perspectives. *Agronomy*, 15(6), 1489. https://doi.org/10.3390/agronomy15061489
42. Manokari, M., Priyadharshini, S., Cokulraj, M., Dey, A., Faisal, M., Alatar, A. A., Alok, A., & Shekhawat, M. S. (2023). Amelioration of morpho-structural and physiological disorders in micropropagation of *Aloe vera* L. by use of an aromatic cytokinin 6-(3-hydroxybenzylamino) purine. Journal of Plant Growth Regulation, 42, 4751–4763. https://doi.org/10.1007/s00344-022-10672-8
43. Marshall JM (1990) Aloe vera gel: what is the evidence? *Pharma Journal,* 24:360–362
44. Meyer HJ, Van Staden J (1991) Rapid in vitro propagation of *Aloe barbadensis* Mill. *Plant Cell, Tissue Organ Culture,* 26(3):167–171
45. Meyer, H. J., & Van Staden, J. (1991). Rapid in vitro propagation of *Aloe barbadensis* Mill. *Plant Cell, Tissue and Organ Culture*, 26, 167–171. https://doi.org/10.1007/BF00039939
46. Molnár, Z., Virág, E., Ördög, V. (2011). Natural substances in tissue culture media of higher plants. *Acta Biologica Szegediensis*, 55(1):123–127
47. Molsaghi, M., Moieni, A., & Kahrizi, D. (2014). Efficient protocol for rapid Aloe vera micropropagation. *Pharmaceutical Biology*, 52(6), 735–739. https://doi.org/10.3109/13880209.2013.868494
48. Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantatum*, 15:473–497
49. Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15, 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
50. Natali, L., Sanchez, I. C., & Cavallini, A. (1990). In vitro culture of *Aloe barbadensis* Mill.: Micropropagation from vegetative meristems. *Plant Cell, Tissue and Organ Culture*, 20, 71–74. https://doi.org/10.1007/BF00034761
51. Nautiyal, P., Dimri, S., Trivedi, V. L., & Gusain, S. S. (2026). Cost-effective plant micropropagation: Constraints, trade-offs, and challenges in scaling. *Plant Cell, Tissue and Organ Culture*, 165, 29. https://doi.org/10.1007/s11240-026-03445-8
52. Ni, Y., Turner, D., Yates, K. M., Tizard, I. (2004). Isolation and characterization of structural components of *Aloe vera* L. leaf pulp. *International Immunopharmacology,* 4(14):1745–1755
53. Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ*, 372, n71. https://doi.org/10.1136/bmj.n71
54. Pandhair, V., Diviya, J., Joginder, S., & Gosal, S. S. (2011). Biochemical studies of Aloe vera (*Aloe barbadensis* Miller) gel of the field grown and micropropagated plants at different developmental stages. *Journal of Plant Biochemistry and Biotechnology*, 20, 283–287. https://doi.org/10.1007/s13562-011-0040-y
55. Paridaa, R., Mohantya, S., Nayak, S. (2013). In vitro propagation of *Hedychium coronarium* Koen: through axillary bud proliferation. *Plant Biosyst*, doi:10.1080/11263504.2012.748102
56. Pożoga, M., Olewnicki, D., & Latocha, P. (2024). A temporary immersion system as a tool for lowering planting material production costs using the example of *Pennisetum advena* 'Rubrum'. *Agriculture*, 14(7), 1177..
57. https://doi.org/10.3390/agriculture14071177
58. Rana, S., Dhar, N., Bhat, W. W., Razdan, S., Khan, S., Dhar, R. S., Dutt, P., Lattoo, S. K., (2012). A 12-deoxywithastramonolide-rich somaclonal variant in *Withania somnifera* (L.) Dunal–molecular cytogenetic analysis and significance as a chemotypic resource. *In Vitro Cell & Dev Biol–Plant*, 48:546–554
59. Rathore, M. S., Chikara, J., Mastan, S. G., Rahman, H., Anand, K. G. V., Shekhawat, N. S. (2011a). Assessment of genetic stability and instability of tissue culture-propagated plantlets of *Aloe vera* L. by RAPD and ISSR Markers. *Applied Biochemistry Biotechnology*, 165:1356–1365
60. Rathore, M. S., Chikara, J., Shekhawat, N. S. (2011b). Plantlet regeneration from callus cultures of selected genotype of *Aloe vera* L. an ancient plant for modern herbal industries. *Applied Biochemistry Biotechnology,* 163:860–868
61. Rathore, M. S., Chikara, J., & Shekhawat, N. S. (2011a). Plantlet regeneration from callus cultures of selected genotype of *Aloe vera* L.: An ancient plant for modern herbal industries. *Applied Biochemistry and Biotechnology*, 163, 860–868. https://doi.org/10.1007/s12010-010-9090-1
62. Rathore, M. S., Chikara, J., Mastan, S. G., Rahman, H., Anand, K. G. V., & Shekhawat, N. S. (2011b). Assessment of genetic stability and instability of tissue culture-propagated plantlets of Aloe vera L. by RAPD and ISSR markers. *Applied Biochemistry and Biotechnology*, 165, 1356–1365. https://doi.org/10.1007/s12010-011-9352-6
63. Reynolds, T., Dweck, A. C. (1999). *Aloe vera* leaf gel: a review update. *Journal of Ethnopham* 68:3–37
64. Rodríguez, E. R., Martín, J. D., Romero, C. D. (2010). *Aloe vera* as a functional ingredient in foods. *Critical Review Food Science Nutrition*, 50:305–326
65. Roy, S. C., & Sarkar, A. (1991). In vitro regeneration and micropropagation of Aloe vera L. *Scientia Horticulturae*, 47, 107–113. https://doi.org/10.1016/0304-4238(91)90032-T
66. Samantaray, S., Maiti, S. (2008). Rapid plant regeneration and assessment of genetic fidelity of in vitro raised plants in *Aloe barbadensis* Mill: using RAPD markers. *Acta Bot Gallica*, 155(3):427–434
67. Sarmiento, J. A. T., Aspuria, E. T., Aguilar, E. A., Sta. Cruz, P. C., & Sanchez, P. B. (2020). Field performance and bioactive content of in vitro-derived *Aloe vera* as influenced by planting distance, fertilization, and plant material. *Journal of the International Society for Southeast Asian Agricultural Sciences*, 26(2), 108–121.
68. Savita, Bhagat, A., Pati, P. K., Virk, G. S., Nagpal, A. (2012). An efficient micropropagation protocol for *Citrus jambhiri* Lush. and assessment of clonal fidelity employing anatomical studies and RAPD markers. *In Vitro Cell & Dev Biol–Plant,* 48:512–520
69. Sharma, A. K., Sharma, A. (1980). Chromosome techniques–theory and practice, 3rd edn. Butterworth-Heinemann Ltd, London
70. Sharma, P., Kharkwal, A. C., Abdin, M. Z., & Varma, A. (2014). Piriformospora indica improves micropropagation, growth, and phytochemical content of *Aloe vera* L. plants. *Symbiosis*, 64, 11–23. https://doi.org/10.1007/s13199-014-0298-7
71. Sharma, S., Sharma, D., & Kanwar, K. (2015). Technology refinement for micropropagated *Aloe vera* L.: A miracle plant. *Research in Plant Biology*, 5(4), 1–10.
72. Shukla, M. R., Jones, A. M. P., Sullivan, J. A., Liu, C. Z., Gosling, S., Saxena, P. K. (2012). In vitro conservation of American elm (*Ulmus americana*): potential role of auxin metabolism in sustained plant proliferation. *Canadian Journal of Forest Research,* 42:686–697
73. Singh, M., Rathore, M. S., Panwar, D., Rathore, J. S., Dagla, H. R., Shekhawat, N. S. (2009). Micropropagation of selected genotype of *Aloe vera* L.: an ancient plant for modern industry. *Journal of Sustainable Forest*, 28(8):935–950
74. Singh, B., & Sood, N. (2009). Significance of explant preparation and sizing in Aloe vera L.: A highly efficient method for in vitro multiple shoot induction. *Scientia Horticulturae*, 122, 146–151. https://doi.org/10.1016/j.scienta.2009.03.023

75. Singh, M., Rathore, M. S., Panwar, D., Rathore, J. S., Dagla, H. R., & Shekhawat, N. S. (2009). Micropropagation of selected genotype of Aloe vera L.: An ancient plant for modern industry. *Journal of Sustainable Forestry*, 28, 935–950. https://doi.org/10.1080/10549810903344660
76. Vig, B. K. (1968). Spontaneous chromosome abnormalities in roots and pollen mother cells in *Aloe vera* L. *Bulletin of Torrey Botanical Club,* 95:89–95
77. Vogler, B. K., Ernst, E. (1999). *Aloe vera*: a systematic review of its clinical effectiveness. *British Journal of General Practice,* 49:823–828
78. Welehaweria, M., & Sbhatu, D. B. (2023). In vitro micropropagation of Aloe elegans Tod. using offshoot cuttings. BMC Research Notes, 16, 215. https://doi.org/10.1186/s13104-023-06490-0
79. Yasmin, S., Hasan, M. J., Hossain, M. S., Saha, S., & Khatun, F. (2022). Auxin and cytokinin synergism in micropropagation for mass production of *Aloe vera*. *BioTechnologia*, 103(3), 301–310. https://doi.org/10.5114/bta.2022.118672
80. Zapata, P. J., Navarro, D., Guillén, F., Castillo, S., Martínez-Romero, D., Valero, D., Serrano, M. (2013). Characterization of gels from different Aloe spp as antifungal treatment: Potential crops for industrial applications. *Industrial Crops and Products*, 42:223–23