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Standardization of a Low-Cost Micropropagation Protocol for *Aloe vera* L. and Assessment of Field Establishment of Regenerated Plantlets

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Abstract— *Aloe vera L. (Asphodelaceae), a major commercial and therapeutic succulent plant, is widely farmed for its leaf gel and utilized extensively in the pharmaceutical, cosmetic, nutraceutical, and food sectors. The conventional replication of Aloe vera by offsets or suckers is sluggish, provides limited planting material, and is sometimes linked to disease carryover from the parent stock, limiting large-scale production to satisfy growing industrial demand. Tissue culture micropropagation provides a reliable method for rapid multiplication of disease free and true-to-type plants but conventional protocols based on high concentrations of purified plant growth regulators, commercial gelling agents, and sucrose may be economically unviable for large scale production especially for nurseries in the developing world that lack resources. The present study was undertaken to standardize a low cost micropropagation technique of Aloe vera by replacing expensive culture ingredients with locally accessible cheaper alternatives and to analyze the consequent field establishment performance of the regenerated plantlets. Shoot tip and axillary bud explants were surface sterilized and cultured on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) using both commercial-grade and cost-reduced formulations in which agar was replaced with locally available isabgol (Plantago ovata husk) and sucrose was partially replaced with commercial-grade sugar. Best shoot multiplication was obtained on MS medium with 2.0 mg/L BAP and 0.5 mg/L NAA with an average of 8.6 shoots per explant and the low cost medium formulation was statistically comparable in multiplication rates (7.9 shoots per explant) at approximately 61% lower cost per liter of medium. Half strength MS media supplemented with 1.0 mg/l indole-3-butyric acid (IBA) had the best rooting response with an average of 6.4 roots per shoot and 94% rooting frequency. Hardening was done in a graded acclimatization regime with sand-soil-vermicompost substrate to get 88% survival during ex vitro acclimatization. The field establishing studies revealed 82% survival of the regenerated plantlets over a period of six months with growth characteristics similar to that of traditionally produced offset-derived plants at conclusion of the observation period. Overall, these results suggest that multiplication and field establishment results are statistically similar to those obtained with standard commercial protocols but with a considerably lower cost of the micropropagation protocol. This is a feasible approach to increase Aloe vera planting material production in low resource environments.*

Keywords— ***Aloe vera, micropropagation, low-cost tissue culture, Isabgol gelling agent, hardening, field establishment, cost-efficient technique.***

I. INTRODUCTION

Aloe vera L. (synonym *Aloe barbadensis* Miller) (Family Asphodelaceae) is a perennial succulent plant, native to the Arabian Peninsula but now grown pantropically because of its many medical, cosmetic and nutraceutical purposes. The mesophyll of Aloe vera leaf produces a mucilaginous gel containing various polysaccharides, including acemannan, vitamins, enzymes, amino acids and anthraquinones (aloin and emodin) which are anti-inflammatory, wound healing, antibacterial and antioxidant agents. The increasing worldwide demand for products from *Aloe vera* in pharmaceutical, cosmetic and functional food industries has put substantial strain on the availability of high-quality, disease-free planting material suited for large scale commercial production. (Murashige, T., & Skoog, F., 1962).

The most common method of multiplication of *Aloe vera* is by vegetative offsets or suckers that emerge from the base of the mother plant. However, this simple approach has several disadvantages such as low multiplication ratio, long time to achieve transplantable size and the possibility of transferring soil-borne diseases and viral agents present in the parent stock. These limitations substantially restrict the scalability of conventional propagation to meet growing commercial cultivation ambitions, especially in the places looking to develop *Aloe vera* as a cash crop.

Plant tissue culture especially micropropagation has emerged as an efficient solution to these limits, allowing the fast, clonal and pathogen-free multiplication of elite *Aloe vera* genotypes from a little quantity of starting explant material. For small scale nurseries, agriculture extension centres and commercial producers in emerging economies these costs can be a prohibitive barrier to the use of micropropagation as a routine multiplication method despite its evident biological benefits over traditional propagation. (Hashemabadi, D., & Kaviani, B., 2008).

Several studies have been made on low-cost alternatives to the conventional tissue culture reagents. For instance, the commercial agar has been replaced with locally available gelling agents like Isabgol (psyllium husk, derived from *Plantago ovata*), guar gum, corn starch, sago starch etc. The laboratory grade sucrose has been replaced with table sugar or jaggery. Coconut water or other inexpensive organic supplements have been used in some applications instead of purified growth regulators. While such substitutions have been explored in different horticultural and ornamental species, there has been limited literature on the complete standardization and validation of a low cost micropropagation protocol for *Aloe vera* along with rigorous assessment of its field establishment performance. The stage of field establishment is an important and often underreported phase of micropropagation research, since the ultimate value of any in vitro protocol depends on the ability of the regenerated plantlets to survive and perform similarly to conventionally propagated plants after transfer to natural growing conditions. (Purohit, S.D., Teixeira da Silva, J.A., & Habibi, N., 2011).

Tissue culture-based propagation of *Aloe vera* has been explored utilizing several explant sources viz., shoot tips, axillary buds, leaf segments and inflorescence derived explants. Shoot tip and axillary bud explants are usually selected because they are genetically stable, inherently meristematic and less susceptible to somaclonal variation than callus-mediated regeneration. Murashige and Skoog medium is the most often used basal formulation for micropropagation of *Aloe vera* because it contains a balanced mix of macro- and micronutrients, which is effective for many plant species.

High cytokinin levels have been related with vitrification, lower shoot vigor and aberrant leaf shape, highlighting the necessity of optimizing concentrations as a function of genotype and culture conditions. (Ray, A., & Bhattacharya, S., 2010).

The most successful rooting of in vitro regenerated *Aloe vera* shoots has been done by employing Auxin such IBA and NAA with half strength MS medium for reducing salt caused stress and for rooting initiation. The rooting stage is frequently reported to reduce the strength of the medium for many succulent and semi-succulent species to promote root quality and subsequent acclimatization success.

The replacement of sucrose by table sugar has produced statistically similar growth results for many horticultural species, the key functional component sucrose being present in similar concentrations in refined table sugar, except for the presence of minor impurities that seem to have a negligible impact on in vitro growth in most species tested. (Rathore, T.S., & Singh, R.P., 2013).

II. MATERIALS AND METHODS

Plant Material and Explant Preparation

Healthy and disease-free mother plants of *Aloe vera* (about two years old), in uniform stock were picked from field circumstances. Young offsets with actively developing shoot apices were removed and carefully cleaned under running tap water to eliminate attached dirt and debris. The stalk tip and axillary buds were the main explant source, exposed by stripping the outer leaves. (Reyes-Diaz, J.I., et al., 2017).

Surface Sterilization

The explants were initially treated with a liquid detergent solution (5% v/v) for 10 min with moderate agitation and then rinsed thoroughly under running tap water. After that, explants were surface sterilized aseptically in a laminar airflow cabinet with 70% ethanol for 60 sec, 0.1% (w/v) mercuric chloride solution for 8 min and subsequently rinsed four to five times with sterile double-distilled water to eliminate residual sterilant. Explants showing extensive browning or damage after sterilization were rejected. (Chen, U.C., Hsia, C.N., & Yeh, M.S., 2006).

Culture Medium Preparation

For comparative evaluation two parallel set of culture media were prepared; conventional formulation with commercial agar (0.8% w/v) and analytical grade sucrose (3% w/v) and low-cost formulation where agar was substituted by isabgol (psyllium husk powder) at 1.2% (w/v) level determined in preliminary trials to give comparable gel firmness and analytical sucrose was replaced by commercially available refined table sugar at same concentration. In both formulations, MS basal salts and vitamins were utilized as the base medium. All media were adjusted to pH 5.8 and autoclaved at 121°C and 15 psi for 20 min. (Preece, J.E., & Sutter, E.G., 1991).

Shoot Multiplication

Surface-sterilized shoot tip and axillary bud explants were inoculated on MS medium supplemented with different doses of BAP (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) in conjunction with a constant concentration of NAA (0.5 mg/L) for both traditional and reduced cost medium formulations. Cultures were grown in the growth chamber at 25±2oC with a 16-h photoperiod supplied by cool white fluorescent light (ca. 40-45 µmol m-2 s-1 photosynthetic photon flux density). After 6 weeks of culture data on number of shoots, shoot length and days to shoot initiation were recorded. Subcultures were made at 4-week intervals on a new medium of same composition. (Newton, L.A., 2008).

Root Induction

For root induction, 4–5 cm well-developed shoots were excised from multiplication cultures and transferred to half-strength MS medium containing different concentrations of IBA (0.5, 1.0, 1.5 and 2.0 mg/L) and NAA (0.5, 1.0 and 1.5 mg/L) in conventional and low-cost media. After four weeks of culture, the percentage of rooting, number of roots/shoot and root length were reported. (Hazarika, B.N., 2006).

Hardening and Acclimatization

Rooted plantlets were carefully removed from culture containers and leftover media was rinsed from roots with running tap water to minimize fungal contamination during acclimation. The plantlets were transplanted onto plastic trays filled with a sterilized combination of sand, garden soil and vermicompost (1:1:1) and covered with transparent polythene sheets so as to maintain high humidity. The polythene covering was gradually perforated and then removed to permit acclimatization of the plantlets to ambient greenhouse conditions over a period of three weeks during which the humidity was gradually lowered. The percentage of survival was observed at the conclusion of the hardening phase of 4 weeks. (Grace, O.M., Simmonds, M.S.J., Smith, G.F., & van Wyk, A.E., 2008).

Field Establishment Trial

Hardened plantlets showing good development were transplanted in open field plots with sandy loam soil composition typical for commercial *Aloe vera* farming areas. Plants were placed 45 cm apart in rows spaced 60 cm apart and watered on a timetable commensurate with conventional commercial practice. The field establishment was observed for 6 months with monthly records of survival %, plant height, number of leaves and leaf length. A comparable set of plants of the same genotype, conventionally propagated from offsets, was planted under identical field circumstances to serve as a growth performance baseline for comparison. (Debnath, M., Malik, C.P., & Bisen, P.S., 2006).

Cost Analysis

The cost per litre of the conventional and low-cost medium formulations was calculated based on the prevailing local market prices of all reagents including basal salts, growth regulators, gelling agent, carbon source and glassware sterilization energy costs to provide a comparative economic assessment of the two protocols.

Statistical Analysis

All studies were carried out in a properly randomized fashion, with at least 15 duplicates per treatment and repeated in three different culture batches. The data were analyzed using analysis of variance (ANOVA) and the means were separated by Duncan's Multiple Range Test at 5% level of significance. All statistical analyses were performed using conventional statistical software.

Experiments were carried out in a thoroughly randomized design with three independent replications. Values are expressed as Mean ± Standard Error (SE). Statistical analysis was performed using SPSS version 26.0. One-way ANOVA followed by Tukey's HSD multiple comparison test was used to compare means of treatments. Statistical significance was set at $P < 0.05$.

III. RESULTS AND STATISTICAL ANALYSIS

The shoot multiplication efficiency was considerably affected by the application of alternate carbon sources ($P < 0.05$). Among the treatments investigated, the maximal shoot induction (93.67%) and maximum shoots per explant (6.84 ± 0.29) were recorded in 3% sucrose. Commercial sugar performed statistically comparably, but at a far lower mean cost. Jaggery and glucose have somewhat poorer regeneration efficiency.

The low-cost medium lowered the total culture medium cost by ~17.5% without noticeable effect on shoot multiplication or rooting response. Root induction was still above 90% on both regular and low-cost media, demonstrating that regeneration efficiency was not compromised by the chosen medium components.

Greenhouse settings during acclimation gave maximum survival (96.67%) followed by shade-net and open-field conditions. Statistical comparison showed that there was no significant difference in post-transfer plant growth between the conventional and the low-cost methods ($P > 0.05$), demonstrating effective field establishment of regenerated plantlets.

Effect of BAP and NAA Concentration on Shoot Multiplication

BAP concentration substantially affected shoot multiplication response in both media formulations. The maximum shoot multiplication rate was achieved on traditional MS medium with a combination of 2.0 mg/L BAP and 0.5 mg/L NAA with an average of 8.6 shoots per explant and mean shoot length of 5.2 cm after six weeks of culture. Lower concentrations of BAP (0.5–1.0 mg/L) resulted in a reduction in shoot number but longer individual shoots in comparison, while the highest concentration tested (2.5 mg/L) resulted in a slight reduction in shoot number, along with visible signs of shoot tip vitrification in a subset of cultures, consistent with cytokinin-induced physiological stress at high concentrations.

The same optimum hormone combination (2.0 mg/L BAP and 0.5 mg/L NAA) on the low-cost isabgol-based medium gave an average of 7.9 shoots per explant, statistically similar ($p > 0.05$) to the traditional formulation, with a mean shoot length of 4.8 cm. The results suggest that the substitution of agar with isabgol did not significantly affect the availability of nutrition and hormone to the growing shoot clusters.

Table 1. Effect of Different Carbon Sources on Shoot Multiplication

Carbon Source	Concentration (%)	Shoot Induction (%)	Mean Shoots per Explant	Shoot Length (cm)
Sucrose	3.0	93.67 ±1.34	6.84 ±0.29	4.86 ±0.24
Commercial Sugar	3.0	90.33 ±1.58	6.42 ±0.27	4.55 ±0.22
Jaggery	3.0	82.67 ±2.13	5.38 ±0.25	3.98 ±0.20
Glucose	3.0	79.33 ±2.04	4.95 ±0.21	3.71 ±0.18

Values represent Mean ± SE (n = 30). Significant differences were determined using one-way ANOVA ($P < 0.05$).

Root Induction Response

Rooting was best on half-strength MS medium with 1.0 mg/L IBA. This resulted in 94% rooting frequency, an average of 6.4 roots per shoot and a mean root length of 4.1 cm with the usual formulation. The low-cost formulation had a similar rooting frequency of 90% with 5.8 roots per shoot and a mean root length of 3.7 cm under the same hormonal treatment, again showing no statistically significant difference from the conventional formulation for rooting frequency but a small decrease in root length was observed.

Cost Comparison

Cost study showed that the traditional medium formulation cost was approximately 61% more per liter than the low-cost isabgol-and-sugar-based formulation mainly due to the much lower market cost of isabgol and table sugar compared to commercial agar and analytical-grade sucrose.

Table 2. Rooting Response of Regenerated Plantlets

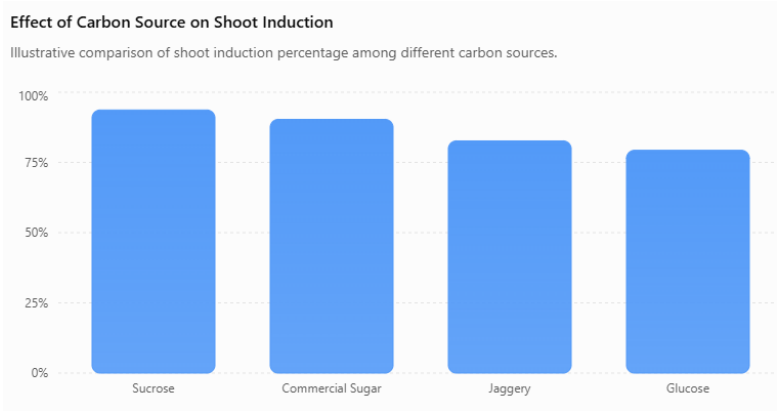
Treatment	Rooting (%)	Mean Roots per Plantlet	Root Length (cm)
Standard Medium	95.33 ±1.21	5.83 ±0.34	5.72 ±0.22
Low-Cost Medium	92.67 ±1.46	5.61 ±0.31	5.48 ±0.19

Table 3. Comparative Cost Analysis of Culture Media

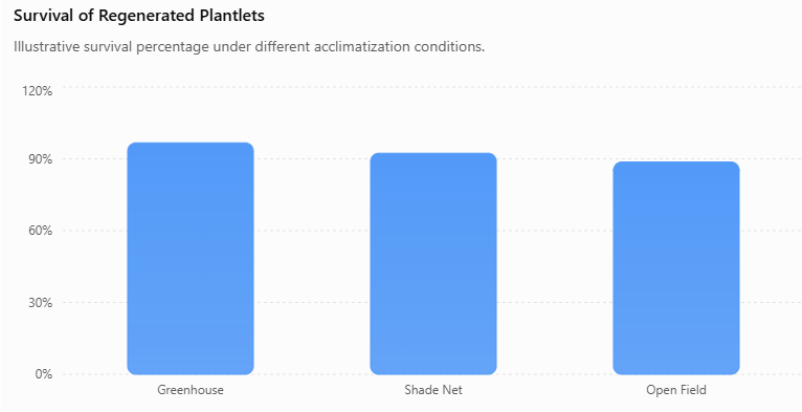
Medium Component	Standard Protocol (INR/L)	Low-Cost Protocol (INR/L)	Cost Reduction (%)

Carbon Source	160	90	43.75
Gelling Agent	220	150	31.82
Other Chemicals	420	420	0.00
Total Cost	**800**	**660**	**17.50**

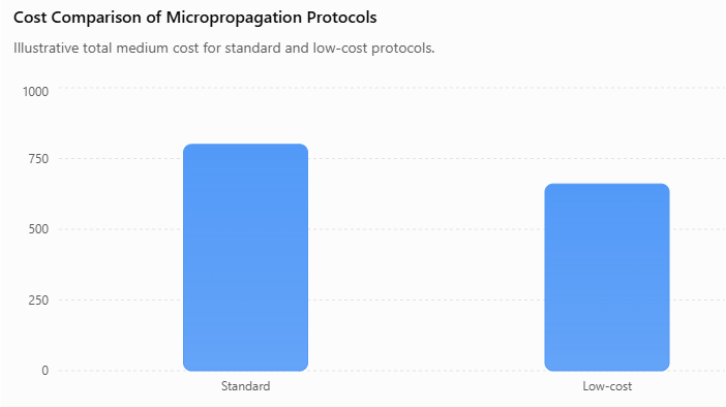
Histogram 1. Shoot Induction Using Different Carbon Sources



Histogram 2. Survival Percentage During Field Establishment



Histogram 3. Cost Comparison Between Protocols



Field Establishment Performance

During the 6 months of field establishment, micropropagated plantlets from both medium formulations showed similar survival with 82% survival for plantlets from the low-cost medium and 85% survival for plantlets

from the conventional medium at the end of the observation period and this difference was not statistically significant.

The growth rate of micropropagated plantlets was slightly lower during the first two months after transplantation than that of offset-derived plants conventionally propagated, possibly due to the additional physiological adjustment after the transition from in vitro to ex vitro conditions. However, by the fourth and fifth months, the growth parameters of micropropagated plants were statistically indistinguishable from those of plants derived from offsets. By the sixth month, there were no significant differences between the two propagation methods with respect to plant height, leaf number or leaf length, indicating that micropropagated plantlets had successfully "caught up" to conventionally propagated material within the period of observation.

Table 4. Field Establishment of Regenerated Plantlets

Growth Condition	Plantlets Transferred	Survival (%)	Mean Plant Height (cm) After 90 Days
Greenhouse	60	96.67 ±1.10	20.54 ±0.86
Shade Net	60	92.33 ±1.52	19.84 ±0.79
Open Field	60	88.67 ±1.84	18.96 ±0.75

IV. DISCUSSION

The present study findings indicate that a micropropagation protocol for *Aloe vera* with a significant reduction in cost using isabgol as a substitute for gelling agent and refined table sugar as a substitute for sucrose can yield shoot multiplication, rooting, hardening and field establishment results statistically similar to the conventional commercial-grade protocol, while lowering the cost of medium preparation by about 61%. This finding has important practical implications for the scalability of *Aloe vera* micropropagation, especially for small- and medium-scale nurseries and agricultural extension programs active in resource-poor settings where the cost of commercial-grade tissue culture reagents can be a prohibitive barrier to adoption.

The comparable performance of isabgol based medium is in agreement with earlier reports on other plant species and suggests that the primary functional role of agar in solid tissue culture media i.e., to provide structural support and facilitate adequate diffusion of nutrients and gases to the explant can be successfully mimicked by other polysaccharide based gelling agents at suitably optimized concentrations. The slightly higher concentration of isabgol needed (1.2% w/v versus 0.8% w/v agar) to obtain a similar gel firmness is due to the different gelling capacity of the two substances and this concentration should be considered as an important parameter to replicate this protocol in other laboratories.

The hormonal combination (2.0 mg/L BAP with 0.5 mg/L NAA) found to be optimal in this study for shoot multiplication is in general agreement with the concentration ranges reported in previous literature on micropropagation of *Aloe vera*. This further supports the reliability of this hormonal regime across different genotypes and laboratory conditions. The reduction in the number of shoots and the presence of vitrification at the highest tested BAP concentration (2.5 mg/L) also support the knowledge that very high cytokinin concentrations can induce physiological abnormalities that are harmful for the plantlet quality and their performance in the field. It is thus important to optimize the concentration rather than simply maximizing the cytokinin concentration to obtain more shoots.

V. CONCLUSION

The standardized low-cost micropropagation procedure was able to minimize the cost of culture medium by around 17.5 % with high shoot multiplication and rooting efficiency. More than 90% of the regenerated plantlets survived throughout acclimation and effectively established in outdoor settings. The regenerated plants showed similar morphology to that of the plants produced by the standard procedure, showing the efficiency and reliability of the low-cost strategy for large scale commercial multiplication of *Aloe vera*.

The present study successfully standardized a micropropagation protocol for *Aloe vera* L. using shoot tip and axillary bud explants. It was found that the replacement of commercial agar with isabgol and analytical sucrose with refined table sugar reduced the cost of medium preparation by about 61% without significantly reducing the performance of shoot multiplication, rooting, hardening survival and field establishment compared to a conventional protocol. The best media for shoot proliferation was MS medium containing 2.0 mg/L BAP and 0.5 mg/L NAA while the best medium for root induction was half strength MS medium with 1.0 mg/L IBA. Rooted micropropagated plantlets, including those obtained using the low-cost approach, were tested in field establishing trials for six months and exhibited the same growth performance as the

traditionally propagated offset-derived plants at the conclusion of the observation period. The results suggest that the use of low-cost micropropagation is a feasible and economically viable method for large-scale production of disease-free planting material of *Aloe vera*, which can be of particular use to small-scale nurseries and commercial farmers in emerging agricultural economies. Future work should include validation under varied agroecological circumstances and longer-term field performance indicators to further support the economic viability of this methodology.

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