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An innovative hardening technology for micro propagated banana plantlets for medium size tissue culture industries

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Abstract

This study explores the use of polypropylene (PP) bags as an innovative alternative to traditional pro-trays in the hardening process of tissue-cultured banana plants. The goal was to reduce the high mortality rates typically seen during acclimatization and improve the growth parameters such as plant height, leaf number, shoot girth, leaf area, root development, and overall biomass. The study compared the performance of banana plants toughened in polypropylene (PP) bags versus pro-trays during primary acclimatization. The results revealed that PP bags significantly lowered mortality rates from 5-8% to 2-3%, resulting in improved plant survival. The better results are attributable to the innate nature of PP bags to maintain high humidity and micro-environmental conditions, which reduces energy inputs and improves plant acclimatization. In addition to increasing plant life and growth, PP bags were shown to be more cost-effective and environment friendly. About 22.72% of overall cost savings were obtained using poly bags. Their flexibility, endurance, lower shipping cost, and one-step acclimatization make it the material of choice. Overall, this study shows that polypropylene bags are a realistic and preferable option for hardening tissue-cultured banana plants, with considerable advantages in terms of efficiency, cost, and environmental effect. This method has the potential to boost banana production on an industrial scale and might be applied to other tissue-cultured crops as it reduces transportation costs. Further research could look into the scalability and wider applicability of this technique across multiple crops and production environments.

Key words: Tissue culture, banana, micropropagation, hardening technology, polypropylene bags.

Introduction

The global banana industry is a vital component of the agricultural economy, with bananas being one of the most widely consumed fruits worldwide. The demand for bananas continues to rise, driven by both domestic consumption and international trade. To cope with this increasing demand, producers have gravitated towards tissue culture technology, which provides several advantages over traditional propagation techniques (Al-Amin *et al.*, 2009). The tissue culture method can produce a good number of uniform and disease-free plantlets in a short period of time. However, like any other process, it is not free of disadvantages, especially during the acclimatization or hardening process, where plantlets are transferred from *in vitro* environment to the environmental surroundings.

Primary hardening of tissue-cultured banana plantlets is the most important step in the production of plantlets from micro propagation to be adapted to natural conditions, including changing humidity and temperature for the plantlets. This phase has recently been done in pro-trays, which have allowed more controlled environments to minimize transplant shock and improve survival rates. Usually made of plastic, pro-trays are designed such that individual plantlets can grow in separate cells filled with a growing medium (cocopeat). Although it has its advantages, this technique comes with a set of drawbacks that can impede the effectiveness of mass banana cultivation.

The main disadvantage of pro trays is the high mortality rate of plantlets during the hardening process, which ranges from 5-8%, particularly where state-of-the-art polyhouses fitted with artificial intelligence (AI) enabled sensors are not available. Over 70% of the mortality that occurs in commercial plant production is due to a lack of a conducive micro-environment, resulting in stress and adverse plantlet growth (Singh *et al.*, 2011). Additionally, this hard structure of pro-trays also restricts and coils the growth of roots. The transshipping of plantlets becomes cumbersome and costly after the production stage.

These limitations imply a need for new hardening strategies that can provide optimal plantlet survival and growth and are cost-effective. Novel methods have been examined, among them the is application of polypropylene (PP) bags, since this alternative method has many likely advantages when compared to using pro-trays. In comparison, PP bags provide more flexibility and sturdiness against mechanical damage and could give a much more stable micro-environment option to facilitate plantlet acclimatization. Their ability to retain more liquid than that of pro-trays results in less frequent watering and more stable humidity of the substrate, which is essential in acclimatizing tissue-cultured plants (Maddah, 2016).

From an economic perspective, PP bags for the user to save a lot of production costs. Lower water needs, in addition to the simpler management and logistics of plantlets, can lead to significant reductions in labour and energy demands. Moreover, packing PP bags is more space-efficient, which reduces transportation space and cuts down costs (Prakash *et al.*, 2002). This has significant economic implications in the regions where bananas are an important agricultural commodity, as improvements in efficiency lead to a marked difference in profitability.

However, from an environmental standpoint, PP bags are deemed to be more sustainable than conventional pro-trays. We propose that the bags are reusable and can be made out of recyclable materials; thus, the total amount of plastic waste and the environmental impact of banana production is reduced (Smith *et al.*, 2022). Additionally, given the enhanced transportation efficiency associated with PP bags, they also contribute to lower carbon emissions, supporting wider goals of reducing the environmental footprint of agricultural practices (Hekkert *et al.*, 2000).

Overall, though pro-trays have been the classical method for primary hardening of micro-propagated banana plants, shortcomings in relation to plantlet survival, cost-effectiveness, and environmental sustainability call for re-thinking concerning their use and post-harvest management and seeking alternative strategies, polypropylene bags are an agri innovation, and the bags can help in economic production of plantlets for mid size (10-25 lakh plants /anumm) tissue culture companies. The objective of this study was to find a cost-effective alternative to pro-trays by evaluating the performance of tissue-cultured banana plantlets in bags of polypropylene (PP) during hardening stage. Reduce death rates, improve growth parameters, and analyze the cost-effectiveness and environmental sustainability of the use of PP bags in acclimatization.

Materials and methods

Plant Material: Plant material used in this study was tissue–cultured banana cv. Grand Naine plantlets (*Musa* spp. collected from ICAR-CISH, Lucknow). Tissue cultured plantlets with at least 4-6 expanded leaves with well-developed roots were chosen.

A total of 600 plantlets were used in this experiment and equally divided into two groups: in one group, plantlets were hardened in PP bags (22 cm × 22 cm), whereas in the other, plantlets were hardened in traditional pro-trays (72-cell trays, each cell 5 cm × 5 cm × 6 cm).

Growing media: Cocopeat (coconut coir dust) was used as the growing medium for both hardening techniques since cocopeat has good water holding capacity as well as aeration. The cocopeat was autoclaved at 121°C for 20 minutes to prevent contamination. Once brought down to room temperature, the cocopeat matrix was enriched with a 50% strength MS salt solution containing the right nutrients necessary for plant growth.

Hardening: In the PP bags method, each bag was filled with approximately 500 g of the prepared cocopeat mixture. A total of 30 tissue-cultured banana plantlets was carefully transferred from the *in vitro* culture to each PP bag, ensuring minimal disturbance to the root system. These bags were subsequently stored for an initial 15 days at 25±5°C in a hardening chamber equipped with fluorescent tubes to achieve a light intensity of 4,000 lux. Relative humidity in the chamber was maintained at ~80–85% using a humidifier. At this stage, the PP bags were moved to a shade net house with 75% shade for 30 days, at the end of which, small holes were made at the top of the bag to allow gaseous exchange and acclimatization to the ambient humidity.

In the pro-trays, the same mix of autoclaved cocopeat as in the PP bags was added for each hole in the pro-tray (99 holes). The tissue-cultured banana plantlets were then transferred into the pro-trays, with one plantlet per hole. The pro-trays were seated on benches in the same hardening room as the PP bags and under the same environmental conditions. Pro-trays were kept in the chamber for 15 days before transferring to the shade net house for 30 days same as performed with PP bags.

Monitoring of survival rate, growth parameters, and signs of stress or disease was performed for both groups. The survival rate, height of the plant (distance from the ground to the terminal leaf of the shortest shoot), number of fully opened leaves in each plantlet, girth of the stem (measured at the base), leaf area (calculated by measuring the length and width of the longest leaf per plantlet and applying a correction factor), root growth (number and length of primary roots), and total biomass (fresh weight of the plantlet including roots and shoots) were recorded after the 45-day hardening period.

Cost analysis: Cost comparison was done between PP bags and pro-trays from the economic perspective. The cost data consisted of the cost of materials, manpower, energy input, and transportation, and based on these, the final cost per hardened plantlet was determined for both methods.

Statistical analysis: The t-test was applied to the data on all the parameters. Statistical significance was inferred at a p-value of <0.05, and analyses were performed using SPSS software (Version 25).

Results and discussion

The growth parameters and survival rate were used to compare the hardening effect of PP bag and Protray treatments on micro-propagated banana plantlets. The t-tests performed for comparisons demonstrated the varying efficacy of treatments across specific traits including plant parameters and survival rate.

The study clearly indicated that tissue-cultured banana plantlets hardened in PP bags had a significantly higher survival rate compared to those hardened in traditional trays. Survival rates were 98% in polypropylene bags and 95% in trays (Fig. 1). The small percentage difference results in a significant reduction in plantlet loss, a major concern in industrial settings where hundreds of thousands of propagules are used simultaneously. PP bags provide optimal micro-environmental conditions for tissue-cultured plantlets, including moisture retention and controlled aeration, which aids in the fragile acclimatization process.

Plantlets hardened in PP bags reached significantly higher heights compared to pro-trays. Plantlet height measured 18.1 cm in PP bags and 16.63 cm in pro-trays (Fig. 1). The increased height could be because the PP bags create a better microclimate and provide more nutrients (nutrients do not wash out owing to continuous misting for the plantlets. The controlled environment within the PP bags aided in stress reduction, leading to longer and stronger plants in the hardening phase.

Results revealed that the PP bag treatment had an average of 6.33 leaves/plant (plant vigor indicator) as compared to the 4.33 leaves/plant in Protrays treatment. While the PP bag produced a greater amount of leaves, the difference was not statistically non

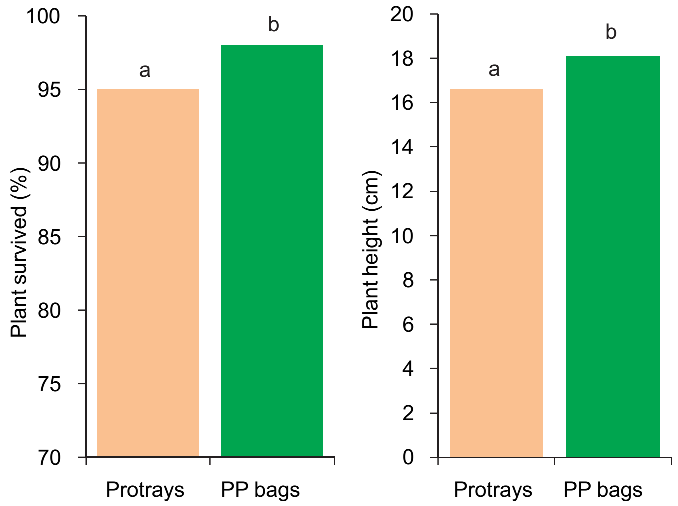

Fig.1. Effect of Protray and PP bag on plant survival and plant height

significant (Fig. 2). This means that, while the PP bag treatment may promote slightly more leaf production, the difference is not significant enough to determine its superiority in enhancing leaf formation.

The shoot diameter, a measure of plant strength, differed significantly between treatments indicating that the PP bag treatment improves shoot development more effectively (Fig. 2, 4). The PP bag treatment produced a 7.4 mm shoot diameter, which was significantly larger than the 5.09 mm observed with the Protrays treatment. The result of the higher thickness of shoot girth prevailed under PP bag indicated that the thickness of shoot girth is an important parameter for early establishment and growth as it indicated the total structural integrity and sturdiness of the plants.

Plantlets had significantly larger leaf area in PP bags (11.92 cm^2 vs. 8.86^2 in pro-trays) (Fig. 2, 4). The PP bags create a favourable microclimate for leaf growth and expansion, resulting in increased leaf area. Larger leaf areas improve photosynthesis, leading to increased growth and biomass output.

The PP bag treatment resulted in a greater number of roots, with an average of 14 roots versus 12 in the Protrays treatment (P = 0.04). This indicates that the PP bag is more effective at stimulating root growth, which is critical for the successful establishment of banana plantlets in the field (Fig. 3, 4). The PP bags appeared to promote the development of longer, more fibrous roots, which are better suited to the post-culture environment. This is significant because stronger, more developed roots help plants remain stable and absorb nutrients more efficiently. As a result improving the survival rate once transplanted to the field.

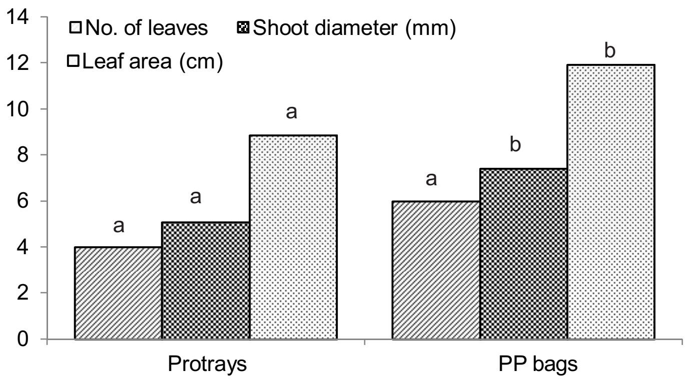

Fig. 2. Effect of Protray and PP bag on plant growth parameters

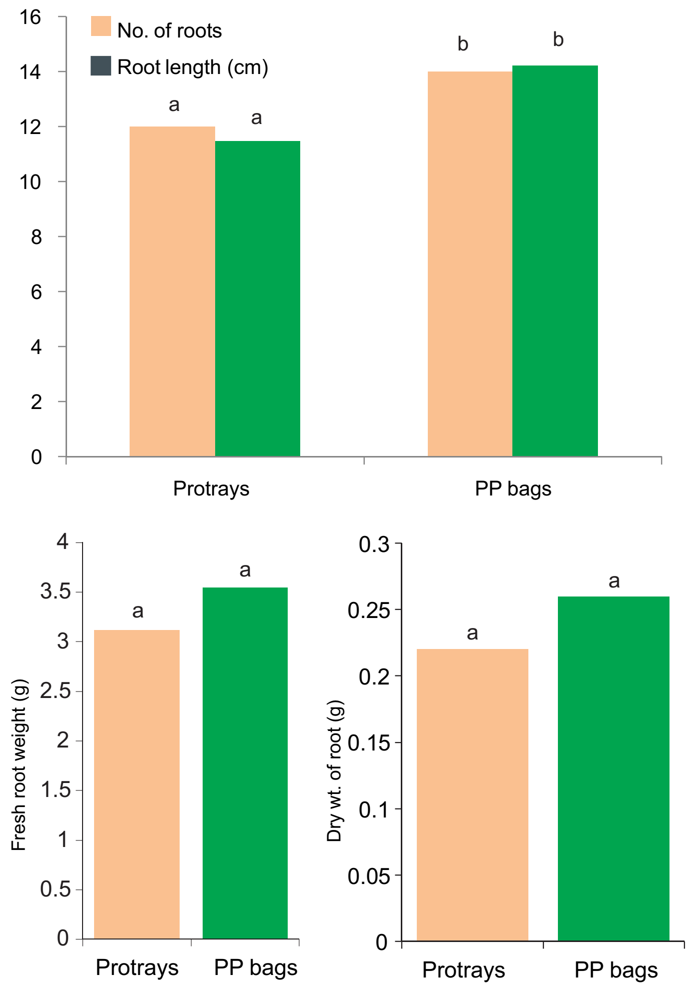

Fig. 3. Effect of Protray and PP bag on root development parameters

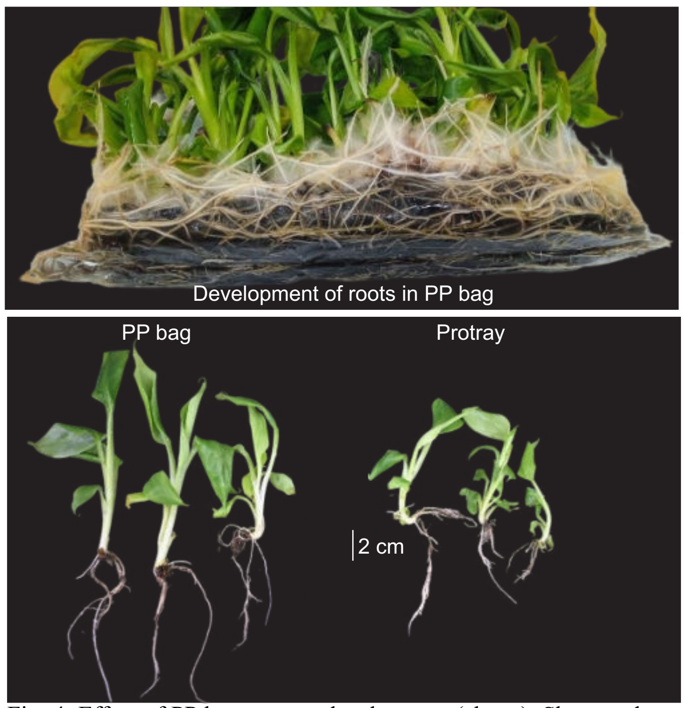

Fig. 4. Effect of PP bag on root development (above). Shoot and root development under PP bag and Protray conditions (below)

The PP bag treatment resulted in plantlets with an average fresh weight of 4.48 g, compared to 3.33 g in Protrays. However, the difference was not statistically significant (Fig. 5).

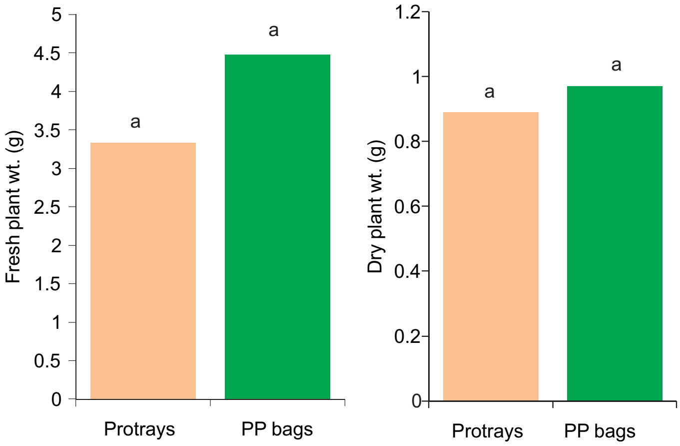

Fig. 5. Effect of Protray and PP bag on biomass

Dry plant weight is an indication of the plants ability to store nutrients and energy. PP bag treatment produced an average dry weight of 0.97 g, somewhat more than observed with Protrays, 0.89 g. The differences was not statistically significant (p-value: 0.43), meaning that both treatments had a similar effect on the accumulation of dry matter in the plantlets or variability with in treatment (Fig. 5).

The results indicate that the PP bags provided a more favourable environment, allowing the plants to grow and develop more effectively. The conditions inside the bags helped to promote healthier, stronger plants. PP bags provide plenty of space for fibrous roots, resulting in increased biomass and better plant health and vigour. Plantlets that have been hardened in PP bags are more likely to thrive in the field, as increased biomass is an important indicator of their readiness.

Table 1. Cost comparison of PP bags and pro-trays hardened tissue cultured banana plantlets

Parameter	PP bags	Pro-trays	Cost savings with PP bags
Cost per plantlet	₹6.00	₹6.00	
Energy costs	₹1.50 per plantlet	₹3.00 per plantlet	50% reduction
Transportation costs	₹1.00 per plantlet	₹2.00 per plantlet	50% reduction
Total cost per plantlet	₹8.50	₹11.00	22.72% overall cost savings

PP bag treatment helped in reducing transfer shock caused by the *in vitro* environment and also very important for the stability of the microenvironment. Enabling adjustment to the external environment and avoiding abiotic stress, which is at its peak for the plantlet, this protective environment ensures smooth transport from tissue culture entry. PP bag provides a high-humidity environment for the initial rooting of the plantlets and helps to form a better root system at an early stage. This ensures the plantlets have access to a well developed root structure allowing for improved water and nutrient uptake leading to improved survival.

Cost analysis: Cost comparison shows that PP bags are more cost-effective than pro-trays. Using poly bags 22.72% overall cost savings was obtained. We found that the lower cost of PP bags can largely be attributed to the reduced energy i.e.₹1.50 per plantlet compared to ₹3.00 per plantlet for Pro-Trays, resulting 50% reduction. Since PP bags are both durable and flexible, they're perfect for packing and moving plantlets to field sites. This not only makes the process more efficient but also helps lower transportation costs approx halved with PP Bags, costing ₹1.00 per plantlet versus ₹2.00 per plantlet for Pro-Trays (Table 1).

Fig. 6. Hardening in tissue-cultured banana plants in PP bags (left) and in protrays (right)

This study's findings show that in tissue-cultured banana plant hardening, polypropylene (PP) bags were far superior to standard pro-trays. The use of PP bags resulted in lower mortality rates, which in turn leads to fewer losses (Mishra *et al.*, 2024). As a result, the production of banana tissue culture plantlets becomes more economically sustainable and competitive on a global scale.

Plantlets grown in PP bags were improved not only in terms of survival rates but also in terms of growth parameters such as plant height, leaf number, shoot girth, and leaf area. The ability of the PP bags to stabilize the microclimate is likely responsible for these advantages; this, in turn, enhances water retention and nutrient availability, leading to greater plant development (Lee *et al.*, 2013). The finding of study suggests that stronger and healthier plants resulted from enhanced photosynthetic activity and nutrient uptake during hardening have lesser mortality are in line with previous research (Hazarika, 2006). Evidence for this can be seen in the increased root development and bigger leaf area (Drouet *et al.*, 2005).

The cost analysis of PP (polypropylene) bags and pro-trays hardened tissue cultured banana plants revealed that the PP bags reduce the overall cost per hardened plantlet to the tune of 22.72% compared to pro-trays due to lower material costs, reduced energy requirements, and less transportation expenses. This is crucial in the hardening process of commercial tissue culture, where cost optimization is essential for small and mid-size tissue culture units and farmers. Fewer energy inputs are needed to maintain the hardening environment, and using PP bags reduces transportation costs, which is the main reason for this reduction. The flexibility and durability of PP bags allow for more efficient packing and transport, which minimizes space requirements and reduces logistical expenses (Mishra *et al.*, 2024). In India, where energy costs can be a substantial burden, this reduction is particularly impactful. It not only lowers operational costs but also aligns with sustainable practices by minimizing energy consumption. Also, the lightweight and flexible nature of PP bags allows for efficient packing and reduced space requirements in trains, trucks or other means of transport, leading to lower fuel and labour costs. This not only makes the process more efficient but also helps lower transportation costs. In India, where transportation infrastructure can be challenging and fuel prices are volatile, these savings are highly beneficial. The reusability of PP bags makes them a sustainable choice in the long term due to their cost-effectiveness. Unlike pro-trays, which may need frequent replacement due to wear and tear, PP bags can be reused multiple times, reducing both material and disposal costs. The regions where banana production is a major sector and where improvements in resource usage efficiency can significantly affect profitability will find these findings particularly important.

With PP bags, we can achieve lower mortality rate, which means fewer losses and more overall output per unit of input. This boosts the economic sustainability of banana tissue culture plantlet production and makes it more competitive on the global market (Mishra *et al.*, 2024). The economic benefits of using PP bags are especially critical in developing countries, where banana cultivation is a major economic activity and where reducing production costs can have significant socio-economic impacts (Fig. 6).

The environmental assessment highlighted the sustainability benefits of using PP bags over traditional pro-trays. Therefore, while PP bags are reusable and may also be recyclable, their impact on the overall plastic waste is lower than that of single-use, non-biodegradable plastic trays (Salem-Al *et al.*, 2009). The moisture retention and decreased irrigation requirements of PP bags also contribute to the lower water and energy requirements, even further reducing the environmental impacts of the hardening phase (Saranya *et al.*, 2024). Moreover, the enhanced bulking and lightweight properties of PP bags contribute to higher transportation efficiency, leading to an average reduction in carbon emissions. This is particularly relevant within the global scope of reducing greenhouse gas emissions from agricultural practices (Alsabri *et al.*, 2021). This study provides evidence that the type of agricultural practices used to increase productivity has the potential for providing broader environmental benefits as long as they are sustained, such as could be the case with use of PP bags. Findings of studies which examined different techniques of plant hardening support the present study. Saranya *et al.*, 2024 have similarly demonstrated the benefits of using flexible, moisture-retentive materials for acclimatizing tissue-cultured plants. However, this study provides additional evidence by directly comparing PP bags with the widely used pro-trays, offering a more comprehensive evaluation of the practical benefits and challenges associated with each method. Although there are clear economic and environmental benefits to using PP bags, there may be potential limitations and issues to solve for the upscale of this method. Secondly, the upfront cost of PP bags compared to pro-trays may be high and PP bags may not perform well under all climatic conditions, which need further investigation. Nonetheless, the long-term benefits in terms of reduced mortality, lower production costs, and environmental sustainability make PP bags a promising alternative for the banana tissue culture industry and potentially beneficial for many other crops.

The study revealed that hardening in polypropylene bags is a much more rational method, leading to enhanced survival growth of tissue-cultured banana plants as well as saving money and contributing waste from used pro-trays towards making a better environment. Collectively, these results indicate PP bags are an important component in the improvement of global banana production in terms of efficiency and sustainability. Research is needed to explore the best utilization of PP bags in varied environmental conditions and their extendibility to other tissue-cultured crops.

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