A Robust Protocol for Managing Microbial Contamination of *In-vitro* Banana Plants

Mark Ochieng Adero¹, *, Easter David Syombua², Richard Oduor¹

¹Department of Biochemistry, Microbiology & Biotechnology, Kenyatta University, Nairobi, Kenya
²Department of Biochemistry, University of Nairobi, Nairobi, Kenya

Email address:
aderomark@gmail.com (M. O. Adero)

*Corresponding author

To cite this article:
Mark Ochieng Adero, Easter David Syombua, Richard Oduor. A Robust Protocol for Managing Microbial Contamination of *In-vitro* Banana Plants. *International Journal of Microbiology and Biotechnology*. Vol. 6, No. 3, 2021, pp. 95-97. doi: 10.11648/j.ijmb.20210603.15

Received: August 5, 2021; Accepted: August 16, 2021; Published: September 27, 2021

**Abstract:** *In-vitro* regeneration of banana (*Musa* spp) is a crucial technique in banana improvement via modern biotechnology like virus indexing, genetic transformation, and genome editing. However, *in-vitro* banana plants are prone to microbial contamination from the environment, leading to the loss of important lines and germplasm. Protocols for disinfecting banana plants before their *in-vitro* culture have been reported; however, there is limited information on strategies for disinfecting *in-vitro* contaminated banana plants, which are more sensitive to most disinfectants. Thus, this study aimed to establish an efficient disinfection protocol that is effective against contaminants and safe on *in-vitro* plants. Contaminated *in-vitro* banana plants (cv. Grand naine) were subjected to commercial bleach (*Jik*®; 3.85% w/v NaClO) at different exposure times (2, 4, 6, and 8 min) with or without rinsing then reinitiated *in-vitro*. *Jik*® exposure time of 2 min with or without rinsing preserved the plant viability by 100% but led to >75% fungal contamination. At 4 min of *Jik*® exposure, the viability of the plants remained at 100%, but >33% fungal contamination was observed. No contamination was observed at 8 min of *Jik*® exposure, but the plants' viability was reduced to below 85%. Notably, 6 min of *Jik*® exposure preserved the viability of the plants by 100% while destroying all the contaminants and is, therefore, recommended as the most efficient treatment. This protocol can save time and other resources and should be applied in banana genetic transformation laboratories.

**Keywords:** *In-vitro* Regeneration, Banana, Microbial Contaminants, Disinfection Protocol

---

1. **Introduction**

Banana (*Musa* spp) is among the top ten most important food crops worldwide in production and consumption [1]. However, several phytopathogens threaten banana production, including viruses, fungi, bacteria, and nematodes [2-8]. This has necessitated its improvement via biotechnology involving *in-vitro* regeneration. The tissue culture of banana is particularly important in virus indexing, production of clean planting material, and genetic transformation procedures [9]. However, contamination of *in-vitro* banana cultures is a serious problem in the micropropagation of the crop [10]. This is especially critical in the propagation of transgenic events, in which losing one line to contamination could mean losing a year's work. In most cases, the contaminants come from the environment during the transfer of banana explants to a new medium due to improper handling, contaminated medium, or equipment failure. However, some contaminants are endogenous and may survive within the plant system for a long time [11]. Most of these contaminants include bacteria and fungi [12]. Even though most bacterial contamination can be controlled by supplementing the tissue culture medium with antibiotics [13], most fungal contaminants are difficult to eliminate and may require the plants to be removed from the culture vessel and disinfected. *In-vitro* plants are typically more sensitive to disinfectants than field grown plants and therefore challenging to disinfect without reducing their viability. Therefore, this study aimed to develop an efficient protocol for rescuing *in-vitro* banana plants from contaminants. Contaminated plants were disinfected with ethanol and commercial bleach (*Jik*®) at
different exposure times to determine the optimum treatment that ensures the highest disinfection and viability of the plants. This study provides a robust protocol for rescuing in-vitro contaminated bananas and should be applied in laboratories dealing with banana micropropagation and genetic transformation.

2. Methods

In-vitro banana plants (cv. Grand naine) in mother food jars were obtained from the GTL company, Nairobi, Kenya. The lids of the bottles were opened overnight to allow contamination of the plants. Subsequently, the lids were closed, and the cultures were left to grow for seven days to allow the contaminants to establish. Next, the contaminated plants were removed from the culture vessels and immersed in 70% (v/v) ethanol for 30 s with slight agitation. After that, the plants were removed from ethanol and rinsed once with sterile distilled water before sub-culturing onto sterile fresh Murashige and Skoog medium [14], while the other batch was sub-cultured without prior rinsing. Control plants were rinsed with water only then, sub-cultured onto fresh sterile banana maintenance medium. The plants were cultured at 26°C under 16 h light/8 h dark for 14 days. The experiment was repeated thrice, and each treatment contained four replicates. Data related to contamination and viability were recorded and subjected to one-way analysis of variance (ANOVA) and the means separated by Turkey’s HSD test at p<0.05.

3. Results and Discussion

This is the first study to report on a protocol for disinfecting in-vitro contaminated bananas to the best of our knowledge. Ethanol and commercial bleach, particularly Jik® (3.85% w/v NaClO) for 2, 4, 6, and 8 min. The treatments were divided into two batches. One batch was rinsed with sterile distilled water before sub-culturing onto sterile fresh bananas and should be applied in the laboratories dealing with banana genetic transformation and germplasm preservation.

Table 1. Effect of different Jik® exposure times on contamination and viability of in-vitro banana plants.

| Time (min) | Rinsing | Percent (%) contamination | Percent viability |
|-----------|---------|---------------------------|------------------|
| 2         | Yes     | 100b                      | 100a             |
| 4         | Yes     | 58.3b                     | 100a             |
| 6         | Yes     | 33.3c                     | 100a             |
| 8         | Yes     | 0.0d                      | 83.3b            |
|           | No      | 0.0d                      | 41.7c            |

Means followed by the same superscript letter in the same column are not significantly different at p<0.05, according to Turkey’s HSD test (n=30).

4. Conclusion

Overall, this study shows that it is possible to save in-vitro contaminated bananas. Thus, this protocol should be applied in the laboratories dealing with banana genetic transformation and germplasm preservation.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This study was funded by the National Research Fund, Kenya, awarded to the first author. We are also grateful to David Geno for his technical assistance.

References

[1] J. Dale, J.-Y. Paul, B. Dugdale, and R. Harding, “Modifying Bananas: From Transgenics to Organics?,” Sustainability, vol. 9, no. 3, Art. no. 3, Mar. 2017, doi: 10.3390/su9030333.

[2] R. C. Ploetz, “Fusarium Wilt of Banana,” Phytopathology®, vol. 105, no. 12, pp. 1512–1521, Jun. 2015, doi: 10.1094/PHYTO-04-15-0101-RVW.

[3] L. Tripathi, J. N. Tripathi, T. Shah, K. S. Muiruri, and M. Katari, “Molecular Basis of Disease Resistance in Banana Progenitor Musa balbisiana against Xanthomonas campestris pv. musacearum,” Sci. Rep., vol. 9, no. 1, Art. no. 1, May 2019, doi: 10.1038/s41598-019-43421-1.

[4] A. C. L. Churchill, “Mycosphaerella fijiensis, the black leaf streak pathogen of banana: progress towards understanding pathogen biology and detection, disease development, and the challenges of control,” Mol. Plant Pathol., vol. 12, no. 4, pp. 307–328, May 2011, doi: 10.1111/j.1364-3730.2010.00672.x.

[5] T. Jekayinoluwa, J. N. Tripathi, G. Obiero, E. Muge, and L. Tripathi, “Phytochemical Analysis and Establishment of Embryogenic Cell Suspension and Agrobacterium-mediated Transformation for Farmer Preferred Cultivars of West African Plantain (Musa spp.),” Plants, vol. 9, no. 6, Art. no. 6, Jun. 2020, doi: 10.3390/plants9060789.
[6] L. Tripathi, M. Mwangi, S. Abele, V. Aritua, W. K. Tushemereirwe, and R. Bandyopadhyay, “Xanthomonas Wilt: A Threat to Banana Production in East and Central Africa,” *Plant Dis.*, vol. 93, no. 5, pp. 440–451, Apr. 2009, doi: 10.1094/PDIS-93-5-0440.

[7] J. N. Tripathi, R. O. Oduor, and L. Tripathi, “A High-Throughput Regeneration and Transformation Platform for Production of Genetically Modified Banana,” *Front. Plant Sci.*, vol. 6, p. 1025, 2015, doi: 10.3389/fpls.2015.01025.

[8] S. P. Raut and S. Ranade, “Diseases of Banana and their Management,” in *Diseases of Fruits and Vegetables: Volume II: Diagnosis and Management*, S. A. M. H. Naqvi, Ed. Dordrecht: Springer Netherlands, 2004, pp. 37–52. doi: 10.1007/1-4020-2607-2_2.

[9] H. Strosse, I. Houwe, B. Panis, S. Jain, and R. Swennen, “Banana cell and tissue culture - review,” 2004, pp. 1–12.

[10] M. Rane, S. Khan, A. Campus, and P. G. Student, “Study of Bacteria and Fungi Isolate From Contaminated Banana Tissue Culture,” vol. 5, no. 3, p. 7, 2007.

[11] A. C. Cassells and B. Doyle-Prestwich, “Contamination Detection and Elimination in Plant Cell Culture,” in *Encyclopedia of Industrial Biotechnology*, American Cancer Society, 2009, pp. 1–14. doi: 10.1002/9780470054581.eib241.

[12] T. Msogoya, H. Kanyagha, J. Mutigitsu, M. Kulebelwa, and D. Mamiro, “Identification and management of microbial contaminants of banana in vitro cultures,” 2012, p. 8.

[13] I. Van den houwe and R. Swennen, “Characterization and control of bacterial contaminants in in vitro cultures of banana (*Musa* spp.),” *Acta Hortic.*, no. 530, pp. 69–79, Sep. 2000, doi: 10.17660/ActaHortic.2000.530.6.

[14] T. Murashige and F. Skoog, “A revised medium for rapid growth and bioassays with tobacco tissue cultures,” *Physiol. Plant.*, vol. 15, no. 3, pp. 473–497, 1962.

[15] M. O. Adero, E. D. Syombua, L. K. Asande, N. O. Amugune, E. S. Mulanda, and G. Macharia, “Somatic embryogenesis and regeneration of Kenyan wheat (*Triticum aestivum* L.) genotypes from mature embryo explants,” *Afr. J. Biotechnol.*, vol. 18, no. 27, pp. 689–694, Jul. 2019, doi: 10.5897/AJB2019.16890.

[16] E. S. Mulanda, M. O. Adero, N. O. Amugune, E. Akunda, and J. I. Kiinyamario, “High-Frequency Regeneration of the Drought-Tolerant Tree *Melia volkensii* Gurke Using Low-Cost Agrochemical Thidiazuron,” *Biotechnol. Res. Int.*, vol. 2012, p. e818472, Nov. 2012, doi: 10.1155/2012/818472.