

Stable clones were produced via regeneration of floral axis organogenesis as demonstrated in this study, to complement vegetative propagation (Vincent & Anushma, 2018). Being as dessert banana, Soka variety is most likely to have AAA genome or probably AAB genome, indicating this variety is closer genetically to its ancestral parent, *M. acuminata* than *M. balbisiana*. Compared to other organs, to use floral axis as *in vitro* culture materials is proven to be successful and rapid, in which to proliferate shoot and root in such banana species with genome type which is usually resulted low proliferation. This result is also in accordance to a previous study which reported a high rate of nodule and shoot on organogenesis of floral axis of AAB and ABB genomes (Lisnandar, Fajarudin, Effendi, & Roostika, 2015). Furthermore, high number of clones could be generated by adding ascorbic acid to reduce browning (Kariyana & Nisyawati, 2013), as revealed in this study. Second cycle of subculture in this study allowed to obtain high similarity of acclimated plant of Soka variety, suggesting that minimal subculture cycle is preferable. As previously reported that high subculture level, the genotype explant, and prolonged light exposure could depict tremendous genetic variation (Poerba, Imelda, & Martanti, 2012; Ray, Dutta, Saha, Das, & Roy, 2006). Phytohormones and repeated *in vitro* subculture and duration may increase mutation and lose the regeneration potential (Muhammad, Rashid, Hussain, & Naqvi, 2007), consequently it is recommended to be eliminated as demonstrated in this study. In addition to the genetic stability of this Soka variety, it was clear that the morphological characteristics of both mother plants and culture plants, were specific to its genomic group (Hapsari & Lestari, 2016), as supported by molecular characterization by SSR markers which were developed. Importantly, the floral axis organogenesis regeneration with low number subcultures is able to provide sufficient clones as important genetic materials of Soka not only for the varietal registration conservation efforts, and other further purposes.

CONCLUSION

Eleven newly developed markers proved their robustness to assess the genetic stability of banana plants produced from *in vitro* regeneration which demonstrated a very low variation (2%).

The genetic similarity coefficient of 0.9-1.0 was observed on both culture plants and mother plants. SSR analysis confirmed that the genetic stability of the culture plants regenerated from floral axis at low level of subcultures of this local variety of Soka. This molecular level complement the same 15 morphological characters of culture plants from floral axis organogenesis and mother plants at 8 old-month age. Three quantitative morphological characters revealed the same between mother plant and culture plants (fruit peel/mesocarp thickness: 2.0-3.0 mm, brix percentage: 28-29%, number of tillers: 2-4), and quite similar on fruit length. The minimum variation among them were detected on plant height, diameter of pseudostem, leaf blade size and male bud size.

REFERENCES

- Ateş Sönmezoğlu, Ö., Bozmaz, B., Yildirim, A., Kandemir, N., & Aydın, N. (2012). Genetic characterization of Turkish bread wheat landraces based on microsatellite markers and morphological characters. *Turkish Journal of Biology*, 36, 589–597. <https://doi.org/10.3906/biy-1202-43>
- Butiuc-keul, A., Farkas, A., & Cristea, V. (2016). Genetic stability assessment of *in vitro* plants by molecular markers. *Studia Universitatis Babeş-Bolyai Biologia*, LXI(1), 107–114. Retrieved from http://studia.ubbcluj.ro/download/pdf/Biologia_pdf/2016_1/28.pdf
- Condello, E., Palombi, M. A., & Tonelli, M. G. (2008). Genetic stability of wild pear (*Pyrus pyraeaster*, Burgsd) after cryopreservation by encapsulation dehydration. *Agricultural and Food Science*, 18(2), 136–143. <https://doi.org/10.2137/145960609789267533>
- Creste, S., Benatti, T. R., Orsi, M. R., Risterucci, A.-M., & Figueira, A. (2006). Isolation and characterization of microsatellite loci from a commercial cultivar of *Musa acuminata*. *Molecular Ecology Notes*, 6(2), 303–306. <https://doi.org/10.1111/j.1471-8286.2005.01209.x>
- Cristea, V., Crăciunaş, C., Marcu, D., Palada, M., & Butiuc-Keul, A. (2014). Genetic stability during *in vitro* propagation of *Dianthus spiculifolius* Schur. *Propagation of Ornamental Plants*, 14(1), 26–31. Retrieved from http://www.journal-pop.org/2014_14_1_26-31.html
- Culley, T. M., Stamper, T. I., Stokes, R. L., Brzyski, J. R., Hardiman, N. A., Klooster, M. R., & Merritt, B. J. (2013). An efficient technique for primer de-

Puji Lestari et al.: Genetic Stability of Banana by Newly Developed SSR Markers

- velopment and application that integrates fluorescent labeling and multiplex PCR. *Applications in Plant Sciences*, 1(10), 1300027. <https://doi.org/10.3732/apps.1300027>
- D'Hont, A., Denoeud, F., Aury, J.-M., Baurens, F.-C., Carreel, F., Garsmeur, O., ... Wincker, P. (2012). The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. *Nature*, 488, 213–217. <https://doi.org/10.1038/nature11241>
- Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12, 13–15. Retrieved from <https://ci.nii.ac.jp/naid/20000864368>
- Elhory, S. M. A., Aziz, M. A., Rashid, A. A., & Yunus, A. G. (2009). Prolific plant regeneration through organogenesis from scapels of *Musa* sp cv. Tanduk. *African Journal of Biotechnology*, 8(22), 6208–6213. <https://doi.org/10.5897/AJB09.1078>
- FAO. (2003). *Chapter 1. Overview of world banana production and trade*. Retrieved from <http://www.fao.org/3/y5102e/y5102e04.htm#bm04>
- François-Xavier, C., Sandoval, J. A., Marie, P., & Au-boiron, E. (1993). Variations in micropropagated bananas and plantains: literature survey. *Fruits*, 48(1), 15–23. Retrieved from <http://agritrop.cirad.fr/397817/>
- Ge, C., Cui, Y.-N., Jing, P.-Y., & Hong, X.-Y. (2014). An alternative suite of universal primers for genotyping in multiplex PCR. *PLoS ONE*, 9(3), e92826. <https://doi.org/10.1371/journal.pone.0092826>
- Govindaraju, S., Saravanan, J., Jayanthi, B., Nancy, D., & Indra Arulselv, P. (2012). In vitro propagation of Banana (*Musa* sp - Rasthali variety) from sword suckers for its commercial production. *Research in Plant Biology*, 2(5), 01–06. Retrieved from <https://updatepublishing.com/journal/index.php/ripb/article/view/2533>
- Hapsari, L., & Lestari, D. A. (2016). Fruit characteristic and nutrient values of four Indonesian banana cultivars (*Musa* spp.) at different genomic groups. *AGRIVITA Journal of Agricultural Science*, 38(3), 303–311. <https://doi.org/10.17503/agrivita.v38i3.696>
- Hapsari, L., Kennedy, J., Lestari, D. A., Masrum, A., & Lestari, W. (2017). Ethnobotanical survey of bananas (Musaceae) in six districts of East Java, Indonesia. *Biodiversitas*, 18(1), 160–174. <https://doi.org/10.13057/biodiv/d180123>
- Hippolyte, I., Bakry, F., Seguin, M., Gardes, L., Rivalan, R., Risterucci, A.-M., ... Glaszmann, J.-C. (2010). A saturated SSR/DArT linkage map of *Musa acuminata* addressing genome rearrangements among bananas. *BMC Plant Biology*, 10, 65. <https://doi.org/10.1186/1471-2229-10-65>
- IPGRI. (1996). *Descriptors for banana (Musa spp.)*. Retrieved from https://www.biodiversityinternational.org/fileadmin/user_upload/online_library/publications/pdfs/326.pdf
- Izquierdo, H., González, M. C., & de la C NÚñez, M. (2014). Genetic stability of micropropagated banana plants (*Musa* spp.) with non-traditional growth regulators. *Biocnología Aplicada*, 31(1), 23–27. Retrieved from http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1027-28522014000100003
- James, E. A., Brown, G. K., Citroen, R., Rossetto, M., & Porter, C. (2011). Development of microsatellite loci in *Triglochin procera* (Juncaginaceae), a polyploid wetland plant. *Conservation Genetics Resources*, 3(1), 103–105. <https://doi.org/10.1007/s12686-010-9301-7>
- Jarda, L., Butiuc-Keul, A., Höhn, M., Pedryc, A., & Cristea, V. (2014). Ex situ conservation of *Dianthus giganteus* d'Urv. subsp. *banaticus* (Heuff.) Tutin by in vitro culture and assessment of somaclonal variability by molecular markers. *Turkish Journal of Biology*, 38, 21–30. <https://doi.org/10.3906/biy-1303-20>
- Kariyana, K., & Nisyawati. (2013). Effect of ascorbic acid, activated charcoal and light duration on shoot regeneration of banana cultivar barangan (*Musa acuminata* L.) in vitro culture. *International Journal of Recent Research and Applied Studies*, 16(1), 118–123. Retrieved from https://www.arpapress.com/Volumes/Vol16Issue1/IJR-RAS_16_1_13.pdf
- Khan, S., Saeed, B., & Kauser, N. (2011). Establishment of genetic fidelity of *in-vitro* raised banana plantlets. *Pakistan Journal of Botany*, 43(1), 233–242. Retrieved from [http://www.pakbs.org/pjbot/PDFs/43\(1\)/PJB43\(1\)233.pdf](http://www.pakbs.org/pjbot/PDFs/43(1)/PJB43(1)233.pdf)
- Kour, B., Kour, G., Kaul, S., & Dhar, M. K. (2014). In vitro mass multiplication and assessment of genetic stability of in vitro raised *Artemisia absinthium* L. plants using ISSR and SSAP molecular markers. *Advances in Botany*, 2014(727020), 1–7. <https://doi.org/10.1155/2014/727020>
- Krikorian, A. D., Irizarry, H., Cronauer-Mitra, S. S., & Rivera, E. (1993). Clonal fidelity and variation in plantain (*Musa AAB*) regenerated from vegetative stem and floral axis tips in vitro. *Annals of Botany*, 71(6), 519–535. <https://doi.org/10.1006/anbo.1993.1068>

Puji Lestari et al.: Genetic Stability of Banana by Newly Developed SSR Markers

- Lakshmanan, V., Venkataramareddy, S. R., & Neelwarne, B. (2007). Molecular analysis of genetic stability in long-term micropropagated shoots of banana using RAPD and ISSR markers. *Electronic Journal of Biotechnology*, 10(1), 106–113. <https://doi.org/10.2225/vol10-issue5-fulltext-12>
- Lisnandar, D. S., Fajarudin, A., Effendi, D., & Roostika, I. (2015). Organogenesis bunga aksis pisang bergenom AAB dan ABB. *Jurnal Hortikultura*, 25(1), 1–8. <https://doi.org/10.21082/jhort.v25n1.2015.p1-8>
- Martin, G., Baurens, F.-C., Cardi, C., Aury, J.-M., & D'Hont, A. (2013). The complete chloroplast genome of banana (*Musa acuminata*, Zingiberales): Insight into plastid monocotyledon evolution. *PLOS ONE*, 8(6), e67350. <https://doi.org/10.1371/journal.pone.0067350>
- Martin, K. P. (2005). Cost effective in vitro propagation of *Musa ornata* Roxb. through floral tip axis segment culture. *Propagation of Ornamental Plants*, 5(2), 84–88. Retrieved from [https://www.journal-pop.org/References/Vol_5_2\(84-88\).pdf](https://www.journal-pop.org/References/Vol_5_2(84-88).pdf)
- Missiaggia, A., & Grattapaglia, D. (2006). Plant microsatellite genotyping with 4-color fluorescent detection using multiple-tailed primers. *Genetics and Molecular Research*, 5(1), 72–78. Retrieved from http://www.funpecrp.com.br/gmr/year2006/vol1-5/gmr0192_abstract.htm
- Muhammad, A., Rashid, H., Hussain, I., & Naqvi, S. M. S. (2007). Proliferation-rate effects of BAP and kinetin on banana (*Musa* spp. AAA group) "Basrai." *HortScience*, 42(5), 1253–1255. <https://doi.org/10.21273/HORTSCI.42.5.1253>
- Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America*, 70(12 Pt 1-2), 3321–3323. <https://doi.org/10.1073/pnas.70.12.3321>
- Panis, B. (2009). Cryopreservation of *Musa* germplasm. (F. Engelmann & E. Benson, Eds.), *Technical Guidelines No. 9* (2nd ed.). Montpellier, France: Bioversity International. Retrieved from https://www.bioversityinternational.org/fileadmin/_migrated/uploads/tx_news/Cryopreservation_of_Musa_germplasm__2nd_edition_1383.pdf
- Poerba, Y. S., Imelda, M., & Martanti, D. (2012). Analisa kestabilan genetik pisang kepok "unti sayang" hasil mikroprogasi dengan marka RAPD dan ISSR. *Berita Biologi*, 11(2), 275–282. Retrieved from http://e-journal.biologi.lipi.go.id/index.php/berita_biologi/article/view/497
- Procházková, D., Boušová, I., & Wilhelmová, N. (2011). Antioxidant and prooxidant properties of flavonoids. *Fitoterapia*, 82(4), 513–523. <https://doi.org/10.1016/j.fitote.2011.01.018>
- Ravishankar, K. V., Vidhya, L., Cyriac, A., Rekha, A., Goel, R., Singh, N. K., & Sharma, T. R. (2012). Development of SSR markers based on a survey of genomic sequences and their molecular analysis in banana (*Musa* spp.). *The Journal of Horticultural Science and Biotechnology*, 87(1), 84–88. <https://doi.org/10.1080/14620316.2012.11512835>
- Ravishankar, K. V., Raghavendra, K. P., Athani, V., Rekha, A., Sudeepa, K., Bhavya, D., ... Ananad, L. (2013). Development and characterisation of microsatellite markers for wild banana (*Musa balbisiana*). *The Journal of Horticultural Science and Biotechnology*, 88(5), 605–609. <https://doi.org/10.1080/14620316.2013.11513013>
- Ray, T., Dutta, I., Saha, P., Das, S., & Roy, S. C. (2006). Genetic stability of three economically important micropropagated banana (*Musa* spp.) cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers. *Plant Cell, Tissue and Organ Culture*, 85(1), 11–21. <https://doi.org/10.1007/s11240-005-9044-4>
- Resmi, L., & Nair, A. S. (2011). Differential effect of cytokinins in the micropropagation of diploid and triploid *Musa* cultivars. *International Journal of Integrative Biology*, 11(1), 35–38. Retrieved from <https://www.cabdirect.org/cabdirect/abstract/20113146632>
- Rohlf, F. J. (2000). *NTSYSpc: Numerical taxonomy and multivariate analysis system, Version 2.1 (Guide user)*. Setauket, NY, USA: Applied Biostatistics Inc. Retrieved from <https://www.exetersoftware.com/downloads/ntsysguide21.pdf>
- Rotchanapreeda, T., Wongniam, S., Swangpol, S. C., Chareonsap, P. P., Sukkaewmanee, N., & Soimana, J. (2016). Development of SSR markers from *Musa balbisiana* for genetic diversity analysis among Thai bananas. *Plant Systematics and Evolution*, 302(7), 739–761. <https://doi.org/10.1007/s00606-015-1274-2>
- Roy, O. S., Bantawa, P., Ghosh, S. K., da Silva, J. A. T., DebGhosh, P., & Mondal, T. K. (2010). Micropropagation and field performance of 'Malbhog' (*Musa paradisiaca*, AAB group): A popular banana cultivar with high keeping quality of North East India. *Tree and Forestry Science and Biotechnology*, 4(Special Issue 1), 52–58. Retrieved from [http://www.globalsciencebooks.info/Online/GSBOnline/images/2010/TFSB_4\(S11\)/TFSB_4\(S11\)52-58o.pdf](http://www.globalsciencebooks.info/Online/GSBOnline/images/2010/TFSB_4(S11)/TFSB_4(S11)52-58o.pdf)

Puji Lestari et al.: Genetic Stability of Banana by Newly Developed SSR Markers

- Rozen, S., & Skaletsky, H. (2000). Primer3 on the WWW for general users and for biologist programmers. In S. Misener & S. A. Krawetz (Eds.), *Bioinformatics Methods and Protocols. Methods in Molecular Biology™*, vol 132 (pp. 365–386). Totowa, NJ: Humana Press. <https://doi.org/10.1385/1-59259-192-2:365>
- Sato, Y. (2009). *Ethnobotanical study of local practices maintaining landrace diversity of bananas (Musa spp.) and enset (Ensete ventricosum) in East African Highland* (G-COE Series No. 59). Kyoto. Retrieved from <http://hdl.handle.net/2433/155765>
- Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*, 18, 233–234. <https://doi.org/10.1038/72708>
- Shankar, C. S., Balaji, P., & Sekar, D. S. (2014). Mass propagation of banana (*Musa* sp.) cv. grand naine through direct organogenesis by benzyl adenine purine and kinetin. *Journal of Academia and Industrial Research*, 3(2), 92–97. Retrieved from <http://jairjp.com/JULY 2014/07 SHIV SHANKAR.pdf>
- Silvarolla, M. B., Mazzafera, P., & de Lima, M. M. A. (2000). Caffeine content of Ethiopian coffee arabica beans. *Genetics and Molecular Biology*, 23(1), 213–215. <https://doi.org/10.1590/S1415-47572000000100036>
- Sultan, M. T., Khan, M. H., Hakim, L., Mamun, A., Morshed, A., Islam, R. M., & Islam, R. M. (2011). In vitro plant regeneration from male flowers of banana. *International Journal of Biosciences (IJB)*, 1(1), 1–11. Retrieved from https://www.researchgate.net/publication/233943404_In_Vitro_plant_regeneration_from_male_flowers_of_Banana
- Tiwari, J. K., Chandel, P., Gupta, S., Gopal, J., Singh, B. P., & Bhardwaj, V. (2013). Analysis of genetic stability of in vitro propagated potato microtubers using DNA markers. *Physiology and Molecular Biology of Plants*, 19(4), 587–595. <https://doi.org/10.1007/s12298-013-0190-6>
- Venkataramana, R. K., Sampangi-Ramaiah, M. H., Ajitha, R., Khadke, G. N., & Chellam, V. (2015). Insights into *Musa balbisiana* and *Musa acuminata* species divergence and development of genic microsatellites by transcriptomics approach. *Plant Gene*, 4, 78–82. <https://doi.org/10.1016/j.plgene.2015.09.007>
- Vincent, L., & Anushma, P. L. (2018). Micropropagation in banana using inflorescence: A review. *Journal of Cell and Tissue Research*, 18(3), 6573–6582. Retrieved from https://tcjournals.com/uploads/7308810._Vinicent.pdf
- Wang, J. Y., Zheng, L. S., Huang, B. Z., Liu, W. L., & Wu, Y. T. (2010). Development, characterization, and variability analysis of microsatellites from a commercial cultivar of *Musa acuminata*. *Genetic Resources and Crop Evolution*, 57(4), 553–563. <https://doi.org/10.1007/s10722-009-9493-4>
- Yu, J., Holland, J. B., McMullen, M. D., & Buckler, E. S. (2008). Genetic design and statistical power of nested association mapping in maize. *Genetics*, 178(1), 539–551. <https://doi.org/10.1534/genetics.107.074245>
- Zerihun, D., Vashist, U., & Boora, K. S. (2009). Molecular characterization of citrus cultivars using DNA markers. *International Journal of Biotechnology and Biochemistry*, 5(3), 271–280. Retrieved from https://www.academia.edu/27333642/Molecular_Characterization_of_Citrus_Cultivars_Using_DNA_Markers?auto=download