



**CENTRAL LUZON STATE UNIVERSITY**



**ESTABLISHMENT OF SIMPLE SEQUENCE REPEAT (SSR) MARKER  
SYSTEM FOR PURITY TESTING OF SELECTED  
THREE-LINE HYBRIDS**

**JENNA MAE CANDELARIA BIENDIMA**

An Undergraduate Thesis Submitted to the Faculty of the Department of  
Biological Sciences, College of Arts and Sciences, Central Luzon  
State University, Science City of Muñoz, Nueva Ecija,  
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Requirements for the Degree

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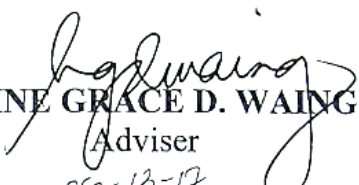
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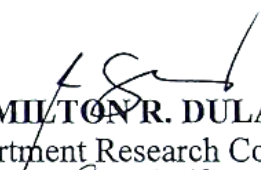
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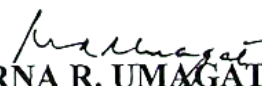
  
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ABSTRACT

**BIENDIMA, JENNA MAE CANDELARIA**, Bachelor of Science in Biology, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, June 2017.  
**ESTABLISHMENT OF SIMPLE SEQUENCE REPEAT (SSR) MARKER SYSTEM FOR PURITY TESTING OF SELECTED THREE-LINE HYBRIDS.**

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Genetic purity assessment is very crucial for varietal identification. To improve these process molecular markers can provide an advance tool for accurate and precise identification. Establishing DNA fingerprinting is essential for testing the genetic purity in order to produce good quality seeds for plant breeders and before it was hand to farmers. This study was conducted to establish unique SSR markers for three-line hybrids, Mestiso 29, Mestiso 48, Mestiso 55, and PR47216H, and to detect the impurities by grow-out test DNA fingerprinting.

A rice panel was assembled which includes the four hybrids, their corresponding A line, B line, and R line, and three inbred varieties, PSB Rc18, PSB Rc82, and NSIC Rc222. DNA was extracted from the panel and the obtained DNA from the samples were used for downstream analyses. Polymorphism survey was conducted and four out of 200 SSR markers evaluated were found to show heterozygous banding patterns for the F<sub>1</sub>



which were distinct from their corresponding parent lines and inbred varieties.  $F_1$  seeds for each hybrid were used for standard grow-out test. Pre-determined off-types from the corresponding A line, R line, and inbred varieties from each hybrid were incorporated and replaced selected  $F_1$  plants from the field. Using morphological characters, the pre-determined off-types were distinguished from the  $F_1$  plants. However, additional off-types were not detected. Using the established SSR markers from the polymorphism survey, the samples from the grow-out test were subjected to molecular genotyping. Pre-determined off-types were distinguished based on their banding patterns and additional off-types were detected. Using RM6100, 5.19% were additionally detected to be off-types for Mestizo 29. For Mestizo 48, additional 10.51% were detected using RM574. For Mestizo 55, 9.86% were detected as additional contaminants using RM216. Lastly, for PR47216H, genotyping using RM440 detected 4.50% additional contaminants. The result showed that the established SSR markers were useful in detecting off-types. Utilization of these markers for genetic purity assessment of hybrids at the production level will help in the production of pure seeds and also for the farmers that depend on hybrid seeds.



LITERATURE CITED

- AGRI INFO.** (2015). Hybrid rice breeding and seed production. Date retrieved: 05-30-16. Available online: [http://www.knowledgebank.irri.org/ricebreedingcourse/Hybrid\\_Rice\\_Breeding\\_&\\_Seed\\_Production.htm](http://www.knowledgebank.irri.org/ricebreedingcourse/Hybrid_Rice_Breeding_&_Seed_Production.htm).
- AKHTAR, S., BHAT, M.A., BHAT, K.A., CHALKOO, S., MIR, M.R. and S.A. WANI.** (2010). Marker assisted selection in rice. *Journal of Phytology*, 2(10): 66-81.
- ALAM, M.S., ISLAM, M.M. and S.N. BEGUM.** (2015). Grow-out-test and determination of physical characteristics of seed of rice landraces. *International Journal Agricultural Research Innovation and Technology*, 5 (1): 15-21.
- BARLEY GROUP.** (2011). Lecture of gene sequence marker. Date retrieved: 05/30/16. Available online: <http://barleygrup/brigid/430/lectures/genseqencemarker/html>.
- BARTLETT, J. and D. STIRLING.** (2003). A short history of the polymerase chain reaction. *Methods in Molecular Biology*, 226: 3-6.
- BHAVSAR, S., SOLANKI, T., AMIN, S. and N. JAIN.** (2015). Assessment of genetic purity of parental lines of hybrid rice using dna-based markers. *Online Journal of Biological Sciences*, 15 (2): 59.69.
- BORA, A., CHOUDHURY, P., PANDE, V. and A. MANDALL.** (2016). Assessment of genetic purity in rice (*Oryza sativa* L.) hybrids using microsatellite markers. *Springer*, 6:50.
- CAMBRIDGE UNIVERSITY PRESS.** (2016). Seed quality testing and certification: resources useful in organic seed production. DR: 06-14-16. AO: Seed Quality Testing and Certification\_ Resource Useful in Organic Seed Production.
- CHAUHAN, A.K. and A. VARMA.** (2009). A textbook of molecular biotechnology. New Delhi: I.K. International Pub. House.
- CHAKRABORTHY, S., VHORA, Z., TRIVEDI, R., RAVIKIRAN, R. and N. SASIDHARAN.** (2013). Molecular studies of aromatic and non-aromatic rice genotypes for quality traits using microsatellite markers. *Research Gate*, 8: 359-362.
- CHU, W.** (2013). The variety and process of production of hybrid rice in the province of iloilo. Retrieved from: <https://decomvergence.wordpress.com/tag/mestizo-rice/>



- CRUZ, I., LEON, J., RIGOR, A., BALDEDARA, L., GRAMAJE, L., ROSARIO, C., DEL, D., ABLAZA, M., CARAMPATANA, J. and D. TABANAO.** (2015). Mestiso 29 (NSIC Rc244H), a PhilRice-bred three-line hybrid rice variety (*Oryza sativa* L.) for irrigated lowland ecosystem. *Philippines Journal of Crop Science*, 39(2): 75-80.
- DANIEL, I., ADETUMBI, J., OELAKIN, O., OLAKOJO, A., AJALA, O. and S. ONAGBEAN.** (2012). Application of SSR markers for genetic purity analysis of parental inbred lines and some commercial hybrid maize (*Zea mays* L.). *Research Gate*, 2:597-606.
- DESHMUKH, U., SAXENA, R., XALXO, M., SHARMA, D. and S. VERULKAR.** (2013). Hybrid purity testing in rice (*Oryza sativa* L.) using microsatellite markers. *Electronic Journal of Plant Breeding*, 4(1): 1021-1026.
- DONGRE, A., RAUT, M., BHANDARKAR, M. and K. MESHRAM.** (2011). Identification and genetic purity testing of cotton F<sub>1</sub> hybrid using molecular markers. *Indian Journal Biotechnology*, 10: 301-306.
- FAO.** (2009). Hybrid rice for food security. Date retrieved: 05-30-16. Available online: [www.rice2009.org](http://www.rice2009.org).
- GALAL, O., ABO-YOUSESF, M., ABDELAZIZ, M., GHARIB, A. and S. DORAL.** (2014). Assessment of genetic purity of some hybrid rice parental lines using protein profile and fertility restorer gene linked markers. *International Journal of Biotechnology Research*, 2(6): 075-088.
- GIBBS, R.A.** (1990) DNA amplification by the Polymerase Chain Reaction. *Analytical Chemistry*, 62:12.
- GUMASI, F., ELFALLEH, W., HANNAHCHI, H., F'ERES, K., TOUIL, L., MARZOUGUI, N., TRIKI, T. and A. FERCHICHI.** (2011). The use of SSR and RAPD markers for genetic diversity among South Tunisian Barley. Available online: <http://www.hindawi.com/journals/isrn/2012/952196/>
- HOSSAIN, M.** (1996). Economic prosperity in Asia: implications for rice research. In: Khush GS (ed) *Rice Genetics III, Proceedings of the Third International Rice Genetics Symposium*, Los Baños, Manila, 16–20 Oct 1995. International Rice Research Institute, Philippines, 3–16.
- IRRI.** (2011). Seed quality. Date retrieved: 06-14-16 Available Online: [Seed quality-IRRI Rice Knowledge Bank.html](http://www.irri.org/seed-quality/).



- IRRI.** (2014). Hybrid rice. Date retrived: 05-30-16. Available online: <http://irri.org/our-work/research/better-rice-varieties/hybrid-rice>.
- ISLAM, M., HOQUE, M., RABBI, S. and M. ALL.** (2011). DNA fingerprinting and diversity analysis of BIRRI hybrid varieties and their corresponding parents. *Plant Tissue Culture And Biotech*, 21(2): 189 - 198.
- JOSHI, M. and J.D. DESHPANDE.** (2011). Polymerase Chain Reaction: methods, principles and application. *International Journal of Biomedical Research*, 2(1) 81-97.
- JONAH, P.M., BELLO, L.L., LUCKY, O., MIDAU, A. and S.M. MORUPPA.** (2011). The importance of molecular markers in plant breeding programmes. *Global Journal of Science Frontier Research*, 6(5): 5-12.
- KATAGI, A., PATIL, R. and P. PARAMAGOUDAR.** (2013). Tools for genetic purity testing of horticultural crops: a review. *HortFlora Res. Spectrum*, 3 (2): 108-113.
- KUMAR, M., VISHWANATH, K., SHIVAKUMAR, N., RAJENDRA, S. and B. RADHA.** (2012). Utilization of SSR markers for seed purity testing in popular rice hybrids (*Oryza sativa* L.). *Annals of Plant Science*, 1(1): 1-5.
- MA, H., YU, Y., ZHI-FU, G., LI-JING, C., LI, Z., MING Z. and S. GUO-JIN.** (2011). Establishment of DNA Fingerprinting of Liaojing Series of *japonica* rice. *Middle-East Journal of Scientific Research*, 8 (2):384-392.
- MAO, C., VIRMANI, S. and I. KUMAR.** (1996). technological innovation to lower the costs of hybrid rice seed production. *rice research*. ISBN: 971-22-011: 111-128.
- MOORTHY, K., BABU, P., SREEDAR, M., SAMA, V., KUMAR, P., BALANCHANDRAN, S. and R. SUNDARAM.** (2011). Identification of informative EST-SSR markers capable of distinguishing popular indian rice varieties and their utilization in seed genetic purity assessment. *Euphytica*, 39(2):282-292.
- MURRAY, M.G. and W.F. THOMPSON.** (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acid Res.*, 8(19): 4321-4325.
- NARSHIMULU, G., JAMALODDIN, M., VEMIREDDY, L.R., ANURDAHA, G. and E. SIDDIQ.** (2011). Potentiality of evenly distributed hypervariable microsatellite markers in marker assisted breeding of rice. *Plant Breeding*, 130: 314-320.



- NSIC.** (2013). National seed industry council crop varieties catalogue. Available online: [www.nseedcouncil.bpinsic.pvpo.ph/seedcatalogue.php](http://www.nseedcouncil.bpinsic.pvpo.ph/seedcatalogue.php).
- OREGON STATE UNIVERSITY.** (2016). Molecular markers. Date retrieved: 05-30-16. Available online: <http://oregonstate.edu/ua/ncs/archives/2014>.
- OXFORD GENE TECHNOLOGY.** (2011). Understanding and measuring variations in DNA sample quality. Date retrieved: 07-09-17. Available online: <http://oxfordgenetechnology.com/UnderstandingandmeasuringvariationsinDNAsamplequality/2017>.
- PETROUDI, S., MAIBODYL, S., NEMATZADEH, G. and A. ARZNIL.** (2010). Semi-random PCR markers for DNA fingerprinting of rice hybrids and their corresponding parents. *African Journal of Biotechnology*, 9(7): 979-985.
- PEREZ, L., PASTOR, H., DOMINGO, J., MANANGHAYA, T., TABANAO, D. and N. MANIGBAS.** (2012). Detecting hybrid admixtures through DNA analysis. *Crop Science*, 37 (1):42-46.
- RAHMAN, M., SOHAG, M. and L. RAHMAN.** (2010). Microsatellite based DNA fingerprinting of 28 local rice (*Oryza sativa* L.) varieties of Bangladesh. *J. Bangladesh Agricultural University*, 8(1): 7-17.
- RANJEKAR, K., JOSHI, S. and V. GUPTA.** (2000). Molecular markers in plant genome analysis. Plant molecular biology group. Retrieved at: [www.iisc.ernet.in/cursci/jul25/article15.htm](http://www.iisc.ernet.in/cursci/jul25/article15.htm).
- REDDY, V.R.P.** (2014). Hybrid and varietal genetic purity testing methods for crop Improvement. *International Journal of Applied Biology and Pharmaceutical Technology*, 5(4):197-197. Retrieved at: <http://www.ijabpt.org/abstract/hybrid-and-varietal-genetic-purity-testing-methods-for-croprniprovement-4312.htm>.
- ROYCHOWDHURY, R., KARMAKAR, J. and N. DEY.** (2012). PCR-compatible genomic DNA isolation from different tissues of rice (*Oryza sativa*) for SSR fingerprinting. *Europe Asian Journal of Biological Sciences*, 6: 85-90.
- SENAN, S., KIZHAKAYIL, D., SASIKUMAR, B. and T. SHEEJA.** (2014). Methods for development of microsatellite markers: an overview. *Notoule Science Biology*, 6(1):1-13.
- SHEHATA, A., AL-GHETAR, H. and A. AL-HOMAIDAN.** (2009). Application of simple sequence repeat (SSR) markers for molecular diversity and heterozygosity analysis in maize inbred lines. *Saudi Journal of Biological Sciences*, 16:57-62.



- SUNDARAM, R.M., NAVEENKUMAR, B., BIRADAR, S.K., BALACHANDRAN, S.M., MISHRA, B., ILYAS AHMED, M., VIRAKTAMATH, B.C., RAMESHA, M.S. and N.P. SARMA.** (2008). Identification of informative SSR markers capable of distinguishing hybrid rice parental lines and their utilization in seed purity assessment. *Euphytica* 163:215–224
- SINGH, A. and R.S. SENGAR.** (2015). DNA fingerprinting based decoding of *indica* rice (*Oryza sativa* L.) via molecular marker (SSR, ISSR, & RAPD) in aerobic condition. *Advance Crop Science Technology*, 3:167.
- TAMILKUMAR, P., JERLIN, N., SENTHIL, N., GANESAN, K., JEEVAN, R. and M. RAVEENDRAN.** (2009). Fingerprinting of rice hybrids and their parental lines using microsatellite markers and their utilization in genetic purity assessment of hybrid rice. *Research Journal of Seed Science*, 2(3): 40-47.
- VARSHNEY, R., HOISINGTON, S. and A. GRANER.** (2011). Molecular plant breeding: methodology and achievements. *Methods Molecular Biology*. 513: 1-22.
- XIE, F. and B. HARDY.** (2009). Accelerating hybrid rice development. Copyright International Rice Research Institute. ISBN 978-971-22-0252-0.
- YASHIOTOLA, J., THIRUMURUNGAN, T., SUNDAAM, R.M., NASEERULLAH, M.K., RAMESH, M.S., SARMA, N.P. and R.V. SONTI.** (2002). Assessment of purity of rice hybrids using microsatellite and STS markers. *Crop Science*, 42: 1369-1373.
- ZHANG, C., PENG, B., ZHANG, W., WANG, S., SUN, H., DONG, Y. and L. ZHAO.** (2014). Application of SSR markers for purity testing of commercial hybrid soybean (*Glycine max* L.). *Journal Agriculture Sciences Technology*, 16: 1389-1396.