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Optimizing reproducibility evaluation for random amplified polymorphic DNA markers

J.R. Ramos[1], M.P.C. Telles[1,2], J.A.F. Diniz-Filho[3], T.N. Soares[2,4], D.B. Melo[2,4] and G. Oliveira[3]

[1]Mestrado em Genética, Universidade Católica de Goiás, Goiânia, GO, Brasil
[2]Departamento de Biologia Geral, Laboratório de Genética and Biodiversidade, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, GO, Brasil
[3]Departamento de Biologia Geral, Laboratório de Ecologia Teórica e Síntese, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, GO, Brasil
[4]Programa de Pós-graduação em Agronomia, Universidade Federal de Goiás, Goiânia, GO, Brasil

Corresponding author: M.P.C. Telles
E-mail: tellesmpc@gmail.com

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ABSTRACT. The random amplified polymorphic DNA (RAPD) technique is often criticized because it usually shows low levels of repeatability; thus it can generate spurious bands. These problems can be partially overcome by rigid laboratory protocols and by performing repeatability tests. However, because it is expensive and time-consuming to obtain genetic data twice for all individuals, a few randomly chosen individuals are usually selected for *a priori* repeatability analysis, introducing a potential bias in genetic parameter estimates. We developed a procedure to optimize repeatability analysis based on RAPD data, which was applied to evaluate genetic variability in three local populations of *Tibochina papyrus*, an endemic Cerrado plant found in elevated rocky fields in Brazil. We used a simulated annealing procedure to select the smallest number of individuals that contain all bands and repeated the analyses only

for those bands that were reproduced in these individuals. We compared genetic parameter estimates using HICKORY and POPGENE softwares on an unreduced data set and on data sets in which we eliminated bands based on repeatability of individuals selected by simulated annealing and based on three randomly selected individuals. Genetic parameter estimates were very similar when we used the optimization procedure to reduce the number of bands analyzed, but as expected, selecting only three individuals to evaluate the repeatability of bands produced very different estimates. We conclude that the problems of repeatability attributed to RAPD markers could be due to bias in the selection of loci and primers and not necessarily to the RAPD technique *per se*.

Key words: Random amplified polymorphic DNA; Repeatability; Optimization; Simulated annealing; Band selection; Primer selection

