Inter-retrotransposon-amplified polymorphism markers for germplasm characterization in *Manihot esculenta* (Euphorbiaceae)

A.M. Oliveira-Silva¹, G.F. Silva², M.C. Dias², C.R. Clement³ and N.R. Sousa²

¹Programa de Pós-Graduação em Biotecnologia, Universidade Federal do Amazonas, Manaus, AM, Brasil
²Laboratório de Biologia Molecular, Embrapa Amazônia Ocidental, Manaus, AM, Brasil
³Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brasil

Corresponding author: N.R. Sousa
E-mail: nelcimar.sousa@embrapa.br

Received February 13, 2013
Accepted July 1, 2013
Published May 16, 2014
DOI http://dx.doi.org/10.4238/2014.May.16.3

**ABSTRACT.** Manioc, *Manihot esculenta*, is economically important in many tropical and subtropical countries. The genetic variability of the species has not been fully explored, and new information may help expand its use. Molecular markers based on retrotransposons have good potential for analysis of genetic diversity given their abundance in the genome. Eight long terminal repeat retrotransposons were selected for the development of inter-retrotransposon-amplified polymorphism markers. To test these primers, we analyzed 32 varieties from Anori, 30 from Manicoré and 10 Mandiocabas from the Manioc Germplasm Bank at Embrapa
Development of IRAP markers for manioc Western Amazonia. The six informative primer pairs yielded 20-60 polymorphic bands, averaging 92% polymorphism (51.7-98.4) and 0.37 heterozygosity (0.17 to 0.40), with a Shannon information index of 0.54 (0.26-0.59). These markers can be used to explore the genetic diversity of manioc.

Key words: Inter-retrotransposon-amplified polymorphism; Polymorphism; Genetic diversity; Varietal discrimination

INTRODUCTION

Manioc (Manihot esculenta Crantz) is a perennial shrub in the Euphorbiaceae that is a major carbohydrate crop in tropical and subtropical countries, where it is mainly used for the production of flour, pure starch, fresh consumption, and assorted industrial uses. Although propagated vegetatively, manioc has great genetic variability because sexual reproduction continues, often resulting in polyclonal varieties (Silva et al., 2001). These local varieties, grown mostly by small-holder farmers, represent the genetic resources conserved and used in breeding programs.

Molecular markers are used to increase the discriminatory power of genetic variability analyses among manioc varieties. Although there are numerous published markers, there is still a need for new and more variable genetic markers, given the polyclonal nature of manioc varieties. Markers based on retrotransposons (IRAP - inter-retrotransposon-amplified polymorphism) generate great quantities of information, making them good tools for detecting genomic changes associated with their activity, because they create large and stable insertions in the genome; they are highly reproducible, show abundant polymorphism, and are easily viewed in a single gel (Kalendar et al., 2011). Retrotransposon polymorphisms are detected using marker systems that rely on PCR amplification between long terminal repeat (LTR) ends and some components of flanking genomic DNA. The IRAP products are generated with one or two primers matching either the 5ꞌ- or 3ꞌ-end of the LTR using outward-facing primers (Kalendar and Schulman, 2006).

Several families of transposable elements have been reported in manioc (Gbadegesin et al., 2008). This study describes the development of eight sets of IRAP primers based on retrotransposons to discriminate between manioc clones and varieties.

MATERIAL AND METHODS

Sequences of LTR retrotransposons were located in Phytozome (http://www.phytozome.net/). LTRs were confirmed with the LTR_Finder software (http://tlife.fudan.edu.cn/ltr_finder/) and cross-checked in GenBank using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Eight LTR retrotransposons were selected and the outward-facing primers of the LTRs were designed using Primer3 (Rozen and Skaletsky, 2000) for amplification of the members of a retrotransposon family in head-to-head, head-to-tail and tail-to-tail orientation. Seventy-two manioc plants (32 from Anori, Amazonas, 30 from Manicoré, Amazonas, and 10 “mandiocabas”, a very sweet variety) maintained in the Manioc Germplasm Bank at Embrapa Western Amazônia, Manaus, Amazonas, were analyzed to test the information
content of these IRAP markers.

Total DNA was extracted with 2% CTAB in the Molecular Biology Laboratory at Embrapa. The 20-μL reaction mixture consisted of 0.2 mM dNTPs, 0.5 μM forward and reverse primers, 2 mM MgCl₂, 1X Taq buffer, 1.5 U Taq GoTaq® DNA polymerase (Promega, USA) and 50 ng template DNA. PCR was performed using the following program: 2 min at 92°C; 40 cycles of 15 s at 92°C, 1 min at 40-60°C (Table 1), 2 min at 72°C; and final extension at 72°C for 10 min. Amplifications were carried out in a Veriti Thermal Cycler (Applied Biosystems, USA). PCR products were resolved by electrophoresis on 1.5% agarose gels in 0.5X TBE buffer stained with ethidium bromide and photographed in a transilluminator (Loccus Biotecnologia, Brazil). Polymorphism was detected by the presence (1) or absence (0) of the PCR product. The percentage of polymorphic loci, expected heterozygosity (Nei’s genetic diversity) and Shannon information index were calculated with PopGene 1.31 (Yeh et al., 1999), for each population and overall.

RESULTS AND DISCUSSION

Two primer pairs (ME_1 and ME_3) produced only six bands and were excluded from further analysis. The others generated 20-60 polymorphic bands with sizes between 100 and 12,000 bp (Tables 1 and 2). With these primers, the two populations and mandiocaba samples showed a mean polymorphism of 92% (range = 51.7 to 98.4%), 0.37 expected heterozygosity (0.17 to 0.40) and a Shannon information index of 0.54 (0.26 to 0.59) (Table 2). Guo et al. (2006) found 86% polymorphism with IRAP and REMAP primers in persimmon (Diospyros kaki Thunb.), very similar to the results found here.

In manioc, 69% polymorphism was previously found with AFLP markers and 56% with RAPD markers in estimating the genetic variability of 54 varieties (Mühlen et al., 2000), demonstrating that these IRAP markers are more informative than other dominant markers.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5'-3')</th>
<th>Length (nt)</th>
<th>Ta (°C)</th>
<th>Amplicon size (bp)</th>
<th>Scaffold location</th>
<th>Phytozome v.9.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME_1</td>
<td>CTGCATTGAAGTTTGGTCCA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21</td>
<td>60</td>
<td>150-1500</td>
<td>00579:8924..18923</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TTTCCAGCTATTGCGGG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME_2</td>
<td>GGTGATGATGTCCCTTTC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20</td>
<td>49</td>
<td>200-4000</td>
<td>06700:127431..132763</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CTAGTGTATACCAATATGCC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME_3</td>
<td>TTCATCAAATGGGTCTCTCA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td>50</td>
<td>200-2000</td>
<td>03122:396999..406998</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACCCATTTCTCCAGTCGG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME_4</td>
<td>TGAGCCTTGGGGGCTTAAGG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td>48</td>
<td>100-5000</td>
<td>00077:42599..52000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TTGATTGCTTCTCTCTTCG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME_5</td>
<td>GCCGGGAGGGGAAAAAG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td>40</td>
<td>100-4000</td>
<td>03413:6000..16000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GCCCTTTCTTACCGGCTG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME_6</td>
<td>TTTTTATTTCATTTCATGCC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27</td>
<td>42</td>
<td>150-1800</td>
<td>09428:1..12686</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CCAAGTTATTGCTACATATTTCTC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME_7</td>
<td>TTCTGTATATCCGAGGGT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td>48</td>
<td>200-3000</td>
<td>01259:102333..122332</td>
<td></td>
</tr>
<tr>
<td>ME_8</td>
<td>GCTGGAATTTCGTATTGGA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21</td>
<td>56</td>
<td>100-12000</td>
<td>03481:28000..36000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CCAAGAATGATTGGTTGAA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Primer designed from 5'-LTR end; <sup>b</sup>primer designed from 3'-LTR end; Ta = annealing temperature; nt = nucleotide.
Development of IRAP markers for manioc

CONCLUSIONS

This is the first report of IRAP markers to characterize manioc varieties. They proved to be efficient in estimating the percentage of polymorphism and genetic diversity, and will likely permit good variety discrimination.

ACKNOWLEDGMENTS

Research supported by EMBRAPA (Project #0106010071406; Manioc Regional Germplasm Bank), the Foundation of the State of Amazonas (FAPEAM; Master’s scholarship to A.M. Oliveira-Silva) and the National Research Council (CNPq; Research fellowship to C.R. Clement). We thank Doriane P. Rodrigues, Federal University of Amazonas, for help with the analysis.

REFERENCES


Table 2. Genetic information of each IRAP primer pair in 72 Manihot esculenta varieties in the Manioc Germplasm Bank at Embrapa Western Amazonia.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Samples</th>
<th>No. of plants</th>
<th>No. polymorphic bands</th>
<th>h ± SD</th>
<th>I ± SD</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME_2</td>
<td>Anori</td>
<td>32</td>
<td>25</td>
<td>0.38 ± 0.17</td>
<td>0.55 ± 0.23</td>
<td>89.3</td>
</tr>
<tr>
<td></td>
<td>Manicoré</td>
<td>30</td>
<td>24</td>
<td>0.32 ± 0.18</td>
<td>0.47 ± 0.24</td>
<td>85.7</td>
</tr>
<tr>
<td></td>
<td>Mandiocaba</td>
<td>10</td>
<td>19</td>
<td>0.26 ± 0.20</td>
<td>0.38 ± 0.28</td>
<td>67.8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>72</td>
<td>25</td>
<td>0.36 ± 0.16</td>
<td>0.52 ± 0.22</td>
<td>89.2</td>
</tr>
<tr>
<td>ME_4</td>
<td>Anori</td>
<td>32</td>
<td>26</td>
<td>0.30 ± 0.18</td>
<td>0.45 ± 0.24</td>
<td>89.6</td>
</tr>
<tr>
<td></td>
<td>Manicoré</td>
<td>30</td>
<td>24</td>
<td>0.30 ± 0.19</td>
<td>0.44 ± 0.26</td>
<td>82.7</td>
</tr>
<tr>
<td></td>
<td>Mandiocaba</td>
<td>10</td>
<td>15</td>
<td>0.17 ± 0.19</td>
<td>0.26 ± 0.27</td>
<td>51.7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>72</td>
<td>26</td>
<td>0.32 ± 0.18</td>
<td>0.47 ± 0.26</td>
<td>89.6</td>
</tr>
<tr>
<td>ME_5</td>
<td>Anori</td>
<td>32</td>
<td>33</td>
<td>0.34 ± 0.16</td>
<td>0.50 ± 0.22</td>
<td>89.2</td>
</tr>
<tr>
<td></td>
<td>Manicoré</td>
<td>30</td>
<td>32</td>
<td>0.31 ± 0.17</td>
<td>0.46 ± 0.24</td>
<td>86.5</td>
</tr>
<tr>
<td></td>
<td>Mandiocaba</td>
<td>10</td>
<td>24</td>
<td>0.25 ± 0.21</td>
<td>0.37 ± 0.30</td>
<td>64.8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>72</td>
<td>33</td>
<td>0.36 ± 0.15</td>
<td>0.52 ± 0.21</td>
<td>89.2</td>
</tr>
<tr>
<td>ME_6</td>
<td>Anori</td>
<td>32</td>
<td>21</td>
<td>0.36 ± 0.17</td>
<td>0.52 ± 0.23</td>
<td>87.5</td>
</tr>
<tr>
<td></td>
<td>Manicoré</td>
<td>30</td>
<td>22</td>
<td>0.34 ± 0.18</td>
<td>0.50 ± 0.24</td>
<td>91.6</td>
</tr>
<tr>
<td></td>
<td>Mandiocaba</td>
<td>10</td>
<td>18</td>
<td>0.27 ± 0.19</td>
<td>0.41 ± 0.27</td>
<td>75.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>72</td>
<td>22</td>
<td>0.39 ± 0.17</td>
<td>0.55 ± 0.22</td>
<td>91.6</td>
</tr>
<tr>
<td>ME_7</td>
<td>Anori</td>
<td>32</td>
<td>19</td>
<td>0.30 ± 0.17</td>
<td>0.45 ± 0.24</td>
<td>82.6</td>
</tr>
<tr>
<td></td>
<td>Manicoré</td>
<td>30</td>
<td>19</td>
<td>0.31 ± 0.19</td>
<td>0.45 ± 0.26</td>
<td>82.6</td>
</tr>
<tr>
<td></td>
<td>Mandiocaba</td>
<td>10</td>
<td>13</td>
<td>0.23 ± 0.22</td>
<td>0.33 ± 0.31</td>
<td>56.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>72</td>
<td>20</td>
<td>0.32 ± 0.18</td>
<td>0.48 ± 0.24</td>
<td>90.0</td>
</tr>
<tr>
<td>ME_8</td>
<td>Anori</td>
<td>32</td>
<td>59</td>
<td>0.39 ± 0.12</td>
<td>0.57 ± 0.15</td>
<td>96.7</td>
</tr>
<tr>
<td></td>
<td>Manicoré</td>
<td>30</td>
<td>60</td>
<td>0.40 ± 0.11</td>
<td>0.59 ± 0.14</td>
<td>98.4</td>
</tr>
<tr>
<td></td>
<td>Mandiocaba</td>
<td>10</td>
<td>52</td>
<td>0.31 ± 0.17</td>
<td>0.46 ± 0.23</td>
<td>85.2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>72</td>
<td>60</td>
<td>0.42 ± 0.09</td>
<td>0.61 ± 0.11</td>
<td>98.4</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>0.37 ± 0.15</td>
<td>0.54 ± 0.20</td>
<td>92.0</td>
</tr>
</tbody>
</table>

h = Nei’s genetic diversity ± standard deviation (SD); I = Shannon information index ± SD; % = percentage of polymorphic loci.


