DNA MICROSATELLITES FOR GENETIC IDENTIFICATION IN BRAZILIAN MURRAH WATER BUFFALOES

[Uso de Microsatélites para identificação genética de búfalos brasileiros da raça Murrah]

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ABSTRACT - Brazilian water buffalo (Bubalus bubalis) population is currently approximately 3,000,000. Despite of this fact, genealogical control is still one of the problems of the Brazilian selection and breeding programs. The DNA test is important to develop a system that allows the animal genealogy certification as well as its undeniable individual and parentage identification. The present study was performed by using a panel of 14 microsatellites markers in Brazilian Murrah buffaloes (n=100) in order to estimate the genetic variability and calculate the parentage exclusion probability. A total of 92 alleles were detected in the whole sample and the number of alleles varied from one (locus D5S2) to 13 (locus CSSM47). The Polymorphism Information Content values ranged from 0.00 (locus D5S2) to 0.845 (locus BM1706). Heterozygosity values ranged from 0.00 (D5S2) to 0.861 (BM1706). The paternity exclusion probabilities when only one and both parents were analyzed was 0.985424 (PE-1) and 0.999541 (PE-2), respectively. Observed a probability of exclusion of 0.999998% when both parents (PE-3) were tested for the set of 14 microsatellites. This panel is already used in Italy for the Mediterranean buffaloes, one of the four breeds raised in Brazil. However, it is highly recommended that new loci are analyzed in order to increase the microsatellites panel repertoire used for genealogical studies.

Keywords: Bubalus bubalis, individual and parentage identification.

INTRODUCTION

Buffaloes (Bubalus bubalis) were introduced in Brazil, Marajó Island, Pará, from the nineteenth century from Asia, Europe (Italy) and the Caribbean (Bernardes, 2007). The great ability of adaptation to various environments, the high fertility and the longevity in production, allowed the herd to evolve. The initial 200 animals into the country resulted in increasing the herd to about three millions heads (ABCB, 2010). Despite of this fact, genealogical control is still one of the weaknesses selection and
breeding programs in Brazilian buffaloes. The DNA test is important to develop a system that allows animal genealogy certification as well as its undeniable individual and parentage identification. Many authors have used microsatellites for molecular genetics characterization of buffalo (Coletta et al., 2010; Muraleedharan et al., 2009; Nagarajan et al., 2009; Elbeltagy et al., 2008; Vij et al., 2008; Zhang et al., 2007; Kumart et al., 2006; Navani et al., 2002; Moioli et al., 2001; Ritz et al., 2000) but only few published papers have included molecular markers in Brazilian populations analysis (Rogberg-Muñoz et al., 2010; Albuquerque et al., 2005).

The aim of this study is to validate a panel of 14 microsatellites markers as a tool of genealogical certification of Brazilian Murrah buffaloes in order to estimate the genetic variability and calculate the exclusion power and the match probability.

MATERIALS AND METHODS

DNA of 100 Brazilian Murrah buffaloes was extracted from hair follicles, consisting of distinct groups of related individuals and unrelated individuals from the states of Minas Gerais, Pará and São Paulo. For DNA extraction by hair follicles was used the technique described by Veterinary Genetics Laboratory at the University of California-Davis, modified by Yves Amigues, INRA, Jouy-en-Josas.

Samples were amplified using a panel of 14 microsatellite markers (BMC1013, BM1706, BM922, CSSM19, CSSM38, CSSM42, CSSM47, CSSM60, CYP21, INRA26, INRA6, MAF65, RM4 and D5S2) recommended by ISAG/2010 – International Society by Animal Genetics/2010. After electrophoresis of PCR products performed by automatic DNA analyzer ABI Model 3130 (AppliedBiosystems), analysis were made using GeneMapper program.

The statistical analysis was performed using software developed by the Stormont Laboratories, Inc- Woodland (UCDavis - CA – USA, 1997), for use under license, expressed in base pairs (bp), representing the specific genotyping. This software estimated parentage exclusion probability (PE), polymorphism information content (PIC) and heterozygosity values (Het.).

RESULTS AND DISCUSSION

A total of 92 alleles were detected in the whole sample and the number of alleles varied from one (locus D5S2) to 13 (locus CSSM47). The PIC values ranged from 0.00 (locus D5S2) to 0.845 (locus BM1706). Heterozygosity values ranged 0.00 (D5S2) to 0.861 (BM1706). Paternity exclusion probabilities ranged from 0.985424 (PE-1) and 0.999541 (PE-2) when only one and both parents were analyzed respectively. The probability of exclusion of both parents (PE-3) reached 0.999998% for the set of 14 microsatellites (Table 1). Our results corroborate with Coletta et al. (2010) and Albuquerque (2005) that estimated gene frequencies for the 13 microsatellite loci and concluded that excellent accuracy of PE (0.99999999% and 0.9998% respectively).

Other studies performed with B. bubalis have described some similar loci to our microsatellite polymorphism results: Muraleedharan et al. (2009) working on Indian water buffalo, Elbeltagy et al. (2008) working on Nile-Delta, Italian and Southern-Egypt buffalo, Zhang et al. (2007) working on Chinese indigenous buffalo and Albuquerque (2005) working on Brazilian buffaloes. Then, they reported number of alleles of 5, 7, 12 vs. 5 (CSSM19). In additional authors, 8, 4, 10, 6 vs. 6 (CSSM38); 9, 12, 16 vs. 13 (CSSM47) to Muraleedharan et al. (2009), Elbeltagy et al. (2008), Zhang et al. (2007) and Moioli et al. (2001) respectively. For this marker (CSSM47), Kumar et al. (2006) have found 9 alleles.

The primers used in the present study have been reported by different authors and also proved to be highly polymorphic in Brazilian Murrah breed: 6, 7 vs. 7 (CSSM42) by Elbeltagy et al. (2008) and Albuquerque (2005); 6, 11, 9 vs. 6 (CSSM60) by Elbeltagy et al. (2008), Zhang et al. (2007) and Moioli et al. (2001); 8, 7 vs. 9 (BM922) and 9, 9 vs. 8 (BM1706) by Muraleedharan et al. (2009) and Elbeltagy et al. (2008); 9, 10 vs. 6 (BMC1013) by Elbeltagy et al. (2008) and Moioli et al. (2001); 7 vs. 9 (INRA6); 10 vs. 7 (INRA26); 4 vs. 5 (CYP21); 6 vs. 4 (MAF65) and 9 vs. 6 (RM4) by Elbeltagy et al. (2008).

Different primers were analyzed and results were also efficient for example, 4, 6 and 8 (CSSM70) by Elbeltagy et al. (2008), Zhang et al. (2007) and Moioli et al. (2001); 11 and 9 (CA004) by Elbeltagy et al. (2008) and Kumar et al. (2006); 9 and 9 (BMS1747); 9 and 9 (BM757); 6 and 6 (BMS1724) by Muraleedharan et al. (2009) and Kumar et al. (2006), respectively.

Many other molecular markers have been tested in buffaloes and the results were polymorphic and highly heterozygosity primers as observed in this study (Muraleedharan et al., 2009; Nagarajan et al.,
Table 1. Genetic variability and probability of exclusion: number of alleles, Exclusion Probability of false parentage (PE), Polymorphic Information Content (PIC) and Heterozygosity value (Het.) in 100 Brazilian Murrah buffaloes

<table>
<thead>
<tr>
<th>LOCUS</th>
<th>ALLELE</th>
<th>PE-1</th>
<th>PE-2</th>
<th>PE-3</th>
<th>PIC</th>
<th>Het.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMC1013</td>
<td>6</td>
<td>0,304</td>
<td>0,476</td>
<td>0,653</td>
<td>0,674</td>
<td>0,723</td>
</tr>
<tr>
<td>BM1706</td>
<td>8</td>
<td>0,556</td>
<td>0,718</td>
<td>0,880</td>
<td>0,840</td>
<td>0,861</td>
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<tr>
<td>BM922</td>
<td>9</td>
<td>0,198</td>
<td>0,371</td>
<td>0,560</td>
<td>0,553</td>
<td>0,590</td>
</tr>
<tr>
<td>CSSM19</td>
<td>5</td>
<td>0,184</td>
<td>0,316</td>
<td>0,471</td>
<td>0,512</td>
<td>0,590</td>
</tr>
<tr>
<td>CSSM38</td>
<td>6</td>
<td>0,088</td>
<td>0,201</td>
<td>0,323</td>
<td>0,036</td>
<td>0,416</td>
</tr>
<tr>
<td>CSSM42</td>
<td>7</td>
<td>0,141</td>
<td>0,305</td>
<td>0,488</td>
<td>0,474</td>
<td>0,502</td>
</tr>
<tr>
<td>CSSM47</td>
<td>13</td>
<td>0,308</td>
<td>0,492</td>
<td>0,697</td>
<td>0,667</td>
<td>0,697</td>
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<tr>
<td>CSSM60</td>
<td>6</td>
<td>0,164</td>
<td>0,331</td>
<td>0,514</td>
<td>0,509</td>
<td>0,543</td>
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<tr>
<td>CYP21</td>
<td>5</td>
<td>0,195</td>
<td>0,348</td>
<td>0,509</td>
<td>0,552</td>
<td>0,614</td>
</tr>
<tr>
<td>D5S2</td>
<td>1</td>
<td>0,000</td>
<td>0,000</td>
<td>0,000</td>
<td>0,000</td>
<td>0,000</td>
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<td>INRA26</td>
<td>7</td>
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<td>0,587</td>
<td>0,771</td>
<td>0,756</td>
<td>0,786</td>
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<tr>
<td>INRA6</td>
<td>9</td>
<td>0,508</td>
<td>0,678</td>
<td>0,850</td>
<td>0,819</td>
<td>0,839</td>
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<tr>
<td>MAF65</td>
<td>4</td>
<td>0,244</td>
<td>0,417</td>
<td>0,599</td>
<td>0,613</td>
<td>0,659</td>
</tr>
<tr>
<td>RM4</td>
<td>6</td>
<td>0,106</td>
<td>0,346</td>
<td>0,397</td>
<td>0,411</td>
<td>0,450</td>
</tr>
</tbody>
</table>

|          |        | 0,985424 | 0,999541 | 0,999998 |

PE-1: Parentage Exclusion Probability when only one parent is typed; PE-2: Parentage Exclusion Probability when both parents are typed; PE-3: Probability of Exclusion both parents; PIC: Polymorphism Information Content when both parents are typed; Het.: Heterozygosity value. Software Stormont Laboratories, Inc - Woodland - CA - USA, developed by Domenico Bernoco (UCDavis - CA - USA), in 1997 (under license).

CONCLUSIONS

The performance of this multiplex panel of markers suggests that it will be useful in parentage DNA test of Brazilian Murrah buffaloes, excepted for one marker (D5S2) which was monomorphic. By presenting only one allele, this marker should be excluded from the test and can be replaced by another marker. These results have similar specificity to those ones reported by Coletta et al. (2010).

This panel is already used in Italy for the Mediterranean buffaloes, one of the four breeds raised in Brazil. However, it is highly recommended that new loci are analyzed in order to increase the microsatellites panel repertoire used for genealogical studies.

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REFERENCES


