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Simple sequence repeat (SSR) markers are effective for identifying pear cultivars and selections

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The present study characterized and identified pear cultivars growing in the southern region of Minas Gerais State, Brazil, using microsatellite markers. Nineteen (19) pear cultivars were collected from two sites of Southern Minas Gerais State: Ribeirão Vermelho and Lavras. DNA was extracted from newly formed leaves and amplified using 21 simple sequence repeat (SSR) markers (NH001c, NH002b, NH005b, NH007b, NH008b, NH009b, NH011b, NH013b, NH012a, NH014a, NH015a, NH017a, KA4b, KA5, KA14, KA16, KB16, KU10, BGA35, BGT23b and HGA8b). The data was analyzed by examining unweighted pair group method with arithmetic mean (UPGMA) clustering, principal coordinate analysis, the Shannon index, and the expected heterozygosity for each primer. The markers used were efficient in separating the Asian and European cultivars as well as hybrids. The primer KA16 was noteworthy in distinguishing the species *Pyrus communis*, *Pyrus pyrifolia* and *Pyrus betulaefolia* by its different banding patterns. Grouping by principal coordinates was similar to UPGMA clustering, forming two distinct groups among the Asian and European pears. The Shannon index and the expected heterozygosity had medium values of 0.440 and 0.300, respectively. The pear cultivars from Southern Minas Gerais usually show low productivity, mainly due to the lack of improved strains for less-demanding weather (warmer) conditions. The selection of the most suitable materials would contribute considerably to overcoming this problem, and the markers selected here can be used in separating cultivars - and presumably contribute to the selection of better matrices.

Key words: *Pyrus* sp., Rosaceae, microsatellite, heterozygosity.

INTRODUCTION

Pears are temperate climate fruits belonging to family Rosaceae (genus *Pyrus*) that are native to Europe and Asia; the most important species are *Pyrus communis*, *Pyrus pyrifolia*, *Pyrus serotina* and *Pyrus bretschneideri*

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(Bell et al., 1996). Pears were probably introduced into Brazil in 1531 and 1532 by Martin Afonso de Souza during a Portuguese colonization expedition. European, Chinese, and Japanese (Asiatic) pears are usually identified using morphological descriptors, but this often results in mistakes in cultivar classification (Erfani et al., 2012). Several studies throughout the world have focused on the classification and allocation of pear species using molecular markers, especially unknown or hybrid materials. Similar studies of material developed in Brazil or derived from the free exchange of genetic material (without controlling the parental stock) still need to be undertaken in that country.

It is estimated that the area planted with pears in Brazil is approximately 1,777 ha (Fachinello et al., 2011). Pears are one of the most imported fresh temperate fruits in the country, and according to the United Nations Food and Agricultire Organization (2014), approximately 210 000 tons were imported in 2011 - representing more than 90% of total consumption of that country. Brazilian production is almost inexpressive globally, largely due to unfavorable weather conditions and issues related to the types of rootstock, plant vigor, floral abortion, low-tech production, and the lack of cultivars suitable to Brazilian environmental conditions (Fachinello et al., 2011).

Southern Minas Gerais state is one of the few regions in Brazil with favorable climatic conditions for planting pears because of its pronounced and well-defined winter season. According to Köppen (1948), the climate in this region is classified as subtropical, with a dry winter, with winter temperatures averaging less than 18°C (reaching 2°C) usually extending for more than three months. Pear cultivation there is largely undertaken by small farmers, and pear trees are commonly found in backyards and near rural dwellings.

Improvements in the production processes of this fruit will be needed, as well as crop management and genetic improvement - to obtain low cold requiring cultivars with superior fruit quality and continuous production (Bettiol Netto, 2013). Genetic diversity with commercially interesting traits is fundamental to any successful breeding program (Amorim et al., 2009), and the agronomic characteristics of existing materials, associated with the use of molecular tools, can provide useful breeding information.

DNA markers represent excellent tools for cultivar identification and the quantification of the available diversity for choosing parents for hybridization through marker-assisted selection and the mapping of potentially interesting genes. Associations between information derived from molecular markers and those obtained from agronomic parameters could maximize genetic intergenerational selection processes. Microsatellite molecular markers are often effective in these efforts as they are highly polymorphic, co-dominant, and abundant in the genome (Varshney et al., 2005).

Simple sequence repeat (SSR) markers can be used to identify and confirm pear cultivars, especially those of unknown origins or the results of natural crossings. They can also be useful in identifying, verifying and quantifying the variability of selected materials. As the present study is limited to cultivars produced in Brazil, it represents an important starting point for their identification. Bao et al. (2007) evaluated the genetic diversity of pear material found in Asia; Brini et al. (2008) described cultivars in Tunisia, and Miranda et al. (2010) and Sehic et al. (2012) evaluated the genetic diversity of materials obtained in Spain and throughout Europe, respectively. New pear cultivars have been developed in Brazil, and breeding programs have been undertaken in that country by the Agronomic Institute of Campinas (IAC) in São Paulo State (Sawazaki et al., 2002). Studies in Southern Minas Gerais State have been scarce, however, in terms of both breeding and crop management. The objective of the present work was therefore to identify and characterize pear cultivars in the southern region of Minas Gerais using microsatellite markers.

MATERIALS AND METHODS

Selection and collection of materials

The leaves of 19 cultivars of *Pyrus* sp. were collected from family farms in two counties in Southern Minas Gerais State, Brazil: Lavras (21° 14' 43" S X 44° 59' 59" W at 919 m a.s.l.) and Ribeirão Vermelho (21°19 '15" S x 45° 59' 59" W at 793 m a.s.l.) (Table 1). Each municipality comprised a collection site, where the cultivars were collected for identification. To collect the plant material, pear plants approximately 8 to 12 years old were selected and a branch ~20 cm long containing newly formed leaves was removed. The branches were then wrapped in sheets of moistened paper, and held in cool boxes for transportation to the Laboratory of Molecular Biology at the Federal University of Lavras - UFLA; DNA extractions were performed on the same day.

DNA extraction and amplification

Genomic DNA was extracted using the 2% CTAB method (2% acetyl dimethyltiethylammonium bromide) (Ferreira and Grataplagia, 1995). After quantitation, the DNA was diluted to a standard concentration of 25 ng/µL.

The amplification reactions of the DNA fragments (PCR) were performed using a Perkin Elmer Gene Amp PCR System Model 2400 ™ in a medium with a final volume of 20 µL, containing: 2 µL of the genomic DNA (50 ng), 0.5 µL of each primer (NH001c, NH002b, NH005b, NH007b, NH008b, NH009b, NH011b, NH013b, NH012a, NH014a, NH015a, NH017a, KA4b, KA5, KA14, KA16, KB16, KU10, BGA35, BGT23b, and HGA8b) (Gianfranceschi et al., 1998; Yamamoto et al., 2002a,b), together with 13.8 µL of sterile ultrapure water, 2.0 µL 10X Buffer (100 mM $MgSO_4$, 100 mM KCl, 80 mM $(NH_4)_2SO4$, 100 mM Tris-HCl), 1.5 mM $MgCl_2$, 10 mM dNTP, and 0.2 µL of Taq polymerase (INVITROGEN Life Technologies™ 5 U/µL). The amplification reactions were performed in a Master Cycler Gradient Thermal Cycler Model 5331™. The amplification program consisted of an initial denaturation step at 95°C for 5 min (denaturation) followed by 35 cycles of 94°C for 30 s, 60°C for 30 s (30 sannealing at 60ºC with the adaptation of Toch Down, decreasing 1.6°C each cycle), and 72°C for 30 s (polymerization), with an extension at 72°C for 5

Table 1. Asian and European pear cultivars growing in two counties in Southern Minas Gerais State, Brazil.

Cultivars	Species	Type	Collection site
Tenra	*P. communis* L.	European	Lavras MG
Red Bartlett	*P. communis* L.	European	Lavras MG
Leconte	*P. communis* x (*P. communis*x *P. pyrifolia)*	Hybrid	Lavras MG
Manshu Mamenashi	*P. betulaefolia* B.	Asian	Lavras MG
D'água	*P. communis* L.	European	Lavras MG
Seleta	*P. communis* x (*P. communis*x *P. pyrifolia)*	Hybrid	Lavras MG
Primorosa	*P. communis* L.	European	Lavras MG
Hosui	*P. pyrifolia*N.	Asian	Lavras MG
Triunfo	*P. communis* L.	European	RibeirãoVermelho MG
Red Sensation	*P. communis* L.	European	Lavras MG
AbatelFetel	*P. communis* L.	European	Lavras MG
Packham's Triumph	*P. communis* L.	European	RibeirãoVermelho MG
Rocha	*P. communis* L.	European	RibeirãoVermelho MG
Williams	*P. communis* L.	European	RibeirãoVermelho MG
Atago	*P. serotina* R.	Asian	RibeirãoVermelho MG
Taiwan Nashi	*P. calleryana* D.	Asian	RibeirãoVermelho MG
Cascatense	*P. communis* x *P. pyrifolia*	Hybrid	Lavras MG
Starkrimson	*P. communis* L.	European	RibeirãoVermelho MG
Shinseiki	*P. pyrifolia* R.	Asian	RibeirãoVermelho MG

Table 2. List of polymorphic primers and the numbers of bands observed in pear genotypes from southern Minas Gerais State, Brazil.

Primer	Number fragments	Number of polymorphic fragments
H01c	9	5
NH02b	5	4
NH05b	10	7
NH07b	7	7
NH08b	10	9
NH09b	16	16
NH011b	8	7
NH012a	12	7
NH013a	11	11
NH014a	4	4
NH015a	8	8
NH017a	10	6
KU10	10	8
KA5	7	6
KA14	8	7
KA4b	5	1
KA16	15	14
KB16	7	6
BGT23	9	7
BGA35	9	7
HGA8b	9	9
Total	189	156

min. The DNA amplification products were then stored in a freezer at ~4°C.

The amplified fragments were separated by polyacrylamide gel electrophoresis at 100 V for 2 h. For comparisons of fragment size, a standard 100 bp DNA ladder was used. After electrophoresis, the polyacrylamide gel was subjected to silver staining and viewed by transillumination in a Hoefer MacroVue model Vis-45 ™.

Statistical analyses

The amplification products produced by each primer and visualized on the gel were used to elaborate a binary matrix of genetic similarity (present [1] or absent [0]) to obtain estimates of genetic similarities (Sgij) using XLSTAT software (Addinsoft ™, version 2014) (2009) and the Jaccard coefficient; genotype clustering was performed using the unweighted pair group method with arithmetic mean (UPGMA).

The genetic similarity between the two municipalities, the Shannon index (I) (Brown and Weir, 1983), and expected heterozygosity (He; Nei 1978) were calculated as described by Lynch and Milligan (1994) and Maguire et al. (2002). These genetic parameters were estimated for each locus using SRR Genalex v.6.3 software (www.anu.edu.au/BoZo/GenAlEx/).

RESULTS AND DISCUSSION

The 21 primers generated 189 fragments; of these, 156 were polymorphic loci and all were used in the analyses. The numbers of polymorphic bands varied from 1 to 16, with an average of 7.42 per primer.

The SSR primers NH09b and KA16 produced the highest total numbers of fragments (16 and 15, respectively) (Table 2). Most researchers throughout the world have used the primers suggested by Yamamotto (2002a), Yamamotto (2002b), and Kimura et al. (2002) to distinguish pear cultivars and quantify genetic diversity.

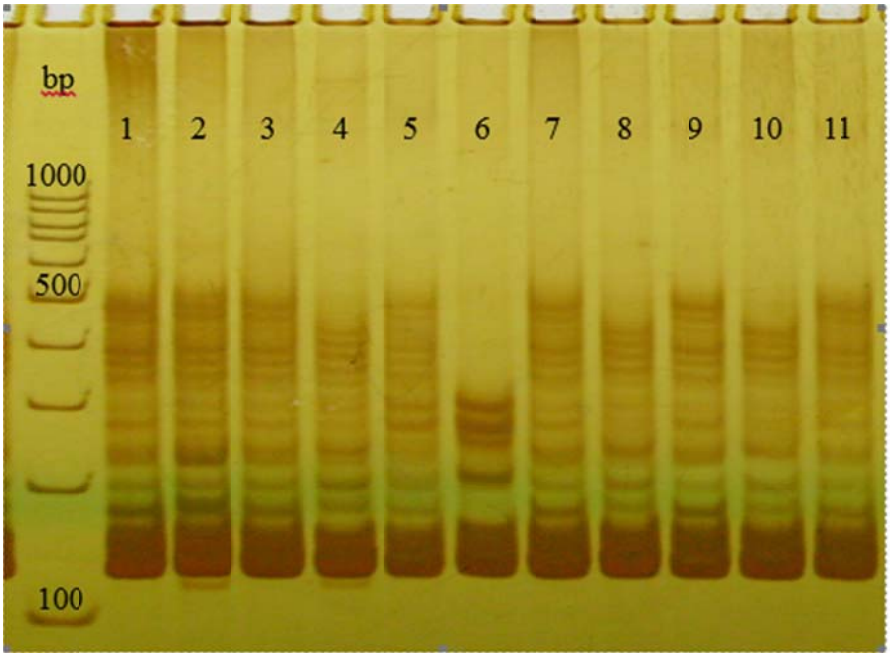

Figure 1. SSR profiles of eleven pear accessions (1 Tenra, 2 D'água, 3 Seleta, 4 Leconte, 5 Primorosa, 6 Hosui, 7 Triunfo, 8 Mainshu Mamenashi, 9 Atago, 10 Red Sensation, and 11 Abatel Fetel) obtained using the primer KA16.

These primers were found to be efficient in the present study in distinguishing European from Asian cultivars and corroborate previously published results. Erfani et al. (2012) studied a large number of SSR primers and were able to classify unknown pear cultivars of European and Asian origin into distinct groups. Among the primers selected for this purpose were KA16, BGT23b, NH011b and NH015a. The dendrogram obtained by Erfani et al. (2012) using the UPGMA method clearly formed a group with cultivars of the species *P. pyrifolia* (Japanese pear). These primers also separated the cultivars in our study. Kimura et al. (2002) analyzed different SSR markers in pear cultivars and suggested using the primers NH004a, KA16, BGA35, BGT23b, NH011b, NH013a, NH014a and NH015a to distinguish Asian material. Some of the genotypes analyzed by Kimura were also evaluated in the present study, including the Atago, Hosui and Shinsei cultivars, and five of the primers used were common to both studies (KA16, BGA35, BGT23b, NH011b and NH014a).

The band profiles demonstrated the efficiency of the microsatellite molecular marker KA16 in distinguishing species of *P. communis*, *P. pyrifolia* and *P. betulaefolia*, and it was possible to observe the band profiles in different individuals: six of the Hosui cultivar and eight of the Mainshu Mamenashi cultivar (all *P. betulaefolia*) (Figure 1). Therefore, among the SSR primers tested in the present study, NH08b, NH017, KA16 and kb16 showed the greatest potential for identifying pear cultivars grown in Brazil.

Note also that the Hosui cultivar demonstrated a distinct banding pattern with the KA16 primer as was also reported by Kimura et al. (2002).

The dendrogram generated indicates the formation of two groups - a larger group composed of 16 cultivars, and another smaller group formed only by the Asian pears Hosui, Atago and Shinseik. The largest group was composed of two smaller groups (1 and 2) - the first subgroup comprising five European cultivars and one Asian cultivar (Manshu Mamenshi), while the second subgroup comprised nine European and one Asian cultivar (Taiwan Nashi).

The cultivars that demonstrated the lowest genetic similarity were Hosui and Williams (49.7%), Hosui and D'agua (47.1%), Hosui and Manshu Mamenashi (47.3%), and Seleta and Manshu Mamenashi (47.6 %). The greatest similarity was observed between the cultivars Leconte and Tenra (77.8%), Tenra and Red Bartlet (76.1%), Leconte and Red Bartlet (74.7%), and Taiwan Nashi and Cascatense (74.5%).

With respect to the Hosui cultivar, its low similarity was due to the fact that it is derived from the species *P. pyrifolia* and not *P. communis* (as most of the genotypes studied here). Hosui is of Japanese origin, and showed many differences in relation to European pears. Japanese pears produce fruits that are characteristically medium to large sized, with a golden brown epidermis and a medium demand for cold, to being incompatible with the quince tree and show low ethylene production (which slows fruit ripening).

Erfani et al. (2012) evaluated 47 Japanese and European pear cultivars and observed an isolated cluster of Japanese pears - indicating that they have unique and inherent characteristics that distinguish them from other cultivars. The floral buds of the productive branches of the Hosui and Kousui cultivars develop in brindilas, lamburdas and, more rarely, in bags (Esumi et al., 2007).

Manshu Mamenashi pears also show low genetic similarity to other genotypes as it was derived from *P. betulaefolia* and not *P. communis* (as were most genotypes studied in the present study). The Taiwan Nashi and Manshu Mamenashi cultivars are widely used as rootstock for pear trees in Brazil (Camellato, 2003). Examinations of hybrids and cultivars in the pear germplasm bank at the Agronomic Institute of Campinas (IAC) using RAPD markers showed that the two cultivars mentioned above are grouped only by the pears used as rootstock (Sawazaki et al., 2002). In the present study, these cultivars were assigned to different groups, probably due to the exchange of genetic material between them.

Many of the cultivars examined were collected in home gardens without any control of their genetic crossings, and it is a common practice among farmers there to exchange cuttings and seeds. Additionally, pears and apples are self-incompatible (and classified into the same sub-family of Pomoideae) - favoring genetic exchange and thus generating genetic variability (Erfani et al., 2012).

Evaluations of cultivars in São Paulo State undertaken by Sawazaki et al. (2002) found that the Hosui, Shinseik, and Atago varieties were likewise grouped as Asian pears, while Williams, Packham's Triumph, Tenra, and D'água were grouped with European pears; our results corroborate those findings. Monte-Corvo et al. (2000) likewise grouped Shinseik cultivars with Japanese pears; the Williams, Packham's Triumph, and Rocha cultivars were also studied and both grouped with European pears. Our studies, however, indicated that the Packham's Triumph and Rocha cultivars were closely related and, as in the work of Monte-Corvo, these cultivars were divided into distinct subgroups.

Fachinello et al. (2000) evaluated pear cultivars grown in Italy using isozyme markers, and reported that the cultivars Abatel Fetel and Packham's Triumph were allocated to a group separate from the Williams cultivar. Although all of these cultivars were clustered within the group of European pears in the present study, the Williams and Abatel Fetel pears grouped very close together, but distant from the Packham's Triumph cultivar.

Dequigiovanni et al. (2012) evaluated pear cultivars using SRR markers and reported that the cultivars Williams, Red Bartlett, and Packham's Triumph corresponded to the European pear variety Bon Chrétien Williams. Bartlett is a mutant that probably originated from seeds derived from 'Bon Chrétien Williams' circa 1880; Packham's Triumph was selected around 1886-1887 from a cross conducted in Australia between 'Uvedale St. Germain' and 'Williams Bon Chrétien'. Our results differ from those obtained by Dequigiovanni et al. (2012) as the Williams cultivar was distant from the cultivars Red Bartlett and Packham's Triumph, although the latter two grouped close together.

With regard to Asian pears, Oliveira et al. (1999) found that RAPD markers were effective in separating the cultivars Shinseiki and Rocha. The dendrogram clearly places Shinseik in the group of Asian pears and Rocha in the group of European pears, corroborating the present research. The distinct genetic backgrounds of these cultivars separated them into different groups.

Other studies performed using different markers with different pear varieties in the world have generally been found to be deficient in separating European from Asian cultivars and hybrids in breeding and regional strains obtained from free exchange programs of genetic material. Brini et al. (2008) evaluated 31 genotypes and 26 Tunisian and European cultivars and concluded that there were no clear differences between the Tunisian and European cultivars due to the great diversity encountered. Erfani et al. (2012) analyzed 47 Asian, European, and regional cultivars using SSR markers and found clear groupings according to their origins and distributions.

The results of this research demonstrated that although some pear materials were classified in a particular group, they differed somewhat in comparison with published data. These variations can be influenced by a number of interacting factors that will define the degrees of divergence of the genotypes. The differences between pear cultivars is probably also a reflection of their self-incompatibility (Erfani et al., 2012), and many authors have noted the consequences of this hypothesis - in the sense that self-incompatibility would tend to force cross-pollination between cultivars.

The principal coordinates analyses presented here (Figure 3) reinforce the positions of both the closest and most distant cultivars identified by UPGMA (Figure 2), with the formation of a group with populations of both Asian and European pears. Grouping by principal coordinate analysis (PCA) explained 36.39% of the existing diversity (Figure 3), and pear cultivars from different municipalities formed two distinct groups according to UPGMA clustering. The first comprised only three Asian cultivars, while the second comprised the remaining 16 (Figure 3).

This grouping indicated that Red Sensation and Shinseik, and Star Krimson and Shinseik were the most distant cultivars. Sawazaki et al. (2002) used some of the same cultivars examined in the present and found that grouping by principal coordinate analysis (PCA) explained 29.83% of the observed variability. Oliveira et al. (1999) reported that 36.89% of the diversity could be explained by principal coordinates analysis grouping.

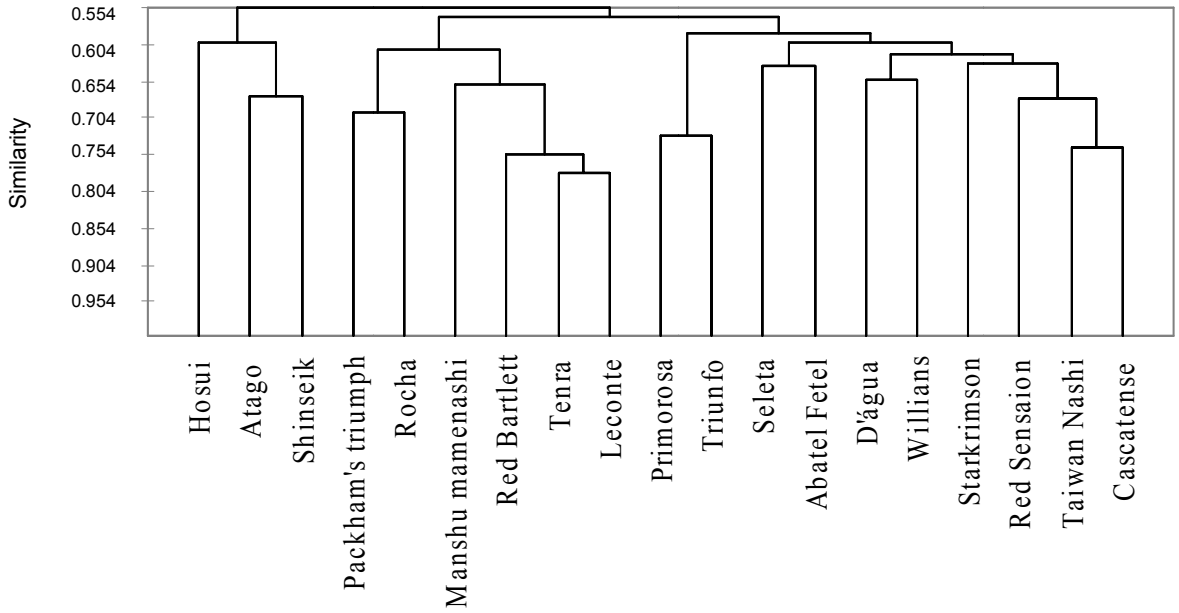

Figure 2. Representation of different cultivars of Asian and European pear derived from two municipalities in Southern Minas Gerais State, Brazil, by UPGMA clustering and genetic similarity using the Jaccard coefficient (1908).

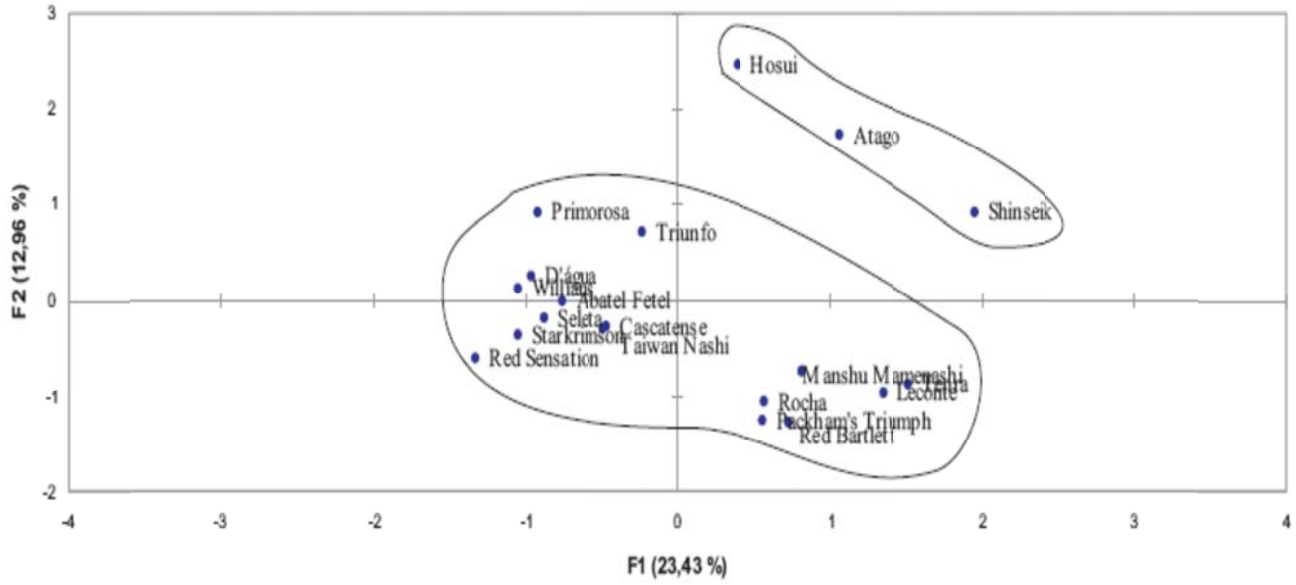

Figure 3. Principal coordinate analysis (PCA) pear cultivars from different municipalities in Southern Minas Gerais.

The estimated Shannon index values were high for most primers, except NH012a, followed by NH01c and NH017a; the highest Shannon index value was observed with NH014a. The heterozygosity of the primers used ranged from 0.150 to 0.488, with an average of 0.300 (Table 3).

Dequigiovanni et al. (2012) reported an average expected heterozygosity of 0.56 for pear cultivars in Brazil, with the numbers of alleles ranging from 3 to 11. Pear cultivars in Spain had an average expected heterozygosity value of 0.74, with the numbers of alleles ranging from 9 to 15 (Miranda et al., 2010). Fernández-Fernández et al. (2006) reported that He ranged from 0.30 to 0.91 for European pear cultivars; Kimura et al. (2002) reported that the numbers of alleles varied from 7 to 20, with an average of 14.8, while the Ho averaged

Table 3. Characterization of pear varieties using 21 SSR loci.

Locus	I[1]	He[2]
BGT23	0.314	0.209
BGA35	0.340	0.226
NH01c	0.236	0.152
NH011b	0.534	0.370
NH02b	0.430	0.288
NH05b	0.388	0.267
NH07b	0.583	0.398
NH08b	0.449	0.302
NH09b	0.569	0.391
NH012a	0.230	0.150
NH013a	0.568	0.390
NH014a	0.681	0.488
NH015a	0.608	0.427
NH017a	0.272	0.183
KU10	0.387	0.252
KA4b	0.430	0.288
KA16	0.559	0.393
KB16	0.426	0.295
KA5	0.388	0.261
KA14	0.366	0.241
HGA8B	0.499	0.338

I[1], Shannon index; He[2], Heterozygosity.

0.86.

Reliable genetic markers are essential for efficient identification of cultivars and for establishing genetic relationships among them. The 21 SSR markers used in this study were effective in identifying cultivars and quantifying their diversity. The results for the number of alleles per locus were within the ranges reported for other studies of *Pyrus* sp. cultivars (Kimura et al., 2002; Fernández-Fernández et al., 2006; Miranda et al., 2010; Dequigiovanni et al., 2012). Heterozygosity summarizes the fundamental variations of genetic diversity in a population (Berg and Harmik, 1997), and is therefore commonly employed in studies of diversity.

The average heterozygosity (He) found in our study (0.300) is considered a reasonable value, although smaller than values reported in other studies of pears using SSR markers. Sosinski et al. (2000) reported values ranging from 0.21 to 0.56 (average 0.45) in their valuation of 28 peach varieties. These low heterozygosity values for peaches are presumably due to their capacity for natural self-pollination.

The average heterozygosity (He) found in our study (0.300) is considered a reasonable value, although smaller than values reported in other studies of pears using SSR markers. Similar values were reported by Ferreira dos Santos et al. (2011) with average heterozygosity values varying from 0.358 to 0.916 among 147 genotypes of Pyrus spp. from Northwestern Spain. Sosinski et al. (2000) reported values ranging from 0.21 to 0.56 (average 0.45) in their valuation of 28 peach varieties. These low heterozygosity values for peaches are presumably due to their capacity for natural self-pollination.

Our studies show that the 21 SSR loci evaluated here have great potential for identifying pear cultivars in Brazil. The pear cultivars examined were grouped into three distinct categories: "European" pears (*P. communis*), "Asian" pears (*P. pyrifolia*), and hybrid pears. These markers showed satisfactory polymorphisms, with high reproducibility.

Research seeking pear cultivars better adapted to the climate of Brazil (especially to that of Minas Gerais State) should be encouraged as the productivity of this crop is currently quite limited in that country and almost all of the fruits consumed there are imported. There are many small producers in Minas Gerais State, but their pear orchards generally occupy only small areas - even though this crop generates income and employment for many families. Brazilian producers are also hampered by the current costs of fruit production, as the low productivity of local cultivars, combined with low technology, raises the price pears produced in Brazil. Future research based on the results of this study should attempt to correlate the agronomic (physiological and morphological) and productive characteristics of these pear cultivars to identify varieties that are most suitable to Brazilian conditions.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Addinsoft (2009). XLSTAT Statistical analysis software, 2014. IOP Publishing PhysicsWeb www.xlstat.com

Amorim EP, Lessa LS, Ledo CAS, Amorim VBO, Reis RV, Santos-Serejo JA, Silva SO (2009). Caracterização agronômica e molecular de genótipos diploides melhorados de bananeira. Rev. Bras. Frutic. 31(1):154-161.

Bao L, Chen K, Zhang D, Cao Y, Yamamoto T, Teng Y (2007). Genetic diversity and similarity of pear (*Pyrus* L.) cultivars native to East Asia revealed by SSR (simple sequence repeat) markers. Genet. Resour. Crop. Evol. 54(5):959-971.

Bell RL, Quamme HA, Layne REC, Skirvin RM (1996). Pears. In: Janick, J., Moore, J.N. (Eds.), Fruit Breeding, Volume I: Tree and Tropical Fruits. John Wiley and Sons, Inc., NY, USA.

Berg EE, Hamrick JL (1997). Quantification of genetic diversity at allozyme loci. Can. J. For. Res. 27(3):415-424.

Bettiol Netto JE (2013). Desempenho produtivo e características agronômicas de cultivares de marmeleiro e pereira em jundaí - SP 96 p.

Brini W, Mars M, Hormaza JI (2008). Genetic diversity in local Tunisian pears (*Pyrus communis* L.) studied with SSR markers. Sci. Hortic. 115(4):337-341.

Brown AHD, Weir BS (1983) Measuring genetic variability in plant populations. In: Tanksley SD, Orton, T J (Eds.). Isozymes in plant genetics and breeding. Part A. Amsterdan: Elsevier Science Publishers. pp. 73-86.

Camellato D (2003). Propagação. In: Nakasu BH, Quezada AC, Herter FG. Pêra. Produção. Pelotas, Embrapa Clima Temperado; Brasília, Embrapa Informação Tecnológica.

Dequigiovanni G, Rech F, Gomes FGG, Cerotti IS, Faoro I, Oliveira PRD, Quecini V, Ritschel P (2012). Identification of a Simple Sequence Repeat molecular-marker set for large-scale analyses of pear germplasm. Crop Breed. Appl. Biotechnol. 12(2):118-125.

Erfani J, Ebadi A, Abdollahi H, Fatahi R (2012). Genetic Diversity of Some Pear Cultivars and Genotypes Using Simple Sequence Repeat (SSR) Markers. Plant. Mol. Biol. Rep. 30(5):1065-1072.

Esumi T, Tao R, Yonemori K (2007). Comparison of early inflorescence development between japanese pear (*Pyrus pyrifolia* Nakai) and Quince (*Cydonia oblonga* Mill.). J. Jpn. Soc. Hort. Sci. 76(3):210-216.

Fachinello JC, Musachhi S, Zuccherelli S, Sansavini S (2000). Polimorfismo enzimático nos tecidos de pereira. Pes. Agrop. Bras. 35(37):1427-1432.

Fachinello JC, Pasa MS, Shmtiz JD, Betemps DL (2011). Situação e perspectivas da fruticultura de clima temperado no Brasil. Ver. Bras. Fruti. 33:109-120.

FAO/Food and Agriculture Organization of the United Organizations (2014). Productio. Crops Primary - Pears. Disponível em http://faostat.fao.org/.

Fernández-Fernández F, Harvey FN, James CM (2006). Isolation and characterization of polymorphic microsatellite markers from European pear (*Pyrus communis* L.). Mol. Ecol. 6(4):1039-1041.

Ferreira dos Santos A, Ramos-Cabrer AM, Díaz-Hernández, Pereira-Lorenzo S (2011). Genetic variability and diversification process in local pear cultivars from northwestern Spain using microsatellites. Tree Genet. Genomes 7:1041-1056

Ferreira ME, Grattapaglia D (1995). Introdução ao uso de marcadores RAPD e RFLP em análise genética. EMBRAPA-CENARGEN, Documento 20, pp. 220

Gianfranceschi L, Seglias N, Tarchini R, Komjanc M, Gessler C (1998). Simple sequence repeats for the genetic analysis of apple. Theor. Appl. Genet. 96(8):1069-1076.

Kimura T, Shi YZ, Shoda M, Kotobuki KM, Atsuta NH, Ayashi T, Ban Y, Yamamoto T (2002). Identification of Asian pear varieties by SSR analysis. Breed. Sci. 52(2):115-121.

Köppen W (1948). Climatologia: con un estudio de los climas de la tierra. México: Fondo de Cultura Economica.

Lynch M, Milligan BG (1994). Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3(2):91-99.

Maguire TL, Pearkall R, Saenger P (2002). Comparative analysis of diversity in the mangrove species *Avicennia marina* (Fosk.) Vierth. (*Avicenniaceae)* detected by AFLPs and SSRs. Theo. Appl. Genet. 1004(2-3):388-398.

Miranda C, Urrestarazu J, Santesteban LG, Royo JB (2010). Genetic Diversity and Structure in a Collection of Ancient Spanish Pear Cultivars Assessed by Microsatellite Markers. J. Amer. Soc. Hort. Sci. 135(5):428-437.

Monte-Corvo L, Cabrita L, Oliveira C, Leitão J (2000). Assessment of genetic relationships among *Pyrus* species and cultivars using AFLP and RAPD markers. Genet. Resour. Crop. Evol.47(3): 257-265.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Oliveira CM, Mota M, Monte-Corvo L, Goulão L, Silva DM (1999). Molecular typing of *Pyrus* based on RAPD markers. Sci. Hortic. 79:63-174.

Sawazaki HE, Barbosa W, Colombo CA (2002). Caracterização e identificação de cultivares e seleções de pereiras através de marcadores RAPD. Rev. Bras. Frutic. 24(2)447-452.

Sehic J, Garkava-Gustavsson L, Fernández-Fernández F, Nybom H (2012). Genetic diversity in a collection of European pear (*Pyrus communis*) cultivars determined with SSR markers chosen by ECPGR. Sci. Hortic. 145:39-45.

Sosinski B, Gannavarapu M, Hager LD, Beck LE, King GJ, Ryder CD, Rajapakse S, Baird WV, Ballard RE, Abbott AG (2000). Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. Theor. Appl. Genet. 101(3):421-428.

Varshney RK, Graner A, Sorrells ME (2005). Genic microsatellite markers in plants:features and applications. Trends Biotechnol. 23(1):48-55.

Yamamoto T, Kimura T, Sawamura Y, Manabe T, Kotobuki K, Hayashi T, Ban Y, Matsuta N (2002a). Simple sequence repeats for genetic analysis in pear. Euphytica 124(1):129-137.

Yamamoto T, Kimura T, Shoda M, Ban Y, Hayashi T, Matsuta N (2002b). Development of microsatellite markers in the Japanese pear (*Pyrus pyrifolia* Nakai). Mol. Ecol. Notes 2(1):14-16.