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Genetic divergence between sub-accessions of the melon using molecular markers[1]

Divergência genética entre subacessos de meloeiros por meio de marcadores moleculares

Clisneide Coelho de Amorim[2]*, Francisco Davi da Silva[3], Patricia do Nascimento Bordallo[4], Fernando Antonio Souza de Aragão[4], Manoel Abílio de Queiróz[5]

ABSTRACT - The melon (*Cucumis melo* L.) is one of the most important horticultural crops in the northeast of Brazil. The characterisation of germplasm is an efficient process for managing plant genetic resources, as it contributes to better conservation and improved use in breeding programs. In the present study, the genetic divergence between sub-accessions of the melon taken from traditional agriculture in the semi-arid region of Brazil was evaluated using ISSR and RAPD markers, verifying the level of agreement with the botanical classification. Twenty-six sub-accessions preserved in the Active Germplasm Bank of Cucurbitaceae for the northeast of Brazil, located at Embrapa Semiárido in Petrolina, Pernambuco, were analysed. The binary matrix of the molecular data was used to obtain the genetic dissimilarities using the complement of the Jaccard index. The genetic dissimilarities were represented in dendrograms generated using the UPGMA method. Twenty-eight ISSR primers and 26 RAPD primers were polymorphic. A total of 686 fragments were obtained, of which 451 were polymorphic. The mean polymorphism of the RAPD markers was greater than that of the ISSR markers. The number of markers was considered sufficient to accurately determine the genetic diversity. The ISSR dendrogram allowed the formation of three groups, the RAPD dendrogram, four groups, and the joint dendrogram, three groups. The study demonstrated the genetic variability between the sub-accessions, and the strong association between the characterisation made by the molecular markers and the taxonomic classification of the genotypes under study.

Key words: *Cucumis melo*. Diversity. Molecular characterisation. Cluster analysis.

RESUMO - O meloeiro (*Cucumis melo* L.) é uma das hortaliças mais importantes para o Nordeste brasileiro. A caracterização de germoplasma é um processo eficiente no manejo dos Recursos Genéticos Vegetais, pois contribui para uma melhor conservação e utilização em programas de Melhoramento. Nesse estudo, avaliou-se a divergência genética entre subacessos de meloeiro procedentes da Agricultura Tradicional do Semiárido brasileiro, por meio de marcadores ISSR e RAPD, verificando o grau de associação com a classificação botânica. Foram analisados 26 subacessos conservados no Banco Ativo de Germoplasma de Cucurbitáceas para o Nordeste brasileiro, localizado na Embrapa Semiárido, em Petrolina-PE. A matriz binária dos dados moleculares foi utilizada para a obtenção das dissimilaridades genéticas, pelo complemento do índice de Jaccard. As dissimilaridades genéticas foram representadas em dendrogramas gerados pelo método UPGMA. Vinte oito *primers* ISSR e 26 *primers* RAPD foram polimórficos. Um total de 686 fragmentos foi obtido, sendo 451 polimórficos. O polimorfismo médio dos marcadores RAPD foi maior que o dos marcadores ISSR. O número de marcadores foi considerado adequado para determinar com precisão a diversidade genética. O dendrograma ISSR permitiu a formação de três grupos, o dendrograma RAPD, quatro grupos, e o dendrograma conjunto, três grupos. O estudo evidenciou a variabilidade genética presente entre os subacessos e a forte associação entre a caracterização feita pelos marcadores moleculares e a classificação taxonômica dos genótipos estudados.

Palavras-chave: *Cucumis melo*. Diversidade. Caracterização molecular. Análise de agrupamento.

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*Author for correspondence
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[1]Parte da Dissertação do primeiro autor, apresentado ao Curso de Pós-Graduação em Recursos Genéticos Vegetais, Universidade Estadual de Feira de Santana (UEFS)
[2]Programa de Pós-Graduação em Recursos Genéticos Vegetais, Universidade Estadual de Feira de Santana (UEFS), Feira de Santana-BA, Brasil, clisamorim@yahoo.com.br (ORCID ID 0000-0002-9363-9561)
[3]Universidade Federal de Viçosa (UFV), Campus Viçosa-MG, Brasil, francisco.davi@ufv.br (ORCID ID 0000-0001-8679-3539)
[4]Embrapa Agroindústria Tropical (CNPAT), Fortaleza-CE, Brasil, patricia.bordallo@embrapa.br (ORCID ID 0000-0003-4032-3106), fernando.aragao@embrapa.br (ORCID ID 0000-0002-4041-7375)
[5]Universidade do Estado da Bahia (UNEB), Campus Juazeiro-BA, Brasil, manoelabiliomaq@gmail.com (ORCID ID 0000-0001-9501-2343)

INTRODUCTION

The melon belongs to the class of Dycotyledoneae, order Cucurbitales, family Cucurbitaceae, genus *Cucumis*, subgenus *Melo*, and species *Cucumis melo* L. (JEFFREY, 1990). It is grown in more than 100 countries on all continents. Brazil produces around 600 thousand tons on 23,000 ha per year, and is the 10th largest producer and 3rd largest exporter in the world (FOOD AND AGRICULTURE ORGANIZATION, 2021). The semi-arid region of Brazil, in the northeast of the country, is responsible for 96% of this production, with the states of Rio Grande do Norte, Ceará, Bahia and Pernambuco being the most important producers (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA, 2021).

The northeast of Brazil has several species of cucurbitaceae that were introduced centuries ago and are still grown under traditional agriculture on small agricultural establishments, which has given rise to numerous traditional or creole varieties. It should be noted that traditional varieties are important sources of alleles, and from the information generated via characterisation and evaluation, it is possible to improve the conservation and management of the germplasm, as well as its use in breeding programs, for example by identifying parents with desirable phenotypes (MAIA; LIMA; LIMA, 2013). Therefore, knowledge of genetic variability and its distribution makes the rational and sustainable use of plant genetic resources possible (DANTAS *et al*., 2012), where genetic divergence is estimated from data obtained through the evaluation and/or characterisation of germplasm, being either morphological, agronomic, chemical or molecular, among others.

Following the classification by Pitrat, Hanelt and Hammer (2000), and using morphological characteristics, Amorim *et al*. (2016) and Macedo *et al.* (2017), found wide genetic divergence between and within melon accessions collected from the traditional agriculture of the state of Maranhão, with sub-accessions of subspecies *agrestis* being identified with the botanical varieties *momordica*, *makuwa* and *conomom*, and of subspecies *melo* with the botanical varieties *cantalupensis* and *chandalak*. The classification of species *C. melo* by Pitrat, Hanelt and Hammer (2000) subdivides the species into sixteen botanical varieties. Subspecies *agrestis* includes *momordica*, *conomom*, *makuwa*, *chinensis* and *acidulus*, and subspecies *melo* includes the varieties *cantalupensis*, *reticulatus*, *adana*, *chandalak*, *ameri*, *inodorus*, *chate*, *tibish*, *flexuosus*, *chito* and *dudaim*.

Characterisation can also be carried out using molecular markers, starting from the identification of genetic marks that directly express the polymorphism of deoxyribonucleic acid (DNA). Dantas *et al.* (2012), Aragão *et al.* (2013) and Andrade *et al*. (2019) conducted a molecular characterisation of accessions collected in the northeast of Brazil, using Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) and Ward-Modified Location Model (Ward-MLM) markers, respectively, and confirmed genetic variability in the individuals found in this region. Alves-Pereira, Viana and Zucchi (2015) reviewed studies that employed molecular markers between 2011 and 2014, and found 43 articles that used RAPD, and 31 that used Inter Simple Sequence Repeats (ISSR). ISSR and RAPD molecular markers are dominant markers, amplified using the Polymerase Chain Reaction (PCR) technique, and are widely used, as they require no prior knowledge of the target DNA, are highly sensitive in detecting polymorphisms, are simple to use, and are of low cost (NADEEM *et al*., 2018).

The aim of this study, therefore, was to estimate, using ISSR and RAPD markers, the genetic divergence between sub-accessions of the melon from traditional agriculture in the semi-arid region of Brazil, verifying the level of agreement with the botanical classification.

MATERIAL AND METHODS

Germplasm

Twenty-six sub-accessions of the melon from traditional agriculture in the state of Maranhão (Table 1) were evaluated, the seeds of which are stored at 10 ºC and a relative humidity of 40% in a cold chamber at the Active Germplasm Bank of Cucurbitaceae for the northeast of Brazil, located at Embrapa Semiárido in Petrolina, Pernambuco. The plant material for molecular characterisation was obtained from a greenhouse, and the molecular analysis was performed at the Laboratory of Molecular Biology of Embrapa Agroindústria Tropical, in Fortaleza, in the state of Ceará.

Fifteen S2 generation seeds from each sub-accession were sown in trays filled with HS Florestal® substrate and powdered coconut fibre at a ratio of 3:1; these were kept in a humidity chamber for 24 h, and then taken to the greenhouse. Ten days after sowing, the seedlings were transplanted into 300 -mL pots filled with HS Florestal®, humus and sand (2:1:1). Eight pots were used for each genotype, each containing one plant, which was irrigated as required.

DNA extraction, amplification, electrophoresis and molecular data

Fifteen days after transplanting, one young leaf was collected per plant to form bulks per sub-accession. These were properly identified and stored in an ultra-low temperature freezer at -80 °C. The

Table 1 - Passport data for the *Cucumis melo* sub-accessions under study, collected in the state of Maranhão, Brazil and deposited in the Active Germplasm Bank of Cucurbitaceae for the semi-arid region of Brazil

Sub-acession	Sub-species*	Botanical variety*	District collected
BGMEL 63.0	melo	cantalupensis	Colinas
BGMEL 66.0	agrestis	makuwa	Colinas
BGMEL 67.0	agrestis	makuwa	Colinas
BGMEL 68.1	agrestis	momordica	Colinas
BGMEL 68.2	agrestis	ND	Colinas
BGMEL 68.3	ND	ND	Colinas
BGMEL 78.0	melo	cantalupensis	Codó
BGMEL 79.0	agrestis	makuwa	Itapecuru Mirim
BGMEL 86.1	melo	cantalupensis	Codó
BGMEL 86.2	agrestis	ND	Codó
BGMEL 86.3	melo	ND	Codó
BGMEL 87.1	agrestis	momordica	São Luis Gonzaga do Maranhão
BGMEL 87.2	melo	cantalupensis	São Luis Gonzaga do Maranhão
BGMEL 87.3	ND	ND	São Luis Gonzaga do Maranhão
BGMEL 97.1	melo	cantalupensis	Caxias
BGMEL 97.2	melo	ND	Caxias
BGMEL 101.0	melo	cantalupensis	Caxias
BGMEL 103.1	melo	cantalupensis	Caxias
BGMEL 103.2	ND	ND	Caxias
BGMEL 108.1	agrestis	makuwa	Caxias
BGMEL 108.2	melo	ND	Caxias
BGMEL 108.3	agrestis	ND	Caxias
BGMEL 108.4	ND	ND	Caxias
BGMEL 111.0	agrestis	makuwa	Colinas
BGMEL 112.0	agrestis	makuwa	Colinas
BGMEL 115.0	agrestis	makuwa	São Vicente Ferrer

* Botanical classification by Amorim *et al.* (2016), based on Pitrat, Hanelt and Hammer (2000). ND: Not defined

DNA was then extracted following the Ferreira and Grattapaglia protocol (1998) with modifications. The DNA was quantified using a Nanodrop® 2000, diluted to a concentration of 10 µg.µL^{-1} and visualised on agarose gel.

Amplification tests were initially carried out with 59 ISSR primers and 33 RAPD primers using DNA from five sub-accessions (BGMEL 68.1, BGMEL 79.0, BGMEL 86.1, BGMEL 86.3 and BGMEL 108.3). From these, the primers with the best results for polymorphism were selected.

DNA amplification reactions with ISSR and RAPD primers were obtained for a final volume of 15 µL and 25 µL, respectively. Each reaction contained 1X PCR buffer (10 mM Tris-HCl pH 8.3 and 50 mM KCl), 4.0 mM MgCl2, 0.2 mM dNTPs, 0.8 µM primer, 30 g of genomic DNA, 1 U of Taq DNA polymerase, and ultrapure sterile water to make up the volume of the respective reaction.

The DNA amplification process, carried out in a thermocycler (Veriti Applies Biosystems®), consisted of five minutes at 94 °C for initial DNA denaturation, followed by 40 amplification cycles. Each cycle consisted of one minute at 94 °C (DNA denaturation), one minute at the annealing temperature of the primers (35 °C for RAPD and varying per primer for ISSR) and one minute at 72 °C (DNA amplification), followed by a final five-minute extension cycle at 72 °C.

After amplification, the products were separated by electrophoresis on 1.8% agarose gel for the ISSR primers and 1.2% for the RAPD primers, using 1X TBE buffer (Tris - Boric Acid - EDTA, pH 8.0), stained with ethidium bromide (10 μL.mL^{-1}) and subjected to 120 volts (ISSR) and 140 volts (RAPD) for 2h30m. The gels were then visualised under UV light and photographed using a photo-documentation system (Loccus L-Pix Chemi®). The molecular data were obtained by visualising the bands in the gels, and polymorphism was verified based on the presence or absence of the bands at each locus. The bands were counted with the aid of the Gelcompar software (Applied Maths NV®).

Statistical analysis

The band patterns obtained with the markers were coded in a binary data matrix, based on the presence (1) or absence (0) of bands. The polymorphism of the markers was calculated from the percentage of polymorphic fragments in the total number of amplified fragments. The genetic distance matrices were constructed using the arithmetic complement of the Jaccard coefficient, and the dendrograms using the unweighted pair group method with arithmetic mean (UPGMA). In the dendrogram, the formed groups were separated using Mojena's criterion (1977), with k = 1.25. The optimal number of markers was estimated by correlation to check whether the number used was sufficient to assess the genetic divergence between the sub-accessions, and the stress value was obtained to establish the fit between the original matrix and the simulated matrix. The number of fragments is considered sufficient when the stress value is less than 0.05 (KRUSKAL, 1964). The cophenetic correlation coefficient (CCC) of each dendrogram and the correlations between matrix pairs (ISSR, RAPD and joint ISSR+RAPD) were also estimated using the Mantel test (1967), with 10,000 permutations and significance calculated by t-test. The quality of the fit of the graphic projection of the original genetic distance was estimated by the stress parameter, considered insufficient, regular, good, excellent or perfect, for the respective stress values of 20%, 10%, 5%, 2.5% and 0% (KRUSKAL, 1964).

The statistical analysis was carried out using the Genes (CRUZ, 2016) and R (R CORE TEAM, 2017) software, with the *dendextend* (GALILI, 2015) and *tidyverse* (WICKHAM, 2019) packages.

RESULTS AND DISCUSSION

The genetic diversity of 26 melon sub-accessions was studied using 28 ISSR primers selected from 59 previously tested primers, giving a total of 327 marks, with 63.91% polymorphism. Of the 33 initial RAPD primers, 26 were selected, and with these, 359 marks were identified, 242 of which (67.41%) were polymorphic, giving an average of 9.31 marks per primer (Table 2).

Table 2 - List of the primers that amplified the DNA of the melon sub-accessions

Primer	Sequence (5' – 3')* ISSR Primers	AT (°C)	TB	PB	P (%)
I 811	GAGAGAGAGAGAGAGAC	46.0	17	8	47.05
I 815	CRCTCTCTCTCTCTCTG	41.8	13	7	53.84
I 823	TCTCTCTCTCTCTCTCC	47.1	12	9	75.00
I 824	TCTCTCTCTCTCTCTCG	43.5	5	5	100.00
I 825	ACACACACACACACACT	46.4	12	7	58.33
I 826	ACACACACACACACACC	47.7	10	6	60.00
I 834	AGAGAGAGAGAGAGAGYT	44.2	7	2	28.57
I 835	AGAGAGAGAGAGAGAGYC	45.2	16	12	75.00
I 840	GAGAGAGAGAGAGAGAYT	42.4	7	4	57.14
I 841	GAGAGAGAGAGAGAGAYC	43.5	14	12	85.71
I 843	CTCTCTCTCTCTCTCTRA	46.0	12	7	58.33
I 844	CTCTCTCTCTCTCTCTRC	47.6	8	6	75.00
I 845	CTCTCTCTCTCTCTCTRG	47.1	8	4	50.00
I 848	CACACACACACACACARG	47.7	9	3	33.33
I 849	GTGTGTGTGTGTGTGTYA	50.4	8	7	87.50
I 852	TCTCTCTCTCTCTCTCRA	48.1	8	6	75.00
I 854	TCTCTCTCTCTCTCTCRG	45.2	9	7	77.77

Continuation Table 2

I 855	ACACACACACACACACYT	48.1	21	14	67.67
I 856	ACACACACACACACACYA	47.7	8	5	62.50
I 857	ACACACACACACACACYG	48.1	13	10	76.92
I 859	TGTGTGTGTGTGTGTGRC	49.5	17	12	70.58
I 860	TGTGTGTGTGTGTGTGRA	48.1	13	9	69.23
I 864	ATGATGATGATGATGATG	42.6	6	3	50.00
I 866	CTCCTCCTCCTCCTCCTC	50.7	16	10	62.50
I 873	GACAGACAGACAGACA	42.4	15	7	46.67
I 880	GGAGAGGAGAGGAGA	43.1	14	11	78.57
I 884	HBHAGAGAGAGAGAGAG	41.1	10	7	70.00
I 888	BDBCACACACACACACA	47.3	19	9	47.36
	Total	---	327	209	63.91
	RAPD Primers				
OPA-2	TGCCGAGCTG	35.0	11	7	63.63
OPA-3	AGTCAGCCAC	35.0	13	4	30.76
OPA-8	GTGACGTAGG	35.0	13	12	92.31
OPA-9	GGGTAACGCC	35.0	10	5	50.00
OPAA-4	AGGACTGCTC	35.0	9	1	11.11
OPAB-7	GTAAACCGCC	35.0	11	8	72.72
OPB-17	AGGGAACGAG	35.0	15	10	67.67
OPB-20	GGACCCTTAC	35.0	16	15	93.75
OPD-2	GGACCCAACC	35.0	19	18	94.73
OPD-20	ACCCGGTCAC	35.0	22	18	81.82
OPF-6	GGGAATTCGG	35.0	13	9	69.23
OPF-12	ACGGTACCAG	35.0	10	9	90.00
OPG-2	GGCACTGAGG	35.0	10	4	40.00
OPG-8	TCACGTCCAC	35.0	10	7	70.00
OPL-9	TGCGAGAGTC	35.0	9	4	44.44
OPN-3	GGTACTCCCC	35.0	15	13	86.67
OPN-13	AGCGTCACTC	35.0	17	5	29.41
OPN-14	TCGTGCGGGT	35.0	11	10	90.91
OPO-10	TCAGAGCGCC	35.0	14	11	78.57
OPP-6	GTGGGCTGAC	35.0	11	8	72.73
OPR-1	TGCGGGTCCT	35.0	14	12	85.71
OPW-3	GTCCGGAGTG	35.0	15	10	67.67
OPW-8	GACTGCCTCT	35.0	14	11	78.57
OPW-9	GTGACCGAGT	35.0	14	6	42.86
OPW-11	CTGATGCGTG	35.0	12	6	50.00
UBC-320	CCGGCATAGA	35.0	10	5	50.00
UBC-322	GCCGCTACTA	35.0	15	13	86.67
UBC-341	CTGGGGCCGT	35.0	6	1	16.67
	Total	---	359	242	67.41

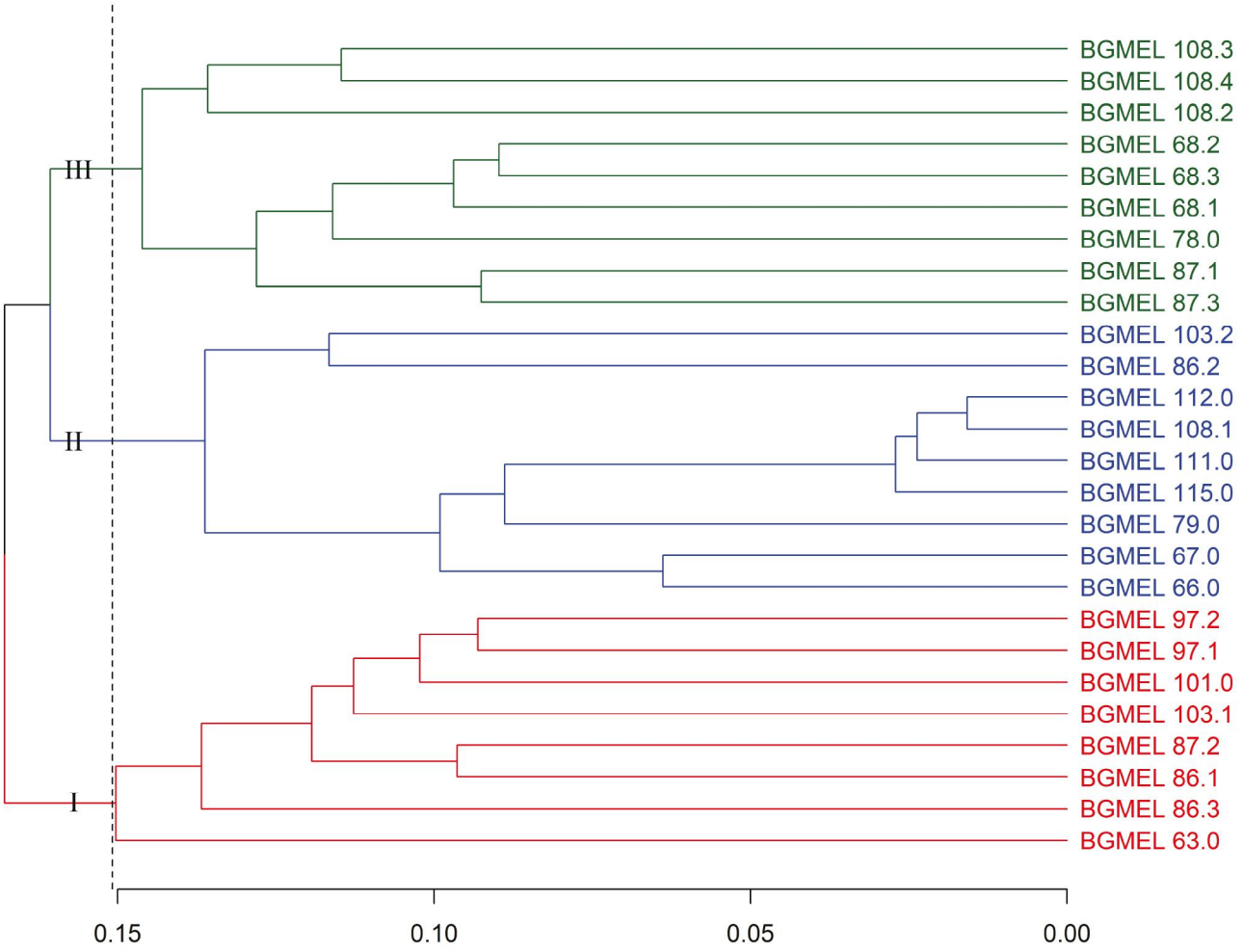
AT - annealing temperature, in °C; TB - total bands; PB - Polymorphic bands; P - Polymorphism, in %. * B-C, G or T; H-A, C or T; R-A or G; Y-C or T

Dantas *et al*. (2012) observed 96.53% of polymorphic bands when analysing 18 RAPD primers in 43 melon accessions collected in the northeast of Brazil. Yildiz, Akgul and Sensoy (2014) detected 63.6% polymorphism in 31 ISSR markers when characterising 24 melon accessions in Turkey. Similarly, Yildiz *et al*. (2011) and Erdinc *et al*. (2013), studying accessions of Turkish melons, found polymorphism of 72.11% and 86.79% in RAPD markers, and 57.50% and 86.76% in ISSR, respectively. Muhammad *et al*. (2020) noted 33.67% polymorphism in six ISSR primers when characterising 12 melon accessions from different regions of Egypt. The polymorphism identified in studies of genetic divergence in the melon using markers is therefore variable, and depends and on the primers used and on the group of genotypes being evaluated, which can have greater or lesser genetic variability. A high degree of band polymorphism is indicative of the wide genetic divergence among melon accessions, and can vary depending on the type of primer (KARIMI *et al*., 2016).

Based on the ISSR loci, and using the UPGMA method, three groups were formed (Figure 1), showing reasonable grouping of the sub-accessions in line with the botanical classification at subspecies level (92.3%), and the respective botanical varieties (96.2%), considering those classified by Amorim *et al*. (2016). Thus, the first group comprised the BGMEL 63.0, BGMEL 86.1, BGMEL 86.3, BGMEL 87.2, BGMEL 97.1, BGMEL 97.2, BGMEL 101.0 and BGMEL 103.1 sub-accessions, all from subspecies *melo*, with a predominance of variety *cantalupensis*, except for the BGMEL 86.3 sub-accessions with *reticulatus* characteristics, and BGMEL 97.2, with *chito* characteristics, both undefined as to botanical variety.

Two subgroups were formed in the second group. The first comprised sub-accessions of subspecies *agrestis* and variety *makuwa* (BGMEL 66.0, BGMEL 67.0, BGMEL 79.0, BGMEL 108.1, BGMEL 111.0, BGMEL 112.0 and BGMEL 115.0). The second subgroup contained the BGMEL 86.2 sub-accession, belonging to subspecies *agrestis* and an undefined botanical

Figure 1 - Dendrogram of genetic dissimilarity (UPGMA) in 26 sub-accessions of *Cucumis melo* from traditional agriculture in Maranhão, obtained by means of the arithmetic complement of the Jaccard coefficient, estimated based on 28 ISSR markers



variety, plus the BGMEL 103.2 sub-accession, which, as it presents great variation in its plant, flower and fruit descriptors (AMORIM *et al*., 2016), is undefined as to both subspecies and botanical variety.

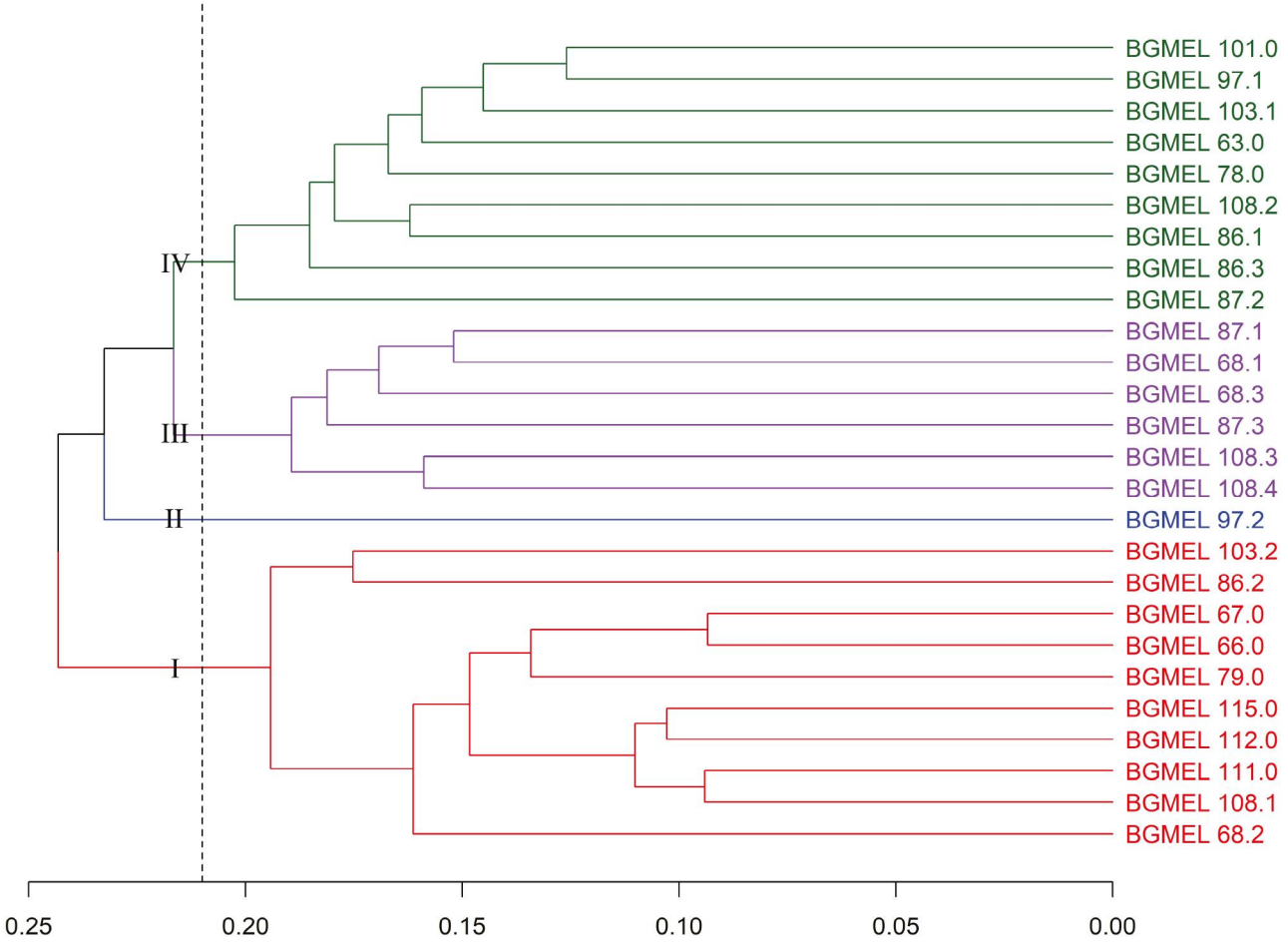
The third group also comprised two subgroups: the first grouping sub-accessions of subspecies *agrestis* and variety *momordica* (BGMEL 68.1, BGMEL 87.1) together with plants of subspecies *agrestis* and an undefined botanical variety, but with the characteristics of *momordica* (BGMEL 68.2); sub-accessions undefined as to subspecies and botanical variety, but also with the characteristics of *momordica* (BGMEL 68.3 and BGMEL 87.3); and, differing from the others, BGMEL 78.0, of subspecies *melo*, variety *cantalupensis*. The second subgroup, formed by three sub-accessions, was botanically more divergent; it was not possible to define the botanical variety of any of the accessions, but only the subspecies of BGMEL 108.2 (*melo*) and BGMEL 108.3 (*agrestis*), in addition to BGMEL 108.4 (undefined).

It should be noted that, in agrobiodiversity, cross-pollination is prevalent in melon germplasm (ARAGÃO *et al*., 2013). Therefore, the fact that the BGMEL 78.0 sub-accession was distant from the other sub-accessions belonging to the same variety may be the result of the introgression of alleles from different botanical varieties (MACEDO *et al*., 2017). However, the efficiency of the ISSR markers in studying variability in melon germplasm from traditional agriculture is confirmed by the formation of different groups and subgroups in the cluster analysis, and in the distribution of botanical varieties at subspecies level.

Based on the RAPD markers, four groups were formed (Figure 2), each comprising only sub-accessions of the same subspecies, *melo* or *agrestis*, considering sub-accessions with a defined botanical classification.

Two subgroups were formed within the first group. The first subgroup contained the BGMEL 68.2, BGMEL 108.1, BGMEL 111.0, BGMEL 112.0, BGMEL 115.0, BGMEL 79.0, BGMEL 66.0 and BGMEL 67.0 sub-accessions, all of subspecies *agrestis* and variety *makuwa*,

Figure 2 - Dendrogram of genetic dissimilarity (UPGMA) in 26 sub-accessions of *Cucumis melo* from traditional agriculture in Maranhão, obtained by means of the arithmetic complement of the Jaccard coefficient, estimated based on 26 RAPD markers



with the exception of BGMEL 68.2, which remained undefined as to botanical variety. The second subgroup comprised the BGMEL 86.2 sub-accessions belonging to subspecies *agrestis* and undefined as to botanical variety, and BGMEL 103.2, of undefined botanical classification.

The second group consists of the BGMEL 97.2 sub-accession only, belonging to subspecies melo, and undefined as to botanical variety, but showing similarities with *chito* (AMORIM *et al*., 2016). In the third group, there was a prevalence of subspecies *agrestis* and the BGMEL 68.1 and BGMEL 87.1 sub-accessions, belonging to variety *momordica*, and BGMEL 108.4, BGMEL 108.3, BGMEL 87.3, BGMEL 68.3, unidentified as to botanical variety. The last group comprised sub-accessions of subspecies *melo*, namely BGMEL 63.0, BGMEL 78.0, BGMEL 86.1, BGMEL 87.2, BGMEL 97.1, BGMEL 101.0, BGMEL 103.1 of variety cantalupensis, and the sub-accessions BGMEL 86.3 and BGMEL 108.2 of unidentified variety, but with the characteristics of *reticulatus* and *inodorus*, respectively.

It should be noted that the RAPD markers were also effective in detecting the genetic variability in *melo* germplasm from family farming, including grouping the sub-accessions by subspecies: *agrestis* (I and III) and *melo* (II and IV), and also by botanical variety: *makuwa* in group I, *momordica* in group III and *cantalupensis* in group IV, when the varieties could be defined, as some sub-accessions showed variations in their plant and fruit characteristics that made identification impossible (AMORIM *et al*., 2016; MACEDO *et al*., 2017). It is worth mentioning here, that this variation was also seen by Pitrat (2016), who proposed a new classification for species *C. melo*, taking into account the variation seen within any one group.

Aragão *et al*. (2013) found no association between the formation of groups and botanical classification; however, those authors followed the classification of Robinson and Decker-Walters (1997), which considers only six botanical groups, two belonging to subspecies *agrestis* (*momordica* and *conomom*) and four to subspecies *melo* (*cantalupensis*, *inodorus*, *flexuosus* and *dudaim*).

Aierken *et al*. (2011), analysing variability in a germplasm of 120 accessions from various parts of the world using RAPD and SSR markers, also found a clear genetic difference between the subspecies *agrestis* and *melo*. Using molecular characterisation with RAPD, ISSR and Sequence-Related Amplified Polymorphism (SRAP) markers, Yildiz *et al*. (2011) also found that most sweet genotypes (*cantalupensis* and *inodorus*) are separated from groups of non-sweet genotypes (*momordica*, *conomom*, *dudaim*). However, those authors also used the Robinson and Decker-Walters classification (1997), which divides the species into six botanical groups. Results obtained by morphological characterisation and ISSR and SSR markers also confirm this difference (MALEKI, SHOJAEIYAN; RASHIDI-MONFARED, 2017; YILDIZ; AKGUL; SENSOY, 2014). In the present study, depending on the ISSR and RAPD markers used, grouping could also be seen between sweet and non-sweet genotypes.

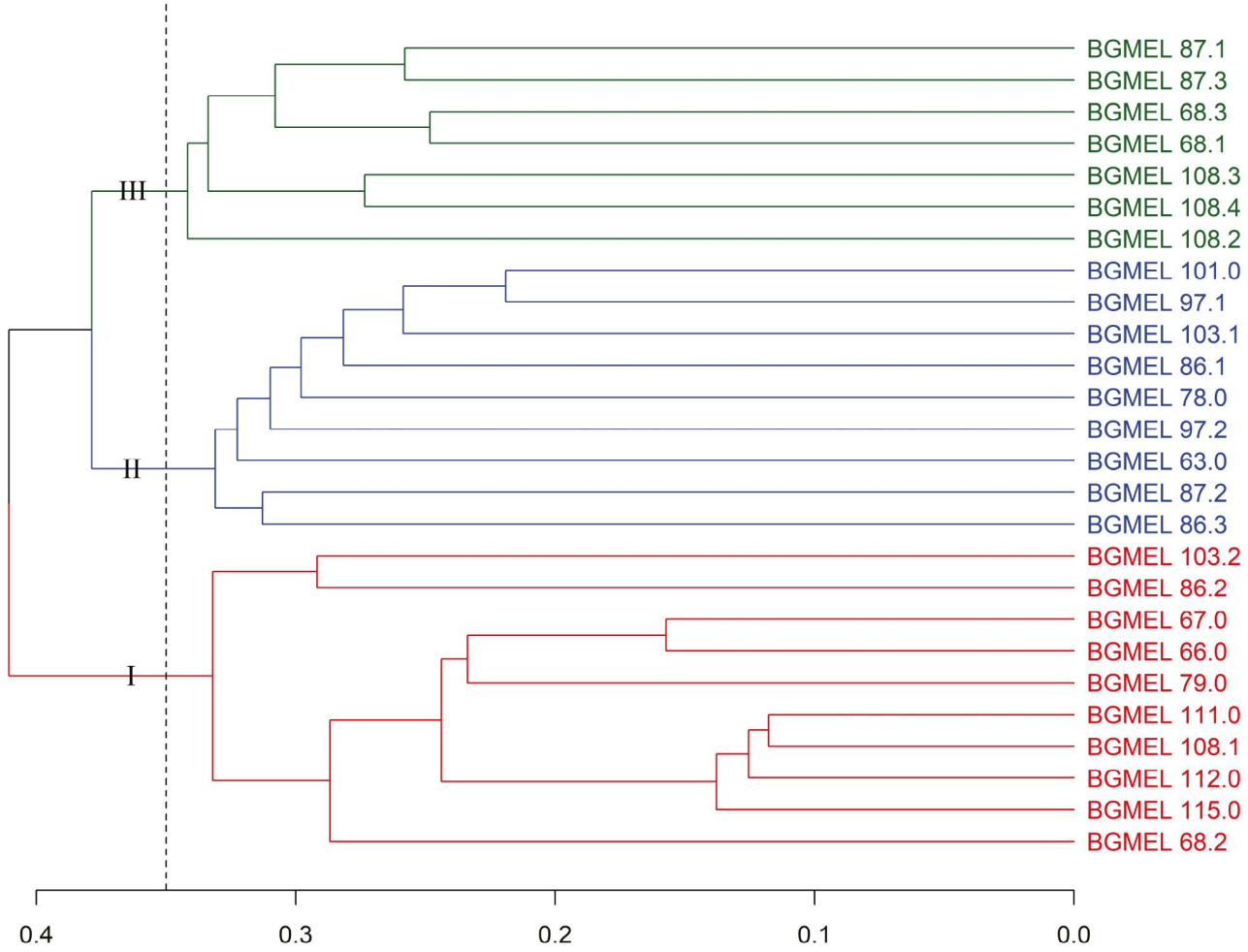
For an analyse of the optimal number of markers, several estimates were obtained for the correlation between the original genetic dissimilarity matrix and the simulated dissimilarity matrix, based on the different number of marks. The optimal number of binary markers for the ISSR data matrix was 210 marks, and 224 for the RAPD data matrix, with a correlation of 0.9540 and 0.9516, and stress value of 0.0261 and 0.0296, respectively. In the study, the number of marks used was well above the estimated optimal number, with 327 marks for the ISSR data matrix and 359 for the RAPD matrix. As such, the number of markers used was considered sufficient to accurately determine the genetic diversity of the individual markers. The CCC of the ISSR dendrogram was 0.82, with 0.83 for the RAPD dendrogram, showing good consistency of the clustering pattern. CCC values of less than 0.7 show the clustering method to be inadequate for representing the information given by the genetic distance matrix (ROHLF, 1970). The stress level was 12.33% and 10.63%, respectively, suggesting high accuracy in adjusting the graphic projection of the genetic distance matrix, based on the Kruskal classification of the adaptability of graphic projection (1964).

The constructed dendrograms were correlated with each other by the Mantel test (1967), using 1% significance and 10,000 permutations, and revealed good genetic correlation between the groups obtained with the ISSR and RAPD markers ($r = 0.72$) evaluated independently.

Given the degree of association between the dendrograms, a joint dendrogram was created (Figure 3) from the sum of all the marks produced by the ISSR and RAPD markers in the sub-accessions under study, giving a total of 686 marks. The optimal number of markers was 382 fragments, with a correlation of 0.9502 between the original matrix and the simulated matrix, and a stress value of 0.03. The values related to CCC (0.86) and stress level (9.62%) were even better in this dendrogram, agreeing with the observed genetic correlation ($r > 0.90$) for the separate results achieved for both markers.

This joint dendrogram allowed the formation of three groups. The first grouped sub-accessions of subspecies *agrestis* (BGMEL 66.0, BGMEL 67.0, BGMEL 68.2, BGMEL 79.0, BGMEL 86.2, BGMEL 108.1, BGMEL 111.0, BGMEL 112.0 and BGMEL 115.0) and BGMEL 103.2, undefined as to subspecies and botanical variety. However, the high genetic similarity of BGMEL 103.2 to the group, corroborated by molecular characterisation, suggests it has the same, or similar, botanical classification as the other sub-accessions (Figure 3).

Figure 3 - Dendrogram of genetic dissimilarity (UPGMA) in 26 sub-accessions of *Cucumis melo* from traditional agriculture in Maranhão, obtained by means of the arithmetic complement of the Jaccard coefficient, estimated based on 26 RAPD and 28 ISSR markers



The second group comprised only sub-accessions of subspecies *melo* (BGMEL 63.0, BGMEL 78.0, BGMEL 86.1, BGMEL 86.3, BGMEL 87.2, BGMEL 97.1, BGMEL 97.2, BGMEL 101.0 and BGMEL 103.1) with a predominance of variety *cantalupensis*. The third group included the BGMEL 68.1 and BGMEL 87.1 sub-accessions (subspecies *agrestis* and variety *momordica*), BGMEL 108.3 (subspecies *agrestis* and undefined variety), BGMEL 108.2 (subspecies *melo* and undefined variety), and the BGMEL 68.3, BGMEL 87.3 and BGMEL 108.4 sub-accessions (undefined subspecies and variety) (Figure 3).

The impossibility of accurately determining the botanical variety of some sub-accesses is probably the result of interbreeding between landraces in areas occupied by the farmers, since there are no barriers to crossings between botanical varieties of the melon. However, the molecular characterisation demonstrated a genetic proximity between sub-accessions, even when complete botanical classification was not possible, increasing the chances of belonging to the same subspecies and/or variety due to similarities in the taxonomic descriptors.

Thus, at the level of subspecies and botanical variety, it was inferred that both markers were efficient in detecting genetic variability in *melo* sub-accessions from traditional agriculture, since only four sub-accessions, representing 15.4%, showed discrepancies between the groups, both individual and joint (Table 3).

It is possible that the allocation of these sub-accessions into distinct groups is the result of the exchange of alleles between different botanical varieties (AMORIM *et al*., 2016; MACEDO *et al*., 2017). Furthermore, the results show that the sub-accessions of subspecies *melo* and *agrestis* are clearly distinct, implying genetic divergence at subspecies level.

It was inferred drom the molecular data that the sub-accessions BGMEL 68.3 and BGMEL 87.3, undefined as to subspecies and botanical variety, may be of variety

Table 3 - Comparison between groups of 26 sub-accessions of Cucumis melo, based on ISSR and RAPD markers, and ISSR+RAPD sets

Group			Sub-acession	Subspecies	Botanical variety*	
ISSR	RADP	ISSR+RAPD			defined	probable
			BGMEL 66.0	agrestis	makuwa	
			BGMEL 67.0	agrestis	makuwa	
			BGMEL 79.0	agrestis	makuwa	
			BGMEL 108.1	agrestis	makuwa	
	1		BGMEL 111.0	agrestis	makuwa	
			BGMEL 112.0	agrestis	makuwa	
			BGMEL 115.0	agrestis	makuwa	
			BGMEL 86.2	agrestis	ND	
			BGMEL 103.2	ND	ND	
			BGMEL 63.0	melo	cantalupensis	
			BGMEL 86.1	melo	cantalupensis	
			BGMEL 87.2	melo	cantalupensis	
	2		BGMEL 97.1	melo	cantalupensis	
			BGMEL 101.0	melo	cantalupensis	
			BGMEL 103.1	melo	cantalupensis	
			BGMEL 86.3	melo	ND	reticulatus
			BGMEL 68.1	agrestis	momordica	
			BGMEL 87.1	agrestis	momordica	
	3		BGMEL 108.3	agrestis	ND	acidulus
			BGMEL 68.3	ND	ND	momordica
			BGMEL 87.3	ND	ND	momordica
			BGMEL 108.4	ND	ND	
2	4	2	BGMEL 97.2	melo	ND	chito
3	1	1	BGMEL 68.2	agrestis	ND	momordica
3	2	2	BGMEL 78.0	melo	cantalupensis	
3	2	3	BGMEL 108.2	melo	ND	inodorus

Botanical classification by Amorim *et al.* (2016), based on Pitrat, Hanelt and Hammer (2000). ND: Not defined

momordica, and as such, of subspecies *agrestis*, the same classification as the other members of the group, as they remained phylogenetically close in the three groups. Amorim *et al*. (2016) were unable to identify the subspecies of these sub-accessions due to segregating by ovarian pilosity.

It is important to highlight that the morphological distinction between the melon subspecies used in the present study was determined from the ovarian pilosity of the flower. However, this classification was questioned by Pitrat (2016), who presented a new classification of melon types based on groups and subgroups, since a large variation in ovarian pilosity was seen between melon plants of the same botanical group, thereby contradicting the earlier classification.

Additionally, the molecular data show close genetic proximity between sub-accessions of variety *makuwa* and the BGMEL 86.2 and BGMEL 103.2 sub-accessions, undefined as to botanical variety (AMORIM *et al*., 2016), indicating that either they belong to the same variety or are related.

CONCLUSION

The study of genetic divergence based on ISSR and RAPD markers showed great genetic variability between sub-accessions of the melon from agrobiodiversity, and confirmed the botanical classification at the level of subspecies and botanical variety.

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