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BOTANICAL IDENTIFICATION AND GENETIC DIVERSITY IN MELONS FROM FAMILY FARMING IN THE STATE OF MARANHÃO[1]

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ABSTRACT – The aim of this work was to perform botanical identification and to estimate genetic diversity in two sequential inbred generations (progenies S_1 and S_2) of melon accessions from traditional agriculture in the state of Maranhão, in order to generate useful information for commercial melon breeding. Two field experiments were carried out in a completely randomized block, using four replicates of 15 accessions from a first selfing cycle in 2013, and three replicates of 25 subaccessions (generation S_2) in 2014. Flower and fruit descriptors were measured to obtain quantitative and qualitative data, in addition to a systematized photographic documentation of fruit for visually comparing the progenies S_1 and S_2. Distance matrices for quantitative and qualitative data were obtained and used to perform a joint analysis and UPGMA method. Large genetic diversity was found in the accessions analysed, since the presence of melon progenies was observed in the *Cucumis melo* ssp. *agrestis*, with its botanical varieties *momordica* and *conomom*, and of the *Cucumis melo* ssp. *melo*, with the botanical varieties *cantalupensis* and *chandalak*. Divergence analysis showed the formation of three groups in generation S_1 and four groups in S_2. However, the groups were not separated either by subspecies or by botanical variety. Thus, in addition to the large genetic diversity among and within melon accessions from family farming in the state of Maranhão, the progenies presented a large introgression of traits of the different subspecies and their botanical varieties due to the reproductive system and seed management of these species.

Keywords: Botanical variety. *Cucumis melo* L.. Endogamic progenies.

CLASSIFICAÇÃO BOTÂNICA E DIVERGÊNCIA GENÉTICA EM MELÕES DA AGRICULTURA FAMILIAR MARANHESE

RESUMO - O trabalho teve como objetivos realizar a classificação botânica e estimar a diversidade genética em duas gerações endogâmicas sequenciais (progênies S_1 e S_2) de acessos de melão da agricultura familiar do Maranhão, visando gerar informações úteis para o melhoramento do melão comercial. Foram conduzidos dois experimentos de campo em blocos casualizados completos, com quatro repetições e 15 acessos em 2013 e, três repetições e 25 subacessos em 2014. Para avaliação dos acessos foram aplicados descritores quantitativos e qualitativos de flor e fruto, além de uma documentação fotográfica sistematizada dos frutos com análise visual, comparando os frutos das gerações S_1 e S_2. Foram obtidas as matrizes de distância nos dois tipos de descritores e se fez a análise conjunta e em seguida realizou-se o agrupamento pelo método UPGMA. Verificou-se que nos acessos analisados, existe uma grande diversidade genética, pois foram encontradas progênies de melão da subespécie *agrestis* e suas variedades botânicas *momordica* e *conomom* e da subespécie melo com as variedades botânicas *cantalupensis* e *chandalak*. A análise de divergência na geração S_1 mostrou a formação de três grupos e na geração S_2 foram formados quatro agrupamentos, entretanto os grupos não foram formados nem por subespécie nem por variedade botânica. Assim, além de se ter encontrado uma grande diversidade genética entre e dentro dos acessos de melão da agricultura familiar maranhense, é provável que tenha ocorrido grande introgressão de alelos das subespécies e das diferentes variedades botânicas na área dos agricultores devido ao sistema reprodutivo da espécie a ao manejo de sementes.

Palavras-chave: Variedade botânica. *Cucumis melo L.*. Progênies endogâmicas.

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INTRODUCTION

Melon (*Cucumis melo* L.), belonging to the Cucurbitaceae family, stands out for its appreciation and growing popularity among consumers both in Brazil and worldwide. In Brazil, commercial hybrids are cultivated and cultivars belong to only two botanical varieties: var. *inodorus* and var. *cantalupensis*, occupying an area of 22,000 ha, of which 19,000 ha are located in Northeast Brazil. Most are found in the states of Rio Grande do Norte and Ceará, followed by the states of Bahia and Pernambuco (IBGE, 2013). The number of commercial cultivars is not large and the types have often been developed for different environmental conditions of the Brazilian semi-arid region.

Tropical Africa has been indicated as the centre of origin of the melon plant (BURGER et al., 2010), although conversely, Sebastian et al. (2010) and John et al. (2012) indicate that Asia is the centre of origin. Regardless of the place of origin of the melon plant, different centres of diversity have been formed (JOHN et al., 2012). In Europe, melon arrived during the decline of the Roman Empire (PURSEGLOVE, 1977). Since then, it was introduced to the Americas by different routes (CORREA, 2010). Subsequently, it was established in family farming in northeastern and southern Brazil, where part of the existing diversity was collected for formation of active germplasm banks (QUEIROZ, 2004; MAPA, 2010).

Extensive polymorphism was found in cultivated melons when compared with wild melons, as reported by Pitrat (2013). Indeed, a large number of polymorphisms was identified in melons grown in China (LUAN; DELANNAY; STAUB, 2008), Turkey (YILDIZ et al., 2014) and for different botanical groups in Tunisia (TRIMECH et al., 2013). Notwithstanding, Roy et al. (2012) found wide variation in wild melons from India and showed that melons of the variety *momordica* were grown there. Thereafter, with the Great Navigations, it is likely that melons of this variety, grown in several states of the Brazilian semi-arid region, have come from India, as shown in the list of accessions from the collection of the Federal Rural University of the Semi-Arid (ALBUQUERQUE et al., 2015). This assumption gains strength because current reports indicate that melons split when approaching maturity in many areas of Northeast Brazil, a descriptor that is characteristic of the variety *momordica*.

Studies by Aragão et al. (2013) and Torres Filho et al. (2009) highlight the existence of a great genetic diversity in melon accessions from family farming in the state of Maranhão. More recently, a study investigated the diversity of melons collected from family farming in Northeast Brazil, based on morphological and molecular characters (DANTAS et al., 2015). However, the two previous works used classifications that did not consider melon subspecies (ARAGÃO et al., 2013; TORRES FILHO et al., 2009), while the last work (DANTAS et al., 2015) used the classification by Pitrat, Hanelt and Hammer (2000), which takes these subspecies into account (*agrestis*, with five varieties, and *melo*, with eleven varieties). Nonetheless, Dantas et al. (2015) only used six accessions from family farming in the state of Maranhão. Thus, the objective of the present work was to study the genetic variability existing in two generations of sequential endogamic progenies of melon (progenies S_1 and S_2) and to make a classification of melon subspecies and their respective botanical varieties in these progenies originating from a sample of melon accessions from family farming in the state of Maranhão.

MATERIAL AND METHODS

Fifteen melon accessions (S_1 progenies) were collected from the traditional agriculture of Maranhão between 1991 and 1997, and were preserved in the Active Germplasm Bank (AGB) of Cucurbitaceae for Northeast Brazil, located at Embrapa Semi-Arid, Petrolina – PE (Table 1).

Table 1. Passport data of melon accessions of the AGB of Cucurbitaceae for Northeast Brazil, collected from traditional agriculture in the state of Maranhão.

Accession	Municipality	Coordinates of the municipality	Collection date
BGMEL 10	São João dos Patos	6° 29′ 43″ South, 43° 42′ 10″ West	05/1991
BGMEL 64	Colinas	7° 6′ 59″ South, 46° 15′ 26″ West	03/1996
BGMEL 72	Arari	3º 27′ 13″ South, 44° 46′ 48″ West	09/1996
BGMEL 77	Coroatá	4° 7′ 31″ South, 44° 7′ 49″ West	03/1997
BGMEL 80	Itapecuru Mirim	3° 23′ 42″ South, 44° 21′ 36″ West	07/1997
BGMEL 82	Itapecuru Mirim	3° 23′ 42″ South, 44° 21′ 36″ West	07/1997
BGMEL 83	Itapecuru Mirim	3° 23′ 42″ South, 44° 21′ 36″ West	07/1997
BGMEL 85	Codó	4° 27′ 19″ South, 43° 53′ 08″ West	07/1997
BGMEL 89	São Luiz Gonzaga	4° 22′ 51″ South, 44° 40′ 14″ West	07/1997
BGMEL 98	Caxias	4° 51′ 32″ South, 43° 21′ 22″ West	07/1997
BGMEL 99	Caxias	4° 51′ 32″ South, 43° 21′ 22″ West	07/1997
BGMEL 109	Caxias	4° 51′ 32″ South, 43° 21′ 22″ West	07/1997
BGMEL 137	São Mateus	4° 2′ 26″ South, 44° 28′ 6″ West	03/1995
BGMEL 139	Itapecuru Mirim	3° 23′ 42″ South, 44° 21′ 36″ West	05/1995
BGMEL 140	Itapecuru Mirim	3° 23′ 42″ South, 44° 21′ 36″ West	05/1995

For the characterization of progenies S_1 and S_2, two experiments were conducted in the experimental field at the Department of Technology and Social Sciences of the University of the State of Bahia (DTCS/UNEB), located in the municipality of Juazeiro-BA, at 09° 24' 50" south latitude and 40° 30' 10" west longitude, with an altitude of 368 metres. The experimental design was a randomized block, with four replicates of 15 S_1 progenies in the experiment performed in 2013, and three replicates of 25 S_2 progenies in the experiment conducted in 2014, using plots of five plants spaced 2.5 m between rows and 0.80 m between plants, with irrigations twice a week in infiltration furrows. However, for some analyses, some progenies could not be included due to the small number of plants available in some plots.

Thus, in 2013, progenies from a selfing cycle (generation S_1) were evaluated within the selected accessions and, simultaneously, inbred progenies were obtained (generation S_2). In 2014, these S_2 progenies were also evaluated, keeping strict control of genealogy.

The descriptor list applied was adapted from IPGRI (2003) and supplemented by descriptors proposed by Pitrat, Hanelt and Hammer (2000) for different phenotypes of the botanical varieties. The qualitative descriptors were: ovary hairiness (short and long) (JEFFREY, 1980), sexual expression (monoecious, andromonoecious and ginomonoecious), fruit shape (globular, flat, elliptical, pear-shaped, oval, elongated, acorn-shaped and malformation), skin colour (light yellow, yellow, yellow-green, intense yellow, light yellow spotted with medium green, yellow spotted with dark green, light green, dark green, light green streaked with dark green, light green streaked with medium green and light green spotted with dark green), presence of furrows (absent, superficial, medium and deep), stripe colour (absent, light green, medium green, dark green) and pulp colour (white, greenish, orange and cream).

Quantitative descriptors were evaluated as follows: AFM - average fruit mass (kg); LD - longitudinal diameter of the fruit (cm); TD - transversal diameter of the fruit (cm); PTS - pulp thickness in the side part of the fruit (cm); PTU - pulp thickness in the upper part of the fruit (cm); PTB - pulp thickness in the bottom part of the fruit (cm); CL - cavity length (cm); CD - cavity diameter (cm); SSL - stylar scar length (mm); SSD - stylar scar diameter (mm); PL - peduncle length (mm); PD - peduncle diameter (mm); SL - seed length (cm); SD - seed diameter (cm); SM - average 100-seed mass (g); SS - soluble solids (°Brix), obtained by extraction of homogenized juice from different parts of the fruit pulp with the aid of a centrifuge, recording the reading with a digital refractometer.

In addition, a systematic photographic record was taken of the internal and external parts of all fruits harvested in generations S_1 and S_2, identifying each plant with respect to its progeny, aiming to capture existing variations between plants within each progeny.

The data of the phenotypic characterization of generations S_1 and S_2 were compared visually with photographic records and consistent qualitative criteria for the classification of melon subspecies and their botanical varieties, according to Pitrat, Hanelt and Hammer (2000).

Following this characterization of phenotypes, it was necessary to establish a subdivision of the accessions into subaccessions, which subsequently received additional codes to accession codes (Table 2). When the accession showed no variation between generations S_1 and S_2, the additional code was 0, indicating that the accession expressed homozygosity in relation to fruit characteristics. When generation S_2 showed variation, for example, exhibiting, fruits with two different characteristics, the accession was then divided into two subaccessions with the additional codes 1 and 2. Thus, for accession BGMEL10 code (Table 2), in cases where there was no variation, it could receive the BGMEL10.0 code, and in the case of segregation, the denomination would be BGMEL 10.1 and BGMEL 10.2, each one being assigned as a subaccession. However, when observing phenotypes that could not be determined by the methodology applied for the classification of subspecies and botanical variety, these subaccessions were allocated to a group of unidentified samples. These were analysed to determine which descriptors contributed significantly to the indeterminacy.

Distance matrices and a joint matrix were obtained for both S_1 and S_2 progenies for quantitative and qualitative descriptors. The genetic dissimilarity for quantitative data was determined from the Mahalanobis distance. To obtain the distance matrix of qualitative descriptors, the method for multicategoric data, based on the simple matching coefficient index, was used. To perform these procedures, GENES software was used (CRUZ, 2013).

The joint matrix was quantified using the Gower algorithm (1971) and cluster analysis was performed by the UPGMA method (Unweighted Pair-Group Method using Arithmetic averages). The cutoff point in the dendrogram was established based on the method of Mojena (1977), and the validation of clusters was tested using the cophenetic correlation coefficient (CCC). To evaluate the significance of the cophenetic correlation, a Mantel test with 10,000 permutations was used. These procedures were performed in the R program (R DEVELOPMENT CORE TEAM, 2012).

RESULTS AND DISCUSSION

From the visual comparison using the photographic documentation of fruits in two generations and the comparison of descriptors applied to generations S_1 and S_2, there was a subdivision of the 15 accessions (S_1 progenies) into 25 subaccessions, even with the presence of accessions which showed no segregation between the two generations. This new categorization was aided by Jeffrey methodology (1980) for classification of melon subspecies and botanical varieties (PITRAT; HANELT; HAMMER, 2000). Thus, a representation of the most contrasting phenotypes is shown in Figure 1. It is worth noting that the fruit colour difference in S_1 and S_2 plants of the subaccession BGMEL 137.0 stems from the different period of harvest.

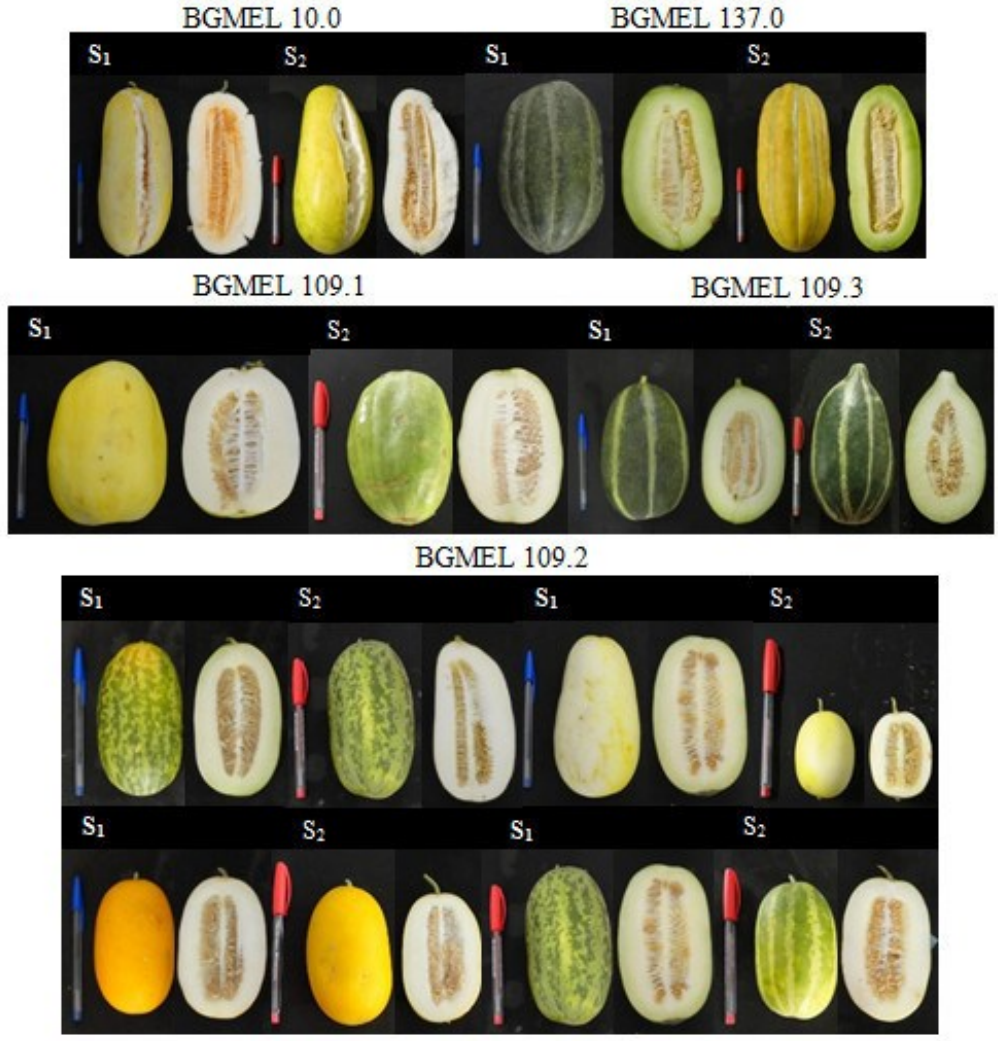

Figure 1. Genetic diversity of generations S_1 and S_2 of the melon progenies evaluated for the morphological descriptors of fruits.

It was found that 47% of genotypes expressed homozygosity when comparing S_1 and S_2 generations for flower and fruit characters. They were identified as the subspecies and botanical variety of the accessions BGMEL 10.0 (ssp. *agrestis* var. *momordica*); BGMEL 72.0; BGMEL 137.0; BGMEL 139.0 and BGMEL 140.0 (spp. *melo* var. *cantalupensis*) (Table 2). It is worth highlighting that these accessions did not show subaccessions and, therefore, zero was added to the accession code (Figure 1 and Table 2).

Table 2. Characterization and classification of the accessions in their respective subspecies (ssp.) and botanical varieties (BV) in inbred generations S_1 and S_2.

Accession	ssp.	BV		OvH	SE	CB	LC	PC
BGMEL 10.0	*agrestis*	*momordica*	S_1	S	M	0	0	1
			S_2	S	M	0	0	1
BGMEL 72.0	*melo*	*cantalupensis*	S_1	L	M	1 2 3	0	2
			S_2	L	M	1 2	0	2
BGMEL 137.0	*melo*	*cantalupensis*	S_1	L	M	2 3	0	2
			S_2	L	M	1 2	0	2
BGMEL 139.0	*melo*	*cantalupensis*	S_1	L	M	1 2	0	2 3
			S_2	L	M	1 2	0	2 3
BGMEL 140.0	*melo*	*cantalupensis*	S_1	L	M	3	0	3
			S_2	L	M	2	0	3
BGMEL 89.0	*melo*	ND	S_1	L	M	0 1 2	0	1 2 3
			S_2	L	M	0 1	0	1 3
BGMEL 98.0	ND	ND	S_1	S L	M	0	0	2
			S_2	S L	M	0 1	0	2
BGMEL 99.0	*melo*	ND	S_1	L	M AN	0 1 2	0	1 2
			S_2	L	M AN	0 1 2	0	1 2
BGMEL 64.1	*agrestis*	*conomon*	S_1	S	AN	0	0	1
			S_2	S	AN	0	0	1
BGMEL 64.2	*agrestis*	ND	S_1	S	M AN	0	0	1
			S_2	S	M AN	0	0	1
BGMEL 77.1	*agrestis*	*momordica*	S_1	S	M	0	0	1
			S_2	S	M	0	0	1
BGMEL 77.2	*agrestis*	ND	S_1	S	M	0 1	0	1
			S_2	S	M	0 1	0	1 3
BGMEL 77.3	ND	ND	S_1	S	M	0 1	0	2
			S_2	S L	M NA	0 1	0	1 2 3
BGMEL 80.1	*melo*	*chandalak*	S_1	L	AN	0	0	2
			S_2	L	AN	0	0	2
BGMEL 80.2	*melo*	*cantalupensis*	S_1	L	M AN	1 2 3	0	2 3
			S_2	L	M AN	1 2	0	2 3
BGMEL 80.3	ND	ND	S_1	S L	NA	0	0	1 2
			S_2	S L	M NA	0 2	0	1 2 3
BGMEL 82.1	*agrestis*	ND	S_1	S	M AN	0	0	2
			S_2	S	M AN	0 2	0	2 4
BGMEL 82.2	ND	ND	S_1	S	M AN	0 1	0	2
			S_2	S L	M AN G	0 1	0	1 2 3
BGMEL 83.1	*agrestis*	ND	S_1	S	M	0 1	0	1 2 3
			S_2	S	M	0 1	0	1 2 3
BGMEL 83.2	ND	ND	S_1	S	AN	0	0	2
			S_2	S L	AN	0	0	2 4
BGMEL 85.1	*melo*	*cantalupensis*	S_1	L	M	2 3	0	2 3
			S_2	L	M	1 2 3	0	2 3
BGMEL 85.2	*melo*	ND	S_1	L	M	0	0	2 3
			S_2	L	M	0	0	2 3

(ssp) – Subspecies: ND – Not defined; (BV) – Botanical Variety: ND - Not Defined; (OvH) – Ovary Hairiness: S – Short, L – Long; (SE) – Sexual Expression: M – Monoecious, AN – Andromonoecious, G – Ginomonoecious; (CB) – Classification of Buds: 0 – absent, 1 – superficial, 2 – medium, 3 – deep; (LC) – Lists Colour: 0 – absent, 1 – light green, 2 – medium green, 3 – dark green; (PC) – Pulp Colour: 1 – white, 2 – greenish, 3 – orangish, 4 – cream.

Table 2. Continuation.

Accession	ssp.	BV		OvH	SE	CB	LC	PC
BGMEL 109.1	*agrestis*	*conomon*	S_1	S	AN	0	0	1
			S_2	S	AN	0	0	1
BGMEL 109.2	*agrestis*	ND	S_1	S	M	0	0 2 3	1 2
			S_2	S	M AN	0 1	0 1 2 3	1 2
BGMEL 109.3	ND	ND	S_1	S	M	0 1	0 1	1 2
			S_2	L	M	0 1	0 1	1 3 4

(ssp) – Subspecies: ND – Not defined; (BV) – Botanical Variety: ND - Not Defined; (OvH) – Ovary Hairiness: S – Short, L – Long; (SE) – Sexual Expression: M – Monoecious, AN – Andromonoecious, G – Ginomonoecious; (CB) – Classification of Buds: 0 – absent, 1 – superficial, 2 – medium, 3 – deep; (LC) – Lists Colour: 0 – absent, 1 – light green, 2 – medium green, 3 – dark green; (PC) – Pulp Colour: 1 – white, 2 – greenish, 3 – orangish, 4 – cream.

Plants of the accessions BGMEL 89 and 99 showed long hairiness of the ovary in both generations (Table 2 and Figure 2), and were therefore identified as *melo* subspecies (JEFFREY, 1980), while accession 98 showed segregation for ovary hairiness in both generation S_1 and S_2, and thus it was not possible to identify the subspecies (Figure 3). In accession 89, despite all plants in generation S_2 showing evidence for belonging to the subspecies *melo*, three fruit phenotypes were present. One fruit showed phenotypic similarity to the botanical variety *momordica* (Figure 3), characteristic of ssp. *agrestis*, giving strong indication of introgression of alleles between subspecies and botanical varieties in plants coming from seeds kept in traditional agriculture. The other two fruits resembled the phenotype of the *cantalupensis* group (Figure 3), nevertheless, the characters did not allow the definition of this botanical variety.

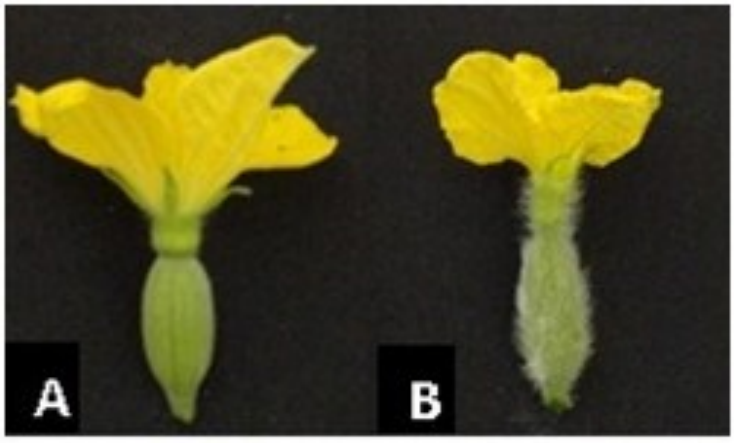

Figure 2. Female melon flowers showing ovary with short and dense hairiness, typical of ssp. *agrestis* (A), and long hairiness, relative to ssp. *melo* (B).

Figure 3. Genetic diversity of fruits in generation S_2 of the accession BGMEL 89.0.

Therefore, defining a botanical variety demands the analysis of various characteristics (PITRAT; HANELT; HAMMER, 2000). The most significant characteristics in generations S_1 and S_2 for determining the subspecies and botanical varieties are presented in Table 2.

The data presented in Table 2 show that some characteristics were expressed in progenies in both

generations. This is possible because the germplasm originates from a species in which some plants have a mixed breeding-system (monoic and andromonoic plants), cultivated predominantly in family farms, where farmers manage seeds over the years and regularly exchange seeds with other farmers. However, among the accessions that had characters which showed to be homozygous in generation S_2, some were collected in different municipalities and only two were collected in different production units within the same municipality (Tables 1 and 2), indicating that the management of seeds is performed at the production unit level.

If selfing of S_2 progenies that showed segregation between generations were to be continued, it would be possible to produce homozygous offspring for ovary hairiness (Figure 3) in the case where alleles for this character are not fixed, although genetic control of this descriptor has not been found from the list of melon genes (DOGIMONT, 2011).

Accessions belonging to the var. *momordica* (Table 2 and Figure 1) are characterized by disruption of the fruit epidermis when reaching maturity, in addition to mealy pulp, absence of sugar and presence of flavour (FERGANY et al., 2011). On the other hand, Manohar and Murthy (2012) found that melon populations of the var. *momordica* had a mild aroma when mature, and were slightly sweet, which was not found in this study. In Brazil, the var. *momordica* is commonly found in street markets in several states, being locally called "caxixi" melon or "pepino" melon. In addition to this botanical variety, var. *cantalupensis* (Table 2) was found in this study, which is characterized by deep furrows (SZAMOSI et al., 2010). However, the accessions studied showed variation in furrow depth, ranging from superficial to deep. It is worth noting that this characteristic is reported as being controlled by a single recessive gene (DOGIMONT, 2011). Thus, the observed furrows gradations may stem from the introgression of alleles responsible for the expression of different types of surfaces of melon fruits managed in family farming.

For other accessions, BGMEL 64, BGMEL 77, BGMEL 80, BGMEL 82, BGMEL 83, BGMEL 85 and BGMEL 109, the formation of a set of progenies that exhibited the same phenotype for ovary hairiness in generations S_1 and S_2 was observed. In some cases, however, segregation for hairiness was observed in sets of progenies in generation S_2 (Table 2). The identification of the subspecies and botanical variety was possible in the subaccessions BGMEL 64.1 and BGMEL 109.1 (ssp. *agrestis* var. *conomon*), BGMEL 77.1 (ssp. *agrestis* var. *momordica*), BGMEL 80.1 (ssp. *melo* var. *chandalak*), BGMEL 80.2 and BGMEL 85.1 (ssp. *melo* var. *cantalupensis*) (Table 2). For another set of progenies of these same accessions, it was only possible to identify the subspecies *agrestis* (BGMEL 64, BGMEL 77, BGMEL 82, BGMEL 83 and BGMEL 109) and *melo* (BGMEL 85) (Table 2). It should be noted that the accession BGMEL 109 illustrates this variation between plants within the accession very well (Figure 1) because there were three groups of progenies. In the first group, as described, the subspecies and botanical variety were identified; in the second group, it was only possible to identify the subspecies; and in the third group, it was not possible to identify either the subspecies or the botanical variety (Table 2). Incidentally, in the second group of progenies, all belonging to the subspecies *agrestis*, the most different fruit phenotypes were observed. This fact again shows strong evidence of introgression of alleles between different botanical varieties. Similar behaviour was observed in the other accessions that showed variation between plants within the same accession. Some were collected in different municipalities (Table 1), but three of them were collected in the same municipality, although in different production units, and thus the diversity found within the same accession seems to be dependent on the genetic constitution.

The var. *conomon*, of Asian origin (ROBINSON; DECKER-WALTERS, 1997), found in the accession BGMEL 109, has been reported to have agronomic characteristics such as low content of soluble solids, smooth, yellow skin, white pulp colour and small seeds (TANAKA et al., 2006). In contrast, Torres Filho et al. (2009) identified significant characteristics for breeding programs in this botanical variety, such as high prolificacy and high firmness. The botanical var. *chandalak*, originally from Central Asia, showed consistent characteristics, with andromonoecious sexual expression, greenish pulp, small seeds and climacteric fruit (PITRAT; HANELT; HAMMER, 2000).

When studying the variability in the two generations of progenies, an important point, in addition to the segregation of ovary hairiness, was the large variation observed for the descriptors identified by Pitrat, Hanelt and Hammer (2000) regarding the definition of each botanical variety. Nonetheless, many descriptors are controlled by a few genes (DOGIMONT, 2011) and, according to Pitrat (2013), there is no barrier to the crossing of different botanical varieties. Hence, the management and exchange of seeds practised by farmers may have favoured the introgression of alleles through cross-pollination of plants of the subspecies and their respective botanical varieties grown nearby. Indeed, Dhillon et al. (2007) confirmed the presence of allelic introgression between different types of melons.

Torres Filho et al. (2009), studying the morphological characterization of melon accessions collected from family farming in the state of Maranhão, used the classification of Munger and

Robinson (1991) and identified 20 accessions of the var. *cantalupensis*, five accessions of the var. *conomon* and nine accessions of the var. *momordica*, and eight remained undefined. However, the classification by Munger and Robinson (1991) is very different when compared to that of Pitrat, Hanelt and Hammer (2000), which was used in the present study. Munger and Robinson (1991) do not consider the subspecies, dividing species into only one wild variety and six grown varieties. Using this system, although working with the same germplasm, the groups identified by Torres Filho et al. (2009) cannot be compared with the data of the present work. Similarly, Aragão et al. (2013) studied the genetic diversity of accessions previously characterized by Torres Filho et al. (2009), but adopted the classification of Robinson and Decker-Walters (1997), which is also very different from that used by Pitrat, Hanelt and Hammer (2000), since it considers the existence of only six varieties. The authors also observed that there was no association between morphological and molecular clusters (ARAGÃO et al., 2013). Although the information available in the Brazilian literature cannot be compared with the results achieved in this study, they invariably corroborate the existence of large variability within and among melon accessions from family farming in the state of Maranhão. This variability was recorded for plant and fruit attributes as well as for tolerance to biotic stresses, such as powdery mildew caused by the fungus *Podosphaera xanthii* (SANTOS, 2011). As detailed by Dogimont (2011), the various botanical varieties have genes that are expressed in many biotic stresses affecting the melon crop and thus this germplasm, although exotic, is strategic for commercial melon breeding in Northeast Brazil. Moreover, this germplasm may be responsible for more than 90% of the Brazilian melon production for the domestic market and, especially, for the foreign market. Furthermore, the melon crop, formed mostly from cultivars of the group *inodorus*, is affected by many biotic agents such as the leafminer *Liriomyza* sp., potyvirus, and nematodes, among others. The deepening of the study of this germplasm containing different botanical groups can be a very valuable source for future studies in breeding programs of commercial types.

It is important to note that this germplasm is only now being studied in Brazil, but it is widely studied in several countries, such as India (FERGANY et al., 2011; MANOHAR; MURTHY, 2012; ROY et al., 2012), Tunisia (TRIMECH et al., 2013), China (LUAN; DELANNAY; STAUB, 2008) and Turkey (SZAMOSI et al., 2010).

Thus, it is observed that in the traditional agriculture of Maranhão, there is a large genetic diversity, since melon progenies of the two subspecies were found, as well as the botanical varieties *momordica* and *conomom* of ssp. *agrestis* and the botanical varieties *cantalupensis* and *chandalak* within ssp. *melo*, as indicated above. It is important to highlight that in some cases, all progenies of a given accession belonged to only one botanical variety (e.g. BGMEL 10.0, BGMEL 72.0, BGMEL 137.0, BGMEL 139.0 and BGMEL 140.0) and in other cases, two botanical varieties were found in the same accession (e.g. BGMEL 80.1, *melo*, *chandalak*; and BGMEL 80.2, *melo*, *cantalupensis*) (Table 2). Therefore, estimating the genetic diversity within and among accessions (subaccessions) in generations S_1 and S_2 becomes important.

The joint analysis of the qualitative and quantitative descriptors in generation S_1 formed three groups (Figure 4). The first group was formed by the accessions BGMEL 82, BGMEL 64 and BGMEL 109. The second was formed by the accessions BGMEL 85, BGMEL 139, BGMEL 99, BGMEL 80, BGMEL 72 and BGMEL 98. The last group was formed by the accessions BGMEL 89, BGMEL 83, BGMEL 10 and BGMEL 77.

In this evaluation, the cophenetic value was high ($r = 0.82$), indicating consistency of the clustering method used, since values close to unity show good representation (CRUZ; CARNEIRO, 2003; VAZ PATTO et al., 2004).

The dendrogram relating to the analysis of S_2 progenies (Figure 5) formed four groups, with $r = 0.61$ and a cutoff point of 0.34. The dendrogram shows that groups two and four comprise different subspecies and different botanical varieties (Table 2), unlike groups one and three, which are formed by a single accession (BGMEL 89.0, ssp. *melo* and variety not identified) and one progeny of the accession BGMEL 83 without definition of the subspecies or botanical variety, respectively.

Thus, in group two, there are 12 groups of progenies: four groups of the subspecies *agrestis*, five groups of the subspecies *melo*, including the botanical varieties *momordica* and *cantalupensis*, and three groups of progenies in which neither the subspecies nor the botanical variety were identified, in accessions BGMEL 77, BGMEL 98 and BGMEL 109 (Figure 5 and Table 2). In group four, there are nine groups of progenies, two of which belong to the botanical variety *conomom* (ssp. *agrestis*), one belonging to the botanical variety *chandalak* and one belonging to the botanical variety *cantalupensis* (both of the ssp. *melo*) and five groups of progenies; totalling five groups belonging to the subspecies *agrestis*, two belonging to the ssp. *melo* and two groups in which neither the subspecies nor the botanical variety were identified (accessions BGMEL 80 and BGMEL 82 - Figure 5 and Table 2).

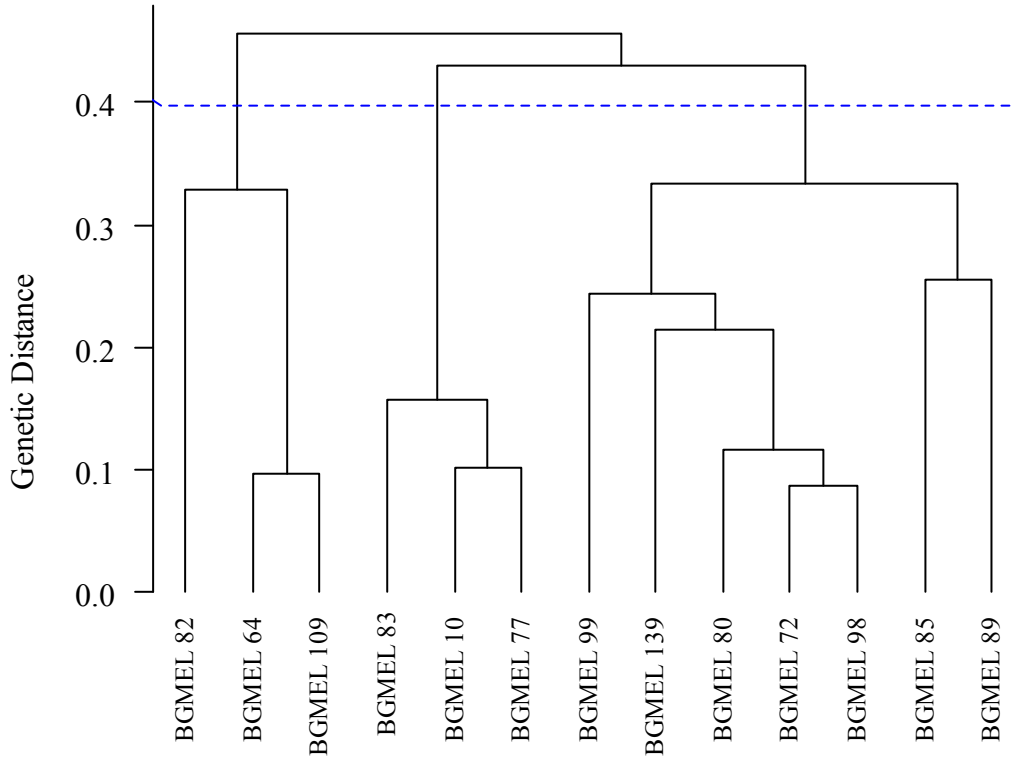

Figure 4. Dendrogram of the genetic dissimilarity of 13 melon accessions (generation S_1) from traditional agriculture in the state of Maranhão, obtained by the UPGMA method.

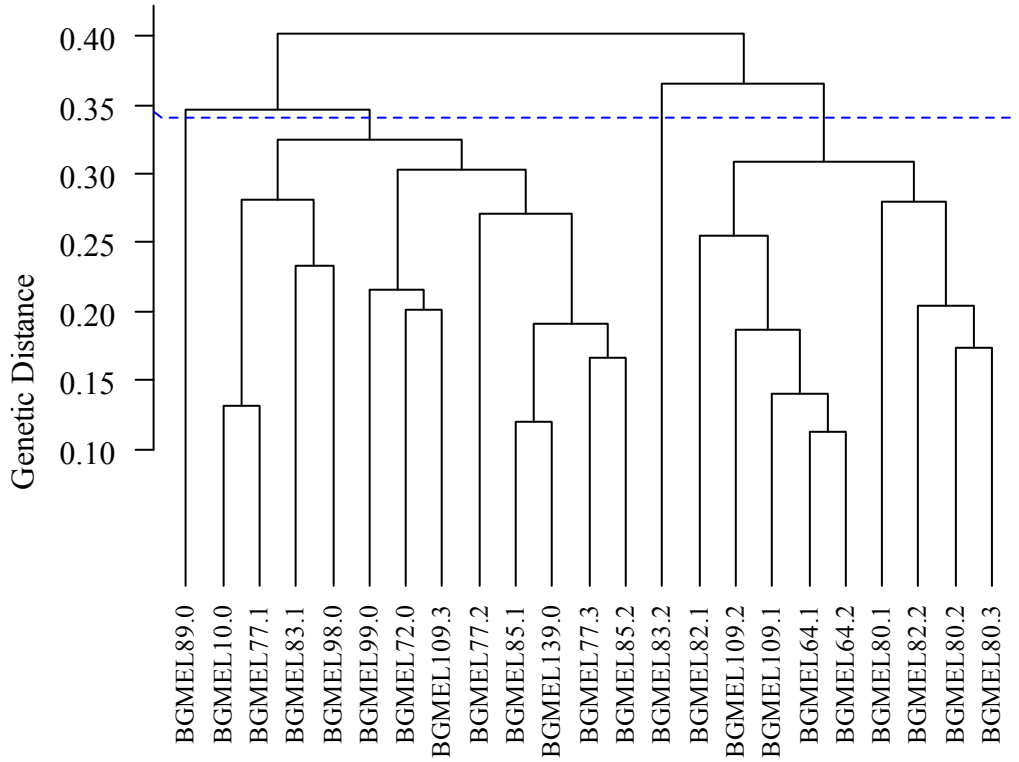

Figure 5. Dendrogram of the genetic dissimilarity between 23 subaccessions (generation S_2) derived from 13 melon accessions (generation S_1) from traditional agriculture in the state of Maranhão, obtained by the UPGMA method.

The introgression of alleles between subspecies and their varieties may have interfered with the genetic diversity observed in generations S_1 and S_2 once the formed groups always had different subspecies and botanical varieties (Figures 4 and 5, Table 2). Although, in the dendrogram of progenies in generation S_2, it was observed that the junction of two identical botanical varieties were within a given subgroup, e.g. subaccessions BGMEL 10.0 and BGMEL 77.1 (ssp. *agrestis*, *momordica*), subaccessions BGMEL 85.1 and BGMEL 139.0 (ssp. *melo*, *cantalupensis*) (Table 2 and Figure 5). However, the reverse situation was also observed, since the botanical variety *cantalupensis* was found in various subgroups within the formed groups. This supports the hypothesis that characteristic introgression takes place between subspecies and their various botanical varieties due to the management of seeds in family farming. Therefore, obtaining further selfing generations in the botanical varieties that could not be identified could result in the expression of more homozygous types. Thus, it would be possible to identify new botanical varieties suitable for the genetic breeding of the species, since most of the characteristics that indicate the botanical varieties are controlled by a few genes (DOGIMONT, 2011). Indeed, among the melon accessions from family farming, the presence of some sources of resistance to pathogens was identified, such as *Rhizoctonia solani* 401 (SALES JÚNIOR et al., 2015), *Myrothecium roridum* (NASCIMENTO et al., 2012), *Macrophomina phaseolina* 402 (AMBRÓSIO et al., 2015) and *Pseudoperonospora cubensis* (ALBUQUERQUE et al., 2015).

On the other hand, it is also important to emphasize that obtaining inbred generations will not necessarily result in the expression of new botanical varieties, as the allelic fixation of the characteristics that define different botanical varieties in one accession may have already occurred. Over the years, these progenies resulting from natural intercrossing were being selected according to the interests of family farmers, and may have rare allelic combinations, useful to melon species breeding programs. This germplasm can be evaluated for its agronomic performance and its reaction to the various biotic and abiotic stressors that affect the culture, in addition to having the attributes of fruit quality and post-harvest conservation well-characterized. Thus, the genetic variability shown can support the development of new cultivars that are adapted to the edaphoclimatic conditions and production systems prevalent in Northeast Brazil, and that may provide innovation and attractiveness to the consumer market.

CONCLUSIONS

There is a large genetic diversity within and between melon accessions from family farming in the state of Maranhão, revealing the existence of two subspecies of melon, different botanical varieties and a large introgression of alleles among different subspecies and botanical varieties. The observed genetic variability and knowledge of the genetic basis of the characteristics that allow the identification of botanical varieties subsidize the adoption and use of this valuable germplasm in commercial melon breeding programs.

REFERENCES

ALBUQUERQUE, L. B. et al. Caracterização morfológica de fontes de resistência de meloeiro a *Pseudoperonospora cubensis*. **Revista Caatinga**, Mossoró, v. 28, n. 3, p. 100-107, 2015.

AMBRÓSIO, M. M. Q. et al. Screening a variable germplasm collection of *Cucumis melo* L. for seedling resistance to *Macrophomina phaseolina*. **Euphytica**, Wageningen, v. 206, n. 2, p. 1-12, 2015.

ARAGÃO, F. A. S. et al. Genetic divergence among accessions of melon from traditional agriculture of the Brazilian Northeast. **Genetics and Molecular Research**, Ribeirão Preto, v. 12, n. 4, p. 6356-6371, 2013.

BURGER, Y. et al. Genetic Diversity of *Cucumis melo*. **Horticultural Reviews**, Westport, v. 36, n. 1, p. 165-198, 2010.

CORREA, S. M. S. Africanidades na paisagem brasileira. **Revista Internacional Interdiscinar Interthesis**, Florianópolis, v. 7, n. 1, p. 96-116, 2010.

CRUZ, C. D.; CARNEIRO, P. C. S. **Modelos biométricos aplicados ao melhoramento genético**. Viçosa, MG: UFV, 2003. 585 p.

CRUZ, C. D. Genes: a software package for analysis in experimental statistics and quantitative genetics. **Acta Scientiarum**. **Agronomy**, Maringá, v. 35, n. 3, p. 271-276, 2013.

DANTAS, A. C. A. et al. Diversity of melon accessions from northeastern Brazil and their relationships with germplasms of diverse origins. **Journal American Society Horticulture Science**, Mount Vernon, v. 140, n. 5, p. 504-517, 2015.

DHILLON, N. P. S. et al. Diversity among landraces of Indian snapmelon (*Cucumis melo* var. *momordica*). **Genetic Resources and Crop Evolution**, Heidelberg, v. 54, n. 6, p. 1267-1283, 2007.

DOGIMONT, C. 2011 Gene list for melon. **Cucurbit Genetics Cooperative Report**, Raleigh, NC, n. 33, p. 104-133, 2011.

FERGANY, M. et al. Variation in melon (*Cucumis melo*) landraces adapted to the humid tropics of southern India. **Genetic Resources and Crop Evolution**, Heidelberg, v. 58, n. 2, p. 225-243, 2011.

GOWER, J. C. A general coefficient of similarity and some of its properties. **Bometrics**, Arlington, v. 27, n. 4, p. 857-874, 1971.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTÁTICA - IBGE. **Produção agrícola municipal**. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística Instituto Brasileiro de Geografia e Estatística, 2013. 102 p. Disponível em: <ftp://ftp.ibge.gov.br/Producao_Agricola/Producao_Agricola_Municipal_[anual]/2013/pam2013.pdf > Acessado em: 02 mar. 2014.

INTERNATIONAL PLANT GENETIC RESOURCES INSTITUTE - IPGRI. **Descriptors for melon (*Cucumis melo* L.)**. Rome: IPGRI. 2003. 65 p. Disponível em: <http://www.bioversityinternational.org/uploads/tx_news/Descriptors_for_melon__Cucumis_melo_L.__906.pdf>. Acessado em: 02 jan. 2013.

JEFFREY, C. Further notes on Cucurbitaceae: V. The Cucurbitaceae of the Indian subcontinent. **Kew Bulletin**, Kew, v. 34, n. 4, p. 789-809, 1980.

JOHN, K. J. et al. On the occurrence, distribution, taxonomy and genepool relationship of *Cucumis callosus* (Rottler) Cogn., the wild progenitor of *Cucumis melo* L. from India. **Genetic Resources and Crop Evolution**, Heidelberg, v. 59, n. 2, p 1-10, 2012.

LUAN, F.; DELANNAY, I.; STAUB, J. E. Chinese melon (*Cucumis melo* L.) diversity analyses provide strategies for germplasm curation, genetic improvement and evidentiary support of domestication patterns. **Euphytica**, Wageningen, v, 164, n. 2, p. 445-461, 2008.

MANOHAR, S. H.; MURTHY, H. N. Estimation of phenotypic divergence in a collection of *Cucumis melo*, including shelf-life of fruit. **Scientia Horticulturae**, Amsterdam, v. 148, n. 1, p. 74-82, 2012.

MINISTÉRIO DA AGRICULTURA E DO ABASTECIMENTO - MAPA. **Acervo do Banco Ativo de Germoplasma de Cucurbitáceas da Embrapa Clima Temperado** — 2002 a 2010. Disponível em: <http://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/884191/1/documentos295.pdf>. Acessado em: 27 fev. 2014.

MOJENA. R. Hierárquical grouping method and stopping rules: an evaluation. **Computer Journal**, London, v. 20, n. 4, p. 359-363, 1977.

MUNGER, H. M.; ROBINSON, R. W. Nomenclature of *Cucumis melo* L.. **Cucurbit Genetic Cooperative Report**, Raleigh, n. 14, p. 43-44, 1991.

NASCIMENTO, Í. J. B. et al. Reaction of melon accessions to crater rot and resistance inheritance. **Horticultura Brasileira**, Brasília, v. 30, n. 3, p. 459-465, 2012.

PITRAT, M.; HANELT. P.; HAMMER K. Some comments on interspecific classification of cultivars of melon. **Acta Horticulturae**, Wageningen, v. 510, n. 1, p. 29-36, 2000.

PITRAT, M. Phenotypic diversity in wild and cultivated melons (*Cucumis melo*). **Plant Biotechnology**, Tokyo, v. 30, n. 3, p. 273-278, 2013.

PURSEGLOVE, J. W. **Tropical crops**: Dicotyledons. 3. ed. London: Longman, 1974. 719 p.

QUEIROZ, M. A. Germplasm of cucurbitacae in Brazil. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 4, n. 4, p. 317 - 383, 2004.

R DEVELOPMENT CORE TEAM. **R: A language and environment for statistical computing**. R Foundation for Statistical Computing, Vienna, Áustria. 2012.

ROBINSON, R. W.; DECKER-WALTERS. D. S. **Cucurbits**. New York: CAB International, 1997, 225 p.

ROY, A. et al. Wild melon diversity in India (Punjab state). **Genetic Resources and Crop Evolution**, Dordrecht, v. 59, n. 5, p. 755-767, 2012.

SALES JÚNIOR, R. et al. Caracterização morfológica de fontes de resistência de meloeiro a *Rhizoctonia solani*. **Horticultura Brasileira**, Brasília, v. 33, n. 2, p. 196-202, 2015.

SANTOS, A. S. **Reação de Acessos de melão do BAG de cucurbitáceas para Nordeste brasileiro ao oídio**. 2011. 49 f. Dissertação (Mestrado em

Horticultura Irrigada: Área de Concentração Melhoramento e Manejo de Plantas Hortícolas) – Universidade do Estado da Bahia, Juazeiro, 2011.

SEBASTIAN, P. et al. Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. **Proceedings National Academy Science USA**, Washington, v. 107, n. 32, p. 14269-14273, 2010.

SZAMOSI, C. et al. Morphological evaluation and comparison of Hungarian and Turkish melon (*Cucumis melo* L.) germplasm. **Scientia Horticulturae**, Amsterdam, v. 124, n. 2, p. 170-182, 2010.

TANAKA, K. et al. Molecular characterization of south and east Asian melon, *Cucumis melo* L., and origin of group conomon var. *makuwa* and var. *conomom* revealed by RAPD analysis. **Euphytica**, Wageningen, v. 153, n. 1. p. 233-247, 2006.

TORRES FILHO, J. et al. Caracterização morfológica de acessos de meloeiro coletados no nordeste brasileiro. **Revista Caatinga**, Mossoró, v. 22, n. 3, p.174-18, 2009.

TRIMECH, R. et al. Genetic variation in Tunisian melon (*Cucumis melo* L.) germplasm as assessed by morphological traits. **Genetic Resources and Crop Evolution**, Dordrecht, v. 60, n. 5, p. 1621-1628, 2013.

VAZ PATTO, M. C. et al. Assessing the genetic diversity of Portuguese maize germoplasm using microsatellite markers. **Euphytica**, Wageningen, v. 137, n. 1, p. 63-72, 2004.

YILDIZ, M. et al. Use of ISSR, SRAP, and RAPD markers to assess genetic diversity in Turkish melons. **Scientia Horticulturae**, Amsterdam, v. 130, n. 1, p. 349-353, 2011.