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Genetic divergence in basil cultivars and hybrids

Rodrigo P Alves [1]; Ana Veruska C Silva [2]; Camila S Almeida-Pereira [1]; Tatiana S Costa [2]; Sheila V Alvares-Carvalho [1]; Maria de Fatima Arrigoni-Blank [1]; Arie F Blank [1]

[1]Universidade Federal de Sergipe (UFS), São Cristóvão-SE, Brazil; rodrigo.pa07@gmail.com; camilaalmeida.agro@gmail.com; carvalhosva@gmail.com; fatima.blank@gmail.com; arie.blank@gmail.com; [2]Embrapa Tabuleiros Costeiros, Aracaju-SE, Brazil; ana.veruska@embrapa.br; tatianaitase@gmail.com

ABSTRACT

Basil is an aromatic herb that stands out for its economic importance. It is consumed *in natura* and used to obtain essential oil. The cultivation of this species in several regions of the world has allowed variations by natural crosses and euploidy, leading to the wide genetic variability found nowadays. Considering the importance of this species, we aimed to analyze the genetic diversity of 27 basil genotypes using ISSR molecular markers. Fourteen primers were employed for DNA amplification, resulting in 86% polymorphism. Based on the Jaccard's dissimilarity index, the highest index (0.80) was observed between the individuals BAS001 and BAS012, while the lowest index (0.18) was detected between the genotypes BAS014 and BAS015. The genetic similarity among individuals was calculated, forming four distinct clusters. Most individuals (40.7%) were allocated in cluster I. The polymorphic information content (PIC) (0.89) indicated considerable levels of genetic diversity among genotypes. In this sense, the ISSR markers were efficient in the detection of polymorphisms between the accessions, suggesting the genetic variability of the collection. This result demonstrates the importance of the use of molecular markers and the advantages that this information provides to the breeding of the species.

Keywords: *Ocimum basilicum*, diversity, molecular characterization, dominant marker.

RESUMO

Divergência genética observada entre cultivares e híbridos de manjericão

O manjericão é uma erva aromática que se destaca por possuir importância econômica. É consumido *in natura* e também utilizado na obtenção de óleo essencial. O cultivo desta espécie em diversas regiões do mundo permitiu que surgissem variações mediante cruzamentos naturais e euploidia, ocasionando a ampla variabilidade genética existente. Considerando a importância desta espécie, este trabalho teve como objetivo analisar a diversidade genética de 27 genótipos de manjericão usando marcadores moleculares ISSR. Quatorze primers foram utilizados para amplificação do DNA, resultando em 86% de polimorfismo. Com base no índice de dissimilaridade de Jaccard, observou-se o maior índice (0,80) entre os indivíduos BAS001 e BAS012, enquanto que o menor índice de dissimilaridade (0,18) foi detectado entre os genótipos BAS014 e BAS015. A semelhança genética entre indivíduos foi calculada, formando quatro grupos distintos. A maioria dos indivíduos (40,7%) foi agrupada no grupo I. O conteúdo de informação polimórfica (PIC) (0,89) indicou níveis consideráveis de diversidade genética entre os genótipos. Neste sentido, os marcadores ISSR foram eficientes na detecção de polimorfismos entre os acessos e confirmaram que é possível inferir a variabilidade genética na coleção. Isso demonstra a importância do uso de marcadores moleculares e as vantagens que esta informação pode oferecer ao melhoramento genético das espécies.

Palavras-chave: *Ocimum basilicum*, divergência, caracterização molecular, marcador dominante.

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Basil (*Ocimum basilicum*) belongs to the Lamiaceae family and originates from South-East Asia (India, Pakistan, Iran, Thailand, and other countries) and Central Africa (Makri & Kintzios, 2008). The species has several culinary, ornamental, and aromatic uses (Blank *et al*., 2004). Besides the culinary use, basil has been traditionally employed as medicinal herb in the treatment of headaches, cough, diarrhea, constipation, warts, and/or kidney malfunction (Politeo *et al*., 2007). The global trade of basil in 2013 presented China, India, Madagascar, Egypt, and Mexico as the major exporters. Conversely, the major importers were China, including Hong Kong, South Africa, USA, Germany, and Madagascar (FAO, 2017).

Due to the economic potential of medicinal and aromatic species, studies have increasingly focused on their germplasm characterization and conservation (Souza, 2015). Conservation of plant genetic resources ensures the maintenance of agro-biodiversity and provides farmers and plant breeders with options to develop, by selection and breeding, new and more productive crops, which are resistant to virulent pests and diseases and adapted to changing environments (Singh *et al*., 2017). The evaluation of diversity levels identifies parental combinations to create segregating progenies with maximum genetic variability and introduces desirable genes to the available genetic base (Samal *et al*., 2011).

Ocimum basilicum has distinct morphological diversity regarding its

pigmentation, leaf shape, size, herbage, and oil yield (Saran *et al*., 2017). The agro-morphological characterization is the evaluation of all observable traits that could identify varieties or accessions in a collection, and it can be carried out in several forms, depending on the objectives (Moche *et al*., 2014). Cultivar identification based on phenotypic traits is usually influenced by the environment, which hinders the classification (Aghaei *et al*., 2012). Conversely, DNA fingerprinting with molecular markers allows precise, objective, and rapid cultivar identification, and has proven to be an efficient tool for germplasm characterization and management (Chakravarthi & Naravaneni, 2006).

For breeders, the use of DNA markers to characterize varieties, lines, or hybrids has been of great importance since it allows verifying duplicity of accessions in germplasm collections, besides assisting in backcross programs (Borém, 2005; Melo *et al*., 2011). ISSR markers stand out among the techniques for DNA markers due to the advantages over other dominant markers, besides their greater applicability to genetic studies. ISSR markers are generated by microsatellite sequences, which are highly variable and distributed over the genome. ISSR markers have higher reproducibility when compared with RAPDs markers (Random Amplified Polymorphic DNA) and are cheaper than the AFLPs markers (Amplified Fragment Length Polymorphism) (Ng & Tan, 2015). This technique has been used to estimate the genetic diversity in different medicinal and aromatic plants, such as Ocimum (Aghaei *et al*., 2012; Chen *et al.*, 2013; Patel *et al.*, 2015), *Varronia curassavica* (Brito *et al*., 2016), and *Croton tetradenius* (Almeida-Pereira *et al*., 2016).

In Brazil, basil breeding programs have been successfully conducted in the state of Sergipe, which is evidenced by the release of a new basil cultivar, Maria Bonita (high yield and essential oil content), and the hybrid Norine (rich in methyl cinnamate and linalool), both for cultivation in the Brazilian Northeast (Blank *et al.*, 2007, 2015). Considering the importance of the subject under study, this work aimed to evaluate the genetic diversity among 27 basil genotypes, using ISSR markers.

MATERIAL AND METHODS

Plant material

Young leaves were sampled from plants collected at the Research Farm of Universidade Federal de Sergipe (UFS)", located in the municipality of São Cristovão, Sergipe, totaling 27 individuals. Out of these 27 individuals, 25 are commercial varieties, and two are hybrids produced in UFS (Table 1). During collection, leaves were stored in sterile gauze and kept on ice to prevent oxidation. Subsequently, all the collected material was stored in a freezer at -80°C until DNA extraction.

DNA extraction and PCR-ISSR amplification

Approximately 1 g of each sample was subject to DNA extraction, based on the method described by Doyle & Doyle (1990), modified by Alzate-Marin *et al.* (2009). After extraction, DNA was quantified using the Nanodrop 2000c spectrophotometer (Thermo Scientific, EUA), followed by DNA dilutions for further use in the PCR reactions. The quality of the extracted DNA was verified by 0.8% agarose gel horizontal electrophoresis. DNA samples were stored at -20**ºC**.

Twenty-three ISSR primers were tested, but only fourteen showed polymorphism higher than 75%; therefore, they were used to verify the genetic diversity of the 27 basil genotypes.The ISSR primers belonged to the University of British Columbia, Vancouver, Canada (Table 2). Each ISSR reaction was performed in a 12 µL pre-sterile microtube containing 5.8 µL autoclaved ultrapure water, 1 µL DNA genomic (5 ng/µL), 0.2 µL Taq polymerase (Ludwig Biotec, Brasil-RS) (5 U/µL), 2 µL 10x buffer (100 mM Tris-HCl, pH 8.5, 500 mM KCl), 0.6 µL $MgCl_2$ (50 mM) (Ludwig Biotec, Brasil-RS), 0.4 µL dNTP (2.5 mM), and 2.0 µL primer (25.0 pmol).

The ProFlex PCR System (Thermo Fisher Scientific, Applied Biosystems, Foster City, CA, USA) was used for DNA amplification, which was programmed with initial denaturation at 95°C for 5 minutes; followed by 35 amplification cycles. In each cycle, samples were subject to denaturation at 94°C for 40 seconds; annealing at different temperatures (depending on the optimum temperature of the primer used) for 1 minute; and extension at 72°C for 1 minute. After the reactions cycle, a final extension was carried out at 72°C for 7 minutes.

Afterward, the products were subject to 2.0% agarose gel horizontal electrophoresis. The 100 bp ladder (Ludwig Biotec, Brasil-RS) was used as a molecular weight standard. Subsequently, the agarose gels were stained with ethidium bromide solution (0.5 µL/mL) for the visualization of the DNA fragments under ultraviolet light, and then photo-documented by the Gel doc L-pix device (Loccus Biotecnologia, Cotia-SP).

Data analysis

PCR-ISSR amplification products on agarose gel were analyzed for all the primers used in this study, generating a binary matrix based on the presence (1) and absence (0) of bands. All analyses used this matrix to study the genetic diversity of basil genotypes. Percentage of polymorphic bands (PBP) was obtained by the Genalex 6.5 software (Peakall & Smouse, 2012). Polymorphic Information Content (PIC) and values of correlation and stress were estimated using the GENES software (Cruz, 2016).

Genetic diversity among individuals was calculated using the Jaccard's distance. The dendrogram and the Bootstrap analysis for 10.000 simulations were constructed by the GENES software (Cruz, 2016), based on the genetic dissimilarity index, using the Ward method. The structure of the population and the identification of admixed individuals were performed using the software Structure, version 2.3.4 (Pritchard *et al*., 2000). The software uses the Bayesian clustering method and assumes the distance of K populations (in which K may be unknown), and each one of these populations is characterized by a set of allele frequencies for each locus. To determine the number of clusters (K) in the samples, the values K= 2 to K=

12 were assigned, and five independent analyses were performed for each value of K. The admixture ancestry model was used, and the results were based on 100.000 simulations with burn-in of 10.000. The ΔK value was used to select the ideal number of clusters (Evanno *et al*., 2005). This value was estimated using the Structure Harvester software (Earl & von Holdt, 2012).

RESULTS AND DISCUSSION

The genetic variability of 27 basil genotypes was estimated using 14 ISSR primers, totaling 147 amplified fragments, of which 86%, on average, were polymorphic. The number of amplified fragments varied from eight (UBC 860) to 14 (UBC 841) bands per primer, totaling a mean of 10.5 bands per primer (Table 2). Primers UBC 823, UBC 827, UBC 841, UBC 861, and UBC 862 had the highest percentage of polymorphic bands (above 90%), comprising 54 of 127 amplified polymorphic bands, and were responsible for 42% of the polymorphism generated (Table 2).

Correlation estimates between basil genotypes were high (0.999), confirming the stability of the number of selected primers and fragments obtained. Also, the stress value (0.007) was lower than 0.05, which indicates the reliability of the results (Kruskal, 1964).

The efficiency of the ISSR molecular marker has been proven in the present study and in several other works on genetic diversity, such as those that analyzed species of the genus *Ocimum* (Aghaei *et al.*, 2012; Chen *et al.*, 2013; Patel *et al.*, 2015) and other medicinal and aromatic species [e.g., *Croton tetradenius* (Almeida-Pereira *et al.*, 2016) and *Varronia curassavica* (Brito *et al.,* 2016)].

The PIC value was 0.89, which is considered as highly informative, according to Botstein *et al.* (1980). Therefore, ISSR markers were considered as informative in the evaluation of the genetic diversity of basil genotypes and reproduced polymorphism suitable for the analyses. The molecular size of the bands ranged from 50 to 1000 bp, and several specific bands were identified (Table 2).

Patel *et al.* (2015) demonstrated the effectiveness of dominant markers using 12 ISSR primers in a study with five species of the genus *Ocimum* (*O. basilicum*, *O. americanum*, *O. sanctum*, *O. gratissimum*, and *O. polystachyon*). Their results totaled 238 fragments with 98.17% polymorphism and high mean

Table 1. Identification of the 27 basil genotypes evaluated. São Cristovão, UFS, 2017.

Code	Cultivar/hybrid	Scientific name	Origin/Company
BAS001	Anise	*Ocimum basilicum*	Richters
BAS002	Ararat	*Ocimum basilicum*	Richters
BAS003	Cinnamon	*Ocimum basilicum*	Richters
BAS004	Dark Opal	*Ocimum basilicum*	Richters
BAS005	Edwina	*Ocimum basilicum*	Richters
BAS006	Elidia	*Ocimum basilicum*	Richters
BAS007	Envigor	*Ocimum basilicum*	Richters
BAS008	Gecofure	*Ocimum basilicum*	Richters
BAS009	Genovese	*Ocimum basilicum*	Richters
BAS010	Green Globe	*Ocimum basilicum*	Richters
BAS011	Italian Large Leaf	*Ocimum basilicum*	Richters
BAS012	Magical Michael	*Ocimum basilicum*	Richters
BAS013	Mrs. Burns	*Ocimum basilicum*	Richters
BAS014	Napoletano	*Ocimum basilicum*	Richters
BAS015	Nufar F1	*Ocimum basilicum*	Richters
BAS016	Osmin	*Ocimum basilicum*	Richters
BAS017	Purple Ruffles	*Ocimum basilicum*	Richters
BAS018	Red Genovese	*Ocimum basilicum*	Richters
BAS019	Sweet Dani	*Ocimum x citriodorum*	Richters
BAS020	Grecco a Palla	*Ocimum basilicum*	ISLA (n° 479)
BAS021	Italian Large Leaf	*Ocimum basilicum*	ISLA (n° 488)
BAS022	Italian Large Red Leaf	*Ocimum basilicum*	ISLA (n° 489)
BAS023	Limoncino	*Ocimum basilicum*	ISLA (n° 499)
BAS024	Red Rubin Purple Leaf	*Ocimum basilicum*	ISLA (n° 491)
BAS025	Maria Bonita	*Ocimum basilicum*	UFS
BAS026	Sweet Dani x Genovese	*Ocimum basilicum*	Hybrid 1
BAS027	Cinnamon x Maria Bonita	*Ocimum basilicum*	Hybrid 2

value of PIC (0.92), which evidences the high genetic diversity and corroborates the result observed in the present study (PIC= 0.89). Aghaei *et al.* (2012) also reported high similarity values (0.60-1.00) for the genetic diversity of Iranian basil (*Ocimum basilicum*) using ISSR markers.

Based on the Jaccard's similarity index, the highest dissimilarity value among the 27 basil genotypes was 0.80 for individuals BAS001 (Anise) and

Table 2. Annealing temperature, primers sequence, and amplified products used for the analysis of the genetic diversity of basil genotypes. São Cristovão, UFS, 2017.

Primer	Sequence (5'-3')	Length (bp)	Annealing temperature (°C)	Total bands	Polymorphic bands	Polymorphism (%)
UBC810	GAG AGA GAG AGA GAG AT	50-500	54.8	11	9	81.8
UBC811	GAG AGA GAG AGA GAG AC	150-650	46.8	9	7	77.8
UBC813	CTC TCT CTC TCT CTC TT	200-750	44.6	9	8	88.9
UBC815	CTC TTC TCT CTC TCT CTG	200-900	47.6	9	7	77.8
UBC823	TCT CTC TCT CTC TCT CC	150-750	57.2	10	10	100.0
UBC827	ACA CAC ACA CAC ACA CG	100-750	57.2	10	9	90.0
UBC835	AGA GAG AGA GAG AGA GYC	50-900	58.8	11	9	81.8
UBC841	GAG AGA GAG AGA GAG AYC	50-600	58.8	14	13	92.9
UBC856	ACA CAC ACA CAC ACA CYA	100-750	56.5	12	10	83.3
UBC857	ACA CAC ACA CAC ACY G	100-900	58.8	9	7	77.8
UBC860	TGT GTG TGT GTG TGT GRA	200-750	46.9	8	7	87.5
UBC861	ACC ACCACCACCACCACC	150-1000	64.5	13	12	92.3
UBC862	AGC AGCAGCAGCAGCAGC	100-1000	64.5	11	10	90.9
UBC866	CTC CTCCTCCTCCTCCTC	250-900	55.7	11	9	81.8
Total				147	127	86.0

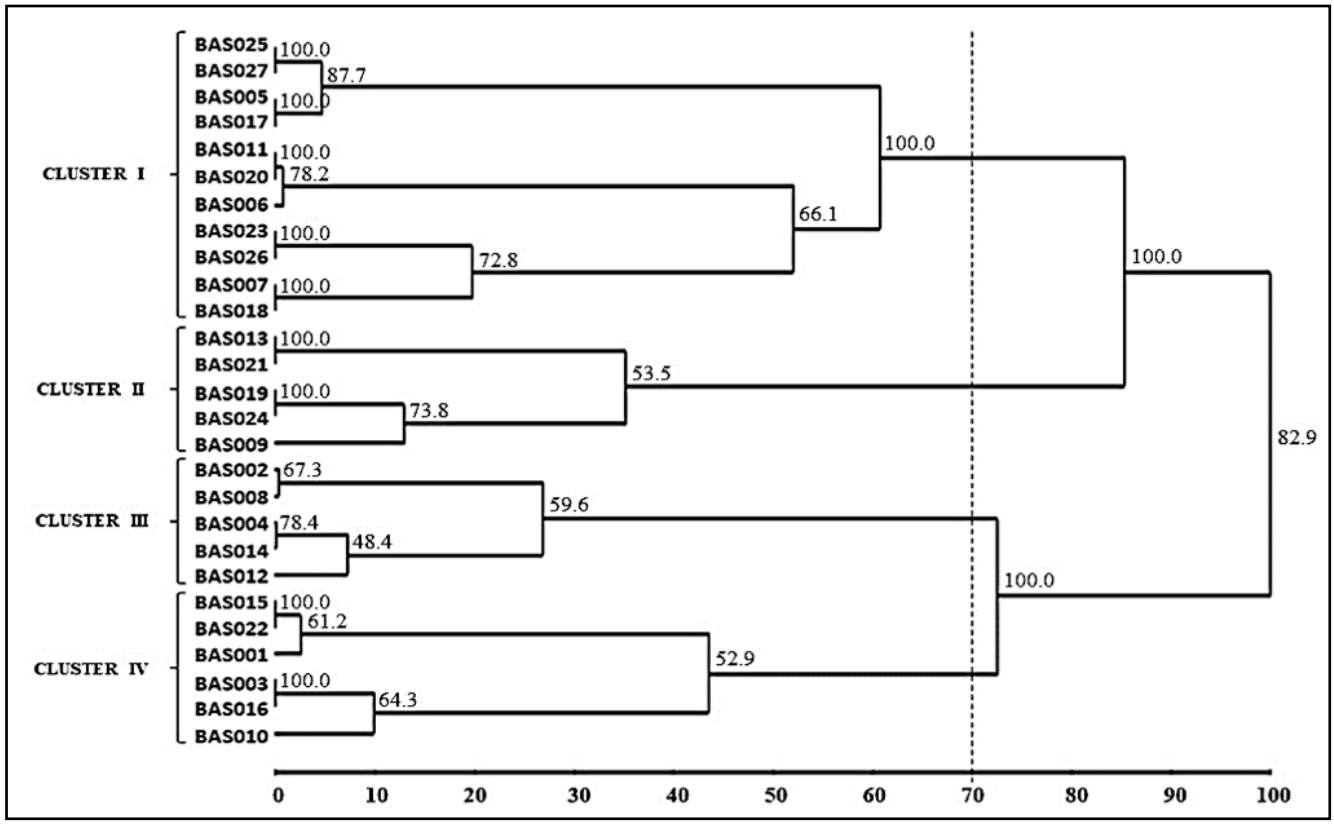

Figure 1. Dendrogram obtained by the WARD method, based on the genetic dissimilarity index by the Jaccard's similarity index, for 27 basil genotypes. São Cristovão, UFS, 2017.

Table 3. Jaccard's dissimilarity index using 14 primers by the ISSR technique among 27 basil genotypes. São Cristovão, UFS, 2017.

	Plant (BAS)																										
Plant	**001**	**002**	**003**	**004**	**005**	**006**	**007**	**008**	**009**	**010**	**011**	**012**	**013**	**014**	**015**	**016**	**017**	**018**	**019**	**020**	**021**	**022**	**023**	**024**	**025**	**026**
BAS002	0.56																									
BAS003	0.56	0.34																								
BAS004	0.54	0.34	0.30																							
BAS005	0.54	0.38	0.25	0.26																						
BAS006	0.51	0.41	0.39	0.42	0.28																					
BAS007	0.48	0.35	0.32	0.34	0.24	0.28																				
BAS008	0.52	0.37	0.34	0.38	0.28	0.35	0.22																			
BAS009	0.55	0.39	0.31	0.31	0.27	0.31	0.27	0.23																		
BAS010	0.57	0.50	0.45	0.46	0.40	0.36	0.29	0.36	0.33																	
BAS011	0.55	0.46	0.41	0.39	0.26	0.30	0.27	0.29	0.31	0.26																
BAS012	0.80	0.49	0.45	0.43	0.53	0.54	0.47	0.51	0.51	0.52	0.52															
BAS013	0.62	0.46	0.42	0.36	0.43	0.50	0.48	0.40	0.48	0.51	0.43	0.52														
BAS014	0.56	0.35	0.40	0.31	0.34	0.43	0.33	0.41	0.38	0.45	0.40	0.53	0.41													
BAS015	0.57	0.34	0.28	0.22	0.26	0.42	0.24	0.31	0.30	0.40	0.34	0.44	0.41	0.18												
BAS016	0.54	0.41	0.44	0.40	0.40	0.39	0.40	0.46	0.36	0.45	0.44	0.47	0.45	0.35	0.33											
BAS017	0.67	0.40	0.36	0.40	0.36	0.45	0.36	0.35	0.41	0.43	0.41	0.51	0.47	0.34	0.24	0.40										
BAS018	0.60	0.42	0.35	0.33	0.39	0.45	0.41	0.46	0.40	0.46	0.43	0.48	0.46	0.34	0.31	0.38	0.29									
BAS019	0.65	0.50	0.46	0.44	0.48	0.54	0.47	0.51	0.48	0.53	0.47	0.57	0.39	0.47	0.47	0.52	0.43	0.41								
BAS020	0.62	0.52	0.39	0.46	0.38	0.42	0.43	0.46	0.44	0.37	0.34	0.52	0.48	0.46	0.43	0.51	0.44	0.38	0.44							
BAS021	0.57	0.40	0.34	0.36	0.26	0.38	0.24	0.35	0.36	0.41	0.33	0.46	0.46	0.34	0.27	0.46	0.37	0.35	0.40	0.40						
BAS022	0.59	0.45	0.38	0.36	0.32	0.40	0.30	0.37	0.38	0.44	0.33	0.42	0.50	0.35	0.29	0.41	0.41	0.32	0.48	0.41	0.24					
BAS023	0.59	0.44	0.43	0.38	0.43	0.48	0.44	0.45	0.45	0.55	0.47	0.53	0.40	0.44	0.42	0.54	0.44	0.47	0.34	0.50	0.41	0.46				
BAS024	0.52	0.41	0.39	0.33	0.35	0.32	0.36	0.37	0.33	0.42	0.39	0.45	0.43	0.35	0.33	0.32	0.36	0.38	0.44	0.42	0.33	0.38	0.36			
BAS025	0.60	0.39	0.35	0.36	0.31	0.37	0.35	0.37	0.34	0.48	0.42	0.51	0.41	0.39	0.33	0.45	0.36	0.41	0.44	0.43	0.32	0.39	0.39	0.26		
BAS026	0.48	0.48	0.49	0.44	0.47	0.50	0.49	0.54	0.44	0.51	0.52	0.55	0.42	0.52	0.51	0.52	0.56	0.50	0.41	0.50	0.49	0.54	0.40	0.42	0.35	
BAS027	0.54	0.43	0.42	0.32	0.40	0.46	0.45	0.46	0.39	0.52	0.46	0.53	0.35	0.45	0.44	0.47	0.46	0.35	0.38	0.44	0.43	0.44	0.36	0.40	0.36	0.34

BAS012 (Magical Michael), indicating higher genetic diversity between these individuals (Table 3). This fact can be explained by the geographical distance between the place of origin of both species since cultivar Anise comes from Iran, and Magical Michael originates from North America. Genotypes BAS014 (Napoletano) and BAS015 (Nufar F1) formed the pair with the lowest genetic dissimilarity index (0.18) (Table 3), suggesting a closer genetic relationship between each other (Sayed *et al.,* 2009). The study describing the variability and/or genetic diversity of individuals within and between populations is mandatory for the genetic characterization of species. This characterization is a common procedure in the conservation of natural resources and breeding programs (White *et al*., 2018).

The number of clusters (K) was identified based on the maximum likelihood and ΔK values. For the 27 basil genotypes, the maximum ΔK was observed for K= 4, and genotypes were separated into four clusters.The genetic diversity between individuals was calculated based on the Jaccard's dissimilarity index, using the Ward method, resulting in four distinct clusters when the mean distance cut-off point was of approximately 0.70. Cluster I was represented by the individuals BAS005, BAS006, BAS007, BAS011, BAS017, BAS018, BAS020, BAS023, BAS025, BAS026, and BAS027 (Figure 1). Genotypes BAS025 (Maria Bonita) and BAS027 (Cinnamon x Maria Bonita hybrid) were included in group 1, and they are respectively the genitor and progeny, which shows the genetic proximity between them. Also, the cluster has the highest number of cultivars of Italian origin, such as Edwina (BAS005), Elidia (BAS006), Envigor (BAS007), Italian Large Leaf (BAS011), and Red Genovese (BAS018). The clustering of a large number of genotypes revealed low genetic variability, which may be due to the autogamous propagation of basil. This fact reduces the genetic variability and the biodiversity in cultivars used in agriculture since farmers utilize fewer genotypes for seed production. Cluster II was represented by the individuals BAS009, BAS013, BAS019, BAS021, and BAS024. Genotypes Ms. Burns (BAS013) and Sweet Dani (BAS019) were possibly inserted in this cluster since they have geranial and neral as the major compounds (Pinto *et al*., 2018). Genotypes Genovese (BAS009), Italian Large Leaf (BAS021), and

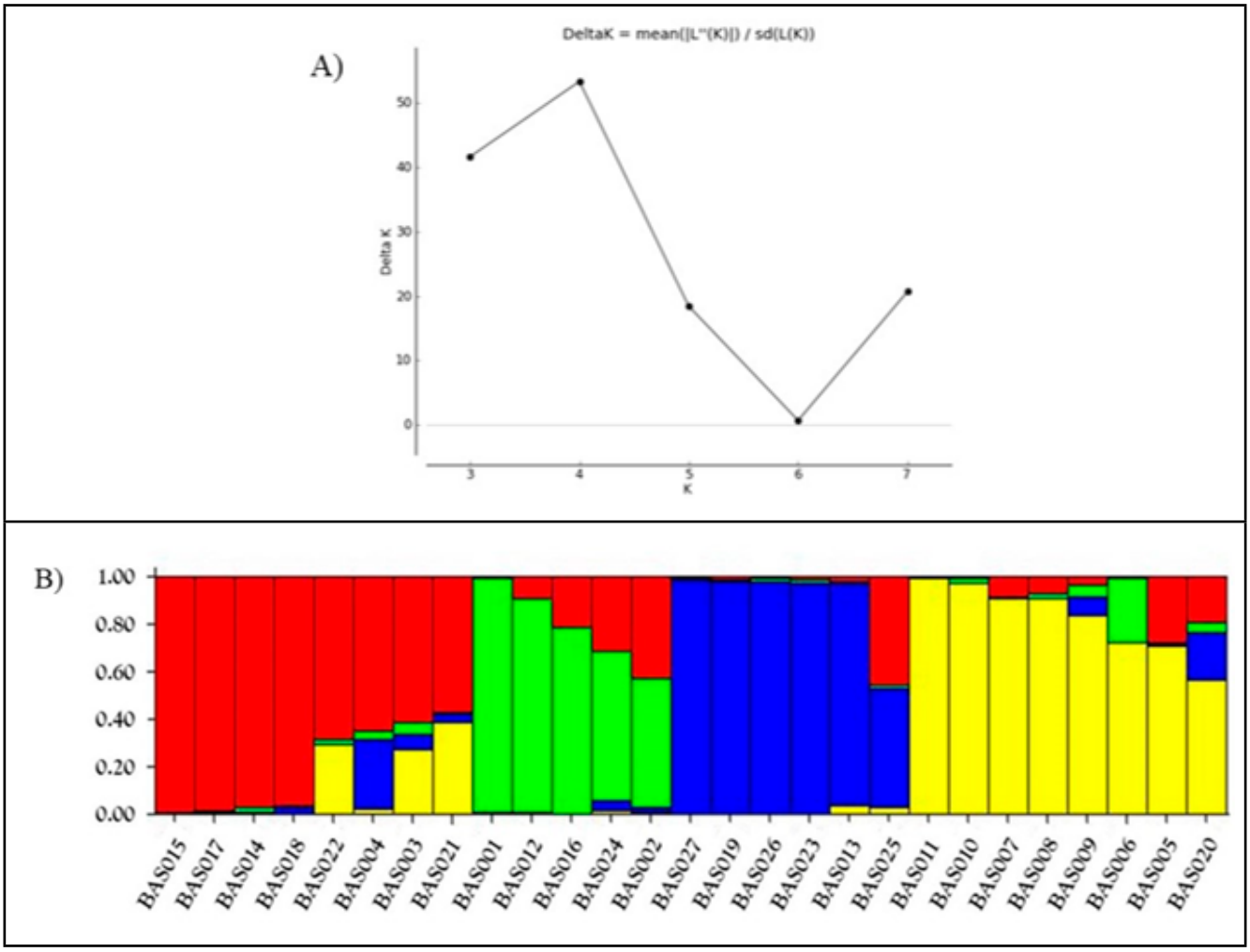

Figure 2. STRUCTURE analysis of the number of populations for K (A), and Bayesian clustering results with K= 4 of 27 basil genotypes (B). São Cristovão, UFS, 2017.

Red Rubin (BAS024) were allocated in the same cluster since they had linalool and 1,8-cineole as the major compounds (Costa *et al*., 2015). Cluster III was represented by the individuals BAS002, BAS004, BAS008, BAS012, and BAS014. This cluster included cultivars that showed high linalool concentrations, such as Dark Opal (BAS004), Magical Michael (BAS012), and Napoletano (BAS014) (Pinto *et al*., 2018). Cluster IV was represented by individuals BAS001, BAS003, BAS010, BAS015, BAS016, and BAS022. Cultivars Anise (BAS001), Cinnamon (BAS003), Green Globe (BAS010), and Nufar F1 (BAS015), allocated in cluster IV, had also been clustered together in another research that evaluated the genetic diversity among *Ocimum* species (Chen *et al.*, 2013).

The Bayesian cluster analysis divided the population into four clusters defined by the colors red, green, blue, and yellow (Figure 2). This analysis revealed similar clusters to those of the WARD method. Genotypes BAS003 (Cinnamon), BAS004 (Osmin), BAS009 (Genovese), BAS020 (Grecco a Palla), BAS024 (Red Rubin Purple Leaf), and BAS025 (Maria Bonita) were the only ones that had the four colors.This result indicates that these plants should be prioritized for preservation since they may be useful in future studies aimed at the genetic breeding of the species. The analysis of plant diversity is fundamental for the germplasm curator as it helps define the variation structure, and thus enable the assessment of genetic erosion, potential exploration, and the priorities for the germplasm conservation (Ojo *et al*., 2012). Conservation helps maintain the genetic base, which is necessary for breeding. During the conservation process, better varieties and crop lines can be selected for food, fuel, and medicine. The leaves and oil of *Ocimum* have many medicinal properties and are highly demanded by the industry. Thus, its cultivation proves to be a good source of income to farmers. The characteristics of the crop, such as short cycle, high biomass production, and suitability to tropical and sub-tropical areas are advantages for large scale production. Moreover, its high cross-pollination provides a high chance for the development of new chemotypes with a unique essential oil profile (Srivastava *et al*., 2018).

The knowledge of the reproductive system of the species of interest is fundamental since it can influence the genetic variability, either by homogenizing or increasing the divergence between individuals and populations (Zanella *et al.*, 2012).

Several other autogamous species had their genetic diversity reduced. This fact might be due to the domestication process, which occurred away from the species' center of origin. Also, it might have been influenced by the genetic breeding the species underwent over the years, which is based on a limited number of genotypes (Saavedra *et al*., 2001). Therefore, the study on the genetic diversity can assist the analysis of the genetic variability of cultivars and identify possible parental combinations for the obtainment of segregating progenies and the introduction of desirable genes (Samal *et al.,* 2011).

The ISSR markers efficiently determined the genetic variation and genetic relationships in basil. The study on the genetic diversity of the selected individuals is paramount for contributing to the knowledge of the species and allowing the selection of genotypes to be included in future breeding programs.

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