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RYDLEY KLAPEYRON BEZERRA LIMA

DIVERSIDADE GENÉTICA DE *Tacinga inamoena* NO SEMIÁRIDO POTIGUAR

MOSSORÓ
2020

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Tese apresentada ao Doutorado em Fitotecnia do Programa de Pós-Graduação em Fitotecnia da Universidade Federal Rural do Semi-Árido como requisito para obtenção do título de Doutor em Fitotecnia.

Linha de Pesquisa: Bioquímica, Fisiologia e Tecnologia Pós-Colheita

Orientadora: Prof[a]. Dr[a]. Patrícia Lígia Dantas de Morais

Coorientador: Prof. Dr. Glauber Henrique de Sousa Nunes

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BANCA EXAMINADORA

Patrícia Lígia Dantas de Morais, Profª. Drª. (UFERSA)
Presidente

Glauber Henrique de Sousa Nunes, Prof. Dr. (UFERSA)
Membro Examinador

Nildo da Silva Dias, Prof. Dr. (UFERSA)
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Membro Examinador

Andréia Mitsa Paiva Negreiros, Drª.
Membro Examinador

Hozano de Souza Lemos Neto, Dr.
Membro Examinador

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ABSTRACT

LIMA, Rydley Klapeyron Bezerra. **Genetic diversity of *Tacinga inamoena* in the Potiguar semiarid.** 2020. 79p. Thesis (Doctorate in Agronomy: Phytotechnics) – Universidade Federal Rural do Semi-Árido/UFERSA, Mossoró, 2020.

The fruit of *Tacinga inamoena* (K. Schum.) [NP Taylor & Stuppy] stands out as a potential source of nutrients for the human diet, however there is little information about this species. Knowing the genetic variability of a species – expressed in morphological, chemical and molecular characters – is fundamental in the development of breeding and domestication programs. The objective of this work was to evaluate the genetic diversity through morphological, chemical, bioactive and molecular compounds of *T. inamoena* individuals between and within the natural populations collected in the semiarid potiguar. In the first experiment, 30 individuals of *T. inamoena* from three different populations were evaluated, in eighteen morphological and chemical characters, whereas in molecular, eight RAPD markers were used. The data were subjected to univariate and multivariate analysis of variance with application of the Snedecor F tests and the Wilks criterion. The individuals were grouped using the UPGMA hierarchical technique for morphological and chemical data and the complement of the Jaccard index for molecular data. In the study of molecular diversity, the percentage of polymorphic loci, the Nei distance, the Shannon index and the gene flow were estimated. There is variability in *T. inamoena* individuals for plant morphological and fruit physicochemical characteristics. The RAPD technique is useful in the characterization and analysis of the genetic diversity of *T. inamoena individuals*. The population from Assú presented greater morphological, physical-chemical and molecular diversity, being chosen for programs of genetic improvement and conservation of genetic resources. In experiment II, the objective was to characterize fruits of *T. inamoena* from different populations regarding the content of bioactive compounds and to evaluate the genetic diversity between and within populations. Fruits were collected from 10 plants in Assú, Apodi and Parelhas, municipalities in the state of Rio Grande do Norte, Brazil. The fruits were analyzed for the contents of bioactive compounds and antioxidant activity by the DPPH and ABTS methods. Univariate and multivariate analysis of variance was performed using the Snedecor F tests and the Wilks criterion. The dendrogram was constructed using the UPGMA hierarchical method and the K Means method was used to group the individuals in the population and Pearson's correlations between the characters were estimated. Parelhas fruits had the highest average of characteristics, although they did not differ from Assú for PET and Apodi for VITC and DPPH. Apodi and Assú did not differ for CARO, BETC, BETX and ABTS. There were no differences between populations in relation to FLAV. In addition, the UPGMA grouping method organized the three populations into two groups, Parelhas in the first and Assú and Apodi in the second. The variation between populations was greater for CARO, BETC, BETX and DPPH, suggesting that most of the total variation of these characteristics occurred among the studied populations. CARO, BETX, BETC and VITC were the traits that most contributed to the variation between individuals. In conclusion, the fruits of *T. inamoena* are rich in bioactive compounds, and these characteristics can vary between and within populations distributed geographically.

Keywords: Molecular markers. RAPD. Bioactive compounds. *Cactaceae*. Characterization

RESUMO

LIMA, Rydley Klapeyron Bezerra. **Diversidade genética de *Tacinga inamoena* no semiárido Potiguar**. 2020. 79p. Tese (Doutorado em Agronomia: Fitotecnia) – Universidade Federal Rural do Semi-Árido/UFERSA, Mossoró, 2020.

O fruto da *Tacinga inamoena* (K. Schum.) [NP Taylor & Stuppy] destaca-se com potencialidade como fonte de nutrientes para dieta humana, porém há poucas informações acerca desta espécie. Conhecer a variabilidade genética de uma espécie – expressada em caracteres morfológicos, químicos e moleculares – é fundamental no desenvolvimento de programas de melhoramento e domesticação. O objetivo deste trabalho foi avaliar a diversidade genética através de compostos morfológicos, químicos, compostos bioativos e moleculares de indivíduos de *T. inamoena* entre e dentro das populações naturais coletadas no semiárido potiguar. No primeiro experimento, foram avaliados 30 indivíduos de *T. inamoena* de três populações diferentes, em dezoito caracteres morfológicos e químicos, ao passo que na molecular foram utilizados oito marcadores *RAPD.* Os dados foram submetidos à análise de variância uni e multivariada com aplicação dos testes F de Snedecor e o critério de Wilks. O agrupamento dos indivíduos foi feito pela técnica hierárquica UPGMA para os dados morfológicos e químicos e o complemento do índice de Jaccard para dados moleculares. No estudo da diversidade molecular, foram estimados a porcentagem de locos polimórficos, a distância de Nei, o índice de Shannon e o fluxo gênico. Há variabilidade nos indivíduos de *T. inamoena* para caracteres morfológicos da planta e físico-química do fruto. A técnica RAPD é útil na caracterização e análise da diversidade genética de indivíduos de *T. inamoena*. A população de Assú apresentou maior diversidade morfológica, físico-química e molecular, sendo escolhida para programas de melhoramento genético e conservação de recursos genéticos. No experimento II, objetivou-se caracterizar frutos de *T. inamoena* de diferentes populações quanto ao conteúdo de compostos bioativos e avaliar a diversidade genética entre e dentro das populações. Foram coletados frutos de 10 plantas ocorridas em Assú, Apodi e Parelhas, municípios do estado do Rio Grande do Norte, Brasil. Os frutos foram analisados quanto aos teores de compostos bioativos e atividade antioxidante pelos métodos DPPH e ABTS. Realizou a análise de variância uni e multivariada com aplicação dos testes F de Snedecor e o critério de Wilks. O dendrograma foi construído pelo método hierárquico UPGMA e utilizou-se o método K Means para agrupar os indivíduos da população e estimou-se as correlações de Pearson entre os caracteres. Os frutos de Parelhas apresentaram as maiores médias das características, embora não tenham diferido de Assú para PET e de Apodi para VITC e DPPH. Apodi e Assú não diferiram para CARO, BETC, BETX e ABTS. Não houve diferenças entre as populações em relação ao FLAV. Além disso, o método de agrupamento UPGMA organizou as três populações em dois grupos, Parelhas no primeiro e Assú e Apodi no segundo. A variação entre as populações foi maior para CARO, BETC, BETX e DPPH, indicando que a maior parte da variação total dessas características ocorreu entre as populações estudadas. CARO, BETX, BETC e VITC foram os traços que mais contribuíram para a variação entre os indivíduos. Concluindo, os frutos de *T. inamoena* são ricos em compostos bioativos, e essas características podem variar entre e dentro das populações distribuídas geograficamente.

Palavras-chave: Marcadores moleculares. RAPD. Compostos bioativos. *Cactaceae*. Caracterização

FIGURE LIST

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SUMMARY

CHAPTER I

1. INTRODUCTION

Brazil has extensive floristic diversity and, thanks to its geographical location and territorial dimension, it has one of the greatest diversities in the world (NASCIMENTO et al., 2011). Among Brazilian biomes, the Caatinga is the least studied, occupying a large area in the Northeastern region (SILVA et al., 2017). This Biome is home to numerous endemic plant species, especially from the Cactaceae family, many of which have been few studied and, consequently, its benefits are not used by man (SILVA et al., 2008; LUCENA et al., 2012).

Fruit intake is no longer based on taste and personal choice, but on the desire to improve health due to its nutritional and therapeutic value (SILVA et al., 2014). Brazil has numerous native and exotic fruit species that have not been explored yet, despite their high nutritional potential and economic value (SCHIASSI et al., 2018). Such little-known species may constitute alternatives to traditional species, in order to meet the new demands of domestic and foreign markets for new flavors, colors, textures, as well as the presence of compounds capable of preventing degenerative diseases and sources of nutritional resources, having considerable amounts of bioactive compounds, such as total phenolic compounds, vitamins, carotenoids, minerals and fibers. However, these species need to be preserved and characterized through the study of their properties, aiming at their use in the functional food market (RUFINO, 2008; ALVES et al., 2008; SCHIASSI et al., 2018).

Several fruits endemic to the Caatinga vegetation have economic potential. Among these fruit trees, with potential for production and commercialization in the Brazilian semiarid, is the species *Tacinga inamoena* (K. Schum.) [NP Taylor & Stuppy], popularly known as cumbeba, gogóia, pêlo or quipá, according to the region. It is a plant native to the Northeastern region, belonging to the Cactaceae family of wide distribution in Northeast Brazil and North of Minas Gerais (LIMA, 1989; LAMBERT, 2009). According to Silva et al. (2009), the fruits of this species have been used as an alternative source of food in the Brazilian semiarid region. The fruit has a relevant amount of bioactive compounds, mainly phenolic compounds with high antioxidant activity (DANTAS et al., 2016; FORMIGA et al., 2016), betalains (DANTAS et al., 2015; LIMA, 2016) and minerals (SOUZA et al., 2007; LIMA, 2016). The physical, chemical and organoleptic characteristics are similar to those of *Opuntia fícus-indica* (SOUZA et al., 2007), resulting in the possibility of having potential value in human food (SILVA et al., 2009; NASCIMENTO et al., 2011; LIMA, 2016). However, production systems for the cultivation of this species are not known and all the collection of plants in this ecosystem has occurred in an extractive and illegal manner, as well as the loss of habitat caused by anthropic action (SOUZA et al., 2012; MEIADO et al., 2012; MANUFACTURER; OLIVEIRA, 2013).

Plant populations that occupy different habitats may have different genetic characteristics, and research on the genetic relationship of these populations is the basis for the preservation of these species (DUFFY et al., 2009; KARIMI et al., 2009). Under the influence of different abiotic factors, such as temperature, light, soil, precipitation, altitude, water stress, nutrient deficiency and geographical position (GRATANI, 2014; PANIAGUA-IBÁNEZ et al., 2015), many species of plants can present morphological and anatomical variations between their populations that allow them to adapt to the environment and survive in different conditions (BOEGER et al., 2008; GRATANI, 2014). In relation to fruits, it is considered that the variables such as season, variety, maturation stage and climatic conditions influence the phytochemical composition (CORDENUNSI et al., 2002). The identification of these genetic materials - which, in addition to being productive, have superior quality for industrial use and / or consumption *in natura* - is essential for the formation of orchards (CHITARRA; CHITARRA, 2005).

Another methodology used to quantify variability in populations is molecular characterization, which is based on the use of molecular markers. Within the class of markers, based on the polymerase chain reaction technique, RAPD (Random Amplified Polymorphic DNA) stands out, as it is practical, fast, less costly and has proved to be efficient in identifying the genetic variability of different groups plant and can be used as an auxiliary tool in breeding programs (PESSANHA et al., 2011; DANTAS et al., 2012). Studies with molecular characterizations of *T. inanoema* are still scarce, and efforts are needed to obtain this knowledge.

Given the above, the study aimed to assess genetic diversity through morphological, chemical, bioactive compounds and molecular of *T. inamoena* individuals between and within the natural populations collected in the potiguar semiarid.

2. THEORETICAL REFERENCE

2.1. General aspects of *Tacinga inamoena*

The Cactaceae family has 127 genera and about 1438 species distributed in tropical and subtropical America, from Canada to Patagonia, with the exception of *Rhipsalis bacífera* (J.S. Muell.) Stearn, which is identified in mainland Africa and the island of Madagascar (HUNT et al., 2006; GONZAGA et al., 2014). In Brazil, cacti are distributed throughout the territory, which makes Brazil the third center of diversity of cacti with 39 genus, occurring 14 endemic, 262 species, of which 188 are endemic (ZAPPI et al., 2015). According to Souza et al. (2006), the most common genus of the Caatinga are: *Cereus* (‘mandacarus’), *Pilosocereus* (‘facheiros’), *Melocactus* (‘cabeças-de-frade’) and *Tacinga* (‘palms’), the latter endemic to the Caatinga.

Several fruit endemic to the Caatinga vegetation have economic potential, with the following species-families: Anacardiaceae - Umbu (*Spondias tuberosa* Arruda); Annonaceae - Bruteira (*Annona vepretorum* Mart.); Passifloraceae - Maracujá-do-mato- (*Passiflora cincinnata* Mast.); Cactaceae - Mandacaru (*Cereus jamacaru*) and Rhamnaceae - Juazeiro (*Ziziphus joazeiro* Mart.) (GIULIETTI et al., 2002; SANTOS et al., 2012). However, others are collected from the native vegetation by the local population and appreciated without the production being registered (GIULIETTI et al., 2002), like *Tacinga inamoena* (K. Schum.) N.P. Taylor & Stuppy, popularly known as quipá, cumbeba, gogóia or small palm, native plant of the Caatinga biome (LIMA, 1989).

The species belongs to the genus *Tacinga* Britton & Rose, subfamily Opuntioideae of Cactaceae, containing eight species, seven of which are endemic to eastern Brazil, and one endemic to Northeastern Venezuela (NEVES et al., 2017). Its distribution is quite wide, occurring throughout the Northeast of Brazil and in the North of Minas Gerais, occurring in different environments, growing from soils in the caatinga areas to numerous rocky outcrops, such as gneiss, granite, quartzite, sandstone and limestone (LAMBERT, 2009; ZAPPI; TAYLOR, 2018).

It is characterized as a shrub plant up to 1 m high, showing a stem formed by elliptical to obovate articles, with a length ranging from 5 to 15 cm and width from 3.5 to 12 cm with irregular branches (MENEZES et al., 2013). It presents an attractive flower with a reddish-scarlet color and small fruits of the berry type and oblong shape, whose color of the bark varies from dark green to yellow-orange during maturation, showing small groups of thorns distributed on its surface, characterizing itself morphologically for presenting a type of thorn, called gloquids, which are short, where the fruits are filled with fleshy mass of yellow-orange color as the ripening progresses, containing small and countless seeds (BARTHLOTT; HUNT, 1993; SILVA et al., 2009; DANTAS et al., 2015). For the species *T. inamoena*, Nascimento et al. (2015) showed that red-footed tortoises can be considered good dispersers of the seeds of this Cactaceae, providing their distribution throughout the environment. However, for Taylor and Zappi (2004), the dispersion of quipá seeds is possibly carried out by small mammals that occur in the Caatinga. The ripening stages for the fruits can be described as follows: 1-Totally green coloring; 2-Coloring with a light green peel; 3-Fruits in the ripening process, predominating green with yellowish tones; 4-Yellowish color with pink nuances; 5-Pink color with yellow tones; 6-Pink color with orange nuances (DANTAS et al., 2016).

T. inamoena stands out for being one of the species botanically close to *Opuntia fícus-indica*, showing great socioeconomic potential. With the appreciation of the fruits of *O. ficus-indica* in the national and international markets, perspectives for other regional cactuses are opening (SOUZA et al., 2007). The fruits and cladodes of *T. inamoena* are used by the Northeastern population in animal feed during periods of drought or exploring its ornamental potential, while in human food it has been used *in natura* or in the production of by-products, such as jelly, sweets and liqueurs (SOUZA et al., 2007; LUCENA et al., 2015; CAVALCANTE et al., 2017). There are records as a medicinal plant: *T. inamoena* can be used to control asthma, various inflammations and to combat worms (CASTRO; CAVALCANTE, 2010). It has growing interest with an appeal to health promotion, as a source of minerals (SOUZA et al., 2007; NASCIMENTO et al., 2011; LIMA, 2016) and bioactive compounds (NASCIMENTO et al., 2011; DANTAS et al., 2015; DANTAS et al., 2016; FORMIGA et al., 2016; LIMA, 2016), arousing interest in the consumer market and allowing it to be used as a healthy food alternative (SILVA et al., 2009).

The nutritional, functional and use potential of these fruits is still limited, failing to gain space in the Brazilian consumer market. However, this scenario has already begun to change in the Brazilian semiarid region, in the central region of Rio Grande do Norte, where fruits are the main source of income for many families, and can be used for various purposes, such as in the manufacture of ice cream. Given the importance of this species to the region, Serviço Brasileiro de Apoio às Micro e Pequenas Empresas (SEBRAE-RN), in partnership with the Universidade Federal Rural do Semi-Árido (UFERSA), in order to generate income and jobs in the region, founded an ice cream factory in the city of Angicos, “Sertão Gelado”, for fruit exploration (MENDONÇA, 2013).

Although consumed regionally, these fruits are an indicator of the agro-industrial potential, which can be explored as a food alternative and/or as a source of complementary income for family farming (SOUZA et al., 2007). In a study carried out in the State of Paraíba regarding the quality of the fruit of *T. inamoena*, Dantas et al. (2016) report a relevant amount of bioactive compounds, mainly phenolic compounds with high antioxidant activity, in addition to the more mature fruits having greater antioxidant activity. Therefore, considering the potential for consumption in the human diet as a possible source of compounds of functional interest (SILVA et al., 2009) it is crucially important to carry out studies to characterize this species.

2.2. Morphological characterization of the plant

The conservation and use of plant genetic resources involve the detailed characterization and classification of genetic diversity (SOLANO et al., 2005), as well as the domestication of plants that need an initial study of the characteristics related to the species involved. Studies on plant morphology have deserved attention, whether through morphoanatomical studies, with the purpose of expanding knowledge about a specific species or systematic group of plants, or aiming at the recognition and identification of species in a given location within an ecological panorama (OLIVEIRA, 1993). Knowledge of morphological characterization is the basis of genetic improvement in breeding programs, therefore the most accessible way to quantify genetic diversity (CROSBY, 1981; SINGH et al., 1991). The characterization of species and cultivars can be determined based on plant morphological differences or differences in protein and DNA molecules (FERREIRA; GRATTAPAGLIA, 1998).

In summary, the characterization of genotypes is one of the main stages of studies with germplasm, as it allows showing cultivars with potential for immediate use by farmers, as well as identifying accessions that have interesting attributes for improvement (SILVA et al., 2009). Mazid et al. (2013) report that this technique is profitable, less time consuming and does not require any technical study. Thus, the characterization process is practiced based on observations or measurements of several naturally differentiable characters, named morphological descriptors, these characters being strongly heritable and controlled by few genes, being expressed in all environments (BURLE; OLIVEIRA, 2010). Erre and Chessa (2009) corroborate that the identifications of highly discriminatory descriptors are important for an efficient and reproducible classification of species and cultivars, as well as adapting the list of descriptors for specific purposes.

Thus, the use of morphological descriptors in the presentation of a new variety has a fundamental role in the dissemination of the agronomic characteristics of new genetic materials, and can decisively influence the choice of varieties by farmers and other interested parties (SILVA et al., 2009).

In order to characterize the genetic diversity of prickly pear, Bendhifi et al. (2013) evaluated 28 accessions of *O. ficus-indica* from Tunisia, using quantitative and qualitative morphological characteristics, indicating that the 20 morphological descriptors based on plant, cladode and fruit characteristics, revealed strong variability between accessions. Peña-Valdivia et al. (2008), in a study on the morphological characterization of 46 accessions of *Opuntia* spp. from Mexico, from 65 morphological descriptors, indicated few morphological characteristics between the accessions, regardless of the place where they were collected. In comparison with the other results, Hadjkouider et al. (2017), in the morphological and phenological characterization in five species of *Opuntia* Argelina using 49 morphological descriptors, observed that 24 morphological descriptors were significant to discriminate *Opuntia* species. Through the analysis of hierarchical clusters, there was the formation of three groups, unlike the analysis of main components, which identified four groups, where the result shows that the species *O. ficus-indica* and *O. amycleae* were identified as very close morphologically.

According to Bressan (2005), the morphological characterization presents a problem that is the quantitative nature of the characters, strongly influenced by the environment, as it depends on the identification and enumeration of visible morphological characteristics, which often become subjective for the evaluator. In addition, some morphological characteristics may have the disadvantage of being influenced by environmental factors, and may not represent the true similarity or dissimilarity between individuals (FERREIRA; GRATTAPAGLIA, 1998). An alternative is the use of molecular tools, as it ensures more information about the genetic diversity of individuals and does not suffer from environmental influence or the development stage of the plant (RABBI et al., 2015).

Information on the morphological aspects in plant species of the genus *Tacinga* is relatively scarce in the literature, despite being an important characterization. According to El Kharrassi et al. (2017), studies on genetic variability in *Opuntia* species using morphological descriptors are scarce.

2.3. Fruit quality

The demand for fruits with good quality standards has increased both domestically and abroad (PRADO et al., 2005), as they are important components of a healthy diet and their consumption in an appropriate fraction can reduce the risk cardiovascular diseases and some types of cancer (LOCK et al., 2005). According to Almeida (2009), the physical, physical-chemical and chemical characteristics of the fruits are fundamental for the acceptance of the consumers, as well as for the insertion in more demanding and promising markets, mainly when referring to native fruits that require more studies on their potentialities. Knowledge of the physical characteristics of the fruits is of paramount importance, both to know the diversity of size and mass in each species and to enable the preparation of packaging for storage and marketing, so that there is no damage to their physical structure and there is better visualization to the consumer (ROCHA et al., 2013).

The physical characteristics of the fruits, such as weight, size, shape of the fruit, color, yield and texture, are important attributes that will be reflected in the choice of a product, such as consumer acceptability and industrial yield (COELHO, 2004; CHITARRA; CHITARRA, 2005). Size and shape are important attributes, as the variation between the individual units of a product can affect their choice by the consumer, handling practices, storage potential, market selection and final destination (fresh consumption or industrialization).

The size of the fruit is an important characteristic, especially when it is aimed at export, for fresh consumption (BALBINO, 2003; AULAR; NATALE, 2013). Products with standardized size and weight characteristics are easier to handle in large quantities, as they present lower losses and better quality (CECCATO; BASSO, 2011). The longitudinal and transverse diameter together represent the size and their relationship, represented by DT/DL, indicating the shape of the fruit. The closer the result to 1, the more rounded the fruit is, with abnormally shaped fruits having little acceptance and low price (SANTOS et al., 2010). In this way, the industries prefer those fruits more rounded, for facilitating the operations of cleaning and processing (CHITARRA; CHITARRA, 2005). It is an important quality characteristic in the classification and standardization, being able to determine the acceptance and valorization of the product for certain markets (QUEIROGA et al., 2008).

Dantas et al. (2015), when working with *T. inamoena* fruits from the State of Paraíba-PB, found average values for fruit mass of 14.2 g. Formiga et al. (2016) found fruit mass values of 13.14 g, 28.68 mm for longitudinal diameter and 30.28 mm for transversal diameter for fruits harvested in the municipality of Pombal-PB. In turn, Lima (2016) found that for the weight of the fruits, longitudinal and transverse diameter values were 16.55 g, 30.32 and 30.29 mm, respectively. For *T. inamoena* fruits, oblong and compressed fruits were observed (SOUZA et al., 2007; SILVA et al., 2009; FORMIGA et al., 2016).

The pulp yield, according to Lira Júnior et al. (2005), it is also an attribute of quality, being an interesting characteristic for the processing industry, whose minimum value required by some industries is 40%. According to Carvalho and Muller (2005), the percentage pulp yield falls into the following categories: very low (equal to or less than 20%); low (between 21% and 40%); medium (between 41% and 60%); high (between 61% and 80%) and very high (above 81%). In addition, these authors confirmed that the low percentage of pulp yield does not constitute a characteristic that makes the use of a particular species unfeasible, either as fresh fruit or for industrial use, constituting fruits of great regional acceptance. Yield of 55.78% of pulp for *T. inamoena* was observed in fruits collected from spontaneous plant populations in the municipality of Angicos-RN (LIMA et al., 2016), ranging from 61.0 to 64.6% in the fleshy portion , which covers the fleshy pericarp and the pulp with seeds for fruits from the Cariri-Curimataú-PB region (SOUZA et al., 2007), and 66.4% for fruits harvested in the semiarid region of the State of Paraíba (DANTAS et al., 2013) According to Souza et al. (2007), the pulp percentage gives this fruit considerable potential as a raw material for industrialization.

The firmness of the fruit is an important quality attribute that influences its commercialization (SILVA et al., 2005), characterized by hardness, softness, fibrousness, juiciness, resistance and elasticity (CHITARRA; CHITARRA, 2005), which can influence the period of conservation and resistance to handling, transport and attack by microorganisms. According to Batista et al. (2017), fruits with low firmness are subject to physical damage, thus reducing their useful life in terms of commercialization. Therefore, firmer fruits are of paramount importance, which will allow greater resistance to damage and probably longer useful life (GARCÍA-CRUZ et al., 2016). Firmness values of the *T. inamoena* fruit varying from 19.93 to 8.11N during ripening (SILVA et al., 2009) and 36.59 N (LIMA et al., 2016) when harvested at the physiological ripening stage.

Among the physical-chemical and chemical characteristics most used in the evaluation of fruit quality, are the content of soluble solids, hydrogen potential (pH), total acidity, SS/ATT ratio, total sugars, reducing sugars, vitamin C, pigments and compounds phenolic (CHITARRA; CHITARRA, 2005). However, these characteristics are influenced by several factors, especially edaphoclimatic conditions, cultivar, time and place of harvest, cultural treatments, maturation stage and post-harvest treatment (FAGUNDES; YAMANISHI, 2001).

Soluble solids reflect the soluble sugars present in the fruits, being used as an indirect measure of the sugar content, as their value increases as they accumulate in the fruit, being expressed as a percentage or in degrees Brix (ºBrix) (CHITARRA, 2006; FORMIGA et al., 2016). According to Chitarra and Chitarra (2005), the sugar content reaches its maximum at the end of maturation, presenting average levels of 8 to 14%, conferring excellence of quality to the product. Fruits with high levels of soluble solids are generally preferred for fresh consumption and for agroindustry, as they provide the advantage of offering higher yield in processing, due to the greater amount of nectar produced per amount of pulp (SANTOS et al., 2010).

In *T. inamoena* fruits harvested at four stages of maturation in the city of Boqueirão, Paraíba, levels between 7.86% and 10.66% were observed, on average (SILVA et al., 2009). Soluble solids contents of 9.46% (LIMA et al., 2016), 14.07% (DANTAS et al., 2015), 7.42% (FORMIGA et al., 2016), 9.4% have been reported (NASCIMENTO et al., 2011) and 9.0 and 10% for pulp and pericarp, respectively (SOUZA et al., 2007).

Total titratable acidity (ATT) and pH are the main analyzes used to measure fruit acidity. While the titratable acidity determines the percentage of organic acids, the pH measures the hydrogen concentration of the solution (KRAMER, 1973). According to Aroucha et al. (2010), acidity indicates acidic or sour taste of fruits, evidenced by the presence of organic acids in vegetables. There are several organic acids, the most abundant in fruits and vegetables are citric, malic, tartaric and others. In fruits, acidity generally represents one of the main components of the flavor, as its acceptance depends on the balance between acids and sugars, and with ripening the organic acid content decreases in most tropical fruits, due to its use as a substrate in the process respiratory system or its conversion into sugars (CHITARRA; CHITARRA, 2005; MORAIS et al., 2009).

The pH is widely used in determining the post-harvest quality of the fruits due to the facility and agility of the analysis (FERNANDEZ, 1996). According to Chitarra and Chitarra (2005), pH is a parameter that measures, in general, the acidity of fruits and food, this being the indicator of the type of treatment necessary to preserve food, since the increase in pH it is related to the decline in acidity and the advance of fruit ripening. Based on the classification by Franco and Landgraf (1996), foods can be subdivided into: low acidity (pH is above 4.5), acidic (pH between 4.0 and 4.5) and very acidic (pH less than 4.0). Higher pH values (low acidity) are preferred for fresh consumption, but it represents a problem for the industry due to the favoring of enzymatic activities and growth of Clostridium botulinum (SANTOS et al., 2010). 0.27% citric acid and pH 4.3 were obtained from *T. inamoena* fruits (FORMIGA et al., 2016); of 0.62 citric acid in 100 g of pulp and pH of 4.33 in fully ripe fruits (SILVA et al., 2009) and 0.53 of citric acid in 100 g of pulp and pH of 4.4 for fruits from Soledade-PB (NASCIMENTO et al., 2011).

The SS/ATT ratio indicates the degree of sweetness of a given product, making it one of the most used indices to analyze fruit ripeness. In this way, both for the table market and for the agribusiness, the high SS / ATT ratio is desirable (ALMEIDA, 2009). The greater this relationship, the greater the degree of sweetness (CHITARRA; CHITARRA, 2005). SS/ATT ratio values of 23.71 were observed for fruits of *T. inamoena* (DANTAS et al., 2015). Nascimento et al. (2011) and Silva et al. (2009) reported an SS/ATT ratio for quipá fruits (*T. inamoena*) of 17.90 and 66.63, respectively. According to Rothman et al. (2012), Cactaceae fruits have a mild and pleasant flavor, with subtle differences in the taste of fruits of different species.

Sugars are present in fruits, in their free or combined form, being responsible for sweetness through the balance with acids, for the attractive color and texture (MENDONÇA, 2011). According to Chitarra and Chitarra (2005) the main total soluble sugars present in the fruits are glucose, sucrose and fructose. Sugars are constituents that undergo changes during fruit ripening, therefore, during the fruit ripening period, there is an increase in sugar levels due to the transformation of starch into simple sugars (glucose and fructose) (ALMEIDA, 2013). Sucrose represents the main non-reducing sugar, while glucose and fructose are the main reducing sugars, with glucose predominating in most fruits (AGUIAR, 2010). Lima (2016) when evaluating fruits of *T. inamoena* from the city of Angicos-RN detected levels of total sugars of 5.46% and 3.3% for reducing sugars.

2.4. Bioactive compounds and antioxidant activity

Several studies have shown that the consumption of tropical fruits is increasing in the national and international market, due to the recognition of its nutritional and therapeutic properties (REIS et al., 2015). In this context, fruit and vegetable intake is associated with a lower incidence of mortality from numerous chronic non-communicable diseases, due to the protection that these foods offer against degenerative diseases, such as cancer, cerebrovascular and cardiovascular diseases, attributed to the presence of high content of chemical constituents with important properties, such as minerals, dietary fibers and phytochemicals with antioxidant action, highlighting the bioactive compounds, such as vitamins C and E, carotenoids, flavonoids, polyphenols and betalains (HINNERBURG et al., 2006; SARMENTO, 2017). Antioxidants are chemical compounds that can prevent or reduce the oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species (ROS's), which include free radicals, that is, antioxidants have the ability to react with free radicals and, thus, restrict its harmful effects to the organism (COUTO; CANNIATTI-BRAZACA, 2010). The main enzymes responsible for the body's antioxidant defense are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), which make up the first endogenous defense of neutralization of reactive oxygen species (EROS) (ROCHA et al., 2013). Thus, there is an increasing demand for products with antioxidant properties from natural sources (RUFINO, 2008).

Vitamins are essential micronutrients for maintaining the health of the body, not representing a source of energy, but they act in a series of relevant metabolic processes (SPÍNOLA, 2011). The most important vitamin found in fruits and vegetables, for human consumption, is vitamin C (MORAES et al., 2010), essential to human health, being essential in the development and regeneration of muscles, skin, bones, in the formation of collagen , regulation of body temperature, in the synthesis of several hormones and in metabolism in general (ANDRADE et al., 2002).

Severe deficiency makes the body more vulnerable to more serious diseases, such as scurvy, for example. However, consumed in high doses, it can cause side effects, such as: diarrhea, abdominal pain and kidney stones in genetically predisposed people (ROCHA et al., 2008). Therefore, vitamin C is an excellent antioxidant and is used as an index of food quality (CHITARRA; CHITARRA, 2005). The daily requirement for vitamin C varies according to age and health conditions, and the Agência Nacional de Vigilância Sanitária (ANVISA) recommended daily intake of vitamin C of 45 mg / day for adults (BRASIL, 2005). Ramful et al. (2011) classify fruits in relation to vitamin C content in three groups: low (<30 mg/100 g), medium (30-50 mg/100 g) and high (> 50 mg/100 g). For *T. inamoena* fruits, contents of 26.85 mg/100 g (FORMIGA et al., 2016), 35.35 mg/100 g (LIMA, 2016), 42.01 mg/100 g (DANTAS et al., 2015), 72.62 mg/100 g (SILVA et al., 2009). In other species, which are also from the Cactaceae family, contents of 22.94 mg/100 g (*Cereus fernambucensis*) (SOUZA et al., 2015), 3.64 mg/100 g (*Hylocereus undatus*) (FERNANDES et al., 2017) and 8 to 14 mg/100 g, with an average of 13 mg/100 g (*Stenocereus stellatus*) (BELTRÁN-OROZCO et al., 2009). According to Vaillant et al. (2005), most cacti contain little vitamin C content. The vitamin C content varies between species and varieties and can be influenced by the type of soil, form of cultivation and climatic conditions (BELTRÁN-OROZCO et al., 2009).

Polyphenols are composed of the secondary metabolism of plants that perform several functions, such as protecting against the attack of pathogens or herbivores or in the pigmentation that helps to attract pollinators. They present structures with aromatic rings and double conjugated bonds from which they exert antioxidant action (PÉREZ-JIMÉNEZ, 2007). They represent one of the most abundant groups of compounds found in nature and are of particular interest in post-harvest, and can influence the nutritional value and sensory quality, conferring attributes such as color, texture, bitterness, astringency, flavor and aroma of vegetables (VILAS BOAS, 2002; ROCHA et al., 2011). They are considered the most abundant antioxidants in the diet (CERQUEIRA et al., 2007), highlighting flavonoids that can be classified into seven groups: flavones, flavanones, flavonols, flavanonols, isaflavones, flavanols (catechins) and anthocyanidins (PEREIRA; CARDOSO, 2012). Flavonoids make up the largest class of plant phenolics and are aromatic substances containing 15 carbon atoms organized by two aromatic rings and an oxygenated heterocycle, forming a C6-C3-C6 system (PEREIRA; CARDOSO, 2012). They are natural pigments widely spread in the plant kingdom, which act to protect the organism from the damage produced by oxidizing agents, such as ultraviolet rays, environmental pollution, chemical substances present in food, stresses, among others (VOLP et al., 2009). They are found in fruits, vegetables, seeds and flowers, as well as in beer, wine, green tea, black tea and soy, which are regularly consumed in the human diet and can also be consumed in the form of nutritional supplements, together with certain vitamins and minerals (MARTÍNEZ-FLÓREZ et al., 2002).

According to Wu et al. (2006) and Juhaimi et al. (2020), phenolic compounds may vary due to environmental factors, post-harvest and handling processes, fruit ripening phase, methods and conditions of phenolic analysis, and may vary in the content of *T. inamoena* genotypes, as observed by Formiga et al. (2016) (80.97 mg/100 g), LIMA (2016) (49.97 mg/100 g) and Dantas et al. (2015) (29.84 mg/100 g). As for flavonoids, Formiga et al. (2016) report content for *T. inamoena* fruits of 1.66 mg/100 g. Lima (2016) found a content of 0.67 mg/100 g for fruits of *T. inamoena* from the city of Angicos-RN.

Carotenoids are important natural pigments found throughout the plant kingdom, responsible for the colors yellow, orange and red, in addition to some of them having pro-vitamin A properties, such as β-carotene, β-cryptoxanthin, α-carotene and β-zeacarotene, zeaxanthin, violaxanthin, neoxanthin and phytene (RODRIGUEZ-AMAYA et al., 2008; ABREU et al., 2020). Of the more than 600 types of carotenoids discovered, about 50 have significant biological activity as provitamin A (PEREIRA et al., 2012). These compounds are important in the diet, acting as antioxidants, protecting cells from oxidative damage and, consequently, decreasing the risk of developing some chronic diseases (ARAÚJO, 2008). During fruit ripening, these pigments can be present, becoming visible after chlorophyll degradation or they can be synthesized concurrently with chlorophyll degradation (CHITARRA; CHITARRA, 2005).

Betalains are natural pigments present in some classes of plants, fruits and flowers, characterized by being a class of pigments that provide attractive and stable colors under the processing conditions of the food industry (LIMA, 2016). They are classified into two groups:

betaxanthines (yellow pigment) and betacyanins (red-purple pigment), depending on the groups R1-N-R2. There are more than 50 betalains and they all have the same basic structure (HAMERSKI et al., 2013). According to Azeredo (2009), betalains are scarce in nature and have not been widely explored as bioactive compounds, but some studies have indicated their potential as antioxidant pigments, being widely used commercially and constituting an important food source, presenting strong antioxidant activity, capable of to reduce the action of free radicals and acting in the prevention of cancer cells and diseases related to the heart (STINTZING; CARLE, 2004; GENGATHARAN et al., 2015). Castellanos-Santiago and Yahia (2008), studying several species of the genus *Opuntia*, reported betaxanthine content of 0.99 mg/100 g (*O. robusta*), 1.04 mg/100 g (*O. streptacantha*), 0.14 mg/100 g (*O. ficus-indica*) and 0.16 mg/100 g (*O. megacantha*) in the fruit pulp. Dantas et al. (2015) obtained content of 0.64 mg/100 g and 1.79 mg/100 g of betaxanthines and betacyanins, respectively; in *T. inamoena* fruits; in turn, a content of 12.83 mg/100 g for betacyanins and 9.63 mg/100 g for betaxanthines was found by Lima (2016) in fruits of *T. inamoena*.

The methods for assessing the total antioxidant activity proposed in the literature are diverse, however some are more appropriate than others, depending on the nature of the compounds present in the constitution of each fruit (SUCUPIRA et al., 2012). Thus, different techniques have been developed in order to determine the antioxidant activity in vitro (MORAIS et al., 2013). Among the most used methods for determining the antioxidant capacity in fruits, ABTS and DPPH stand out, due to the ease of use, stability and simplicity of procedure (ROGINSKY; LISSI, 2005; SÁ, 2008).

ABTS (2,2 '-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt or TEAC (Trolox Equivalent Antioxidant Activity) is a method based on the ability of long-term capture antioxidants in the radical ABTS cation · +. This capture produces a decrease in absorbance, which is read from the mixture of the radical with the antioxidant at different times being represented graphically (PÉREZ-JIMÉNEZ; SAURA-CALIXTO, 2006). Antioxidant activity value of 0.67 µmol of Trolox/g was obtained by Lima (2016) for fruits of *T. inamoena* from Angicos-RN. The total antioxidant capacity of the aqueous, alcoholic and acetonic extracts of the pulp of *T. inamoena* by the $ABTS^{\cdot +}$ method was 2.69 µmol of Trolox/g, 2.43 µmol of Trolox/g and 2.22 µmol of Trolox/g, respectively (SOUZA et al., 2016). Beltran-Orozco et al. (2009) verified antioxidant activity for pulp of four varieties pitaya from Acatlán de Osorio, Mexico, considering 11.0 µmol Trolox/g for pitaya red, 16.8 µmol Trolox/g yellow, 12.2 µmol Trolox/g for cherry and 17.3 µmol Trolox/g for white, belonging to the genus *Stenocereus stellatus*.

DPPH (2,2 - diphenyl - 1 - picrilidrazil) is a method used to determine the antioxidant capacity of a compound to sequester free radicals (SUCUPIRA et al., 2012). The DPPH free radical scavenging method can be used to evaluate the antioxidant activity of specific compounds or an extract in a short period of time (PRADO, 2009). The antiradical activity expressed by parameter EC50 is defined as the amount of antioxidant needed to reduce 50% of the initial DPPH concentration. Some modifications to this method are necessary in order to adapt them to fruits, due to the reaction mechanism between the antioxidant and DPPH, which depends on the structural conformation of each antioxidant evaluated (ALVES et al., 2006). Dantas et al. (2015), evaluating fruits of different species of Cactaceae, found values for pulp of *T. inamoena* of 2153 g fruit/g DPPH and *O. stricta* of 1730 g fruit/g DPPH. Red and purple fruit varieties of the *Opuntia* genus have greater antioxidant activity by the DPPH method, highly correlated with the content of vitamin C and phenolic compounds (SUMAYA-MARTINEZ et al., 2011). Genotypic characteristics and environmental factors, such as location, soil type, growing season, geographic location, mineral composition, plant maturity and post-harvest, can interfere with the biosynthesis of the antioxidants produced (TSAO et al., 2006).

2.5. Molecular characterization

The characterization of plant germplasm has been benefited by the development and use of molecular methods that allow to identify sequence variation in the DNA molecule (FERREIRA et al., 2007). For molecular characterization methods, molecular markers are used, useful tools whose function is to reveal polymorphism in DNA sequences between different individuals, in expressed or unexpressed regions of the genome (AZEVEDO, 2010). Among the main advantages of using molecular markers, we can evidence the achievement of a practically unlimited number of polymorphisms, the obtaining of the genotype without influence of the environment and the detection of polymorphism at any stage of plant development (FALEIRO, 2007). In addition, the use of molecular markers in this type of study is widespread, due to its relative simplicity and the need for small amounts of sample DNA (AMARAL et al., 2019). According to Faleiro (2007), the principle of using these markers is based on the central dogma of molecular biology, which assumes that genetic differences in DNA correspond to phenotypic differences.

Several types of molecular markers are available to analyze genetic diversity, allowing each method to have different types of information. In this way, the use of the different markers will depend mainly on the objective to be answered. Of course, other factors-such as available resources, financial resources for investment, level of knowledge of the molecular genetics of the species to be studied and analysis infrastructure - will also influence the definition of the type of molecular marker to be used in the study (FERREIRA et al ., 2007; FALEIRO, 2007).

There are several types of molecular markers that can be used and can be classified according to the type into dominant and codominant (TURCHETTO et al., 2017). The main dominant markers used in the analysis of genetic diversity are RAPD (Random amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism) and ISSR (Inter Simple Sequence Repeats) (FALEIRO, 2011). The dominant markers are biallelic, since the alleles of the same locus reveal the presence or absence of a band. Consequently, it is not possible to know whether the amplified locus is homozygous or heterozygous (LOPES et al., 2002).

Among the various types of markers, RAPD is used because it is a technique with simplicity of use, fast, low cost, using a small amount of plant material and for identifying a high number of polymorphic loci of DNA (ZOGHLAMI et al., 2007; DANTAS et al., 2012). Silva et al. (2019) reported that the characterization and quantification of the genetic variability accessed through molecular markers makes it possible to establish genetic relationships between selected plants and without environmental interference, which is very useful in breeding programs. In the literature there are several studies using this technique for studies of genetic diversity in different species, with emphasis on studies with plants of the genus *Opuntia* (ZOGHLAMI et al., 2007; BENDHIFI et al., 2013; VALADEZ-MOCTEZUMA et al., 2014 ; TÜTÜNCÜ et al., 2016; EL KHARRASSI et al., 2017), pitaya (JUNQUEIRA et al., 2010a; JUNQUEIRA et al., 2010b; LIMA et al., 2013).

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CHAPTER II

Genetic diversity of *Tacinga inamoena* individuals through morphological, chemical and molecular characteristics

Abstract The objective of the present work was to study the genetic diversity of *Tacinga inamoena* individuals by plant morphological, physical-chemical character of the fruit and RAPD markers between and within the natural populations of the species collected in the semiarid region of Brazil. Thirty individuals of *T. inamoena*, from three different populations, were evaluated. The characterization was carried out in eighteen morphological and chemical characters, whereas in the molecular one eight RAPD markers were used. Univariate and multivariate analysis of variance was performed using Snedecor's F tests and Wilks' criterion for morphological and chemical data. The grouping of individuals was performed using the UPGMA hierarchical technique using the standardized Euclidean distance for morphological data and the complement of the Jaccard index for molecular data. In the study of molecular diversity, the percentage of polymorphic loci, the Nei distance, the Shannon index and the gene flow were estimated. There is variability in *T. inamoena* individuals for plant morphological and fruit physicochemical characteristics. The RAPD technique is useful in the characterization and analysis of the genetic diversity of *T. inamoena* individuals. In fact, the OPM7 primer proved to be the most informative and recommended in studies of divergence with *T. inamoena*. The greatest genetic divergence was 78% within populations, revealing greater variability within that of *T. inamoena* populations. The population of Assú presented greater morphological, physical-chemical and molecular diversity, being chosen for programs of genetic improvement and conservation of genetic resources.

Keyword: Semi-arid, Morphological characteristic, Physico-chemical characteristics, Molecular markers

Resumo O objetivo do presente trabalho foi estudar a diversidade genética de indivíduos de *Tacinga inamoena* por caracteres morfológicos da planta, físico-química do fruto e marcadores RAPD entre e dentro das populações naturais da espécie coletadas no semiárido do Brasil. Foram avaliados 30 indivíduos de *T. inamoena*, de três populações diferentes. A caracterização foi realizada em dezoito caracteres morfológicos e químicos, ao passo que na molecular foram utilizados oito marcadores RAPD. Realizou a análise de variância uni e multivariada com aplicação dos testes F de Snedecor e o critério de Wilks para os dados morfológicos e químicos.

O agrupamento dos indivíduos foi feito pela técnica hierárquica UPGMA utilizando a distância euclidiana padronizada para dados morfológicos e o complemento do índice de Jaccard para dados moleculares. No estudo da diversidade molecular, foram estimados a porcentagem de locos polimórficos, a distância de Nei, o índice de Shannon e o fluxo gênico. Há variabilidade nos indivíduos de *T. inamoena* para caracteres morfológicos da planta e físico-química do fruto. A técnica RAPD é útil na caracterização e análise da diversidade genética de indivíduos de *T. inamoena*. De fato, o iniciador OPM7 mostrou ser o mais informativo e recomendado em estudos de divergência com *T. inamoena*. A maior divergência genética foi de 78% dentro das populações, revelando maior variabilidade dentro do que entre as populações de *T. inamoena*. A população de Assú apresentou maior diversidade morfológica, físico-química e molecular, sendo escolhida para programas de melhoramento genético e conservação de recursos genéticos.

Palavras-chave: Semiárido, Característica morfológica, Características físico-química, Marcadores moleculares

1. INTRODUCTION

Several non-traditional fruit species are used as a source of income by local populations in Brazil. Of the native fruit species with potential for commercialization in the Brazilian semiarid, the species *Tacinga inamoena* (K. Schum.) [NP Taylor & Stuppy], belonging to the family Cactaceae, stands out. However, the potential of the fruits of these species as a source of nutrients for the human diet is still very little known and the benefits are little used by man (Silva et al. 2008).

The species *T. inamoena*, known as cumbeba, gogóia or quipá, depending on the region, is a cactus native to the Northeastern region of Brazil, with distribution that corresponds to the limits of the Caatinga biome (Lambert, 2009). Its fruits are of the berry type, slightly oblong in shape, whose skin color varies from dark green to yellow-orange during ripening, with small clusters of thorns occupying its surface (Dantas et al. 2015). The fruits are consumed fresh or incorporated into confectionery specialties, such as jelly, sweets and liqueurs. Despite being used for various purposes, natural populations of *Tacinga inamoena* have been suffering from habitat degradation due to anthropic action and uncontrolled extraction for ornamental purposes, posing a threat to their natural distribution (Coelho et al. 2015).

Knowledge about genetic diversity provides valuable data for the formulation of management strategies and conservation of native plants, helping access to information regarding the genetic basis of species, for a better understanding of the status of genetic variability (Amaral et al. 2019). Thus, characterization is an important activity, as it allows new materials of interest to be inserted in genetic improvement programs, serving as a basis for the design of conservation strategies (Souza, 2015). According to Cruz et al. (2011), different methods are used for the characterization of genotypes, which can be evaluated from morphological, biochemical and molecular descriptors.

The study of morphological characteristics is necessary because it allows information about individual genotypes, the relationship between the characteristics and the structure of the collections, in addition to being a simple technique, easy to perform and of low cost (Nasution and Hadiati, 2014). However, these characteristics can be studied subjectively and still suffer environmental influences, making it impossible to reliably detect polymorphism between species, varieties and individuals (Souza, 2015).

With the recent advances in biotechnology, they opened new perspectives for research with the advent of molecular markers, enabling genotypic discrimination, in a skillful way, as it allows the study of genetic variation at the DNA level (Ferreira and Grattapaglia, 1998). According to Souza et al. (2008), molecular markers can be used for studies of genetic diversity among individuals, within and between populations or related species. Among the various types of markers, RAPD (Random Amplified Polymorphic *DNA*) has several advantages when compared to other techniques, such as simplicity of use, fast, low cost, uses little amount of plant material and identifies a high number of polymorphic loci of DNA (Dantas et al. 2012). The RAPD markers have been used to investigate genetic diversity in plants of the genus *Opuntia* spp. (Zoghlami et al. 2007, Bendhifi et al. 2013, Valadez-Moctezuma et al. 2015, Tütüncü et al. 2016, El Kharrassi et al. 2017).

Thus, this study aimed to study the genetic diversity of *Tacinga inamoena* individuals by plant morphological, physical-chemical character of the fruit and RAPD markers between and within the natural populations of the species collected in the semiarid region of Brazil.

2. MATERIAL AND METHODS

2.1. Collection of plant material

For the development of the work, 30 individuals of *Tacinga inamoena* were used, previously identified, randomly selected and georeferenced (latitude and longitude) with the aid of GPS-Garmin, model GPSMAP® 60CSx, and collected in their natural environments spontaneously between January and April 2017 in the State of Rio Grande do Norte, Brazil, in the municipalities of Parelhas (10 individuals), Assú (10 individuals) and Apodi (10 individuals) all from the semiarid region of Brazil, with each plant constituting an individual.

The climate of the regions is BSw'h', according to the Köppen-Geiger classification, which is a very hot semiarid region, with a rainy period between the months of February to April (Köppen and Geiger 1928). The average climatic data from July 2016 to April 2017 and the geographic coordinate for each location are presented in Table 1. The data were obtained from meteorological stations in the region and provided by the National Institute of Meteorology (INMET) and the Agrometeorological Monitoring System (Agritempo), Brazil.

Table 1 Place of collection and monthly average of temperature (T, ºC), rainfall (R, mm), relative humidity (RH, %) of three populations of *T. inamoena*, with their respective geographical coordinates.

Population	T (ºC)	R (mm)	UR (%)	Geographic coordinates
Parelhas (PA)	26.14	91.3	58.6	6º 40.372' S 36˚ 38.838′ W
Apodi (AP)	28.44	109.9	66.3	5º 34.950' S 36º 56.681′ W
Assú (AS)	27.12	141.3	61.8	5º 36.547' S 37º 55.805′ W

In the field, these individuals were characterized morphologically and the cladodes and fruits with predominantly green color with yellowish nuances were collected. The materials were stored in identified plastic bags and transported to the Laboratory of Physiology and Post-harvest Fruit Technology and the Plant Biotechnology Laboratory of the Universidade Federal Rural do Semi-Árido (UFERSA), in Mossoró/RN. In the laboratory, the fruits were subjected to physical evaluations, and the pulp was subsequently separated from the skin (epicarp). The pulp fraction (mesocarp + seeds) was homogenized in Ultra-Turrax® equipment (T25 - IKA, Germany), originating a single sample, conditioned in plastic pots and stored in a freezer (-20ºC) for the evaluations. The packaged cladodes were stored in a freezer (-20ºC) until the moment of molecular extractions.

2.2. Morphological characterization of the plant

Plant height (APL) was determined from the ground level to the highest cladode, with the aid of a measuring tape, a result expressed in cm. The longitudinal diameters (DLC), transverse diameters (DTC) and thickness of the cladodes (ESC), using a digital caliper, with results expressed in cm. The count of the number of areolas by cladodes (NAR) was performed with the aid of a wire frame with a total area of 25 cm² (5.0 cm x 5.0 cm), superimposed on the central portion of one of the faces of the cladode. The cladode area (ACL) was determined according to the methodology proposed by Cortázar and Nobel (1991), by the expression: AC = longitudinal diameter x transverse diameter x 0.632, the results being expressed in cm².

2.3. Physico-chemical characterization of fruits

The average fruit weight (PMF), obtained from weighing the fruits on an analytical balance, was evaluated, with the results expressed in g; the longitudinal (DLF) and transverse (DTF) diameter of the fruit, obtained with a digital caliper, with the results expressed in cm; fruit shape (IFF), corresponding to an index derived from the relationship between longitudinal diameter and transverse diameter, classifying them as: compressed (RF <0.9), spherical ($0.9 \leq RF \leq 1.1$), oblong ($1.1 \leq RF \leq 1.7$) and cylindrical ($RF \geq 1.7$) (Lopes, 1982); the pulp yield (REN) was determined by the difference between the total mass of the fruit and the mass of its peel, a value expressed as a percentage (%). The firmness (FPO) was determined using a digital texture analyzer, from the brand Stable Micro Systems, model TA.XTExpress / TA.XT2icon, equipped with a 5 mm diameter probe. The results were expressed in Newton.

The total soluble solids (TSS) were determined directly in the homogenized juice of the pulp in a digital refractometer (model PR - 100, Palette, Atago Co, LTD., Japan) (AOAC, 2002), with the results expressed in ºBrix. Total titratable acidity (TTA) by volumetric titration, using 1 g of the pulp, expressing the results in % citric acid (IAL, 2008). The hydrogen potential (pH) was estimated using a potentiometer (Model mPA-210 Tecnal®, Brazil), the measured data were expressed in real pH values (IAL, 2008) and the SS/ATT ratio (RSA) was determined by the division direct measurement of total soluble solids and total titratable acidity. Total sugars (ACT) were quantified by the Antrona method (9.10-dihiro-9-oxoanthracene), according to Yemn and Willis (1954). Reducing sugars (ACR) were determined according to the dinitrosalicylic acid (DNS) method, according to Miller (1959). The results were expressed as a percentage (%).

2.4. Molecular characterization

The cladodes of 30 individuals were macerated in liquid nitrogen to obtain a fine powder using pre-cooled mortar and pestle. Subsequently, genomic DNA was isolated from approximately 100 mg of powdered cladodes using the NucleoSpin® Plant II commercial extraction kit (Macherey Nagel), according to the manufacturer's recommendations. A set of 20 RAPD primers were used, of which, eight primers were selected based on their reproducibility and levels of polymorphisms. The primers used with their respective sequences and annealing temperature are shown in Table 2. The DNA amplification reactions were carried out in a final volume of 12 µl, containing the following reagents: sterile milliQ water, 30 ng of genomic DNA, 1 X buffer reaction, 0.25 g / mL purified BSA, 0.33 µM of each RAPD primer, 0.16 mM DNTPs, 1 U / µL Taq DNA polymerase and finally 10 µl of mineral oil to prevent evaporation during the PCR process.

Table 2 List of primers used for RAPD amplification, sequences and annealing temperature.

Primer name	Sequence (5'-3')	Annealing temperature (ºC)
OPM-04	GGCGGTTGTC	40º
OPM-06	CTGGGCAACT	40º
OPM-07	CCGTGACTCA	40º
OPM-11	GTCCACTGTG	40º
OPM-12	GGGACGTTGG	40º
OPM-15	GACCTACCAC	40º
OPM-16	GTAACCAGCC	40º

The reactions were carried out in a thermocycler (Amplitherm Thermal Cyclers TX96 Plus) programmed for 40 amplification cycles, with an initial denaturation step at 92 ºC for 1 minute, 40 ºC for 1 minute, 72 ºC for 2 minutes, followed by 72 ºC for another 5 minutes, for the complete extension of the amplified products. The amplification products were applied on 1.5% agarose gel, stained with ethidium bromide (10 µg / ml), plus 3 µl of a solution of bromophenol blue and subjected to horizontal electrophoresis at 115V for 120 minutes in a solution of 1X TBE buffer. The molecular marker o 1Kb ladder (BioLabs / New England) was used to estimate the size of the amplified fragments. Subsequently, the gels were photographed under ultraviolet light, using the photo-documentation system (AlphaImager® Mini, ProteinSimple). From the photo-documented gels, the amplified products were analyzed, to elaborate a binary matrix considering the presence (1) or absence (0) for the amplified fragments.

2.5. Statistical analysis

For the morphological and chemical data, univariate and multivariate analysis of variance was performed using the Snedecor F tests and the Wilks criterion, respectively. The population means were compared with each other using the t test. All analyzes were performed using a nominal significance level of 5% probability using the Exp.Des.Pt package from the R program (R Core Team, 2019). For each marker, the content of polymorphic information (PIC) (Roldán-Ruiz et al. 2000), the resolution power of the marker (RP) (Prevost and Wilkinson, 1999), the index of the marker (Powell et al. 1996), number of marks, number of polymorphic marks and percentage of polymorphic loci. For each population, the percentage of polymorphic loci, the Nei distance (1978), the Shannon index and the gene flow were estimated via POPGENE version 3.2 (Yeh et al. 1999). AMOVA was performed using the GenAlEx software version 6.5 (Peakall and Smouse, 2012).

The dissimilarity matrices of the individuals in the populations were estimated by the standardized Euclidean distance for morphological and chemical data and the complement of the Jaccard index for molecular data. The dendrograms for the matrices for morphological, chemical and molecular data were constructed using the hierarchical UPGMA method (Unweighted Pair-Group Method Analysis) and the cophenetic correlation was used to verify the quality of grouping using the packages. All of these analyzes were performed by the program R (R CORE TEAM, 2019) using the PheatMap and Biotools packages. The cutoff point used for the formation of the groups followed the methodology of Mojena (1977) and the contribution of each characteristic to the divergence was estimated by the method of Singh (1981). These analyzes were processed using the genes program (CRUZ, 2016).

3. RESULTS

3.1. Plant morphological and physicochemical characteristics of the fruit

An effect was observed between populations ($p < 0.01$; $p < 0.05$) in practically all the evaluated characters, the only exceptions were cladode thickness (ESC), fruit shape index (IFF) and total titratable acidity (ATT) (Table 3). Considering all the characters studied at the same time, there were genotypic differences between the genotypes (FWilks = 12.94, p <0.001).

For the plant characters, it was found that the population in Assú had the highest average for plant height (APL), longitudinal diameter of the cladode (DLC), transversal diameter of the cladode (DTC), area of the cladode (ACL) and thickness cladode (ESC), although for the latter character it did not differ from the Parelhas population. The highest number of areolas per cladode (NAR) was found in Apodi. In this population, the lowest averages for the other characters were found (Table 3).

The Parelhas population stood out with the highest average for the characters longitudinal diameter of the fruit (DLF), transversal diameter (DTF), average fruit weight (PMF) and pulp yield (REN), although it did not differ from the Apodi population in last two characters (Table 3). The highest firmness of pulp was observed in the Apodi population and the lowest in Parelhas. The populations did not differ for the fruit shape index (IFF). In the population of Assú, average intermediate estimates were observed for all characters except the IFF.

The Apodi and Parelhas populations had the highest average pH value and soluble solids and total titratable acidity (RSA) ratio (Table 3). The highest soluble solid (TSS) was observed in Apodi, while the highest content of total sugars (ACT) and reducing sugars (ACR), in Assú. The populations did not differ for total titratable acidity (TTA).

The variance between populations was greater in twelve of the eighteen morphological characters evaluated, indicating that most of the total variation is among the populations studied (Table 3, Fig. 1). The characters with the highest variance within were ATT, DTC, ESC, IFF, REN and RSA (Fig. 1).

Table 3 Snedecor F estimates, variances between and within and population averages for eighteen plant and fruit morphological characters and fruit chemistry evaluated in three *T. inamoena* populations.

Population	Average					
	Plant (Morphological characters)					
	APL	DLC	DTC	ESC	NAR	ACL
Apodi	41.70b	7.89b	3. 78b	0.77b	30.90a	19.65b
Assú	55.73a	10.06a	4.62a	0.92a	25.00b	29.55a
Parelhas	31.71c	7.15b	3.85b	0.81ab	16.90c	17.45b

F	24.98^{**}	16.44^{**}	7.01^{*}	2.38^{ns}	52.56^{**}	14.16^{**}
$\hat{\sigma}^2_{pop}$	139.517	2.146	0.188	0.003	48.463	38.629
$\hat{\sigma}^2_{d}$	58.159	1.389	0.312	0.025	9.400	29.344
	Fruit (Physical characters)					
	PMF	DLF	DTF	IFF	REN	FPO
Apodi	11.71a	2.82c	2.51c	1.13a	36.78a	47.91a
Assú	10.19b	3.06b	2.70b	1.14a	31.79b	39.56b
Parelhas	12.27a	3.40a	2.98a	1.15a	37.09a	33.34c
F	13.65^{**}	35.41^{**}	29.17^{**}	2.74^{ns}	8.56^{*}	50.71^{**}
$\hat{\sigma}^2_{pop}$	1.071	0.084	0.056	3.6×10^{-5}	7.825	52.389
$\hat{\sigma}^2_{d}$	0.846	0.024	0.019	2.1×10^{-4}	10.353	10.538
	Fruit (Chemical characters)					
	SST	pH	ATT	RSA	ACT	ACR
Apodi	7.13a	4.95a	0.13a	53.08a	5.02b	4.84b
Assú	6.18c	4.21b	0.14a	44.92b	5.52a	5.32a
Parelhas	6.55b	4.95a	0.13a	52.71a	4.33c	3.83c
F	21.34^{**}	36.06^{**}	2.00^{ns}	5.82^{*}	16.52^{**}	31.93^{**}
$\hat{\sigma}^2_{pop}$	0.222	0.177	1.9×10^{-5}	17.609	0.339	0.564
$\hat{\sigma}^2_{d}$	0.109	0.050	1.8×10^{-4}	36.569	0.219	0.182

Means followed by the same letter do not differ from each other by the t test ($p > 0.05$). F (Wilks) = 12.94**.

APL: plant height (cm); DLC: longitudinal diameter of the cladode (cm); DTC: transverse diameter of the cladode (cm); ESC: cladode thickness (cm); NAR: number of areolas per cladode; ACL: cladode area (cm^2); PMF: average fruit weight (g); DLF: longitudinal diameter of the fruit (cm); IFF: fruit shape index; RED: pulp yield (%); FPO: firmness of pulp (N); SST: soluble solids (ºBrix); pH: hydrogen potential; ATT: total titratable acidity (%); RSA: ratio of soluble solids to total titratable acidity; ACT: total sugars (%); ACR: reducing sugars (%).

Fig. 1 Percentage of variances between (σ ̂_pop^2) and within (σ ̂_d^2) for eighteen morphological characters of the plant and fruit and fruit chemistry evaluated in three populations of *Tacinga inamoena*. APL: plant height (cm); DLC: longitudinal diameter of t the cladode (cm); DTC: transverse diameter of the cladode (cm); ESC: cladode thickness (cm); NAR: number of areolas per cladode; ACL: cladode area (cm^2); PMF: average fruit weight (g); DLF: longitudinal diameter of the fruit (cm); IFF: fruit shape index; RED: pulp yield (%); FPO: firmness of pulp (N); SST: soluble solids (ºBrix); pH: hydrogen potential; ATT: total titratable acidity (%); RSA: ratio of soluble solids to total titratable acidity; ACT: total sugars (%); ACR: reducing sugars (%).

The individuals from the three populations were divided into four groups based on morphological and chemical data (Fig. 2). The first group corresponded to all individuals in Assú, while the second group corresponded to individuals from Parelhas. In general, the individuals in the first group had higher values for DTF, DLF, IFF, ATT, ACR, ACT, APL, ACL, DLC, DTC and ESC, and lower values for pH, REN, PMF, RSA and SST. On the other hand, individuals in the second group have higher mean values for DTF, DLF, IFF, pH, REN and PMF while the lowest values were found in the characters SST, FPO, NAR, ATT, ACR, ACT, APL, ACL, DLC and DTC (Fig. 2).

The individuals from Apodi were allocated to the third and fourth groups, with one group consisting of the individual AP-01 and the other of the other individuals. The AP-01 individual had the highest ATT content, while the individuals in group four had lower DTF, DLF and IFF values and higher values for pH, REN, PMF, RSA, SST, FPO and NAR.

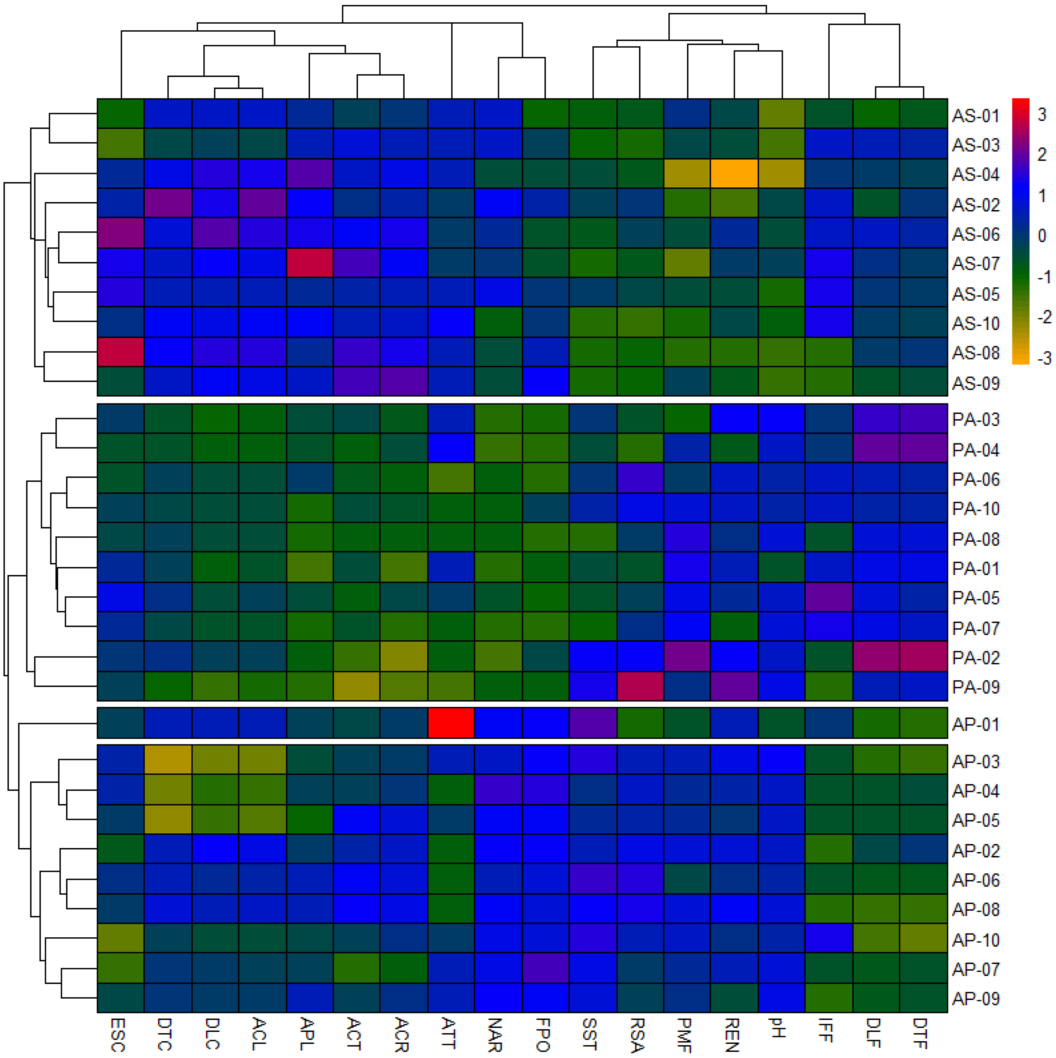

Fig. 2 Dendrogram of individuals from three populations of *T. inamoena* generated by the UPGMA method from the matrix of standardized Euclidean distances obtained in eighteen morphological characters of the plant and fruit and fruit chemistry. APL: plant height (cm); DLC: longitudinal diameter of the cladode (cm); DTC: transverse diameter of the cladode (cm); ESC: cladode thickness (cm); NAR: number of areolas per cladode; ACL: cladode area (cm²); PMF: average fruit weight (g); DLF: longitudinal diameter of the fruit (cm); IFF: fruit shape index; RED: pulp yield (%); FPO: firmness of pulp (N); SST: soluble solids (ºBrix); pH:

hydrogen potential; ATT: total titratable acidity (%); RSA: ratio of soluble solids to total titratable acidity; ACT: total sugars (%); ACR: reducing sugars (%). Cophenetic correlation (r = 0.81**).

The variable that most contributed to the genetic divergence was the plant height with 41.63% followed by cladode area (15.10%), total sugars, soluble solids, number of areolas per cladodes and pulp firmness (Table 4). The combined contribution of these characters was 98.24%, while the other characters accounted for only 1.76% of the genetic divergence.

Table 4 Contribution of plant and fruit morphological characters and fruit chemistry to the genetic divergence of individuals from three populations of *T. inamoena*.

Character	S_j	S_j (%)
Plant high (cm)	134309.00	41.63
Longitudinal diameter of the cladode (cm)	2496.24	0.77
Cross diameter of the cladode (cm)	384.33	0.12
Cladode thickness (cm)	23.73	0.01
Number of halos per cladode	37256.00	11.55
Cladode area (cm²)	48706.92	15.10
Average fruit weight (g)	1378.90	0.43
Longitudinal diameter of the fruit (cm)	71.70	0.02
Fruit shape index	51.00	0.02
Pulp yield (%)	0.20	0.01
Pulp firmness (N)	13701.95	4.25
Soluble solids (ºBrix)	40601.89	12.58
Hydrogen potencial	227.72	0.07
Total titratable acidity (%)	149.71	0.05
Soluble solids ratio and total titratable acidity	0.17	0.01
Total sugars (%)	42380.94	13.14
Reducing sugar (%)	393.80	0.12
Total	322630.82	100.00

Sj: Contribution to genetic divergence (Singh, 1981).

3.2. Molecular data

Of the 20 primers, eight produced clear patterns of polymorphic bands in the three populations. The total number of fragments generated ranged from 9 (OPM12) to 19 (OPM4) in a total of 113 fragments and an average of 14.13 per primer. Ninety polymorphic primers were produced, with OPM6, OPM7 and OPM15 those with the highest polymorphism and OPM20 and OPM11 the least polymorphic (Table 5). The primers had similar and relatively high polymorphism information (PIC) content (≥0.360) indicating that the markers were polymorphic and efficient in the present study. The marker index (MI) fluctuated from 9.41 (OPM20) to 13.27 (OPM7), while the resolving power (PR) from 3.33 (OPM12) to 9.53 (OPM7). The OPM7 primer, with a higher percentage of polymorphism (P%) and higher MI and RP, can be considered the most informative and recommended for studies of divergence with *T. inamoena*.

Table 5 Random Amplified Polymorphic DNA (RAPD) primers selected for amplification of *T. inamoena* individuals, with respective sequences, annealing temperature (TA), total number of amplified fragments (NTFA), total number of polymorphic fragments (NTFP), percentage polymorphism (P%), polymorphism information contente (PIC), marker index (IM) and marker resolving power (PRM).

Primer	NTFA	NTFP	P (%)	PIC	IM	RP
OPM4	19	14	73.68	0.361	10.47	9.47
OPM6	11	10	90.01	0.363	12.86	3.80
OPM7	16	15	93.75	0.360	13.27	9.53
OPM11	14	10	71.43	0.360	10.10	5.80
OPM12	9	7	77.78	0.361	11.04	3.33
OPM15	15	13	86.67	0.362	12.35	6.07
OPM16	14	11	78.57	0.360	11.11	5.13
OMP20	15	10	66.67	0.360	9.41	4.47

The number of alleles (na) varied from 1.53 (Apodi) to 1.78 (Assú and Parelhas) while the effective number of alleles (ne) from 1.42 (Apodi) to 1.60 (Assú) (Table 6). The population of Apodi showed the lowest values for genetic diversity (h = 0.23), Shannon's diversity index (I = 0.33), number of polymorphic loci (Np = 48.0) and percentage of polymorphic loci (Pp = 53.33%). The highest values for the four parameters were found in Assú (Table 6).

Table 6 Genetic diversity within three populations of *T. inamoena* based on RAPD markers.

Population	n	na	ne	*h*	*I*	Np	Pp (%)
Apodi	10	1.53	1.42	0.23	0.33	48.00	53.33
Assú	10	1.78	1.60	0.33	0.47	70.00	77.78
Parelhas	10	1.78	1.49	0.28	0.42	70.00	77.78

n: sample size; na: number of alleles observed; ne: number of effective alleles; *h*: Genetic diversity; *I*: Shannon's diversity index; Np: number of polymorphic loci; Pp: percentage of polymorphic loci.

The shortest genetic distance (d = 0.125) and the highest gene flow (Nm = 3,164) were found between the Assú and Parelhas populations (Table 7). The population of Apodi was the most divergent in relation to the other two populations. The greatest distance (d = 0.173) and the lowest gene flow (Nm = 1,975) occurred between the Apodi and Parelhas populations.

Table 7 Genetic distance (Nei, 1978) (above the diagonal) and Gene flow (below the diagonal) between three populations of *T. inamoena* based on RAPD markers.

Population	Apodi	Assú	Parelhas
Apodi		0.172	0.173
Assú	2.170		0.125
Parelhas	1.975	3.164	

Nm: gene flow [Nm = 0.5 (1 - Gst) / Gst]

In the cluster analysis based on the molecular data, four groups were verified (Fig. 3). The first group consisted of 22 accesses, which corresponded to (73.33%) of the individuals. In this group, most individuals have the presence of amplified marks (blue color in the dendrogram). In that group are 90% of the individuals of the Apodi population, 80% of Assú and 50% of Parelhas. The second group was composed of five individuals from Parelhas and only one from Apodi. Individuals AS-03 and AS-10, both from Assú, were not grouped and each formed a unitary group. These individuals had few electrophoresis gel bands.

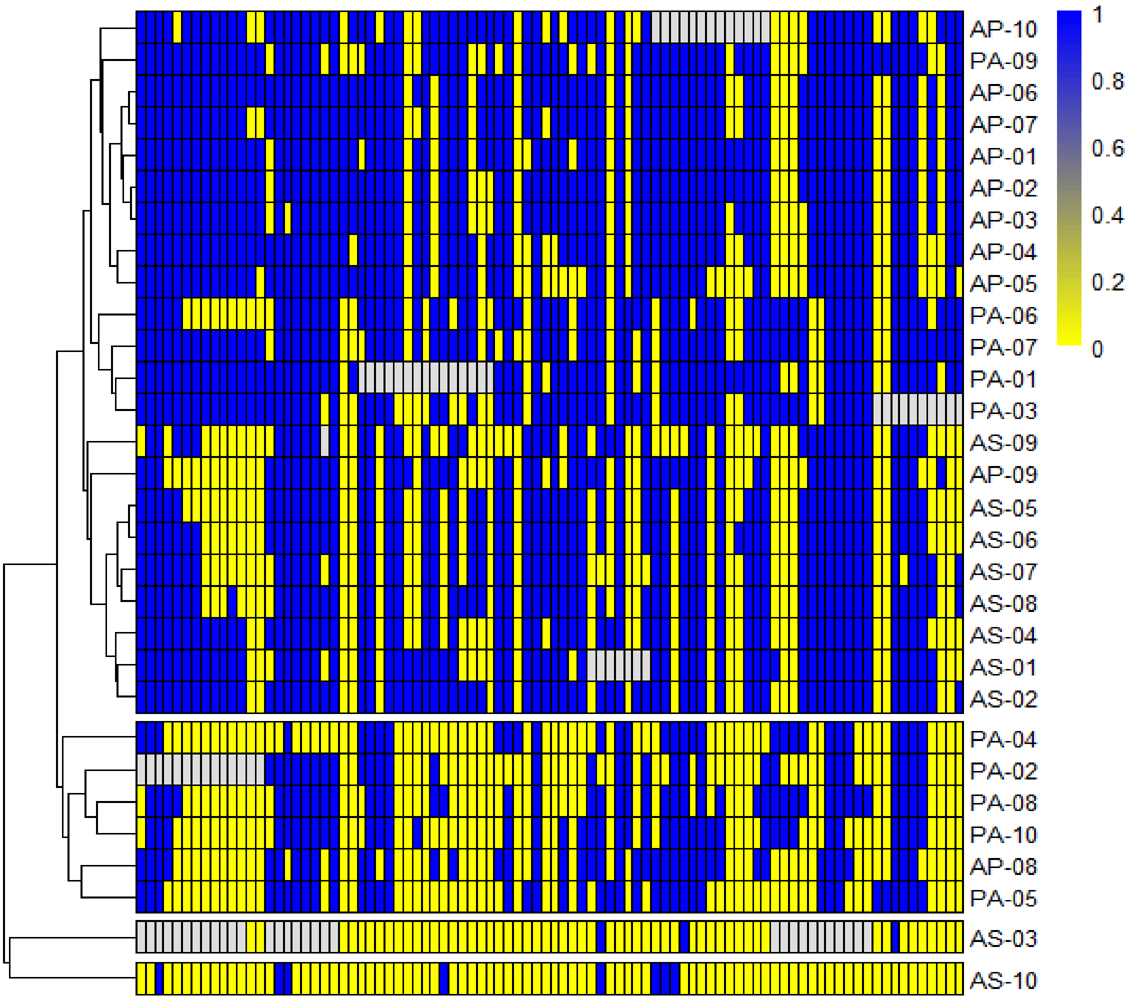

Fig. 3 Dendrogram of individuals from three populations of *T. inamoena* generated by the UPGMA method from the Jaccard index complement matrix obtained from RAPD markers. Cophenetic correlation (r = 0.95**).

The evaluation of the structuring of genetic variability, carried out by AMOVA (Table 8), provided an overall estimate of ΦST = 0.219 (P <0.01, with 5,000 permutations), that is, 21.90% of the total variation is found between populations. The results indicate that the largest source of molecular variation is within the populations of *T. inamoena*.

Table 8 Analysis of molecular variance (AMOVA) of three populations of *T. inamoena*.

FV	gl	SQ	QM	Variance	%Variation	Φ_{ST}
Among population	2	110.40	55.20	4.07	22%	0.219
Within population	27	391.00	14.48	14.48	78%	
Total	29	501.40		18.55	100%	

4. DISCUSSION

4.1. Plant morphological and physicochemical characteristics of the fruit

The genetic improvement of any crop requires the use of well-characterized wild relatives and breeding techniques, so the assessment of genetic diversity is a requirement for selecting high-yield genotypes (Jacinto et al. 2020). The morphological characteristics of the plants of the population of Assú, such as plant height (APL), longitudinal diameter of the cladode (DLC), transversal diameter of the cladode (DTC), area of the cladode (ACL) and thickness of the cladode (ESC) stood out probably due to the greater rainfall that occurred in that location. For Merwer et al. (1997) report that the increased availability of water for the palm (*O. ficus-indica*) influences the growth and yield of the crop. Where vegetative growth is related to water content in the soil, due to the fact that the main biochemical and physiological processes are dependent on water, such as photosynthesis, transpiration and nutrient absorption (Sampaio, 2005). According to Aguilar (1991) despite the fact that the cactacea (*O. ficus-indica*) has a good water reservoir inside the cladodes, in the parenchyma cells and in the chlorenchyma vacuoles, the longitudinal and transversal growth of the cladodes varies according to the availability of water in the soil.

In view of this, one of the attributes of CAM plants is turgidity, which in *Opuntias,* is expressed morphologically through thick cladodes with the presence of water-filled vacuoles in photosynthetic cells, and several water storage cells, commonly presenting greater thickness of mature cladodes (Nobel, 2001), as observed in the population of Assú, which presented greater thickness (Table 3). It was also revealed to the population of Assú, the largest area of the cladode, so the larger the area, the greater the water content of the cladode, therefore the water deficit is more resistant to the plant, as this characteristic is of importance for the palm improvement aiming at the selection of genotypes more tolerant to water deficit (Alves et al. 2015). The morphological characteristics of plants are defined by the genotype, however, they are strongly influenced by the environment, by crop and soil management, as observed in the present work. Species with greater phenotypic plasticity play an important role in the ability to adapt to climatic conditions in the semiarid region (Peixoto, 2009).

The physicochemical characteristics of the fruits were influenced by genotype and locality. Corroborating with Fagundes and Yamanishi (2001) who report that these characteristics are influenced by edaphoclimatic conditions, harvest time, cultural treatments, genetic makeup, maturation stage and post-harvest treatment, among others. Such characteristics are quality factors essential to the use and commercialization of fruit pulp and the elaboration of industrialized products (Chitarra and Chitarra, 2005). According to Vieira and Gusmão (2008) and Santos et al. (2009), these fruit variations may be due to genetic variability or phenotypic plasticity, due to different geographical locations. Considering the content of soluble solids, the fruits of the Apodi population showed higher soluble solids (Table 3), and it was the location that had a higher temperature during the formation of the fruit (Table 1). According to Brighenti et al. (2014) the environmental factor temperature, can influence the quality of the fruit, where high temperatures result in higher levels of soluble solids. With regard to *Tacinga inamoena*, the use of physical-chemical characters to assess the genetic diversity in its individuals is very scarce.

The UPGMA dendrogram showed the distribution of *T. inamoena* individuals, confirming the variability found through the morphological characters of the plant and fruit, as well as the physicochemical characters of the fruits, providing important information for the conservation of genetic material for genetic improvement programs. Therefore, it was possible to separate the individuals studied according to their region of origin. A possible explanation for this result is in the height of plants, since, it was the character that most contributed to the genetic divergence between individuals, with 41.63% of the divergence (Table 4). Crossbreeding between individuals from different groups provides superior strains for improving traits of interest (Silva et al. 2011).

Table 4 shows the relative contribution of each characteristic to the Singh method (1981), in which the variable that most contributed to the genetic divergence was the plant height with 41.63% followed by the cladode area (15.10%), total sugars, soluble solids, number of areolas per cladodes and firmness of pulp. According to Cruz et al. (2012) and Conceição et al. (2014), the contribution of the characters used in the characterization of genotypes are important to select the characteristics that most discriminate the genotypes and variables that contribute with a very low percentage, are less informative in the characterization, and can be discarded in studies of genetic divergence. Lima et al. (2013), in their study with fruits of 21 accessions, of two species of pitaya (Cactaceae), obtained the greatest relative contributions, for the characters diameter of the fruits (27.45%), which was the characteristic that had the greatest contribution, followed by the mass total fruit (25.43%) and the fruit pulp mass (24.67%), being the important characteristics for germplasm characterization works. Paixão (2012) cites the width of the cladode (27.35%) and length of the cladode (20.97%) as the most important characters for the genetic divergence in the *Opuntia* and *Nopalea* genera, which together contributed 48.42% to the genetic divergence.

4.2. Molecular diversity

The results of this study demonstrated that the RAPD markers allowed to detect a higher level of polymorphism (93.75%) (Table 5) among *T. inamoena* individuals who were characterized by a high degree of genetic variability within populations, since the proportion of loci polymorphs is a factor used to quantify genetic diversity in plant populations (Pereira et al. 2009). Valadez-Moctezuma et al. (2014), analyzing the genetic diversity in 52 varieties of the genus *Opuntia* from Mexico, obtained a total of 149 fragments amplified with the RAPD technique, of which 86 were polymorphic. The percentage of polymorphic markers in his study was 58.3%, lower than in the present study (93.75%). This genomic variation detected in these plants with molecular markers is probably caused by frequent mutations and also favored by the exchange and dispersion of individuals between different geographic areas, which is the main source of variation in this species of asexual reproductive plant (Valadez-Moctezuma et al. 2014). According to Oliveira et al. (2007) there is a trend in the germplasm of the species, mainly little improved in having high polymorphism.

The greatest genetic diversity was found in Assú (h = 0.33 and I = 0.37) (Table 6). These results confirm high genetic diversity within the population of *T. inamoena* and contribute to the determination of genetic relationships between materials to determine the crosses to be used in breeding programs. Rifat et al. (2019), evaluating the genetic diversity in *Hylocereus* spp. from Bangladesh, found mean values for heterozygosity (*h*) and Shannon index (*I*), respectively 0.327 and 0.482, and concluded that germplasms showed high genetic diversity.

The results obtained show that the maximum value (0.173) (Table 7) found for the comparison of Nei's genetic distance was between the populations of Apodi and Parelhas. This can be attributed to the location of the two populations, considering that they are more distant geographically. On the other hand, a high level of migrants was found among the populations of Assú and Parelhas (3,164). This indicates that the populations are more similar to each other and suggests a greater gene flow between them. Several factors affect the rate of gene flow between different populations, which can be attributed to the pollen flow mediated by hummingbirds. Such genera *Tacinga* and *Melocactus* are exclusively visited by hummingbirds (*Chlorostilbon lucidus*), suggesting that they are the main pollen vector of these species (Nolasco et al. 2011). In relation to the dispersion of seeds of this cactus it can be practiced by ants of the genera *Camponotus Mayr*, 1861 and Pheidole Westwood, 1839 (Leal, 2003), possibly by small mammals (Taylor and Zappi, 2004) and tortoises of the species *Chelonoidis carbonaria* (Spix, 1824) that can be considered good seed dispersers for the Caatinga areas

(Nascimento et al. 2015). The UPGMA grouping (Fig. 3) with RAPD molecular information showed the formation of four groups based on the Jaccard index. The result obtained in the dendrogram corroborates the result obtained previously by the gene flow (Nm), since most individuals were not grouped by region of collection. Individuals AS-03 and AS-10, both from Assú, were the ones that presented the highest dissimilarity indexes. According to Rotili et al. (2012), groups originated by only one individual evidence that these individuals are more divergent in relation to the others, as observed in this case.

Through AMOVA, a higher percentage of genetic variation attributed to diversity within the population was found, with 78% and the remaining 22% of variation was observed between populations (Table 8). As it is a species with populations threatened by habitat degradation and exploratory extraction, there is a high genetic variability, possibly in the reproductive strategies adopted by the species during its evolution. This indicates that genetic variation within populations is essential in the use, conservation and formation of breeding programs in genetic resources (Li et al. 2011). Similar results have been reported in other species of the Cactacea family, as verified in a study conducted by Valadez-Moctezuma et al. (2014) in accessions of *Opuntia ficus-indica*, where 70% of the total variation was accounted for within the group and 30% between groups and in studies conducted by Morillo-Coronado et al. (2017) in yellow pitayas genotypes (*Selenicereus megalanthus* Haw.) That 69% of the variation was explained by the component within groups and only 31% between groups, proposing management strategies and conservation of these plant genetic resources.

5. CONCLUSIONS

This is the first study to assess genetic diversity within and among populations of *Tacinga inamoena* in the Brazilian semiarid region using morphological characters and RAPD molecular markers. There is variability in individuals of *T. inamoena* for plant morphological and physicochemical characteristics of the fruit. The characteristics that most contribute to the genetic divergence were the plant height, followed by the area of the cladode, total sugars and soluble solids of the fruit, number of areolas per cladodes and firmness of the pulp.

The RAPD technique is useful in the characterization and analysis of the genetic diversity of *T. inamoena* individuals. In fact, the OPM7 primer proved to be the most informative and recommended in studies of divergence with *T. inamoena*. The population of Assú showed greater genetic diversity with higher values of h = 0.33; I = 0.47; Np = 70.0 and Pp = 77.78%.

The greatest genetic divergence was 78% within populations, revealing greater variability within that of *T. inamoena* populations. The population of Assú presented greater morphological, physical-chemical and molecular diversity, being chosen for programs of genetic improvement and conservation of genetic resources.

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CHAPTER III

Bioactive compounds content in *Tacinga inamoena* fruits vary among and within populations

Abstract This study characterized *T. inamoena* fruits from different populations regarding the bioactive compounds content and assessed the genetic diversity among and within populations for these traits. Fruits were collected from 10 plants occurring in Assú, Apodi and Parelhas, municipalities in Rio Grande do Norte State, Brazil. Then, fruits were analyzed for the contents of vitamin C (VITC), carotenoids (CARO), betacyanin (BETC), betaxanthin (BETX), total exctratable polyphenols (PET), and antioxidant activity by the DPPH and ABTS methods. Fruits from Parelhas had the highest trait averages, although it did not differ from Assu for PET and from Apodi for VITC and DPPH. Apodi and Assu did not differ for CARO, BETC, BETX, and ABTS. There were no differences among populations regarding FLAV. Moreover, the UPGMA grouping method arranged the three populations into two groups, Parelhas in the first, and Assú and Apodi in the second. Variance among populations was higher for CARO, BETC, BETX, and DPPH, indicating that most of the total variation for these traits were among populations studied. CARO, BETX, BETC, and VITC were the traits that most contributed to variation among individuals. In conclusion, fruits from *T. inamoena* are rich in bioactive compounds, and these traits can vary either among and within populations geographic distributed.

Keywords: Caatinga. *Cactaceae*. Genetic divesity. Native species. Antioxidant activity.

Resumo Este estudo caracterizou frutos de *T. inamoena* de diferentes populações quanto ao conteúdo de compostos bioativos e avaliou a diversidade genética entre e dentro das populações para essas características. Foram coletados frutos de 10 plantas ocorridas em Assú, Apodi e Parelhas, municípios do estado do Rio Grande do Norte, Brasil. Os frutos foram analisados quanto aos teores de vitamina C (VITC), carotenóides (CARO), betacianina (BETC), betaxantina (BETX), polifenóis extratáveis totais (PET) e atividade antioxidante pelos métodos DPPH e ABTS. Os frutos de Parelhas apresentaram as maiores médias das características, embora não tenham diferido de Assú para PET e de Apodi para VITC e DPPH. Apodi e Assú não diferiram para CARO, BETC, BETX e ABTS. Não houve diferenças entre as populações em relação ao FLAV. Além disso, o método de agrupamento UPGMA organizou as três populações em dois grupos, Parelhas no primeiro e Assú e Apodi no segundo. A variação entre as populações foi maior para CARO, BETC, BETX e DPPH, indicando que a maior parte da variação total dessas características ocorreu entre as populações estudadas. CARO, BETX, BETC e VITC foram os traços que mais contribuíram para a variação entre os indivíduos. Concluindo, os frutos de *T. inamoena* são ricos em compostos bioativos, e essas características podem variar entre e dentro das populações distribuídas geograficamente.

Palavras-chave: Caatinga, *Cactaceae*, Diversidade genética, Espécies nativas, Atividade antioxidante

1. INTRODUCTION

Brazil owns high biodiversity of plant species widely distributed over the country, composing distinct and well-defined biomes. Such ecosystems are rich in native and exotic plant species, whose nutritional and economic value are little known (Schiassi et al. 2018). Caatinga, an exclusive biome from Brazil, harbors numerous endemic plant species, especially from the *Cactaceae* family. Among them, *Tacinga inamoena* (K. Schum.) [NP Taylor & Stuppy] is a native species widely distributed in the Northeastern region and North of Minas Gerais, along which the biome is distributed (Lambert, 2009).

Popularly known as cumbeba, gogóia, pêlo, or quipá, *T. inamoena* is a perennial shrub that can reach up to one meter in height. With a succulent and prickly stem, typical of cacti, the plant produces yellowish fruits up to 9 cm in diameter, weighing around 13 g, with 7-14% soluble solids content, and 0.27-0.62 g citric acid g^{-1} fresh weight titratable acidity. Also, fruits are rich in bioactive compounds, such as vitamin C, betalains, flavonoids, and polyphenols, which confer high antioxidant activity (Souza et al. 2007; Dantas et al. 2015; Dantas et al. 2016; Formiga et al. 2016). Thus, fruits from this species have high antioxidant properties for human health and good potential for fresh consumption and processing.

With physicochemical traits similar to that of *Opuntia fícus-indica* (Souza et al. 2007), *T. inamonea* fruits have potential value for human nutrition. Fruits have already been used for fresh consumption and processing, but over still restricted areas in Brazil (Silva et al. 2009). Also, cactus species is not cultivated, and exploitation has been extractive and illegal, cause of its habitat destruction (Fabricante and Oliveira, 2013).

It has been shown that populations occupying different habitats may have different genetic characteristics, and research on the genetic relationship among these populations is the basis for the conservation of species (Duffy et al. 2009). Under different environmental conditions, such as temperature, light, soil, rainfall, altitude, water stress, nutrient availability and geographical position, plants show genetic variation in morphological and anatomical traits, which allow them to adapt and survive to different environmental conditions (Gratani, 2014).

Genetic variation may also occur in the fruit physicochemical traits. Studies have reported differences in the morphological and nutritional characteristics of *T. inamoena* fruits from plants occurring in different locations (Silva et al. 2009; Dantas et al. 2015; Formiga et al. 2016). Also, the composition of bioactive compounds in fruits seems to differ drastically among populations, which promotes studies to know whether these differences are genetically inherited or influenced by the environmental conditions where plants grow. Thus, this work characterized *T. inamoena* fruits, collected from plants occurring in different locations, regarding the content of bioactive compounds and studied the genetic diversity among and within populations for these traits.

2. MATERIAL AND METHODS

2.1. Plant material

Fruits from thirty *Tacinga inamoena* genotypes in their natural environments were collected from January to April 2017. The genotypes were distributed in three populations, with ten individuals each, located in Apodi, Assú, and Parelhas, Rio Grande do Norte State, Brazil. Geographic locations are shown in **Erro! Fonte de referência não encontrada.**.

Table 1 Collection site of *T. inamoena* fruits in different locations in Brazil.

Population no.	State in Brazil	Collection site	Sample size	Altitude (m)	Latitude	Longitude
1	Rio Grande do Norte	Parelhas	10	290	6° 40.36′ S	36° 38.83′ W
2	Rio Grande do Norte	Assú	10	87	5º 34.95′ S	36º 56.68′ W
3	Rio Grande do Norte	Apodi	10	124	5º 36.55′ S	37º 55.80′ W

The climate in the region is BSw'h, according to the Köppen-Geiger classification, which is very hot tropical semiarid with rainfall concentrated from February to April. Climatic data from July 2016 to April 2017 are shown in Table 1. Data were obtained from automatic meteorological weather stations in the region and available at the National Institute of Meteorology (INMET) and Agrometeorological Monitoring System (Agritempo), Brazil.

Table 2 Climatic data in Parelhas, Apodi and Assú, Rio Grande do Norte State, Brazil.

Month	Temperature (ºC)			Rainfall (mm)			Air relative humidity (%)		
	Parelhas	Apodi	Assú	Parelhas	Apodi	Assú	Parelhas	Apodi	Assú
Jul/16	28.07	28.20	27.94	0	0	0	54.93	57.26	57.18
Aug/16	28.54	28.12	28.45	0	0	0	52.95	58.72	57.46
Sept/16	29.20	28.56	20.08	3.40	0	0	55.96	65.07	54.75
Oct/16	29.75	29.23	20.39	0	0	0	56.18	64.57	56.15
Nov/16	30.10	29.35	29.33	0	0	0	50.40	68.33	57.72
Dec/16	30.18	28.91	29.68	7.10	54.40	0	50.55	67.69	59.17
Jan/17	30.40	29.14	29.69	17.10	15.30	16.00	55.25	60.30	63.75
Feb/17	28.80	28.21	29.00	216.70	217.70	123.10	63.63	67.91	65.60
Mar/17	27.60	27.25	28.34	172.80	117.60	307.70	76.88	77.68	73.92
Apr/17	28.00	27.41	28.38	131.00	144.70	118.30	68.92	75.80	72.15
Mean	26.14	28.44	27.12	91.3	109.9	141.3	58.6	66.3	61.8

Fifty fruits from each individual were collected showing a predominantly green color with yellowish nuances in the peel. Then, the fruits were packed in plastic bags and transported to the Physiology and Postharvest Laboratory, Federal Rural University of Semiarid, Mossoró, RN, Brazil, for evaluation of their bioactive compounds content. At the laboratory, pulp was separated from the peel (epicarp), and the edible fraction (mesocarp + seeds) was homogenized in Ultra-Turrax® equipment (T25 - IKA, Germany). Then, the processed was packed in plastic pots and stored in a freezer for further evaluation of vitamin C (VITC), flavonoids (FLA), carotenoids (CARO), betacyanins (BETC), betaxanthin (BETX), and polyphenol contents (PET), and antioxidant activity by capture of the ABTS and DPPH radicals.

2.2. Bioactive compounds evaluation

Vitamin C content was estimated by titration with Tilman's solution (DFI - 2.6 dichloro-phenol 0.02% indophenol), according to the methodology proposed by (Strohecker and Henning, 1967) and the results were expressed as mg ascorbic acid (AsA) 100 g^{-1} fresh weight (FW). The contents of yellow flavonoids and carotenoids were determined by spectrophotometry according to (Higby, 1962; Francis, 1982), respectively, and results were expressed as mg 100 g^{-1} FW.

Betacyanins and betaxanthins were quantified directly on homogenized edible fraction. Citrate-phosphate buffer solution (0.05 M, 6.5 pH) was used when necessary to dilute samples so that the readings at 538 nm for betacyanin and 480 nm for betaxanthin remained between 0.8 < Absorbance < 1.0 (Stintzing et al. 2005), and quantification was performed according to

(Schwartz and von Elbe, 1980; Trezzini and Zrÿb, 1991). Results were expressed as mg 100 g^{-1} FW.

To determine total extractable polyphenols (PET) and antioxidant activity (AA), an extract in methanol and acetone was obtained from pulp according to (Larrauri et al. 1997). PET was determined by spectrophotometry using the Folin-Ciocalteau reagent as described by (Obanda et al. 1997), and results were expressed as mg gallic acid equivalent (GAE) 100 g^{-1} FW. In turn, AA was determined by sequestration of ABTS (2,2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid) and DPPH (2,2-diphenyl-1-picryl-hydrazil) radicals as proposed by (Rufino et al. 2007a, b). ABTS results were expressed as µmol Trolox g^{-1} FW, and DPPH as g pulp g^{-1} DPPH to be reduced in 50% (EC50) by the radical.

2.3. Statistical analyses

Data were submitted to uni and multivariate analysis of variance with the application of Snedecor's F test and Wilks' criterion, respectively. Then, means among populations were compared by the t-test. All analyzes were performed using a nominal significance level of 5% probability.

The distance matrix between individuals was estimated by the standardized Euclidean distance ($\sim Z$, $\mu = 0$, $\sigma = 1$). The dendrogram was constructed using the UPGMA (Unweighted Pair-Group Method Analysis) hierarchical method and the co-phenetic correlation was used to verify the quality of the cluster using the PheatMap and Biotools (Silva, 2019) packages in R software. The K-mean method was used to group individuals and Pearson's correlations between traits were estimated.

All statistical analyzes were performed in R software (R CORE TEAM, 2019) using the ExpDes.pt and PheatMap packages.

3. RESULTS

A significant effect was observed among populations ($p < 0.01$; $p < 0.05$) for almost all traits evaluated, except for yellow flavonoids (FLAV) (Table 3). By considering all traits simultaneously, genotypes showed genotypic differences among them ($F_{Wilks} = 25.85$, $p < 0.001$).

Parelhas population showed the highest trait averages, although it did not differ from Assú for total extractable polyphenols (PET) and from Apodi for Vitamin C (VITC) and DPPH

(Table 3). Apodi and Assú populations did not differ for CARO, BETC, BETX, and ABTS. There were no differences among populations regarding FLAV (p> 0.05).

Table 3 Estimates of Snedecor's F, variance among and within populations, and means for eight fruits traits evaluated in three *Tacinga inamoena* populations.

Population	Mean (Trait)							
	VITC	FLAV	CARO	BETC	BETX	PET	ABTS	DPPH
Apodi	50.11a	8.91a	0.18b	1.96b	2.31b	15.83b	1.59b	7451.63a
Assú	42.51b	9.09a	0.20b	1.85b	2.27b	17.97a	1.62b	4918.07b
Parelhas	51.89a	9.19a	0.74a	3.34a	3.66a	16.95ab	1.89a	6688.35a
F	9.39^{**}	0.09^{ns}	291.57^{**}	16.64^{**}	26.08^{**}	4.09^{*}	3.73^{*}	16.21^{**}
$\hat{\sigma}^2_{pop}$	22.190	0.000	0.102	0.643	0.603	0.868	0.020	1585.004
$\hat{\sigma}^2_{d}$	26.459	2.120	0.004	0.411	0.240	2.809	0.074	1042.270
CV (%)	10.68	16.60	15.80	26.92	17.85	9.91	15.94	16.07

Means followed by the same letter in columns do not differ by the t-test (p > 0.05). F (Wilks) = 25.85**.
VITC: Vitamin C; FLAV: Yellow flavonoids; CARO: Total carotenoids; BETC: Betacyanin; BETX: Betaxanthin; PET: Total extractable polyphenols; ABTS: Total antioxidant activity by the ABTS method; DPPH: Total antioxidant activity by the DPPH method.

Variance among populations was higher for CARO, BETC, BETX, and DPPH, indicating that most of the total variation for these traits were among populations studied (Table 3). For the other traits, the variance within population was higher.

Cluster analysis by the UPGMA method from the standardized Euclidean distance arranged samples from the three populations into two groups (Fig. 1). The first group was formed by the ten individuals from Parelhas and the second group by individuals from Apodi and Assú.

Most individuals in the first group have higher values for BETX, BETC, CARO, and, although to less extent, for VITC (green and red areas), while individuals in the second group have lower estimates for these traits (blue area) (Fig. 1).

The cophenetic correlation estimate (r = 0.81**) indicated that matrices of original and final distances are similar, that there was little loss of information when individuals were hierarchized in groups, showing the reliability of the method used (UPGMA).

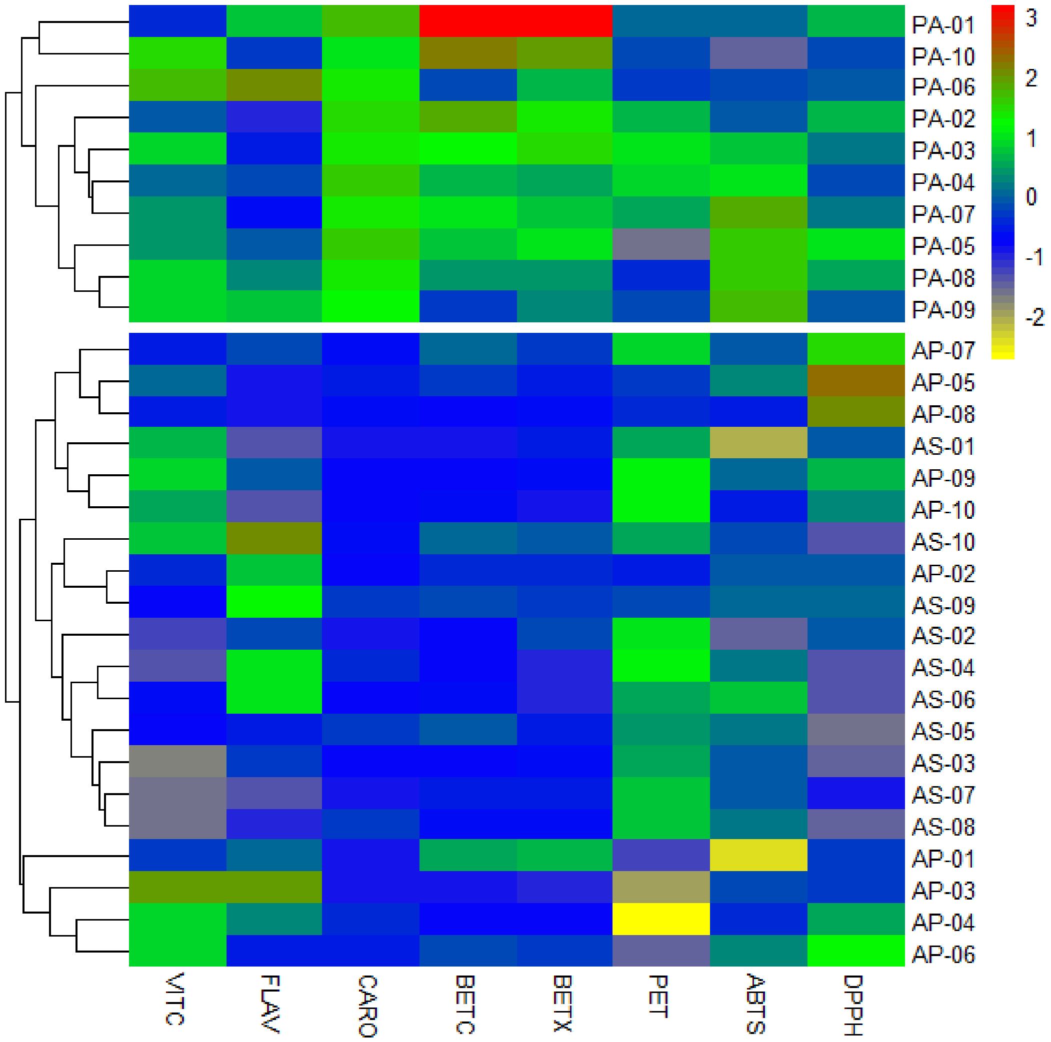


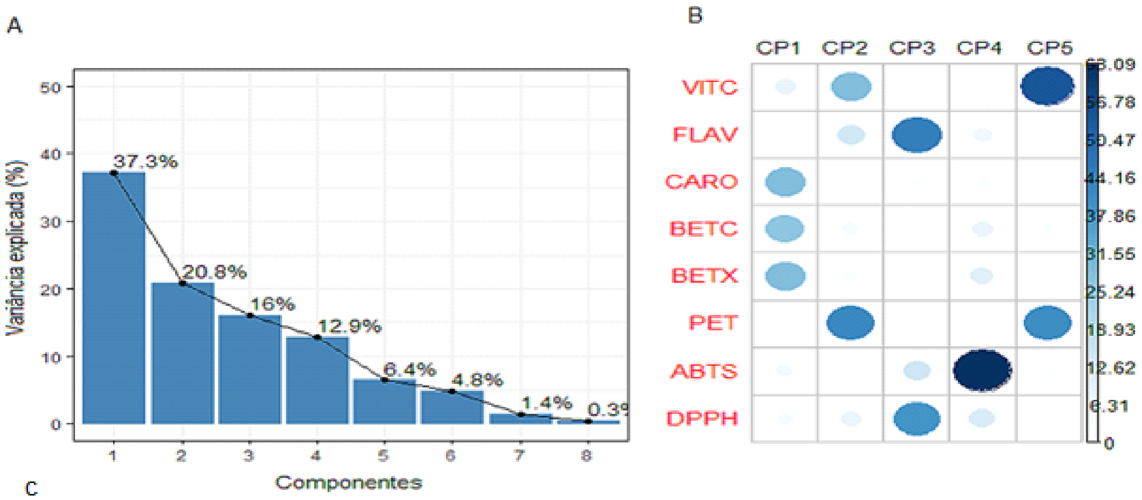
Fig. 1 Dendrogram of individuals from three *Tacinga inamoena* populations generated by the UPGMA method from the standardized Euclidean distance matrix obtained in eight fruit traits. (r = 0.81**). VITC: Vitamin C; FLAV: Yellow flavonoids; CARO: Total carotenoi ds; BETC: Betacyanin; BETX: Betaxanthin; PET: Total extractable polyphenols; ABTS: Total antioxidant activity by the ABTS method; DPPH: Total antioxidant activity by the DPPH method.

In the principal components analysis, the first two principal components (CP1 and CP2) explained, respectively, 37.3 and 20.8% of the total variation, accounting for approximately 60% total (Fig. 2A). That result indicated the method was adequate to discriminate genotypes in the groups formed.

Variables that most contributed to the first main component were CARO (28.28%), BETX (27.80%), and BETC (26.35%) (Fig. 2B). To the second main component, variables that most contributed were PET (41.52%) and VITC (27.89%) while to the third were FLAV

(43.90%) and DPPH (38.31%). ABTS (63.09%) was that most contributed to the fourth main component. In the fifth main component, VITC (53.81) and PET (40.15%) most contributed. Regarding the correlation estimates between traits, CARO positively correlated with BETC, BETX, and ABTS (Fig. 2C). The highest correlation coefficient was between BETC and BETX (0.95**). VITC and PET were negatively correlated. When correlating positively, traits grow in the same direction, because they are directly proportional. On the other hand, negative estimates indicate inversely proportional quantities.

In the PCA and K-means analyses, two groups were formed (Fig. 3A, B), confirming the UPGMA hierarchical grouping (Fig. 1). The first group consisted of individuals from Parelhas that had higher values of BETC, BETX, and CARO, variables correlated with the first main component. In the second group are the individuals associated with PET.



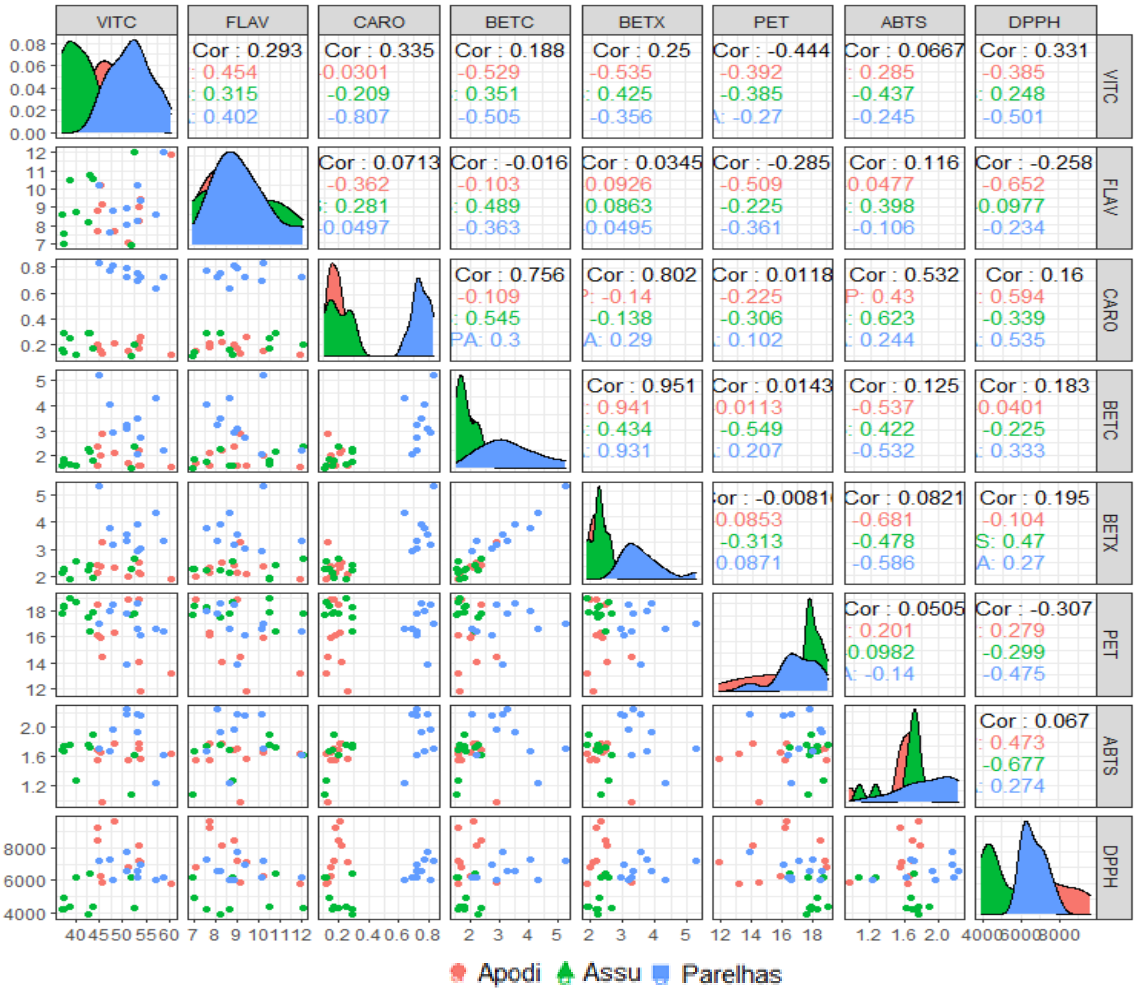


Fig. 2 Explained variance (%), contribution of variables for main components, and correlation coefficients between fruits traits evaluated in three *Tacinga inamoena* populations. VITC: Vitamin C; FLAV: Yellow flavonoids; CARO: Total carotenoids; BETC: Betacyani n; BETX: Betaxanthin; PET: Total extractable polyphenols; ABTS: Total antioxidant activity by the ABTS method; DPPH: Total antioxidant activity by the DPPH method.

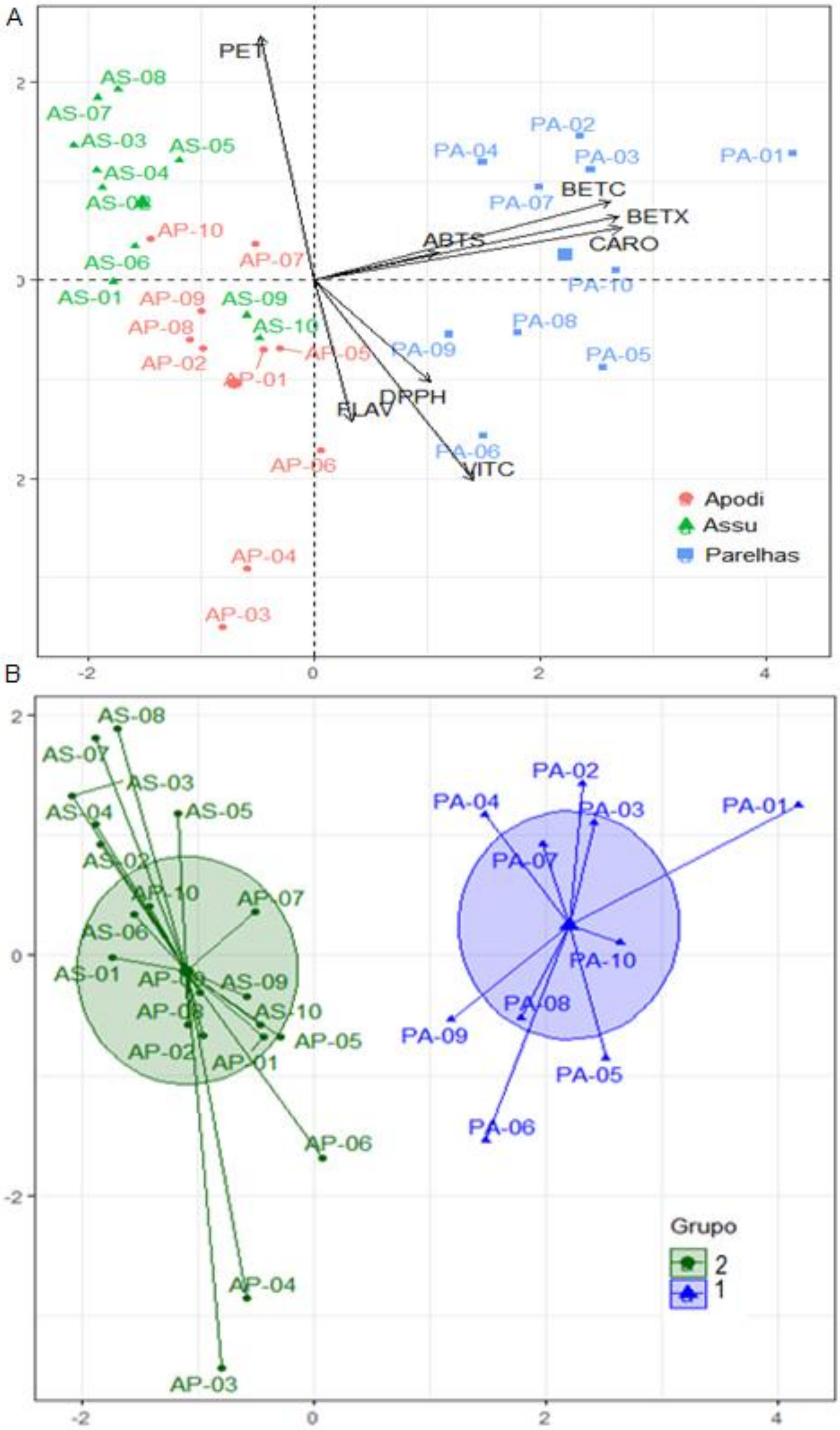


Fig. 3 Groups of individuals from three *Tacinga inamoena* populations formed by the K-Means method from eight fruit traits. VITC: Vitamin C; FLAV: Yellow flavonoids; CARO: Total carotenoids; BETC: Betacyanin; BETX: Betaxanthin; PET: Total extractable polyphenol;

ABTS: Total antioxidant activity by the ABTS method; DPPH: Total antioxidant activity by the DPPH method.

4. DISCUSSION

In this study, fruits from 30 *T. inamoena* individuals distributed in three populations located on different geographic sites in Northeastern Brazil were characterized regarding bioactive compounds content. Results revealed wide genotypic diversity in fruits traits, evidenced by differences in betalains, carotenoids, flavonoids, polyphenols, and vitamin C contents (Table 3, Fig. 1). Higher values of σ^2_{pop} for BETC, BETX, CARO, and DPPH relative to σ^2_d, indicate that most variance for these traits were among populations. On the other hand, the other bioactive compounds had higher σ^2_d, and most variantion was within population. Also, as shown in the dendrogram, fruits from plants in Parelhas had more contents of BETX, BETC, CARO, and VITC than those from Assú and Apodi.

Among individuals, vitamin C content ranged from 37.11 to 60.43 mg AsA 100 g^{-1} FW. And most variation occurred within population, which may be due differences in environmental temperature, light intensity, and air relative humidity even in the same geographic location (Rufino et al. 2009). Nonetheless, we found values higher than those found by (Dantas et al. 2015) and (Formiga et al. 2016), 42.01 and 26.85 mg AsA 100 g^{-1} FW, respectively, in other regions in Brazil. Regardless of different contents reported, vitamin C content in some *T. inamoena* fruits exceeded that of widely consumed fruits, such as citrus (16-58 mg 100 g^{-1}; Najwa and Azrina, 2017) and strawberry (42.45 mg 100 g^{-1}; Cardoso et al. 2011). Thus, *T. inamoena* fruits can contribute to dietary vitamin C to humans, and daily consumption of 100 g its fruits aid in meeting the recommendation of this vitamin (45 mg) for adults in order to prevent diseases (Brasil, 2005).

Besides vitamin C, *T. inamoena* fruits also presented considerable contents of carotenoids, flavonoids, betacyanins and betaxanthins. The contents highly varied, considering populations from where fruits were harvested. Such variation can be attributed to genetic variability, but also to the different response of the plant to environmental conditions. Irrespectively to sites locations, flavonoids ranged from 6.95 to 12.04 mg 100g^{-1}, content five times greater than that found by (Formiga et al. 2016), 1.77 mg 100 g^{-1}. In turn, carotenoids content ranged from 0.12 a 0.83 mg 100 g^{-1}, similar to Souza et al. (2007), 0.47 mg 100 g^{-1}.

Betacyanin and betaxanthin, respectively red to red-violet and yellow betalain compounds, were 2.38 and 2.75 mg 100 g^{-1} edible fraction in *T. inamoena* fruits. These values were superior to found by (Dantas et al. 2015), 0.64 and 1.79 mg 100 g^{-1}, respectively. Variation in these contents in fruits among populations may occur due to differences in environmental conditions among locations where plants grow, since betalains are sensitive to light and pH (Gandía-Herrero et al. 2010). Also, the content can be affected by fruit ripening stage (Dantas et al. 2016) and due to genetic variation (Stintzing et al. 2005). Such betalain compounds can contribute to human health since studies have shown diets rich in these substances are not only non-toxic but proved to be a promising alternative to supplement therapies in oxidative stress-, inflammation-, and dyslipidemia-related diseases in humans, such as stenosis of the arteries, atherosclerosis, hypertension, and cancer (Rahimi et al. 2019). Moreover, betalains have potential use as a food colorant, which may allow the development of new food products without using artificial colorants (Stintzing et al. 2005; Fernández-López et al. 2018). Furthermore, in the present study, *T. inamoena* fruits showed equivalent or even higher betalain contents than fruits of other cacti, such as *Opuntia* species, which varied from 0.50 to 20.95 mg 100 g^{-1} for betacyanin and 1.08 to 11.49 mg 100 g^{-1} for betaxanthin among varieties (Aparicio-Fernández et al. 2017).

Total extractable polyphenols (PET) ranged from 11.83 to 19.0 mg GAE 100 g^{-1} FW, lower than those found by (Formiga et al. 2016) in plants from another region in Brazil. Nevertheless, PET content in *T. inamoena* fruits is higher than in other more consumed fruits, such as apple (15.63; Petkovska et al. 2016), litchi (1.60; Su et al. 2019), and peach (2.68; Liu et al. 2019). Therefore, given the importance of polyphenols for human health, studies must focus in search of non-extractable polyphenols in *T. inamoena* fruits, which is the fraction not extracted during the aqueous-organic treatments commonly performed to evaluate polyphenol content in foods. This fraction includes low molecular weight polyphenols associated with macromolecules (proteins, dietary fiber), thus acting as macromolecular antioxidants. Non-extractable fraction were found in a higher amount than PET in a range of fruit and vegetable species (Pérez-Jiménez and Saura-Calixto 2015, 2018).

PCA showed (Fig. 2B) that the bioactive compounds that most contributed to genetic diversity among individuals were CARO, BETC, and BETX, followed by PET and FLAV. These traits also contributed to arranging groups that separated individuals from Parelhas to individuals from Apodi and Assú (Fig. 3A-B). Thus, individuals from Parelhas (first group) distinguished from Apodi and Assú by higher BETC, BETX, CARO and lower PET contents (Fig. 1 and Fig. 3). Although in the same group, individuals from Assú had higher PET while individuals from Apodi had higher FLAV and DPPH. These results are consistent with most genetic variability among than within populations, as shown in **Erro! Fonte de referência não**

encontrada.. Moreover, as showm by K-Mean grouping method (**Erro! Fonte de referência não encontrada.**), populations from Assú and Apodi were more genetically similar and distinct from Parelhas. Geographically separated populations may share common genetic structures as a result of having common ancestors. Moreover, *T. inamoena* plants are pollinated by hummingbirds and ants are seed dispersors (Leal et al. 2018). Such plant-animal interactions in Caatinga may contribute to disperse the species over the region.

T. inamoena is still little-known species from Caatinga but its fruits show to be as potential alternatives to meet demands of domestic and foreign markets for new flavors, colors, textures, and bioactive compounds content capable of preventing degenerative diseases.

5. CONCLUSION

Fruits from *T. inamoena* are rich in bioactive compounds content such as betalains, flavonoids, polyphenols, and vitamin C contents.

Carotenoids, betacyanin, betaxantin and antixiodant activity by the DPPH method most vary among populations, while vitamin C, flavonoids, polyphenols and ABTS most vary within populations.

Fruits from plants in Parelhas show higher carotenoids, betacyanins and betaxanhthins contents than fruits from Assú and Apodi. Assú and Apodi present more genetic similarity, differing from Parelhas.

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