

## Genetic diversity in *Campomanesia* (MYRTACEAE) estimated by multivariate analysis of the phenotypic characteristics

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### ABSTRACT

The *Campomanesia* sp. is a native fruit in the *strict sense* Cerrado. Although being traditionally used for either *in natura* consumption and the preparation of ice-creams, sweets and liqueurs, it is insufficiently explored under the economical viewpoint. Twenty one genotypes from a population representing the *Campomanesia* genus were studied in the *strict sense* Cerrado at Patrocínio county, State of Minas Gerais- Brazil. The objective of this study was to evaluate either the phenotypic diversity estimated by multivariate analysis and the morphological characteristics that are promising for improvement programs and germoplasm conservation collections of this shrub fruit. So, two species were identified in the area under study: *C. adamantium* and *C. pubescens*. The *C. pubescens* individuals showed more variations than the *C. adamantium*. In this case, the *C. pubescens* species can provide more possibilities to be explored in the genetic breeding program. Although their few variations, the *C. adamantium* individuals also showed interesting characteristics to be evaluated.

**Key words:** *Campomanesia adamantium*, *Campomanesia pubescens*, Gabiroba, Native fruit, Cerrado

### RESUMO

## Diversidade genética em *Campomanesia* (MYRTACEA) estimada por análise multivariada de características fenotípicas

A gabioba (*Campomanesia* sp.) é uma fruta nativa do Cerrado, utilizada tradicionalmente tanto no consumo *in natura* quanto para fabricação de sorvetes, doces e licores, mas ainda pouco explorada economicamente. Vinte e um genótipos de uma população representante do gênero *Campomanesia* foram estudados em uma área de Cerrado sentido restrito, no município de Patrocínio, Minas Gerais, Brasil. Objetivou-se avaliar a diversidade fenotípica estimada por análise multivariada, bem como as características morfológicas promissoras para programas de melhoramento e coleções de conservação de germoplasma dessa fruteira. Duas espécies foram identificadas na área de estudo: *C. adamantium* e *C. pubescens*. Os indivíduos de *C. pubescens* apresentaram mais variações que os de *C. adamantium*. Nesse caso, a espécie *C. pubescens* pode oferecer mais possibilidades de ser exploradas em um programa de melhoramento genético. Apesar de poucas variações, os indivíduos de *C. adamantium* também mostraram interessantes características a serem exploradas.

**Palavras chave:** *Campomanesia adamantium*, *Campomanesia pubescens*, Gabiroba, Fruta Nativa, Cerrado.

Recebido para publicação em setembro de 2007 e aprovado em janeiro de 2009

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## INTRODUCTION

The genus *Campomanesia* Ruiz & Pav. (Myrtaceae) is a group of trees and shrubs member of the tribe Myrteae D.C., the fleshy fruited Myrtaceae (Landrum, 1982). There are 29 species in the *Campomanesia* genus distributed throughout neotropics from northeastern Argentina to the Trinidad island and from eastern coast of Brazil to the Peruvian Andes, Ecuador and Colombia (Rotman 1976; Landrum, 1986, 1987, 2001; Kawazaki, 2000).

According to Landrum (1986) only the species *Campomanesia speciosa* Landrum, but is found in the Peruvian Andes. Most are found on northeastern and southeastern Brazil (Arantes & Monteiro, 2002). The Brazilian Cerrado presents a great variety of this genus that is popularly known as Gabiroba, Guaviroba, Guabiroba or Guavira. It is widely distributed in the states of Bahia, Distrito Federal, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, São Paulo, Tocantins, Espírito Santo, Paraná and Rio Grande do Sul (Almeida *et al.*, 1998).

The *Campomanesia* planting has been recommended for spoiled-area reforestation projects (Hardt *et al.*, 2006), and the species is observed in the floristic composition of natural reclamation (Caldato *et al.*, 1996). At least one species (*Campomanesia xanthocarpa*) shows antiulcerogenic effects already confirmed (Markman *et al.*, 2004). *Campomanesia* individuals range from trees about 15 m high to bushes shorter than 1 m (Landrum, 1986). Studies concerning to the reproductive biology of *C. pubescens* and *C. velutina* point out those species to be selfcompatible, although the fruit establishment mainly occurs for crossed pollination. The flowers exhale sweetened perfume and are always visited by bees of both Apidae and Halictidae families, by distinguishing the *Apis mellifera* and *Melipona quadrifasciata*, therefore indicating the melliferous potential of the gender (Proença & Gibbs 1994; Torezan & Del-Claro, 1998; Andena *et al.*, 2005). The fruit are usually yellowish green, yellow or orange in color. Populations from North and South America have been reported as having black fruit (Landrum, 1986).

The *Campomanesia* fruits are agriculturally important because they are wild hosts of the fruit flies (Thum & Costa, 1999), and they are distinguished for their feeding use. They can be consumed *in natura* or used to produce juices, liqueur, ice cream and jam (Almeida, 1998), but they have been used in an extractive and often predatory manner that shows the need to include these fruit trees in genetic breeding programs that can serve as a base for rational cropping.

To start a program of genetic breeding of any plant species it is necessary to join the available genetic variability. Ferreira (2002) presents the Genebank network for tropical and subtropical fruits by the Brazilian

Agriculture Research Corporation (EMBRAPA) that gather collections of the Brazilian native species, inclusive the *Campomanesia* gender. According to Paiva and Valois (2001), when the species is wild genetic material has to be collected in the region of natural occurrence of the species. Those collects can contribute for the enrichment of information about the existent genebanks besides turning possible the formation of new genebanks.

In comparative *stricto sensu* studies of the savannah vegetation in central Brazil, the Patrocínio and Paracatu regions (MG) are distinguished for their natural biodiversity; however, they are subjected to strong anthropic action due to the wide areas cropped with coffee and soybean mainly, therefore suggesting the need for the establishment of conservation units in this region (Felfili *et al.*, 1998; Felfili & Felfili, 2001).

The objective of this study was to assess the genetic diversity in *Campomanesia*, estimated by multivariate analysis of phenotypic characteristics, in an area of *strict sense* Cerrado in Patrocínio/MG, as well as to evaluate the morphological characteristics that are promising to the improvement programs and conservative collections of this fruit shrub.

## MATERIAL AND METHODS

The area under study is an altered *strict sense* Cerrado in Patrocínio county- MG, Brazil, localized between the coordinates (18°57'04.94"S/46°59'01.64"W and 18°57'21.33"S/46°58'22.64"W). The area was chosen because it presents variability of the *Campomanesia* phenotypic characteristics, little human disturbance, easy access and the possibility of routine observation. The plants were marked and identified with numbered tags. The data collection visits to the field occurred monthly between May/2004 and September/2004, weekly between October/2004 and December/2004, daily at the peak of flowering and fruiting and monthly between January/2005 and May/2005. The phenotypic characteristics were recorded to represent the variability of the genotypes.

Forty characteristics were observed by assessing plant and reproductive structures. For the statistical analyses, twenty seven characteristics were evaluated as quantitative variables and thirteen as multicategorical ones (Table 1). Field visualization was performed with the naked eye and the leaf and fruit pilosity were visualized using a stereomicroscope. To obtaining the measures of the leaves, flower buds, open flowers, fruits and seeds, the calculation of the arithmetical averages of eight leaves, three buds, five open flowers, 12 ripen fruits and 10 seeds in each plant were accomplished. The data collected were used for the multivariate analysis using the GENES program version 2006.4.1 (Cruz, 2006).

**Table 1.** Listing the 40 phenotypic characteristics under evaluation, as being 27 quantitative variables and 13 multicategorical ones

Quantitative variables		Multicategorical variables	
1	Height of the largest plant branch	28	Leaf color
2	Diameter of this highest branch at 10 cm from the soil	29	Sprouting color
3	Brush area	30	Color of the main leaf nervures
4	Average leaf size	31	Color of the flower bud calyx
5	Leaf pilosity in the adult stage	32	Color of the flower bud corolla
6	Leaf waxiness	33	Color of the stigma base in the flower bud
7	Sprouting season	34	Color of the flower stigma base
8	Bracteam size	35	Color of the stigma tip in the flower bud
9	Flower peduncle size	36	Flower peduncle color
10	Floral calyx size	37	Flower calyx color
11	Flower stigma size	38	Flower corolla color
12	Flower corolla size	39	Color of the flower stigma tip
13	Largest stamen size	40	Fruit skin color
14	Smallest stamen size		
15	Flower bud appearance season		
16	Flower appearance season		
17	Ovary size		
18	Flowering pattern		
19	Average fruit size (larger diameter)		
20	Average fruit weight		
21	Average weight of the fruit skins		
22	Average weight of the seeds		
23	Average number of viable seeds		
24	Average seed size		
25	Fruit skin pilosity		
26	Fruit ripening season		
27	Germination time		

To estimate the dissimilarity, by taking into account the quantitative variables together, the standardized average Euclidian distance was used, where: considering  $Y_{ij}$  as the observation in the  $i$ -eth genotype ( $i = 1, 2, \dots, n$ ) for the  $j$ -eth characteristic ( $j = 1, 2, \dots, n$ ), the average Euclidian distance is defined between the genotype pair  $i$  and  $i'$ , by using the expression:

$$d_{ii'} = \sqrt{\frac{1}{v} \sum_j (Y_{ij} - Y_{i'j})^2}, \text{ where } v \text{ is the number of the}$$

characteristics under study and the standardization of the

values by:  $y_j = \frac{Y_j}{\bar{\sigma}_j}$ , where  $\bar{\sigma}_j$  is the standard deviation associated to the  $j$ -eth characteristic. Considering the multicategorical variable group, the dissimilarity was estimated by the arithmetic complement of the similarity

index given by:  $d_{ii'} = 1 - S_{ii'}$ , as being,  $S_{ii'} = \frac{C}{C+D}$

where C: category agreement and D: category disagreement (Cruz, 2006). The generated matrices were all standardized and added, whereas the response matrix was used for composing the groupings. To form the clusters the Tocher optimization method, the UPGMA (Unweighted Pair-Group Method using Arithmetic Averages) clustering method and

graphic dispersion analysis in three dimensional space were used (Cruz *et al.*, 2004).

## RESULTS AND DISCUSSION

The final sample for biometric analyses consisted of 21 genotypes. Two species were identified in the location based on Landrum (1986) and Arantes & Monteiro (2002): *Campomanesia pubescens* (A.P. de Candolle) Berg and *Campomanesia adamantium* (Cambessèdes) Berg. Four of the 21 genotypes were identified as *C. adamantium* (genotypes 2, 8, 9 and 21) and the others were *C. pubescens*. Concerning to pilosity of the leaves and fruits, shrub sizes, weight and coloration of the fruits, the individuals of the *C. pubescens* species showed more variations than those of *C. adamantium*.

In this study the *C. pubescens* representatives were bushes that ranged from 98 cm to 1.60 m in height, and could reach up to 3.54m<sup>2</sup> in canopy area. The flowers were white, with a light cream tone when adults, or even with pinkish spots, ranging from 1.5 cm to 4.1 cm in diameter. The leaves and fruits presented some degree of pilosity. The flowerings were always significant and very intense on some plants, covering all the plant structure with flowers, as for example genotype 19. The fruits ranged

from 0.9 cm to 3.8 cm in diameter, and were greenish, yellow or greenish yellow in color and pilosity varied from very little to very intense.

The *C. adamantium* representatives were bushes 36 cm to 92 cm in height, 61 cm average height and 2.0m<sup>2</sup> mean canopy area.

The leaves were glabrous, grayish green, with reddish sprouting and some red tones in the reproductive structures and the flowers were white with light red marks or spots and ranged from 1.4 cm to 3.4 cm in diameter. The reddish yellow fruits ranged from 2.4 cm to 3.23 cm in diameter. Table 2 shows the average and standard deviation for the main agronomically interesting characteristics.

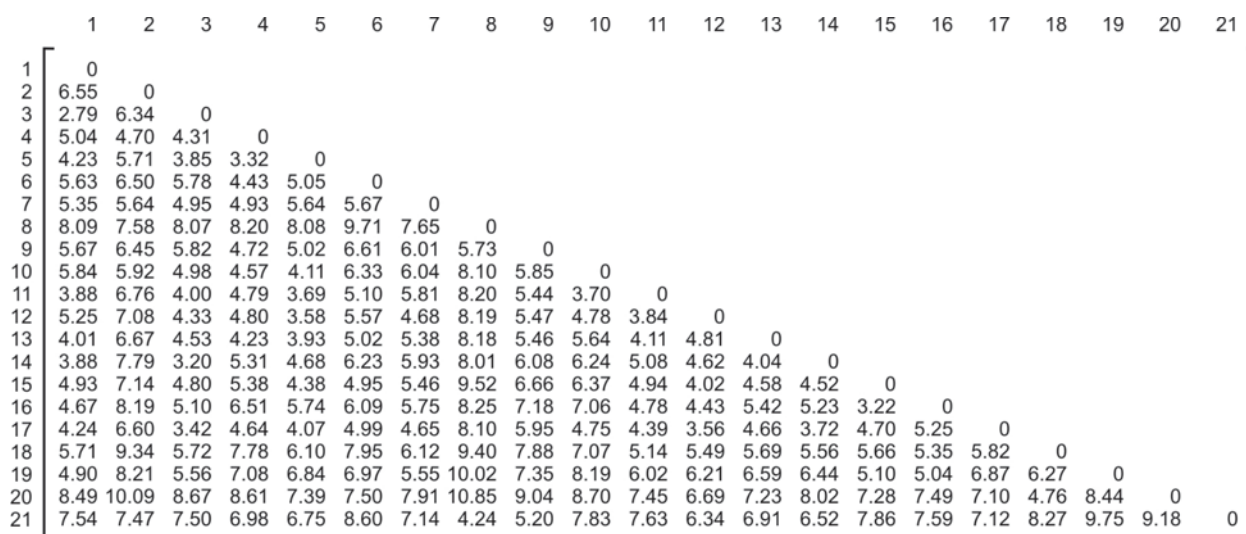
### Distance matrix

The genetic distance matrix generated for 21 genotypes based on 40 phenotypic characteristics (Figure 1) presented genotypes 1 and 3 as least divergent and as most dissimilar genotypes 8 and 20. Genotypes 1 and 3 presented plant and reproductive similarities. They are

bushes of similar size, with the same budding, flowering, fruiting and fruit ripening seasons, with flowers and fruits of the same color, varying in fruit size and mean weight (1-1,82cm e 1,45 g. / 3-2,88cm e 2.96 g.). The germination time was also different: genotype 1 took 22 days to germinate while genotype 3 germinated 14 days after sowing. The most dissimilar genotype 8 identified as *C. adamantium* did not present pilosity on any plant or reproductive structure. The leaves were gray green and glabrous, the flowers had reddish petals in the flower bud that deepened in color as the flower aged and the glabrous fruit was reddish-yellow in color. Genotype 20, identified as *C. pubescens*, presented abundant pilosity that gave an opaque appearance to the leaves. When young they were intensely pilose, with white hairs that remained on the mature leaf with a more yellowish coloring. The flowers were large compared to the other genotypes, white in color both in the bud and in the open flower, and had a long bract. The same characteristics were observed in genotype 18. The fruit were yellow in color, with an intensely pilose skin. These genotypes (18 and 20) are

**Table 2.** Average and standard deviation of the main quantitative variables. The values of the leaves, fruits and seeds refer to the arithmetic average of eight leaves, 12 ripe fruits and 10 seeds from each plant

Quantitative variables	Average	Standard Deviation
Height of the highest plant branch	89.14 cm	30.72
Diameter of this highest branch at 10 cm from the soil	1.26 cm	0.65
Brush area	1.46 m <sup>2</sup>	2.11
Average leaf size	20.93 cm <sup>2</sup>	6.35
Average fruit size (larger diameter)	2.52 cm	0.76
Average fruit weight	2.07 gr	0.97
Average weight of the fruit skins	0.49 gr	0.10
Average weight of the seeds	0.06 gr	0.03
Average number of viable seeds	2.26	1.00
Average seed size	0.26 cm <sup>2</sup>	0.10
Germination time	16 days	6.26



**Figure 1.** Genetic distance matrix generated for 21 genotypes based on 40 phenotypic characteristics expressed by the standardized average Euclidian distance and arithmetic complement of the similarity index.

known in the region by the popular names of “Gabirola Peluda” or “Peludinha” (“Little Hairy”).

### Clustering analyses

The Tocher clustering based on the genetic distance matrix identified 10 groups (Table 3). Group A included genotypes of the *C. pubescens* species, Group B consisted of genotypes of the *C. adamantium* species and Group C was formed by *C. pubescens* genotypes that differed from the other genotypes of the species because of their intense pilosity on plant and reproductive structures. The others groups isolated genotypes with distinct characteristics that could have contributed to increasing the mean distances of these groups, separating them from

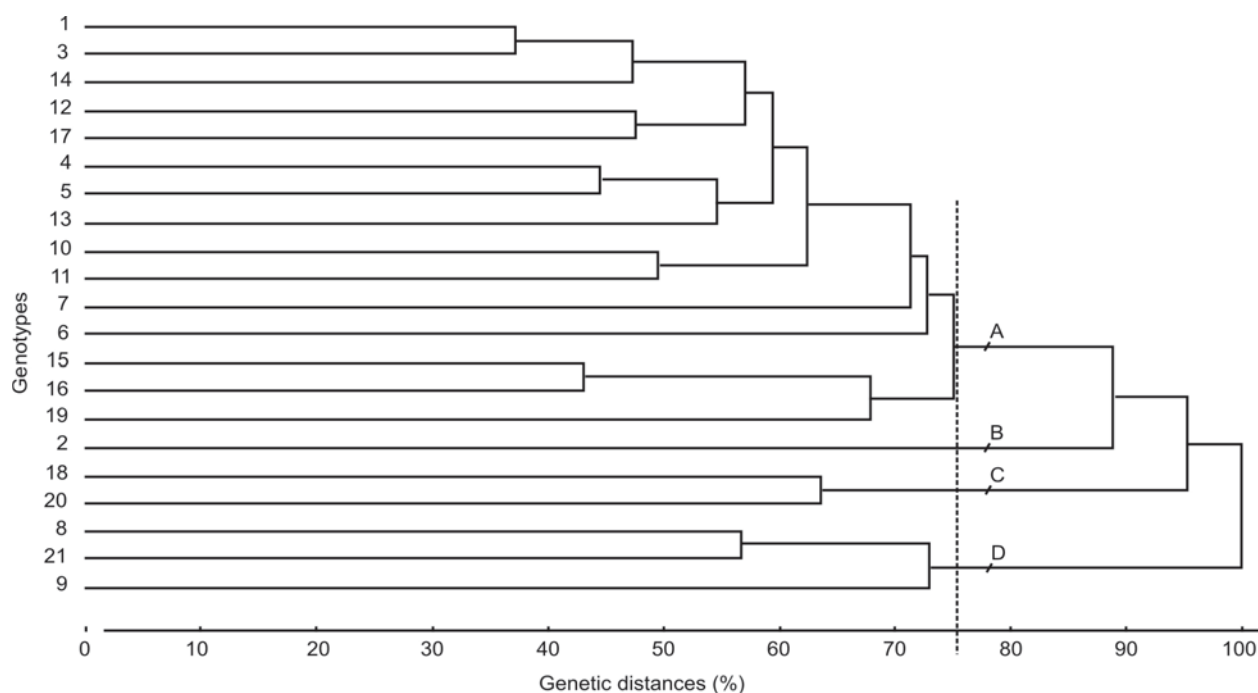
**Table 3.** Groups of genotypes established by the Tocher clustering method among 21 genotypes from the genetic distances expressed by the standardized average Euclidian distance and arithmetic complement of the similarity index.

Group	Genotypes
A	1 3 14 17 5 11 12 13 4 15
B	8 21
C	18 20
D	16
E	7
F	19
G	6
H	10
I	9
J	2

the others. Genotype 10 stood out because it presented the largest fruit (means of 3.8 cm and 4.13 g.). Genotype 19 presented the smallest fruit (means 0.9 cm and 0.53 g) the general mean of the fruits was 2.5 to cm in size and 2.07 g weight. In spite of the small fruits, the yield was superior to the other genotypes’ under observation, since they almost occupied the whole extension of the branches. Genotype 2 presented intermediate characteristics between the *C. adamantium* and *C. pubescens* species. The main outstanding characteristic of genotype 9 was intense reddish coloring on plant and reproductive structures.

The dendrogram obtained from the clustering analysis based on the genetic distances using the UPGMA method (Figure 2) at the level of 75% similarity defined four groups: group “A” consisting of the *C. pubescens* genotypes, group “B” with genotype 2, group “C” with genotypes 18 and 20 reinforced the distancing of these genotypes from the other *C. pubescens* species, probably due to very intense pilosity on plant and reproductive structures, and group “D” of the *C. adamantium* genotypes.

The position of the genotype 2 on the dendrogram represented coherently its set of characteristics compared to the others. When compared, *C. adamantium* individuals were smaller bushes that produced few flowers, few fruits and had reddish coloring on different parts of the plants, while *C. pubescens* individuals were larger bushes, with many flowers and fruits, and were yellowish green in color on reproductive and plant structures. Genotype 2 (*C. adamantium*) presented characteristics common to its species regarding the reddish coloring of the flowers and



**Figure 2.** Dendrogram obtained by the UPGMA (Unweighted Pair-Group Method using Arithmetic Averages) among 21 genotypes from the genetic distances expressed by the standardized average Euclidian distance and arithmetic complement of the similarity index. A – D : groups defined at the level of 75% similarity.

fruits, glabrous, grayish leaves and glabrous fruit. However, it showed *C. pubescens* characteristics, such as larger bush stand, much flowering and fruiting that were not observed in any other *C. adamantium* species.

The genetic distances between *C. pubescens* representatives were greater than the distances observed among *C. adamantium* representatives and as more *C. pubescens* individuals were assessed the variation within this group was better represented. The small flower and fruit production of the species during the year of study made assessment difficult of a greater number of *C. adamantium* individuals.

The dispersion graph at three planes (Figure 3) suggests the separation of three main groups, so in agreement with those shown by both UPGMA and Tocher methods. Cruz and Carneiro (2004) reported that the results expressed by graphic dispersion analysis in three dimensional space could be considered satisfactory when the distortion and stress coefficients were less than 20%. Although stress and distortion overcame 20%, the correlation between the original matrix and the data plotted on the graph was high (92.30%) and the results correspond to the groups formed by the other grouping techniques

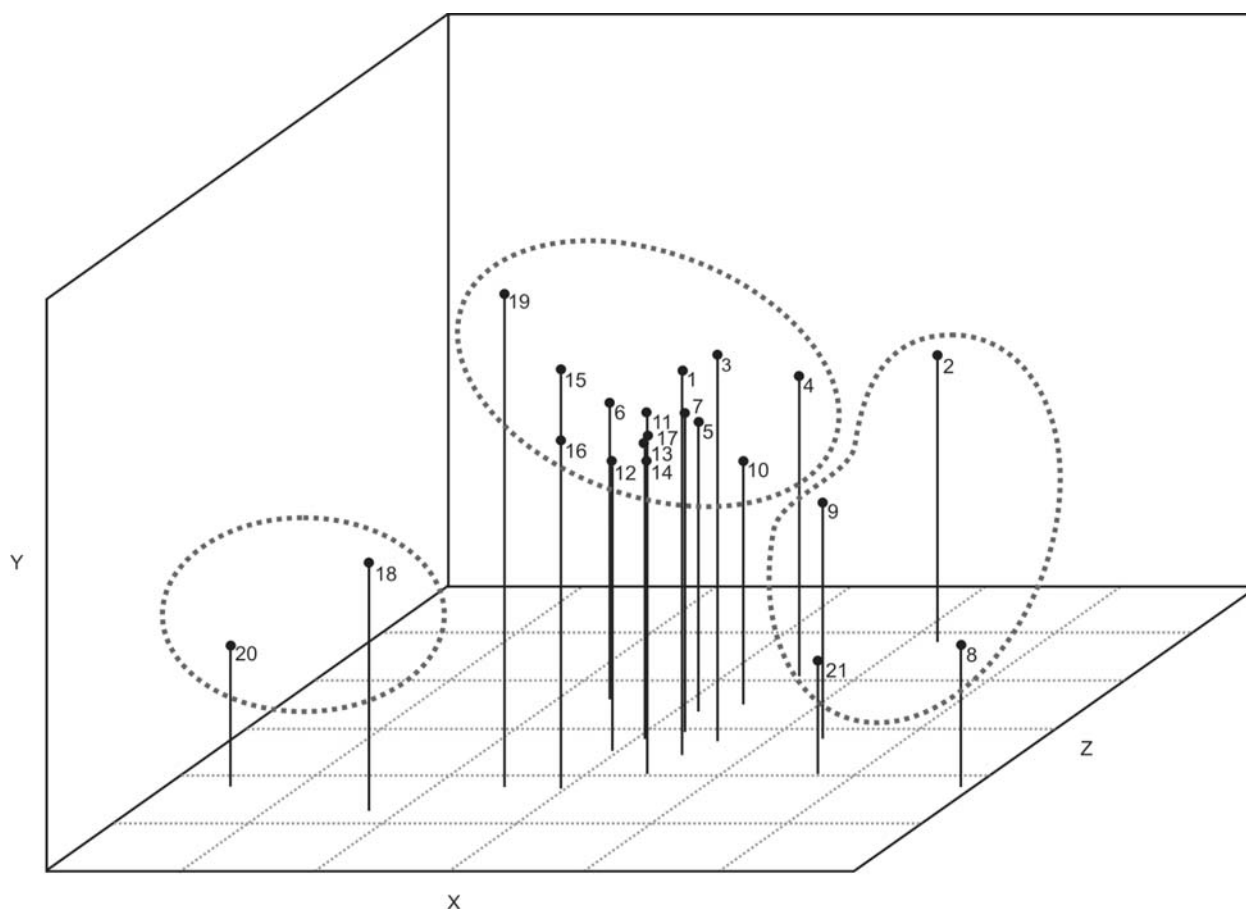
already discussed. Collects for the formation of germplasm collections should include those genotypes representing the three main groups suggested by the clustering methods, by sampling the differences within and among the groups.

### Genetic Breeding Programs

Certain characteristics are of greater economic interest for Genetic breeding programs. Larger fruits, with more attractive coloring, more pulp and more resistance, would be fruits with greater commercial advantages. Skin color, average size and fruit pilosity were important characteristics for the genetic distances. These characteristics should be observed for breeding programs seeking hybrid vigor that can associate these characteristics for commercial interests.

Characteristics recommended for the *Campomanesia* genetic breeding program include large fruit with thin skins, resistance and reddish yellow color, with a large quantity of pulp, on an individual with high yield.

Genotypes in this study were promising for *Campomanesia* genetic breeding programs: genotype 2 because of the glabrous fruit, genotype 9 because of its



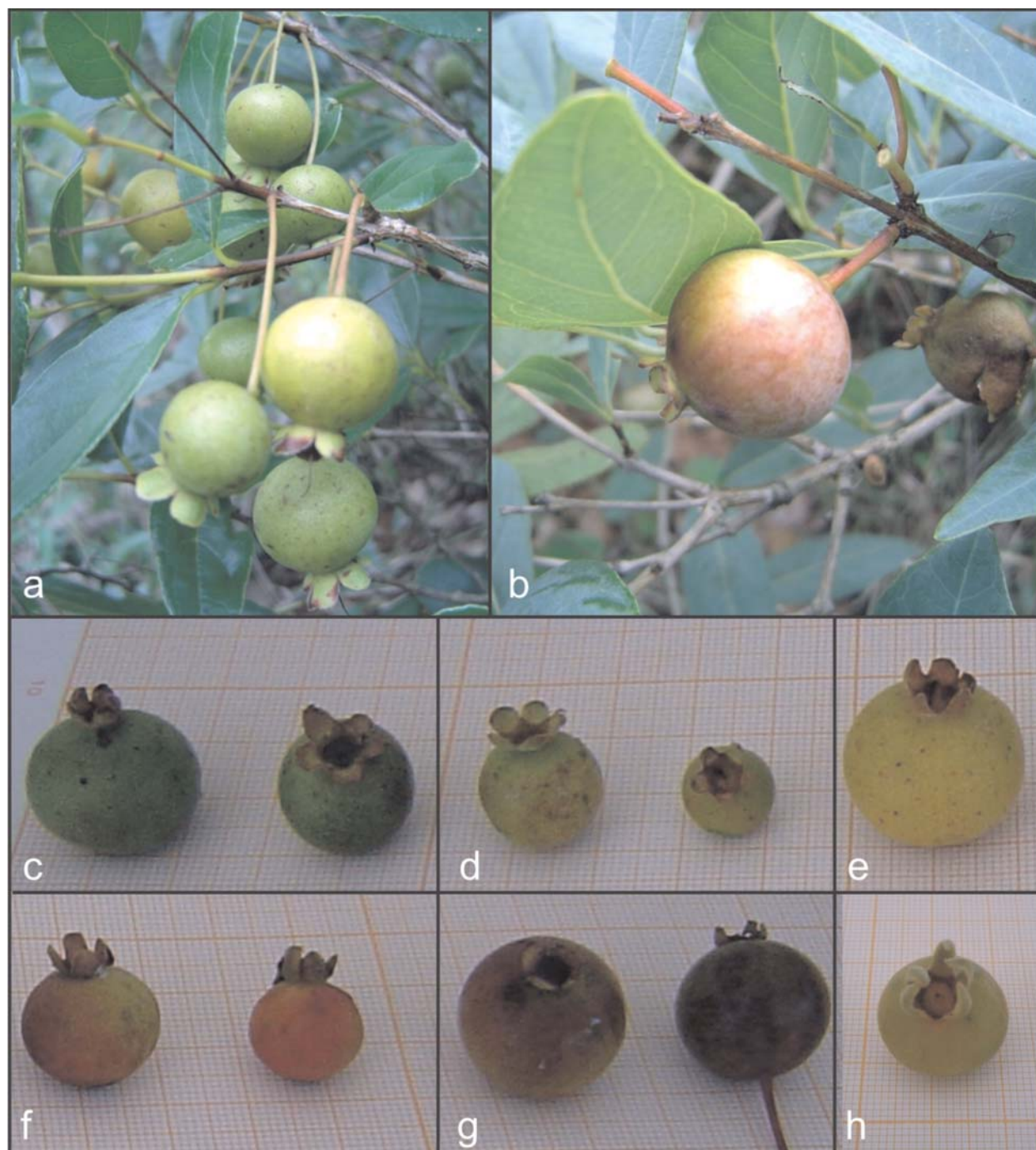
Correlation: 0.923 - Distortion(%): 31.765 - Stress(%): 34.676

**Figure 3.** Projection of the dissimilarity among 21 genotypes from the genetic distances expressed by the standardized average Euclidian distance and arithmetic complement of the similarity index in the three-dimensional space.

intense red coloring, genotype 2, 10 and 17 because of the fruit size, genotype 18 because of pilosity of the fruit, genotype 19 because of the intense fruiting (Figure 4).

Preference for pilose or glabrous fruit in a breeding program should be tested. References were not found for the genus in question. Penna (1999) studied cotton breeding and the relationship of some insect resistance traits and assessed that the presence of pilosity had negative effect for boll weevil and *Empoasca* spp because

it functioned as a mechanical barrier and antibiosis, and positive effect for *Heliothis* spp because it facilitated oviposition. Glabrous cotton plants presented negative effect for apple and pink caterpillars because they hindered oviposition and had positive effect for *Tripés* and *Epoasca* spp. A preliminary observation showed that, in the study location, *C. pubescens* plants were more attacked by some type of rust especially in the drought, from March to July, before leaf fall prior to



**Figure 4.** Size diversity and coloration of the *Campomanesia* sp. fruits under evaluation and the promising genotypes for *Campomanesia* genetic breeding programs. A – The *Campomanesia pubescens* fruits; B – The *Campomanesia adamantium* fruits; C – genotype 17; D - genotype 19; E - genotype 10; F - genotype 9; G – genotype 2; H – genotype 18.

sprouting, while the *C. adamantium* plants remained green in the same period until the leaves also began to fall. Specific tests for resistance or susceptibility to diseases linked to pilosity and an analysis of the physical and chemical characteristics of the fruit such as acidity, sugar index and macro and micronutrients would be useful in breeding programs of this fruit tree.

## CONCLUSION

This study showed that a small area of natural Cerrado still has significant genetic diversity of species of the genus *Campomanesia* (Gabiroba) a native fruit much appreciated in its area of natural occurrence. The fruits evaluated in this study show characteristics of interest for genetic breeding programs aiming to the commercial use of this native fruit. We provide relevant information to the conservation of germplasm banks that have genotypes of *Campomanesia* in their collections.

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