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Genetic Divergence among Camu-Camu Plant Populations Based on the Initial Characteristics of the Plants

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Abstract

The objective in the present work was to evaluate the genetic diversity among 15 indigenous populations of camu-camu plants, identifying important characteristics in the evaluation of genetic divergence, based on the initial characteristics of the seedlings. Seeds extracted from fruits deriving from fifteen indigenous populations of camu-camu were collected. The experimental design was entirely random, with fifteen treatments (populations), and fifteen repetitions (each sub-sample), considering 30 seeds per subsample as an experimental unit. At 40 days after sowing the following were evaluated: the percentage of emergence, the index of emergence velocity, the average time of germination, the height of the seedling and the number of leaves. The data obtained was submitted to variance analysis, and the averages were grouped by the Scott and Knott (1974) test. The genetic diversity was studied according to the Tocher grouping method, based on the Mahalanobis distance (D^2_{ii}) and canonical variables. The fifteen populations are divergent among themselves and the Rio Branco Estirão do Veado, Rio Branco Onofre and Igarapé Agua Boa populations are indicated to have hybridization with other populations due to the high divergence, as well as the rates of emergence and vigor of the seedlings. The height of the seedlings, percentage and speed of emergence, are those that most indicate genetic divergence. The measuring techniques of genetic divergence, canonical variables Mahalanobis distances are useful and corroborating in the evaluation of genetic divergence of the camu-camu plant.

Keywords: Amazonia, canonical variables, genetic variability, multivariated analysis, *Myrciaria dubia*

1. Introduction

The camu-camu (*Myrciaria dubia* (kunth) McVaugh), is an Amazonian fruit species of the Mirtaceae family, that stands out for its elevated level of vitamin C, which can reach from 3 to 8 g per 100 g of pulp, exceeding values presented by the majority of plants cultivated in Brazil (Bardales et al., 2014; Chagas et al., 2015), in addition to containing diverse antioxidant and nutritional composites (Zanata & Mercadante, 2007; Chirinos et al., 2010; Akter, Oh, Eun, & Ahmed, 2011; Imán, Pinedo, & Melchor, 2011).

In Amazonia, the potential for camu-camu is in its use in the preparation of foods like juices, sweets and ferments (Rodrigues et al., 2004; Teixeira et al., 2004; Chirinos et al., 2010; Akter, Oh, Eun, & Ahmed, 2011). It also constitutes a raw material for the cosmetic, chemical, pharmacological industries, food preservation and production of aerated beverages (Correa, 2000; Yuyama, 2011). Thus, the production and the utilization of the fruit appear to be viable alternatives in regional development, as a means of aggregating value from the natural resources available in the region (Welter et al., 2011; Chagas et al., 2015).

The principal function of genetic improvement of the camu-camu is selecting the genotypes which maximize yield from the first phases of their development (Pinedo, Linares, Mendoza, & Anguiz, 2004; Yuyama & Valente, 2011). Genetic divergence is one of the most important parameters evaluated by improvers of the plant in the initial phase of a genetic improvement programme, because, adequately explored, it may accelerate the genetic progress of particular characteristics (Negreiros et al., 2008). In relation to the germination and emergence of

seeds of the camu-camu plant, the beginning and the end occur in an irregular fashion, with this period possibly being from 15 to 120 days, making it difficult to form and produce shoots, as they are not uniform (Pinedo, Linares, Mendoza, & Anguiz, 2004; Bardales et al., 2014).

The genetic diversity may be investigated precociously by the physiological quality of the seeds, utilizing vigour tests (Dias & Marcos Filho, 1995, Pinedo, Linares, Mendoza, & Anguiz, 2004). This way, the average time for germination, emergence and uniformity can facilitate the production of shoots on the commercial scale in a more efficient manner (Negreiros et al., 2008).

In the prediction of genetic divergence, various multivariated methods can be applied such as analysis of principle components, canonical variables and the agglomerated methods. The choice of the most appropriate method should be done in relation to the level of precision desired, the ease of analysis and the form in which the data was obtained (Cruz, Carvalho, & Vencovsky, 2004). These multivariated techniques to estimate the genetic divergence among populations have been utilized in various works and with diverse species, such as the coconut (Ribeiro, Soares, & Ramalho, 1999), açaí plant (Oliveira, Ferreira, & Santos, 2007), passion fruit plant (Negreiros et al., 2008), castanheira-do-gurgueia (Ribeiro, Souza, & Lopes, 2012) and pupunha plant (Negreiros, Bergo, Miqueloni, & Lunz, 2013).

In this context, the objective of the present work was to evaluate the genetic diversity among populations of the camu-camu plant, separating the more important characteristics in genetic divergence, based on the initial characteristics of the plants.

2. Methods

The work was done at the Fruticulture Sector of Embrapa Roraima with seeds extracted from fruits derived from fifteen indigenous populations of the camu-camu (*M. dubia*) of the State of Roraima (Figure 1).

At the moment of collection, the fruits presented stage seven (green redish) and eight (red-wine coloured) maturation, according to Inga et al. (2001). In each collection location, the average size of the samples (n) was 15, and for each subsample, 60 fruits were collected, conditioned in polypropylene sacks, maintained in expanded polystyrene boxes, with ice, and carried to the laboratory. Each population received a code with the abbreviation of the name of the river or creek of origin (Table 1).

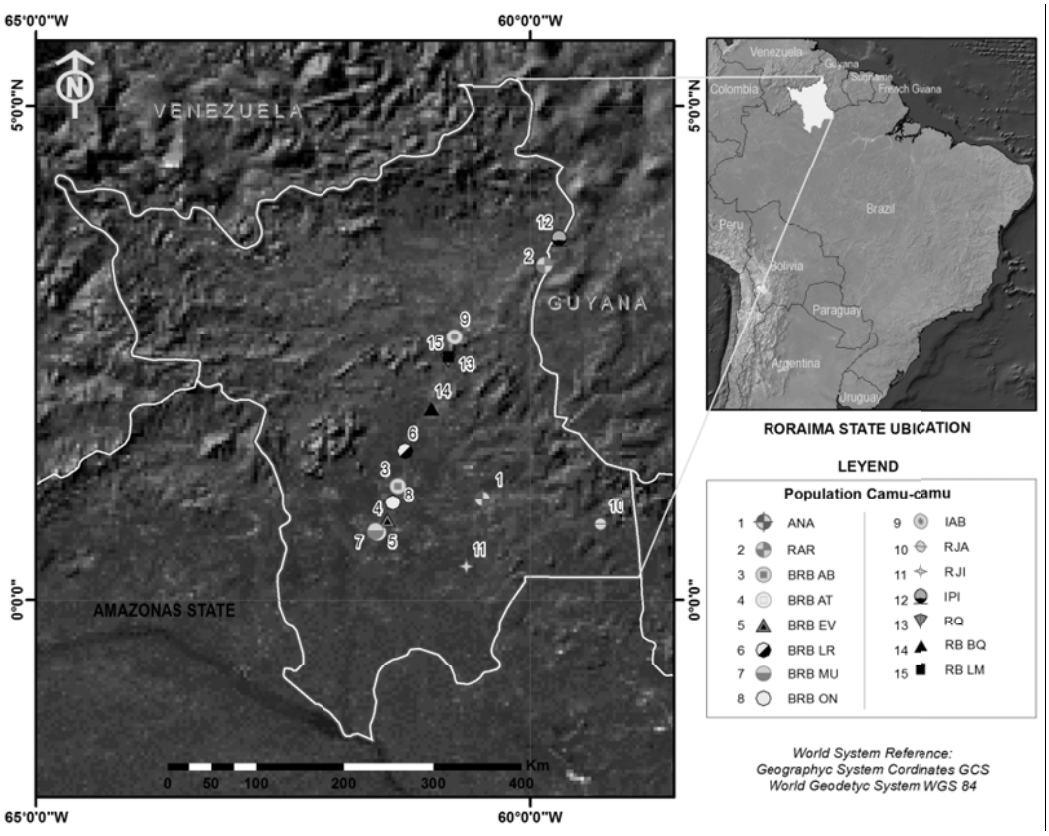

Figure 1. Location of sampling points of 15 camu-camu populations in the State of Roraima

The seeds were manually separated from the fruit and the residual pulp, by friction in a fine grade sieve. After removal, the seeds were washed in running water and treated with sodium hypochlorite solution at 10% for five minutes. The seeds were not submitted to artificial drying. Thereafter, the seeds were stored for 15 days in cold chamber at 10 ºC in polypropylene sacks, and the relative humidity of the air was maintained at 50%. Subsequently, they were planted in seedbeds containing sand substrate and sawdust in 1:1 proportions. The seedbed was placed in a nebulization chamber, with irrigation intervals at four times per day, for a period of 10 minutes. The seeds were distributed at a distance of 2 cm between rows and 1 cm between them in line, and at 1.5 cm of depth. The evaluation criteria adopted was counting as the epicotyl appeared 1.5 cm above the surface of the substrate, in emergence stage.

Table 1. Natural camu-camu populations prospected in the State of Roraima in different locations, municipalities and Hydrographical Regions

Population	Location	Municipality	Region Hydrographic
RAR	Rio Arraia	Bonfim	Alto Rio Branco
IPI	Rio Tacutu- Igarapé (Ig.) Pirara	Normandia	Alto Rio Branco
RB LM	Rio Branco- Lago da Morena	Cantá	Alto Rio Branco
IAB	Rio Mucajaí- Ig. Água Boa	Mucajaí	Alto Rio Branco
RQ	Rio Quitauaú	Cantá	Alto Rio Branco
RB BQ	Rio Branco- Bem Querer	Caracaraí	Médio Rio Branco
RAN	Rio Anauá	Rorainópolis	Baixo Rio Branco
BRB AB	Rio Branco- Ig. Água Boa	Caracaraí	Baixo Rio Branco
BRB AT	Rio Branco- Ig. Açaí Tuba	Caracaraí	Baixo Rio Branco
BRB EV	Rio Branco- Ig. Estirão do Veado	Caracaraí	Baixo Rio Branco
BRB LR	Rio Branco- Lago do Rei	Caracaraí	Baixo Rio Branco
BRB UM	Rio Branco- Lago Muçum	Caracaraí	Baixo Rio Branco
BRB ON	Rio Branco- Ig. Onofre	Caracaraí	Baixo Rio Branco
RJI	Rio Jauaperí	Rorainópolis	Sub-Bacia Rio Negro
RJA	Rio Jatapu	Caroebe/Entre Rios	Sub-Bacia Rio Amazonas

The experimental delineation was entirely random, with fifteen treatments (indigenous populations) and fifteen repetitions (subsamples), considering thirty seeds per subsample as an experimental unit, totalling 450 seeds per treatment.

At 40 days after planting, the experiment was finalized as two populations had already presented 100% emergence. The percentage of emergence was evaluated, the speed emergence of seedling (SES, index) (Maguire, 1962), the average time of emergence (Yuyama, Mendes, & Valente, 2011), the height of the plant shoots (cm) and the number of leaves emitted. The emergence percentage data were transformed in square root of arcsine "x/100" and the SES in square root "x + 0.5" (Gotelli & Ellison, 2011).

$$arcosine\sqrt{x/100} \quad (1)$$

$$\sqrt{x + 0.5} \quad (2)$$

The data from the rest of the variables was not transformed. The SES was established from the emergence test, with daily evaluations being done upon the emergence of the first plants up until the 40th day.

The data was submitted to variance analysis in order to verify the existence of genetic variability among the populations, being that their averages were grouped in accordance with the Scott and Knott (1974) test, at 5% probability. Thereafter, multivariated analyses were used, applying the grouping and canonical variable techniques with the assistance of INFOGEN software, version 2013 (Balzarini & Di Rienzo, 2013).

In the grouping technique, Mahalanobis generalized distance was utilized (D2ii) (Mahalanobis, 1936) as a dissimilarity measure. In the group delimitations, the Tocher optimization method was used, adopting the criteria that the average of the measurements of genetic divergence within each group aught to be less than the average distances between groups (Cruz, Regazzi, & Carneiro, 2004).

Additionally, the relative contributions of the characteristics to genetic divergence was quantified by Mahalanobis generalized distances, utilizing the criteria proposed by Singh (1981), analyzed with the assistance of GENES software, version 2005 (Cruz, 2008).

3. Results

By univariated variance analysis, there were significant differences between the population averages ($p < 0.01$), through testing of F, for all the evaluated characteristics (Table 2), indicating at least divergence among the populations.

Based on the grouping of averages, it was verified that only two populations (RJI and RAN) presented percentages of emergence (EP) below 50%, while the population average of emergence had been 73.8%. The BRB EV and IAB populations obtained 100% EP, indicating that these materials are promissory for future work in improvement. The populations which presented the highest indices of emergence velocity, plant shoot height and number of leaves were those that obtained values above 80% emergence, with the stand out populations being the BRB EV, RB LM and IAB. The shortest emergence times were registered in the RB LM and BRB ON populations with values less than 33 days (Table 2).

Table 2. Average values for emergence of plants (EP, %), speed emergence of seedling (SES, index), average time of emergence (ATE, days), height of plants (HP, cm) and number of leaves (NL) obtained for 15 indigenous populations of the camu-camu plant

Treatments	Initial Characteristics									
	EP (%)		SES (index)		ATE (days)		HP (cm)		NL	
BRB AB	56*	c	0.83	b	33..52	b	15..03	b	15..67	c
BRB AT	60	c	1.28	b	33..55	b	15..90	a	18..55	a
BRB EV	100	a	2.09	a	33..30	b	15..39	a	16..89	b
BRB LR	87	b	1.35	b	34.64	d	11.36	c	16.21	b
BRB UM	69	c	1.10	b	34.90	d	9.67	d	15.40	c
BRB ON	84	b	1.45	b	32.91	b	15.60	a	17.20	b
IAB	100	a	2.08	a	34.39	d	15.48	a	18.60	a
IPI	75	b	1.38	b	33.07	b	12.70	b	14.00	c
RAN	49	c	0.92	b	33.20	b	13.02	b	16.40	b
RAR	80	b	1.36	b	33.35	b	8.85	d	13.33	c
RB BQ	77	b	1.32	b	33.80	c	9.79	d	15.00	c
RB LM	82	b	2.06	a	32.02	a	10.44	c	17.17	b
RJA	64	c	1.18	b	33.09	b	10.67	c	14.44	c
RJI	45	c	1.04	b	33.32	b	12.83	b	17.17	b
RQ	78	b	1.47	b	33.41	b	9.97	d	14.62	c
F (population)	5.18	**	7.23	**	9.30	**	39.78	**	6.99	**
Overall average	73.80		1.39		33.50		12.45		16.04	
CV (%)	29.07		11.28		3.35		11.61		13.14	

Note. *: Averages with the same letter, in column, belong to the same Scott-Knott grouping at 5% probability; **: Significant in F testing at 1% probability; CV = variation coefficient %.

With a basis in the relative magnitude of D2ii values, the formation of seven distinct groups was verified using the Tocher grouping method, being that the largest concentration of populations was in the first group (Table 3).

Table 3. Grouping of 15 indigenous populations of the camu-camu plant by the Tocher optimization method, based on the Mahalanobis generalized distance (D2ii)

Group	Population				
< 1 >	BRB MU	RB BQ	BRB LR	RQ	RJA
< 2 >	RAN	RJI			
< 3 >	BRB EV	BRB ON	IAB		
< 4 >	BRB AB	BRB AT			
< 5 >	IPI				
< 6 >	RB LM				
< 7 >	RAR				

Note. Greatest distance between the minimums: 3.43.

Group 1 was composed of five populations that were grouped according to those that presented the lowest average values in the plant height characteristic (< 11.5 cm). Of these, the BRB LR and BRB MU populations presented the most time for emergence (> 34.6 days). Group 2 was composed of two populations that presented emergence percentages of less than 50%.

Group 3 was composed of populations that presented the highest values in SES, height of plant shoots and number of leaves, the same obtained values higher than 80% of emergence, and therefore, is indicated for future work in improvement such as crossing, with an eye to obtaining progenies with high heterosis in emergence and plant vigour. Group 4 was made up of two populations, grouped according to the following characteristics: percentage of emergence (≤ 60%) and ATE (33.5 days).

The populations that remained isolated, those are the RB LM and RAR populations, which presented considerable divergence, with percentage of emergence of 80 and 82%, ATE of 32.02 and 33.35 days, and plant shoot height of 10.40 and 8.85 cm, respectively (Table 2). These results were similar to those of the formation of some groups through canonical variance analysis (Figure 2), where a possible structuring of the group was observed, similar to the Tocher method (Table 3), which resulted in two auto-values or canonical variables, of which the first two constituted 86.07% of original variance of the data (CV1 = 68.31%; CV2 = 17.76%).

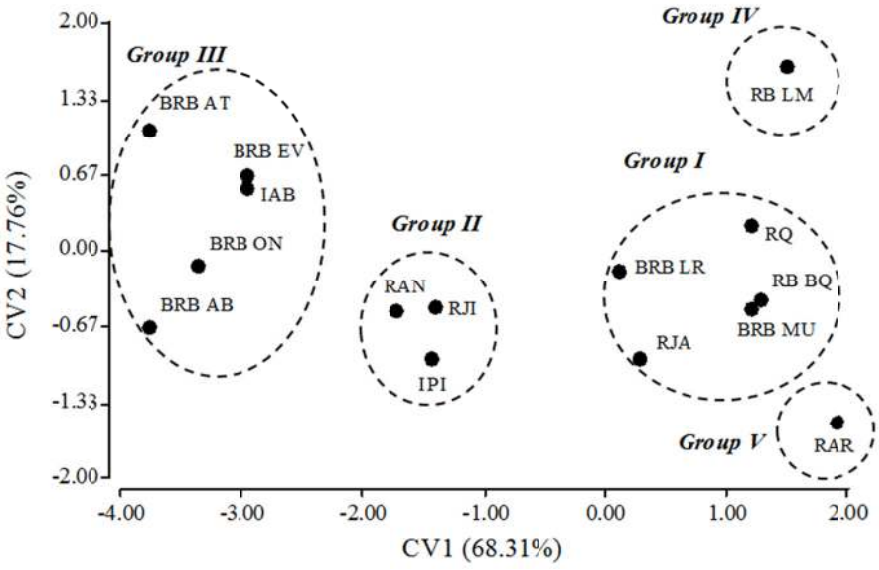

Figure 2. Graphic dispersal by scores of 15 indigenous camu-camu populations and respective groupings, in relation to the representative axes of the canonical variables (CV1 and CV2)

4. Discussion

According to Tekrony and Egli (1991), the vigour of plants, observed in the field by the ability of the seed to emerge and grow rapidly and vigorously, is a factor which can influence the productivity of cultures. According to Pinedo, Linares, Mendoza, and Anguiz, (2004), part of genetic improvement of the camu-camu is related to the selection of genotypes based on their rapidity, percentage of emergence and vigour of plant shoots, among other agronomic characteristics.

According to Cruz, Regazzi, and Carneiro (2004), when the first two canonical variables are above 80% of total variation, their utilization is satisfactory in the study of genetic divergence by way of the evaluation of the graphic dispersal of the scores in relation to the canonical variables (CV1 and CV2). This constant structure, through the formation of group coincidence in the utilization of complementary methods for morpho-agronomic characteristics, generates greater confidence in the results (Sudré et al., 2005; Oliveira, Ferreira, & Santos, 2007; Negreiros, Bergo, Miqueloni, & Lunz, 2013). Thus, the BRB AB and BRB AT populations that had to be classified as one group (Group 4), with the Tocher method, were classified by canonical variables within group III, together with the BRB EV, BRB ON and IAB populations. However, the IPI population, isolated in the Tocher method, was classified, by canonical variables, within group II, together with the RAN and RJI populations (Figure 2).

In group III, the populations of the lower Branco river BRB AB, AT, EV and ON, are interconnected by the same hydrographical region, which probably is related to the high values registered in the initial plant characteristics, representing a region of great potential for obtaining promissory genotypes for future improvement works.

The plant shoot height variable was the most important in distinguishing the populations in the first canonical variable (CV1) (68.31%), followed by the percentage of emergence and the average time of emergence, being that the relative contribution of these three characteristics to the genetic diversity among the 15 populations is confirmed, with a basis in the criteria proposed by Singh (1981) (Table 4). Thus contribution was verified in descending order as follows: plant shoot height, percentage of emergence, average time of emergence and SES. The number of leaves presented the lowest estimate of genetic diversity between the populations (S.j), not being important for the evaluation of genetic divergence between populations.

Table 4. Estimates of relative contribution of each characteristic (S.j) to the genetic divergence between the camu-camu plant populations, based on the partition of the total D^2_{ii}

Variable	S.j	Value (%)
Plant height (cm)	972.35	75.99
Plant shoot emergence (%)	131.11	10.25
Average time for emergence (days)	86.18	6.73
speed emergence of seedling(index)	70.1	5.48
Number of leaves	19.9	1.56

Note. S.j = S is the average relative importance for each variable; j = for the study of genetic diversity.

The percentage of emergence and plant shoot height characteristics contributed to 86.24% of divergence, which could be the initial agronomic parameters to be considered in the selection of genotypes in future works in genetic improvement in the species, bearing the values observed in mind.

5. Conclusions

There is genetic divergence among the fifteen populations and the BRB EV, BRB ON and IAB populations can be indicated for hybridization with other populations due to high genetic divergence, rate of emergence and vigour of the plant shoots.

The height of the plant shoots, percentage of emergence and the ATE are the characteristics of highest contribution to genetic divergence identified in the camu-camu plant.

The canonical variables and the Mahalanobis distance are useful and complementary in the evaluation of genetic divergence of the camu-camu plant.

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