


ORIGINAL ARTICLE

Genetic diversity in creole genotypes of Amazon chicory

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ABSTRACT

Amazon chicory is still a little-known vegetable despite its great agronomic potential. The characterization of chicory genotypes concerning genetic divergence is a key step for breeding programs, as it allows the selection of superior individuals and to explore the variability and complementarity of characteristics via interbreeding between newly generated genotypes. In this context, this study aimed to evaluate the genetic divergence among Amazon chicory creole genotypes from the northern Brazilian states of Pará and Rondônia based on morpho-agronomic traits. We conducted an experiment in a randomized block design with eight chicory genotypes (treatments) and four replications. Both quantitative and qualitative characteristics were evaluated. Genetic divergence was estimated via squared generalized Mahalanobis distance (D^2), considering only quantitative characters, and the genotypes were subsequently clustered via the UPGMA method. Analysis of variance showed significant differences among genotypes for all studied characteristics, except shoot fresh weight. The UPGMA grouped the genotypes into three clusters, which demonstrated that the genotypes from Colares and Santarém Novo (Pará) (Chic-02 and Chic-04) were the most divergent as compared to the genotypes from Castanhal and Santa Isabel do Pará (Pará). Qualitative characteristics showed a monomorphic behavior and, therefore, were not used to assess genetic divergences. To obtain segregating populations with complementary characteristics, crossbreeding between the two most divergent clusters is recommended.

KEYWORDS: *Eryngium foetidum*, non-conventional food plants, genetic variability, plant breeding

Diversidade genética em genótipos crioulos de chicória da Amazônia

RESUMO

A chicória da Amazônia é uma hortaliça ainda pouco conhecida, mas com grande potencial agrônomo. A caracterização dos genótipos de chicória quanto a divergência genética é um importante passo para programas de melhoramento genético, pois permite selecionar indivíduos superiores e explorar a variabilidade e a complementariedade de características, a partir dos novos genótipos gerados. Nesse contexto, o objetivo deste estudo foi avaliar a divergência genética de genótipos crioulos de chicória da Amazônia dos estados do Pará e Rondônia, com base em caracteres morfoagronômicos. Realizou-se um experimento em delineamento de blocos ao acaso, com oito genótipos de chicória (tratamentos) e quatro repetições. Foram avaliadas características quantitativas e qualitativas. A divergência genética foi estimada a partir da distância quadrada generalizada de Mahalanobis (D^2), levando em consideração apenas os caracteres quantitativos, e os genótipos foram agrupados pelo método UPGMA. A análise de variância evidenciou diferença significativa entre os genótipos para todas as características, exceto massa fresca. O UPGMA agrupou os genótipos em três grupos, sendo os genótipos de Colares e Santarém Novo (Pará) (Chic-02 e Chic-04) os mais divergentes em comparação com os genótipos da região de Castanhal e Santa Isabel do Pará (Pará). As características qualitativas apresentaram padrão monomórfico, não sendo, portanto, utilizadas para avaliar a divergência. Para obter populações segregantes com complementariedade de características, recomenda-se o cruzamento entre os dois grupos mais divergentes.

PALAVRAS-CHAVE: *Eryngium foetidum*, plantas alimentícias não convencionais, variabilidade genética, melhoramento vegetal

INTRODUCTION

The Amazon chicory (*Eryngium foetidum* L.) is a perennial Apiaceae that has the Central and Latin Americas as likely centers of origin (Ekpong and Sukprakarn 2006). It is characterized as an aromatic tropical vegetable, mainly due

to its pungent aroma, and is widely used as a spice in some Asian countries like India (Paul *et al.* 2011).

In Brazil, the Amazon chicory is considered an unconventional vegetable, as it is not part of the standard urban culinary tradition and is mostly consumed in the

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northern region of the country. Although the consumption of Amazon chicory is restricted, its socioeconomic potential has increased constantly (Hossain *et al.* 2017). Yet this is still a semi-domesticated crop and studies on this vegetable are still incipient, and its genetic potential is not well analyzed and explored to benefit both crop breeding and commercialization.

To make possible the production of Amazon chicory on a large scale, it is necessary to better understand its genetic variability, which remains unknown since it is a semi-domesticated crop. Knowledge of genetic variability provides a theoretical basis not only for genetic material preservation but also for studies that seek to better exploit the genetic base and select divergent individuals (Aragão *et al.* 2013).

This knowledge can be obtained via morpho-agronomic trait assessment, which assesses signatures of genetic divergence within or between populations, enabling the identification of the most promising parents with desirable characteristics for interbreeding and the development of hybrids that lead to higher segregation in recombination and the occurrence of transgressive segregating genotypes that hold parental characteristics (Morales *et al.* 2011; Cruz *et al.* 2020).

Studies concerning genetic divergence in vegetables have been addressed to select individuals with desirable agronomic characteristics. For example, the analysis of genetic divergence in another Apiaceae, coriander (*Coriandrum sativum* L.) identified potential genotypes to be used in genetic breeding programs for early flowering coupled with higher fruit set production (Bertine *et al.* 2010). Such results suggest that these genotypes are not recommended for producers that seek to obtain a higher fresh weight. Likewise, Viana *et al.* (2016) observed a variability in garlic genotypes (*Allium sativum* L.) from the state of Piauí, in northeastern Brazil, which is relevant for the internal market dynamics related to this culture that competes with overseas production.

Currently, no studies have yet reported genetic divergence in Amazon chicory, which makes the genetic basis of this crop little explored. This assessment is important for the development of genetic breeding programs, since the characterization of plant morphology, as well as the quantification of genetic variability, are important premises in breeding programs when the selection of groups for interbreeding is intended (Andrade *et al.* 2019). In this context, we aimed to evaluate the genetic divergence among Amazon chicory creole genotypes based on morpho-agronomic characteristics.

MATERIAL AND METHODS

The experiment was conducted at the horticulture sector of Universidade Federal Rural da Amazônia (1°11'26.82"S; 47°09'36.31"W; 25 m altitude), in Capanema, Pará state, Brazil, from August 2019 to July 2020. According to the Köppen-Geiger classification, the region's climate is Ami-type, characterized as humid mega-thermal, with annual average

temperature of 25 °C, average relative humidity of 85%, and average annual rainfall of 1900 mm (Alvares *et al.* 2013).

The experimental design was in randomized blocks, with eight treatments and four replications. The treatments consisted of eight Amazon chicory creole genotypes, directly collected from local producers. Seven genotypes were collected in seven municipalities in Pará state and one in the state of Rondônia (Table 1). Each experimental plot encompassed 12 plants. We considered only the five central plants as useful area.

Table 1. Collection sites of Amazon chicory (*Eryngium foetidum*) creole genotypes in Pará and Rondônia states (Brazil) for genetic divergence assessment.

Genotype	Municipality	Geographic coordinates
Chic-01	Primavera-Pará	00°56'36"S; 47°07'06"W
Chic-02	Colares-Pará	00°55'38"S; 48°17'04"W
Chic-03	São João de Pirabas-Pará	00°45'59"S; 47°10'21"W
Chic-04	Santarém Novo-Pará	00°55'45"S; 47°23'55"W
Chic-05	Castanhal-Pará	01°17'38"S; 47°55'35"W
Chic-06	Bragança-Pará	01°03'15"S; 46°46'10"W
Chic-07	Alto Alegre do Parecis-Rondônia	12°07'42"S; 61°51'05"W
Chic-08	Santa Isabel-Pará	01°17'55"S; 48°09'38"W

Seeds were extracted from inflorescences in transition from green to brown color. The inflorescences were collected randomly and in all orders, following the casualization criteria. The inflorescences were placed in paper bags and then dried at room temperature for five days. After drying, the seeds were moved to the laboratory, prepared by removing the remaining tissues from the inflorescences with a sieve, and stored in 50-mL plastic containers in a refrigerator at 3-5 °C until sowing.

Before sowing, the seeds were immersed in a solution of gibberellic acid (GA₃) at 100 ppm, for 7 h, seeking to increase the rate of germination. For seedling production, the seeds were sown in polystyrene trays with 128 cells, filled with commercial substrate Tropstrato®, containing two seeds per cell. After sowing, the trays were kept in a protected environment with a 50% shading screen and were irrigated daily. After emergence, the seedlings were subjected to thinning, leaving only one seedling per cell.

At 120 days after sowing (DAS), the seedlings that displayed four well-developed definitive leaves were transplanted to beds 7 m long x 1 m wide, with a spacing of 0.20 m between rows and between plants, composing a quadrangular spatial arrangement. Plants were grown in dystrophic yellow latosol of medium texture. The soil analysis was carried out in the arable layer (0-20 cm), and presented the following characteristics: pH_{CaCl2} = 5.7; P = 7.7 mg dm⁻³; K = 1.1 mmol_c dm⁻³; Ca⁺² = 31 mmol_c dm⁻³; Mg⁺² = 10 mmol_c dm⁻³; Al⁺ = 0 mmol dm⁻³; H+Al = 20 mmol_c dm⁻³; SB = 42.7 mmol_c dm⁻³; CTC = 62.7 mmol_c dm⁻³; V = 68%.

Based on the soil chemical analysis, fertilization was accomplished via furrow seven days after transplanting (DAT) by using urea, simple superphosphate, and potassium chloride as sources of nitrogen, phosphorus, and potassium, respectively, as recommended by Cravo *et al.* (2020) for leafy vegetables. During the experiment, weeding and manual irrigations were performed weekly.

The plants were pruned at 54 DAT on the developing floral tassels to avoid competition with leaf differentiation. After 90 DAT, five plants per plot were collected to assess quantitative and qualitative morpho-agronomic characteristics according to Mozumder *et al.* (2013), in addition to the most representative descriptors for Apiaceae.

The following quantitative parameters were measured for each plant: leaf length; leaf width; number of leaves; shoot fresh weight; number of tillers; number of floral tassels; and days to bolting. The following qualitative parameters were determined as described by Vidal and Vidal (2003): leaf limb shape (lanceolate or spatulate); leaf apex shape (truncated or rounded); leaf base shape (derived or decurrent); and bract consistency (membranous or coriaceous). We also determined the type of leaf margin (dentate or serrate) according to Gonçalves and Lorenzi (2007) and leaf phyllotaxy (alternate spiral or spiral alternate rosette) according to Almeida and Almeida (2008).

Statistical analysis

Assumptions for parametric analysis were evaluated by *fbasics* (Wuertz *et al.* 2017), *RVAidemoire* (Hervé 2021), *car* (Fox and Weisberg 2019), *mclust* (Scrucca *et al.* 2016) and *psych* (Revelle 2021) packages in the R platform. All variables met the assumptions and were compared between genotypes

by using analysis of variance (ANOVA) and the Scott-Knott test, at a 5% probability level, with the *ExpDes.pt* package in the R platform (Ferreira *et al.* 2021).

Genetic divergence among genotypes was determined with the generalized Mahalanobis distance (D^2), both as a measure of dissimilarity and for clusters formation. To avoid scale divergence among traits, a feature standardization by zero-mean and unit-variance was applied (Hair Jr *et al.* 2009).

Genotypes were clustered by the unweighted pair group method with arithmetic mean (UPGMA) using the following R packages: *cluster* (Maechler *et al.* 2017), *circlize* (Gu *et al.* 2014), *dendextend* (Galili 2015) and *fpc* (Hennig 2015). The probable number of clusters was determined based on the maximum average silhouette width (ASW), derived from the method of partitioning around medoids (PAM) within the cophenetic distance matrix of the dendrogram (Galili 2015). To infer whether the cluster generated by the UPGMA corroborates the observed distance matrix, the cophenetic correlation coefficient was used. The relative contribution of each trait to the genetic divergence among genotypes was evaluated using the method of Singh (1981). Characteristics that presented a relative contribution below 3% were used as a criterion for variable exclusion.

Qualitative variables were analyzed as multicategorical characteristics, expressed as the mode of each genotype within each descriptor. All data were analyzed using GENES software (Cruz 2013) and R platform (R core team 2022).

RESULTS

Leaf length and width, number of leaves per plant, number of floral tassels per plant and days to bolting varied significantly among genotypes (Table 2). The Chic-03, Chic-

Table 2. Summary of the analysis of variance (F test) of leaf length (Length), leaf width (Width), number of leaves per plant (NL), shoot fresh weight (SFW), number of floral tassels per plant (NFT) and days to bolting (DB) among eight Amazon chicory (*Eryngium foetidum*) creole genotypes.

UPGMA cluster	Genotype	Length (cm)	Width (cm)	NL	SFW (g)	NFT	DB
I	Chic-01	17.04 b	4.43 b	26.58 b	70.84 a	1.74 b	45.06 b
III	Chic-02	16,04 b	4.24 b	31.58 a	62.08 a	2.72 a	40.06 b
I	Chic-03	18.14 a	4.65 a	26.08 b	63.33 a	1.66 b	47.20 a
III	Chic-04	14.66 b	3.83 b	35.58 a	55.33 a	2.75 a	43.55 b
I	Chic-05	18.72 a	4.86 a	21.41 b	60.65 a	1.47 b	48.94 a
I	Chic-06	15.43 b	4.24 b	24.50 b	49.58 a	1.43 b	50.30 a
I	Chic-07	18.60 a	5.21 a	21.49 b	58.93 a	0.83 b	53.77 a
II	Chic-08	20.38 a	5.21 a	23.58 b	82.85 a	1.31 b	46.88 a
F test		7.27**	7.66**	5.78**	2.18 ^{ns}	7.75**	5.54**
Overall mean		17.38	4.58	26.35	62.95	1.74	46.97
Standard error		0.39	0.11	1.09	2.98	0.14	0.90
CV (%)		8.21	7.73	15.63	21.78	27.68	7.61

Means followed by the same letter do not differ from each other by the Scott-Knott test, at $P < 0.05$ (** = $P < 0.01$; ^{ns} = not significant; CV = coefficient of variation (%)).

05, Chic-07 and Chic-08 genotypes had significantly longer and wider leaves than the other genotypes (Table 2).

The Chic-02 and Chic-04 genotypes had significantly more leaves per plant, however, there was no significant difference among genotypes in shoot fresh weight, which had an overall mean of 63.32 g (Table 2). These genotypes also had significantly more floral tassels per plant than the other genotypes. Chic-01, Chic-02 and Chic-04 had significantly

fewer days to bolting (overall average of 43 d) as compared to their counterpart genotypes (Table 2).

The Mahalanobis distance (D₂) values ranged between 3.87 and 106.62, indicating a moderate degree of dissimilarity among the genotypes (Table 3). The greatest genetic distances were observed between genotype pairs Chic-04 and Chic-08 (D₂ = 106.62), Chic-04 and Chic-07 (D₂ = 77.20), and Chic-04 and Chic-05 (D₂ = 70.07). Chic-02 showed highest

Table 3. Genetic dissimilarity matrix of Amazon chicory (*Eryngium foetidum*) creole genotypes, based on generalized Mahalanobis distance (D²) for quantitative traits.

Genotype	Chic-02	Chic-03	Chic-04	Chic-05	Chic-06	Chic-07	Chic-08
Chic-01	24.94	6.96	47.65	13.53	7.36	21.34	16.14
Chic-02	-	22.30	7.73	40.61	16.40	53.45	68.40
Chic-03		-	43.16	3.87	5.47	9.79	16.19
Chic-04			-	70.07	28.33	77.20	106.62
Chic-05				-	15.09	7.20	9.21
Chic-06					-	16.62	31.26
Chic-07						-	14.17
Chic-08							-

dissimilarity to Chic-07 (D₂ = 53.45) and Chic-08 (D₂ = 68.40) (Table 3). The smallest dissimilarity was observed between Chic-03 and Chic-05 (D₂ = 3.87), and Chic-03 and Chic-06 (D₂ = 5.47) (Table 3).

Genotypes from Group III (Chic-02 and Chic-04) differed considerably from Group II, due to the significant differences in length and width of leaves, number of leaves, number of floral tassels per plant and days to bolting (Table 2).

The cophenetic correlation coefficient generated via dissimilarity data was 0.74 for all genotypes, which corroborates the genetic divergence among genotypes observed in the dendrogram (Figure 1).

UPGMA grouped the genotypes into three clusters (Figure 1). The distance between genotypes from Group I (Chic-01, Chic-03, Chic-05, Chic-06 and Chic-07) and Group II (Chic-

08 in the dissimilarity matrix (D₂) was not high (Table 3). This was likely influenced by the similarities observed in the number of leaves, number of floral tassels and days to bolting. On the other hand, the genotypes from Group III (Chic-02 and Chic-04) differed considerably from Group II due to the significant differences in length and width of leaves, number of leaves, and number of floral tassels per plant and days to bolting (Table 2).

The analysis of the relative contribution of each character to genetic divergence, as proposed by Singh (1981), showed that leaf length, number of leaves, shoot fresh weight and number of floral tassels per plant contributed the most to dissimilarity among genotypes (Table 4). Leaf width presented a relative contribution below 3% and was excluded from the analysis.

Qualitative characters were mostly monomorphic, with only one difference in leaf margin for Chic-08 genotype (Table 5).

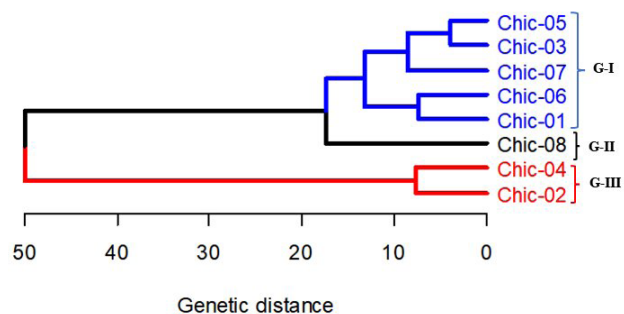


Figure 1. Dendrogram generated by the UPGMA method based on the genetic dissimilarity between Amazon chicory genotypes (*Eryngium foetidum*) via generalized Mahalanobis distance (D²). This figure is in color in the electronic version.

Table 4. Estimates of the relative contribution of quantitative morpho-agronomic characters to genetic divergence among Amazon chicory (*Eryngium foetidum*) creole genotypes.

Character	Value (%)
Leaf length	26.49
Leaf width	0.00
Number of leaves	30.29
Shoot fresh weight	16.65
Number of floral tassels	17.66
Days to bolting	8.91

Table 5. Multicategorical qualitative descriptors of morphological characters of Amazon chicory (*Eryngium foetidum*) creole genotypes. LS = limb shape; AS = apex shape; BF = base format; LM = leaf margin; LC = leaf consistency; LP = leaf phyllotaxy.

Genotypes	LS	AS	BF	LM	LC	LP
Chic-01	spatulate	rounded	attenuate	serrate	membranaceous	alternate spiral
Chic-02	spatulate	rounded	attenuate	serrate	membranaceous	alternate spiral
Chic-03	spatulate	rounded	attenuate	serrate	membranaceous	alternate spiral
Chic-04	spatulate	rounded	attenuate	serrate	membranaceous	alternate spiral
Chic-05	spatulate	rounded	attenuate	serrate	membranaceous	alternate spiral
Chic-06	spatulate	rounded	attenuate	serrate	membranaceous	alternate spiral
Chic-07	spatulate	rounded	attenuate	serrate	membranaceous	alternate spiral
Chic-08	spatulate	rounded	attenuate	dentate	membranaceous	alternate spiral

DISCUSSION

Although leaf length and width are also influenced by environmental conditions, they can express a certain degree of variability within the same lot, which makes them promising markers. In the present study, the generally larger leaves in genotypes from Groups I and II suggest a genetic influence on leaf size, which is economically relevant, as the leaves are the commercialized organs of the Amazon chicory.

The significantly higher number of leaves in Chic-02 and Chic-04 suggest genetic variability among genotypes, which should be evaluated in further studies to ensure genetic gains by recombination. For Amazon chicory producers, the high number of leaves per plant is an important feature, as this trait increases the number of bunches per m², thus providing higher income and profits.

The lack of significant differences in shoot fresh weight is likely due to this trait being strongly influenced by cultivation conditions and the hydric status of the plants at the time of harvest. The higher number of floral tassels in genotypes Chic-02 and Chic-04 is not suitable for chicory quality, as leaf size and number decrease with the increase of floral tassels. However, these genotypes are suitable for seed production, as more tassels lead to more inflorescences (umbels in this case) and a higher number of seeds.

The six genotypes that showed an increased time to bolting (Chic-01, Chic-03, Chic-05, Chic-06, Chic-07 and Chic-08) are promising for *in natura* commercialization, as the production of floral tassels may drain the energy of Amazon chicory plants (Gomes *et al.* 2013). On the other hand, delayed flowering maintains the flux of assimilates towards leaf development for a longer time.

The dissimilarity among some of the genotypes indicates a high degree of divergence, which suggests that they can be used in breeding programs for the development of segregating populations. The dissimilarity matrix revealed a high frequency of elevated distances between genotypes when paired with Chic-02 or Chic-04. These genotypes were collected in the geographically close municipalities of Colares and Santarém

Novo, which facilitates the exchange of seeds between producers, thereby explaining the overall similarity between genotypes. These genotypes differed from the others due to three main divergent characteristics, namely the number of leaves, number of floral tassels per plant and days to bolting.

Genotypes in Group I showed a higher degree of similarity and are not promising for breeding programs. The unique genotype from Group II, Chic-08, was collected in Santa Isabel, Pará state, a region characterized by an important production of vegetables that supply the metropolitan region of Belém, the state capital. There is also a high concentration of Amazon chicory producers in this region, which leads to a more intense cycle of selection. As no cultivars are available, producers select the best plants within local materials to produce their own seeds, resulting in a population that is genetically differentiated from other locations.

The higher the cophenetic correlation coefficient, the greater the reliability of the cluster formed (Silva and Dias 2013; Cruz *et al.* 2020), so that the coefficient value observed in this study is in agreement with the dendrogram generated by the dissimilarity matrix related to the analyzed materials.

A moderate genetic variability was observed among clusters, and an elevated similarity within groups. This similarity may indicate that the genotypes of the same group are likely duplicates, suggesting a narrow genetic base for this material (Silva *et al.* 2011). As the Amazon chicory does not have a registered cultivar, all available genotypes are creoles and produced predominantly in rural areas distant from larger commercialization centers, where a high rate of seed exchange among producers may occur. The genotype obtained in the state of Rondônia was similar to the genotypes from Pará, further evidencing that these materials are duplicates with a narrow genetic base, as observed in the intra-group dissimilarity measures.

Among the evaluated characteristics with highest percentages of contribution, leaf length, number of leaves, shoot fresh weight and number of floral tassels per plant are highlighted. This result is an important indicator of characteristics underlying the genetic variability of Amazon

chicory. More detailed studies are necessary, as the vegetative development of plants is strongly influenced by edaphoclimatic factors. As the relative contribution of leaf width was below 3%, this parameter was not used to indicate promising genotypes for intercrossing or recommended to producers.

The monomorphic behavior observed in the analysis of qualitative descriptors suggests similarity among the genotypes; therefore, they did not contribute to indicate the most divergent for intercrossing, which were defined by quantitative characteristics. In another study that evaluated Amazon chicory creole genotypes via multicategorical descriptors, a variability related to leaf margin characteristics was also observed (Gomes *et al.* 2020), which demonstrates that this trait varies among genotypes of this species, but it was not enough to express more variability in the present study.

The conservation of Amazon chicory germplasm may improve the potential for studies on this unconventional crop, as it allows for easier access to the genetic variability of the species. We suggest that the genotypes from Group II (Chic-08) and Group III (Chic-04 and Chic-02) be used as a base population for Amazon chicory interbreeding, as they hold a high genetic variability and complementary characteristics, especially those related to leaf production.

CONCLUSIONS

Based on quantitative descriptors, the eight analyzed Amazon chicory genotypes were grouped into three clusters. Overall, leaf length, number of leaves and number of floral tassels per plant were the characteristics that most contributed to the observed divergence. We thus recommend crosses between genotype Chic-08 (Group II) and Chic-04 or Chic-02 (Group III) for implementation of genetic breeding programs, as they provide complementarity characteristics to obtain superior hybrids, which increases the probability of transgressive genotype occurrence.

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