Research Article

Morphoagronomic divergence among Amazonian spinach genotypes[1]

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ABSTRACT

Amazonian spinach is an unconventional food plant with great production potential and excellent nutritional properties. This study aimed to verify the genetic divergence among 16 Amazonian spinach genotypes based on morphoagronomic traits. The experiment was conducted in a randomized block design, with 16 treatments and four replicates. The AM-7 and AP-1 genotypes stood out for presenting superior values for important traits, making them promising for the formation of superior genotypes in future genetic breeding studies. The Tocher's optimization method led to the formation of distinct groups, corroborating the existence of genetic variability among the genotypes.

KEYWORDS: *Alternanthera sessilis* (L.) DC, genetic variability, unconventional food plant, genetic dissimilarity.

RESUMO

Divergência morfoagronômica entre genótipos de espinafre-da-amazônia

O espinafre-da-amazônia é uma planta alimentícia não convencional com grande potencial de produção e excelentes propriedades nutritivas. Objetivou-se verificar a divergência genética entre 16 genótipos de espinafre-da-amazônia com base em caracteres morfoagronômicos. O experimento foi conduzido em delineamento de blocos ao acaso, com 16 tratamentos e quatro repetições. Os genótipos AM-7 e AP-1 se destacaram por apresentar valores superiores para características importantes, o que os torna promissores para a formação de genótipos superiores em futuros trabalhos de melhoramento genético. O método de agrupamento de Tocher levou à formação de grupos distintos, corroborando a existência de variabilidade genética entre os genótipos.

PALAVRAS-CHAVE: *Alternanthera sessilis* (L.) DC, variabilidade genética, planta alimentícia não convencional, dissimilaridade genética.

INTRODUCTION

Amazonian spinach [*Alternanthera sessilis* (L.) DC] belongs to the Amaranthaceae family and is rich in vitamins A, B9 (folic acid), C and K. It also contains carotenoids, flavonoids, fiber and minerals such as iron, calcium and potassium, as well as antioxidant properties that protect cells from free radical damage and reduce the risk of chronic diseases (Klein et al. 2018, Ferreira et al. 2021, Sommai et al. 2021, Wuni et al. 2022).

Its wrinkled leaves and upright or decumbent habit make it a herb up to 30 cm tall. Propagation is carried out using cuttings, which may or may not be rooted, and cultivation should be done in the shade to ensure the development of larger and tender leaves (Silva et al. 2022, Teatrawan et al. 2022, Aragão et al. 2023).

Studies conducted on the species focus on crop density and spacing, organic fertilization, use of shade screens, weeds, bromatology and hydroponics, but there is a lack of studies in the area of genetic breeding.

Genetic divergence analysis is essential in plant breeding, as it allows promising genotypes to be identified based on genetic variability. Measures such as the Euclidean distance and Mahalanobis' distance (D^2), which considers variances and covariances among characteristics, are effective for this quantification, especially in experiments with designs (Cruz & Carneiro 2006, Santos et al. 2022).

The choice of genetically divergent parents increases variability in segregating populations, as well as the chances of obtaining favorable gene combinations (Barbieri et al. 2005, Arriel et al. 2007).

The search for divergent genotypes, aiming at the formation of populations with broad genetic variability, contributes to the development of new cultivars with desirable traits. Thus, this study aimed to quantify the genetic divergence among 16 genotypes of Amazonian spinach.

MATERIAL AND METHODS

The experiment was conducted at the Universidade Federal do Acre, in Rio Branco, Acre state, Brazil (67º42'18"W, 10º01'30"S and altitude of 136 m), from March to June 2020, in an unheated greenhouse with arched metal structure covered with 150 mm of low-density polyethylene. According to the Köppen classification, the region's climate is classified as AWI (hot and humid), with average temperature of 25.5 ºC and annual rainfall of 1,648 mm.

A randomized block design was adopted, consisting of 16 treatments and four replicates. The treatments corresponded to 16 genotypes of *Alternanthera sessilis* L. obtained from the germplasm bank of the Universidade Federal do Acre (UFAC) and collected from farmers and open markets in the northern region in 2019 (Table 1).

Soil sampling of a Red-Yellow Argissol (USDA 2022, Santos et al. 2025) (0-20 cm layer) was carried out in April 2020, for the purpose of chemical characterization. Twenty samples were collected in the experimental area, which were combined to form a representative sample of approximately 1,000 g. From this sample, a subsample of 500 g was sent for analysis (Table 2).

Dolomitic limestone was applied at a rate of 1,000 kg ha^{-1} to the beds and incorporated into the soil to raise the base saturation to approximately 70 %. The soil preparation consisted of turning the beds and distributing fertilizer.

The seedlings were prepared in a substrate composed of a mixture of soil, sawdust and poultry manure at a ratio of 3:1:1 (v/v). Propagation was carried out using the cutting method, with 10-cm-long cuttings placed in disposable 500-mL plastic containers filled with the substrate. The seedlings were placed in a nursery with screens that blocked 50 % of the light, for a period of 15 days. After this period, the seedlings were transplanted to the greenhouse, in beds with 20 x 20 cm spacing among plants. Each plot consisted of 16 plants, and the four central plants were evaluated at the end of the experiment.

Weeding management was performed manually, and insect pests controlled by applying neem extract (*Azadirachia indica* A. Juss) at the concentration of 1 %, as needed. The water supply was ensured by a micro-sprinkler irrigation system, using nebulizers with a flow rate of 30 L h^{-1}, pressure of 1.5 kg cm^{-2} and range of 2.4-2.7 m.

Table 1. Identification and origin of *Alternanthera sessilis* (L.) DC genotypes that comprise the Germplasm Bank of the Universidade Federal do Acre (Rio Branco, Acre state, Brazil).

Genotypes	Collection location	Origin
AM-1	Modern Manaus Fair	Manaus - AM
AM-2	Producer's Fair	Manaus - AM
AM-3	Rural vegetable garden	Manaus - AM
AM-4	Urban vegetable garden	Novo Airão - AM
AM-5	Urban backyards	Envira - AM
AM-6	Japanese site	Rio Preto da Eva - AM
AM-7	Japanese site	Rio Preto da Eva - AM
AC-1	Colônia Esperança	Tarauacá - AC
AC-2	Rural vegetable garden	Rio Branco - AC
AC-3	Urban vegetable garden	Mâncio Lima - AC
AC-4	Agroforestry hub	Xapuri - AC
RR-1	Sítio Primavera	Rorainópolis - RR
RR-2	Garimpeiro Fair	Boa Vista - RR
RR-3	Sítio Açaí	Bonfim - RR
RO-1	Free market	Porto Velho - RO
AP-1	Urban vegetable garden	Porto Grande - AP

Table 2. Soil chemical attributes in the experimental area.

pH H$_2$O	OM dag kg^{-1}	P-rem mg L^{-1}	V %	P[1]	K[1]	Ca[2]	Mg[2]	Al[2]	SB	Al + H[3]	t	T
				mg dm^{-3}		cmol$_c$ dm^{-3}						
4.6	1.2	43	41	2	62	0.9	0.5	0	1.6	2.2	1.6	3.8

[1] Mehlich extractor 1; [2] KCl extractor 1 mol L^{-1}; [3] Ca (OAC)$_2$ extractor 0.5 mol L^{-1}, pH 7.0. OM: organic matter; P-rem: remaining phosphorus; V: base saturation; SB: sum of bases; t: effective cation exchange capacity (CEC); T: CEC at pH 7.

At 45 days after transplanting the seedlings, the four central plants in the useful area of each plot were selected to evaluate the following characteristics: average diameter of the plant collar (mm; calculated from the circumference measured with a caliper); number of branches; number of leaves; plant height (cm; measured with a graduated ruler from the plant collar to the tip of the last leaf); plant width (cm; measured with a graduated ruler from one end to the other of the plant side); leaf area (cm²; using a CI 202 potable laser-leaf area meter); shoot fresh mass (g; determined by weighing the plants on a precision scale); and shoot dry mass (g; determined by weighing on a precision scale after drying the plants in a forced-air circulation oven at 55 ºC, until a constant mass was reached).

The data were submitted to analysis of variance and the means grouped using the Scott-Knott test (p < 0.05; equivalent to an accuracy of 0.86, according to Resende & Alves 2022) with the aid of the Genes software (Cruz 2016). To estimate genetic divergence, the Selegen-REML/BLUP model 20 (Resende 2007) was initially used to generate the genotype value matrix. Based on this matrix, multicollinearity was assessed using the genotypic correlation matrix, through the calculation of the condition number and the variance inflation factors (Montgomery & Peck 1981). Then, the genetic divergence procedure among accessions (within populations) was used for quantitative variables, with the aid of the Genes software (Cruz 2016).

To evaluate the relative contribution of traits to the divergence among genotypes, the Singh's method (1981) was used. Then, multivariate analysis was performed based on the Mahalanobis' generalized genetic distance matrix (D2), and grouping was performed using the Tocher's optimization method (Rao 1952).

RESULTS AND DISCUSSION

Highly significant differences (p < 0.01) were observed among the genotypes for all evaluated traits (Table 3), showing a considerable genetic variability among the genotypes, enabling the formation of contrasting groups. The coefficients of variation ranged from 2.02 to 5.73 %, demonstrating consistency of data, good experimental precision and reliability of the obtained measurements (Pimentel-Gomes 2009).

The method proposed by Singh (1981) presents the relative weight that a given variable has in the composition of the generalized Mahalanobis' distance matrix. Plant height, leaf area, shoot fresh and dry mass were the main traits responsible for explaining the genetic variation among the genotypes, contributing with 93.26 % of the observed diversity (Table 4). These characteristics proved to be highly informative in distinguishing the genotypes, indicating that they are important for the study of genetic divergence.

On the other hand, the variables plant width and collar diameter, number of leaves and number of branches showed relatively low contribution values, in relation to genetic diversity, with relative contribution (S.j) of only 0.86, 7.76, 3.08 and 3.04 %, respectively (Table 4). These results suggest that plant width, collar diameter, number of leaves and number of branches can be considered characteristics of lesser importance in assessing the overall divergence among the studied genotypes. Scientific literature has already pointed out that characteristics that do not vary significantly among the analyzed individuals, or that are highly correlated with others, can be considered redundant and, therefore, be discarded in subsequent analyses (Cruz et al. 2012).

Table 3. Summary of the analysis of variance and coefficients of variation for collar diameter (CD), number of branches (NB), number of leaves (NL), plant height (PH), plant width (PW), leaf area (LA), shoot fresh mass (SFM) and shoot dry mass (SDM) of 16 genotypes of Amazonian spinach.

Source of variation	DF	CD mm	NB	NL	PH	PW cm	LA cm²	SFM	SDM
								g plant⁻¹	
Blocks	3	0.02	0.02	100.19	6.89	1.44	80.29	343.33	20.49
Treatment	15	8.42**	10.64**	185.13**	110.27**	91.52**	44,378.30**	17,648.83**	954.20**
Error	45	0.04	0.02	45.00	2.22	1.70	43.16	304.03	14.53
CV (%)		3.07	2.02	4.08	4.05	3.46	3.63	5.66	5.73

** Significant at 1 % of probability, according to the F-test. DF: degrees of freedom; CV: coefficient of variation.

Table 4. Relative contribution to traits, according to Singh (1981), of 16 genotypes of Amazonian spinach.

Category	Contribution (S.j)	Amount (%)	Contribution (S.j)	Amount (%)
Plant height	15,319.74	17.12	15,319.74	18.71
Leaf area	41,544.54	46.42	41,544.54	50.74
Plant width	774.37	0.87	0.00	0.00
Number of branches	2,721.60	3.04	2,721.60	3.32
Number of leaves	2,755.49	3.08	2,775.49	3.39
Shoot fresh mass	10,642.24	11.89	10,642.24	13.00
Collar diameter	6,949.81	7.77	0.00	0.00
Shoot dry mass	8,779.81	9.81	8,879.81	10.84
Total	89,487.60	100.00	81,883.42	100.00

Knowing this information is essential to advance the understanding of genetic variability and develop more efficient and targeted genetic breeding strategies. The importance of plant height and leaf area was verified in other studies of genetic divergence in leafy vegetables such as lettuce (*Lactuca sativa* L.) (Azevedo et al. 2013), kale (*Brassica oleracea* var. *acephala*) (Azevedo et al. 2016, Brito et al. 2021) and jambu [*Acmella oleracea* (L.) R. K. Jansen].

It was observed that the AP-1, AM-7 and AM-3 accessions stood out with better values, in relation to the average plant height, indicating that these genotypes have a promising potential for the development of varieties with larger size and, possibly, higher yield (Table 5). AP-1 and AM-7 showed a superior performance, in terms of fresh mass, dry mass and leaf area. These characteristics are extremely important for plant yield and quality, since fresh mass is directly related to the ability to accumulate biomass, and dry mass is associated with the accumulation of important substances such as nutrients and bioactive compounds. The leaf area is an indicator of the plant's photosynthetic capacity, which can significantly impact its growth and development.

The characteristics highlighted in the AM-07 and AP-01 genotypes are of fundamental importance for decision-making, regarding the genetic breeding of Amazonian spinach. By selecting and working with these genotypes, breeding programs have the opportunity to develop more productive varieties that are better suited to market demands and local environmental conditions (Shi et al. 2017).

Understanding these genetic relationships and identifying the most promising genotypes allow efforts to be directed toward maximizing the agronomic potential of Amazonian spinach and contributing to food security and agricultural sustainability. In this sense, evaluations of morphoagronomic traits are fundamental for determining genetic diversity and selecting criteria for spinach breeding (Shi et al. 2017).

The evaluation of multicollinearity among the variables revealed a condition number (CN) of 3,453.68, which, according to Montgomery & Peck (1981), characterizes severe collinearity (CN > 1,000). This condition indicates a high degree of linear dependence among variables, which can compromise the accuracy of certain multivariate analyses (Hair et al. 2014). The variance inflation factors reinforced this diagnosis, showing high values for shoot fresh (297.66) and dry mass (314.46), followed by leaf area (29.45), number of leaves (17.77), plant height (13.52), collar

Table 5. Average plant height (PH), leaf area (LA), shoot fresh (SFM) and dry mass (SDM) of 16 genotypes of Amazonian spinach.

Genotypes	PH (cm)	LA (cm²)	SFM (g plant⁻¹)	SDM (g plant⁻¹)
AM-01	29.50 e*	429.48 f	266.47 f	52.04 f
AM-02	37.33 c	629.35 c	347.58 c	77.31 c
AM-03	43.23 a	700.00 b	391.55 b	87.99 b
AM-04	34.78 d	543.93 e	270.74 f	59.56 e
AM-05	39.13 b	692.10 b	385.44 b	85.40 b
AM-06	29.50 e	429.48 f	258.05 f	50.06 f
AM-07	45.09 a	750.67 a	433.73 a	93.30 a
AC-01	33.08 d	535.61 e	261.05 f	57.43 e
AC-02	36.08 c	548.84 e	270.39 f	59.48 e
AC-03	40.07 b	587.68 d	288.80 e	63.54 e
AC-04	31.26 e	577.02 d	274.69 f	60.43 e
RR-01	29.50 e	429.48 f	266.47 f	52.04 f
RR-02	35.43 c	516.16 e	235.43 g	51.79 f
RR-03	39.56 b	634.61 c	315.94 d	69.51 d
RO-01	35.85 c	533.01 e	240.71 g	52.96 f
AP-01	45.09 a	742.32 a	424.38 a	91.02 a

* Averages followed by the same letter do not differ (p > 0.05) from each other according to the Scott-Knott test.

diameter (12.62), plant width (11.55) and number of branches (7.57).

Despite the severe collinearity, all variables were retained in the analysis. This decision was based not only on the agronomic relevance of the traits, but also on the exploratory nature of the study and its central aim of understanding the genetic diversity among Amazonian spinach genotypes. This is a species with very few studies reported in the scientific literature, especially within the Amazon context, making it essential to preserve phenotypic information as much as possible.

As discussed by Cruz & Carneiro (2006), in diversity studies, the exclusion of variables should take into account their biological importance, even in the presence of high collinearity. The authors illustrate this approach with the example of plant height and grain yield in maize, which should be retained due to their relevance to genetic breeding. Similarly, in the present study, retaining traits related to morphology and shoot biomass production allowed for a more accurate representation of the phenotypic variability within the evaluated germplasm. This methodological choice aligns with the need to generate robust baseline knowledge about the species, contributing to future strategies for the conservation and sustainable use of local genetic resources.

Genetic dissimilarity analysis using the Mahalanobis' distance (D2) was performed among the 16 genotypes of Amazonian spinach based on morphoagronomic traits (Table 6). The dissimilarity values varied widely, ranging from 0.43 to 2,632.03, indicating the presence of considerable genetic diversity among the studied genotypes (Cruz et al. 2012, Zeist et al. 2022).

It was observed that the combination of the AM-01 and AP-01 genotypes presented the highest dissimilarity ($D^2 = 2632.03$), whereas the lowest was found between AM-01 and RR-1 ($D^2 = 0.43$) (Table 6). These genetic differences are crucial for the selection of parents in breeding programs, since more distant genotypes tend to produce hybrids with a greater potential for heterosis, which may result in more productive and high-quality offspring (Paterniani et al. 2008, Rotili et al. 2012, Santos et al. 2014).

It is noteworthy that the AP-1 genotype was present in most combinations with greater distances, suggesting that it is the most divergent and, therefore, a promising candidate for future crosses (Almeida et al. 2011). The identification of genotypes with desirable traits and high dissimilarity is essential for obtaining a progeny that accumulates the benefits of both traits (Silva et al. 2015).

The cluster analysis of the 16 Amazonian spinach genotypes using the Tocher's optimization method resulted in the formation of five distinct groups (Table 7). Groups II and IV had a higher number of representatives, comprising, together,

Table 6. Estimated dissimilarity of 16 genotypes of Amazonian spinach based on morphoagronomic traits using the Mahalanobis' distance (D^2).

Genotype	Greater dissimilarity	Genotype	Lower dissimilarity	Genotype
1 - AM-1	2,632.03	(16)	0.43	(12)
2 - AM-2	1,317.77	(16)	49.13	(5)
3 - AM-3	937.88	(1)	117.66	(14)
4 - AM-4	1,963.00	(16)	44.81	(13)
5 - AM-5	1,501.66	(16)	49.13	(2)
6 - AM-6	2,610.99	(16)	0.93	(1)
7 - AM-7	2,472.70	(1)	22.69	(16)
8 - AC-1	2,437.11	(16)	17.13	(13)
9 - AC-2	2,360.05	(16)	8.76	(13)
10 - AC-3	1,483.00	(16)	37.19	(15)
11 - AC-4	1,430.56	(16)	52.43	(10)
12 - RR-1	2,632.03	(16)	0.61	(1)
13 - RR-2	2,388.02	(16)	8.76	(9)
14 - RR-3	854.42	(16)	98.22	(2)
15 - RO-1	1,605.93	(16)	37.19	(10)
16 - AP-1	2,632.03	(1)	22.69	(7)
Lower dissimilarity			0.43	(1 and 12)
Greater dissimilarity			2,632.03	(1 and 16)

Table 7. Grouping of 16 genotypes/accessions of Amazonian spinach (*Alternathera sessilis* L.) using the Tocher's optimization method, based on morphoagronomic traits, according to the dissimilarity expressed by the generalized Mahalanobis' distance.

Groups	Access						%
I	AM-1	RR-1	AM-6				18.75
II	AC-2	RR-2	AC-1	AM-4	AC-3	RO-1	37.50
III	AM-7	AP-1					12.50
IV	AM-2	AM-5	AC-4	RR-3			25.00
V	AM-3						6.25
Total			16				100.00

62.5 % of the studied genotypes. It is interesting to note that the composition of the groups includes genotypes from different states, indicating the geographical diversity of the analyzed sample.

Among the formed groups, group III, composed of the accessions AM-7 and AP-1, stands out, what can be considered promising due to the high averages of desirable traits of the crop, such as dry weight (Table 7). This combination of genotypes with advantageous characteristics may be valuable for future crossbreeding, aiming to obtain offspring with potential for higher yield and quality, as confirmed in studies on other leafy vegetables such as lettuce (Candido et al. 2018) and kale (Azevedo et al. 2016).

The Tocher's optimization method is a strategy that seeks to optimize the partitioning of genotypes, looking for groups where the internal distances are smaller than the distances among groups (Cruz et al. 2021). This makes it possible to identify and group genotypes with greater similarity, facilitating the selection of parents in genetic breeding programs.

Studies evaluating genetic distance in plant populations have been of great importance for germplasm bank management and parent selection in genetic breeding (Rêgo et al. 2012). The analysis of genetic diversity among genotypes contributes to the identification of promising materials and the targeting of breeding strategies aimed at developing more adapted and productive varieties.

Cluster analysis is a fundamental tool for the conservation and breeding of plant species, as it allows the identification of promising genetic materials and the selection of the most favorable genetic combinations for the development of new varieties. Based on knowledge of the genetic similarity among genotypes, breeding programs can be more efficient and targeted, aiming at the development of more adapted, productive and high-quality cultivars.

CONCLUSIONS

1. There is genetic diversity among the studied Amazonian spinach genotypes;
2. Promising combinations were obtained for the development of new cultivars with desirable cultivation traits;
3. The AP-1 and AM-7 genotypes stand out with higher values for the averages of the main evaluated traits, such as plant height, shoot fresh mass and leaf area.

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