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Stability of genetic divergence among five mentrasto accessions in two environments

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ABSTRACT - *The stability of genetic divergence in five accessions of mentrasto (*Ageratum conyzoides*) was estimated in two environments (field and greenhouse) and for five harvest times. At each harvest, the following botanical agronomical traits were evaluated: fresh biomass of the aerial part, dry biomass, leaf area, flowering and height in three replications. The grouping analysis (by Tocher's method) of the first, fourth and fifth harvest on the field formed two groups, and three groups for the second and third harvest. In the greenhouse, the first and fourth harvests formed two groups and the second, third and fifth formed three. Variation was observed in the group constitutions with the harvesting times. The genetic divergence among mentrasto accessions is related to the development stage as well as the environment where it was estimated.*

Key words: Genetic plant resources, genetic divergence, *Ageratum conyzoides*, multivariate methods.

INTRODUCTION

Genetic variation in natural plant and animal populations is the basis of their resistance against environmental pressure and represents the raw material for natural selection. Plants that occur along an environmental gradient, for instance, also vary in their genetic constitution and physiological activity, conditioned by the natural selection process. In spite of belonging to a same species, they can respond in very different ways to a given degree of environmental pressure (Piris and Gripp 1988).

Studies on the genetic divergence are important in improvement programs since they provide information for the identification of those parents which in crosses allow a greater heterotic effect in the progeny and higher probability of recovering superior genotypes in the segregating generations (Silva et al. 2001, Castro et al. 2004).

Mentrasto (*Ageratum conyzoides)* is a native plant of the Americas with adaptation to diverse environmental conditions, established in various tropical and subtropical climate regions around the world (Ladeira et al. 1987).

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Studies on the essential oil of *Ageratum conyzoides* in rats demonstrated its considerable analgesic, anti-inflammatory and antipyretic effect while gastric toxicity was not observed (Abena et al. 1996, Magalhães et al. 1997, Bowers et al. 1976, Okunade 2002). The consumption of mentrasto has increased in Brazil and in other countries, since it was included in the list of the Central de Medicamentos, with analgesic and anti-inflammatory properties (Oliveira et al. 1993).

This study had the objective to study the stability of genetic divergence in mentrasto accessions, originated from different geographical regions but cultivated under the same environmental conditions, through botanical agronomical traits and using multivariate methods.

MATERIAL AND METHODS

Five mentrasto accessions were studied, collected in the counties of Mariana-MG (AMA), Piranga-MG (API), Visconde do Rio Branco-MG (ARB) and Viçosa-MG (AVB and AVP). The accessions from Viçosa county were collected in the rural regions Bom Sucesso (AVB) and Paraíso (AVP). Seeds from 15 plants were collected in each county and later the same quantity of seeds per plant removed to form the "bulk" and establish the experiment.

The stability of the genetic divergence was analyzed in two environments, field and greenhouse, on an experimental area of the Universidade Federal of Viçosa; the accessions were seed-propagated.

The experimental design was completely randomized, in a split plot arrangement. The plots consisted in five accessions of mentrasto and the subplots were represented by five sampling periods. Samples were collected every 21 days (destructive method), with three replications. Five plants per subplot were used in the experiment realized in the field, and in the greenhouse experiment three plants per subplot.

Five botanical agronomical traits were evaluated: fresh biomass of the aerial part, dry biomass, leaf area, flowering, and height.

To obtain the total leaf area (LA) per plant a regression equation was initially adjusted in function of the data of leaf length (C) and leaf width (L): $LA = -10.0945 + 0.5853\,C + 6.4263\,L$ ($R^2 = 0.9483$).

The dynamics of flowering were determined using the following stages of flower development:

E0: plants without flowers (grade = 0); E1: initial inflorescence development (grade = 1); E2: open inflorescence (grade = 2); E3: darkening inflorescence (grade = 3); E4: seed dropping (grade = 4).

Five cluster analyses were realized separately for each period of sampling, using software Genes. To calculate the genetic distance the generalized distance of Mahalanobis (D^2) was obtained and to discriminate the dissimilarity groups of accessions Tocher's method was adopted (Cruz and Regazzi 1997).

The mean distance within the group was estimated by calculating the mean of the distances between pairs of accessions of one and the same group; the distance between groups is estimated by the mean of the distances between all pairs of accessions of the groups. The criterion of Singh (1981) was used to identify the relative contribution of each trait to the diversity between the accessions.

The correlation of Spearman was estimated between the genetic distances obtained in each environment in the harvest periods.

RESULTS AND DISCUSSION

Field

The groups formed by the method of Tocher in the five harvest periods are diplayed in Table 1, and the intra and inter-groups distances (D^2) in Table 2. The relative contribution of the traits fresh biomass (FB), dry biomass (DB), height (ALT), flowering (FLOR), and leaf area (LA) to genetic divergence in percentage is shown in Table 3.

First harvest period (21 days after transplanting)

Two groups were formed (Table 1). The most divergent pair was formed by accessions ARB and AVB, with $D^2 = 69.71$, while the least divergent pair were the accessions AMA and AVP, with $D^2 = 3.33$. The trait that contributed least to genetic divergence was height, and the one that contributed most was fresh biomass (Table 3).

Second harvest period (42 days after transplanting)

Three groups were formed in this harvest (Table 1), presenting a higher potential for differentiation of the accessions than the other harvests, with exception of the third period, in which three groups were formed also. The mean inter-group distances were higher than those obtained in the first and third harvest (Table 2), demonstrating greater capacity of distinction of the traits. The most divergent accessions were ARB and AVB ($D^2 = 331.91$), and the least divergent pair consisted in the accessions AMA and AVP ($D^2 = 16.96$). The trait leaf area contributed least and the trait dry biomass most to the genetic divergence (Table 3).

Table 1. Groups of similar accessions of mentrasto (*Ageratum conyzoides)* established by means of the method of Tocher in five harvest periods (field)

Groups	Harvests				
	21 dat	42 dat	63 dat	84 dat	105 dat
1	AMA, API, ARB and AVP	AMA, API and AVP	AMA, ARB and AVB	AMA, API, AVB and AVP	AMA, API, ARB and AVP
2	AVB	AVB	AVP	ARB	AVB
3		ARB	API		

dat: days after transplanting

Table 2. Intra and inter-group distance of Mahalanobis (D^2) between five accessions of mentrasto (*Ageratum conyzoides)* in five harvest periods (field)

Group	I	II	III
I	13.09 (1)	57.20	-
	20.59 (2)	89.19	106.39
	11.37 (3)	30.76	47.90
	48.86 (4)	271.55	-
	42.47 (5)	177.05	-
II		0	-
		0	331.91
		0	134.36
		0	-
		0	-
III			-
			0
			0
			-
			-

(1) **:** harvest on the 21st dat
(2) **:** harvest on the 42nd dat
(3) **:** harvest on the 63rd dat
(4) **:** harvest on the 84th dat
(5) **:** harvest on the 105th dat

Third harvest (63 days after transplanting)

Three groups were formed (Table 1). The most divergent pair of accessions was formed by the accessions API and AVP (D^2 = 134.36), and the least divergent pair by accessions AMA and AVB (D^2 = 5.24). The trait that contributed least to the genetic divergence among accessions was flowering, and the one that contributed most was dry biomass (Table 3).

Fourth harvest period (84 days after transplanting)

Two groups were formed (Table 1). In this harvest the greatest distance between groups I and II was obtained (Table 2). The most divergent pair of accessions was formed by ARB and AVP (D^2 = 395.40), and the least divergent by the accessions AMA and AVB (D^2 = 18.97). The trait that contributed most to genetic divergence among accessions was dry biomass, and the one that contributed least was flowering (Table 3).

Fifth harvest period (105 days after transplanting)

Two groups were formed in this harvest (Table 1). The most divergent pair was formed by the accessions ARB and AVB (D^2 = 291.42), and the pair that presented least divergence by the accessions API and AVP (D^2 = 5.41). The trait that contributed least to genetic divergence among accessions was flowering, and the one that contributed most was fresh biomass (Table 3).

The pairs of accessions AMA and API, AMA and AVP and API and AVP were grouped in the same clusters in four harvests, showing similarities between accessions. The pairs of accessions with least genetic similarity were API and AVB, ARB and AVB and AVB and AVP, which formed the same groups in only one harvest. Accessions ARB and AVB represented the most divergent pair in three harvests (first, second and fifth harvest).

When analyzing the structure and composition of the groups formed in the harvests, one notes variation in the constitution of the accession groups (Table 1). Except for group 1 in the first and fifth harvests and for the groups formed exclusively by accessions ARB and AVB, all groups varied in composition. This fact demonstrates the inconsistent composition of the groups formed in the harvests.

Greenhouse

The results of the cluster analysis, the intra and inter-group distances and the relative contribution in percent of the analyzed traits are shown in Tables 4, 5 and 6, respectively.

Table 3. Relative contribution to the genetic divergence (D^2) in percent, analyzed based on the criterion of Singh (1981) of the traits fresh biomass (FB), dry biomass (DB), height (ALT), flowering (FLOR), and leaf area (LA) of five accessions of mentrasto (*Ageratum conyzoides)* in five harvest periods (field)

	Harvests				
Variables	21 dat	42 dat	63 dat	84 dat	105 dat
FB	69. 50	28. 10	15. 75	19. 99	3. 17
DB	3. 64	62. 08	72. 63	74. 54	92. 65
ALT	1. 20	3. 58	7. 87	3. 87	2. 11
FLOR	17. 21	4. 21	3. 76	1. 60	2. 08
LA	8. 45	2. 04	-	-	-

dat : days after transplanting

First harvest period (21 days after transplanting)

Two groups were formed in this harvest (Table 4). The most divergent pair of accessions was AMA and ARB (D^2 = 1010.62), while API and AVB formed the least divergent pair (D^2 = 23.74). The trait that contributed least to genetic divergence was flowering, and the one that contributed most was dry biomass (Table 6).

Second harvest period (42 days after transplanting)

The formation of three groups was observed in this harvest (Table 4), with higher mean inter-group distances (Table 5) than in the first, third and fourth harvests, presenting a greater capacity of discrimination of traits. The inter-group distance in this harvest was only smaller than the inter-group distance of the fifth harvest between groups I and III. The most divergent pair of accessions was API and AVP (D^2 = 4950.69), while the least divergent was AMA and AVB (D^2 = 28.83).

Compared to the groups formed (Table 4) in the first two harvests, only accessions AMA and AVB remained in the same group. Accession ARB, which had formed an isolated group from all other accessions in the first harvest, participated in a group with accession API in the second harvest. The trait height was the one that contributed least to genetic divergence while the trait dry biomass contributed most (Table 6).

Third harvest period (64 days after transplanting)

Three groups were formed in this harvest (Table 4) that presented, together with the second and fifth harvests, a greater potential for differentiation of the accessions than the other periods. The most divergent pair of accessions was ARB and AVP (D^2 = 1718.92) and the least divergent was AMA and AVP (D^2 = 21.35). The trait that contributed most to genetic divergence was dry biomass, and the one that contributed least was flowering (Table 6).

Fourth harvest period (84 days after transplanting)

Two groups were formed in this harvest (Table 4). The most divergent pair of accessions was formed by API and AVB (D^2 = 502.71) and the least divergent by the accessions AMA and ARB (D^2 = 8.87). The trait that contributed most to the genetic divergence in accessions was fresh biomass, and the one that contributed least was flowering (Table 6).

Fifth harvest period (105 days after transplanting)

Three groups were formed (Table 4). The most divergent pair of accessions was formed by accessions AMA and AVP (D^2 = 462.34) and the least divergent pair by AMA and API (D^2 = 4.05). The trait that contributed most to the genetic divergence in accessions was dry biomass, and the one that contributed least was height (Table 6).

The pairs of accessions AMA and API, AMA and AVP and API and ARB grouped together in the same clusters in three harvests, evidencing the genetic similarity among them. The pairs of accessions AMA and ARB, API and AVB, ARB and AVB, ARB and AVP and AVB and AVP formed

Table 4. Groups of similar accessions of mentrasto (*Ageratum conyzoides)* established by the method of Tocher in five harvest periods (greenhouse)

	Harvests				
Groups	21 dat	42 dat	63 dat	84 dat	105 dat
1	AMA, API, AVB and AVP	AMA and AVB	AMA and AVP	AMA, API, ARB and AVP	AMA and API
2	ARB	API and ARB	API and ARB	AVB	ARB and AVB
3		AVP	AVB		AVP

dat: days after transplanting

a group together in only one harvest, thus demonstrating less genetic similarity.

Analyzing the structure and composition of the groups formed in the harvests, we observed that the accessions formed different groups in each harvest. Only the groups containing accessions API and ARB (second and third harvests), AVB (third and fourth harvests) and AVP (second and fifth harvest) did not vary in their composition. This fact characterizes the inconsistence of the composition of the groups formed under experimental greenhouse conditions.

The highest inter-group (I and II) values of Mahalanobis distance in the greenhouse compared with the field results 21, 42, 63, and 84 days after transplanting, demonstrates a greater capacity of distinction of the traits. The same is true for the highest inter-group (I and III; II and III) values in the greenhouse.

A tendency of reduction of the relative contribution to the genetic divergence of the fresh biomass was observed on the field (Table 3) which is related with the capacity of absorbing and maintaining the moisture content in the tissues of the accessions. In this sense, the mentrasto accessions presented greater differences in relation to the moisture content in tissues at the beginning of the development, tending to a gradual reduction of this difference until the last harvest, in which the least contribution to genetic divergence of the fresh biomass was observed.

On the other hand, a tendency of increasing relative contribution to genetic divergence of dry biomass was observed in the field (Table 3), a fact that is related with the photosynthetic efficiency and fiber formation in the stems, leaves and flowers, as well as lignin accumulation.

No tendency to a reduced contribution of fresh biomass to genetic divergence was observed in the greenhouse, however only 84 days after transplanting a higher value was observed than for the first harvest (Table 6). In relation to dry biomass, an increase in the values of 21 to 63 days after transplanting was verified, with a reduction 84 days and an increase 105 days after transplanting (Table 6).

The results of the contribution to genetic divergence of flowering differed from each other on the field and in the greenhouse. A reduced contribution to genetic divergence of flowering was verified on the field since the reproductive phase of the accessions began in different periods, and in the last period all accessions were in the stage of seed maturation (Table 3). Low values were observed in the greenhouse in all harvests, indicating a greater uniformity in the dynamics of flowering among accessions (Table 6).

Spearman Correlation

Based on the distances between accessions, a coefficient of positive and significant correlation was observed only between the following harvests: first harvest in the field and fifth harvest in the field (Table 7); second harvest in the field and third harvest in the field (Table 7); and second harvest in the greenhouse and third harvest in the greenhouse (Table 8). Only the second and third harvest presented significant correlation in the field and the greenhouse. Genetic divergence must therefore be estimated separately for each harvest.

Table 5. Intra and inter-group distance of Mahalanobis (D^2) between five accessions of mentrasto (*Ageratum conyzoides)* in five harvest periods (greenhouse)

Group	I	II	III
	117.75 (1)	718.66	-
	28.83 (2)	2633.94	310.29
I	21.35 (3)	1204.24	223.92
	30.57 (4)	342.61	-
	4.05 (5)	150.14	433.35
		0	-
		31.39	4701.63
II		147.50	427.82
		0	-
		16.62	85.72
			-
			0
III			0
			-
			0

(1) = harvest on the 21st dat
(2) = harvest on the 42nd dat
(3) = harvest on the 63rd dat
(4) = harvest on the 84th dat
(5) = harvest on the 105th dat

Table 6. Relative contribution to the genetic divergence (D^2) in percent, analyzed based on the criterion of Singh (1981) of the traits fresh biomass (FB), dry biomass (DB), height (ALT), flowering (FLOR), and leaf area (LA) of five accessions of mentrasto (*Ageratum conyzoides)* in five harvest periods (greenhouse)

Variables	Harvests				
	21 dat	42 dat	63 dat	84 dat	105 dat
FB	45.552	12.08	25.56	50.08	31.34
DB	52.0617	84.49	73.28	44.20	62.45
ALT	0.334	0.44	0.23	0.40	3.44
FLOR	0.013	1.60	0.94	5.33	2.77
LA	2.04	1.39	-	-	-

dat: days after transplanting

Table 7. Coefficients of correlation (Spearman) between the distances of five accessions of mentrasto for five harvest periods in the field

	2c	3c	4c	5c
1c	0.4545	-0.2606	-0.3455	0.7818 **
2c	-	-0.6121*	0.2606	0.3455
3c	-	-	-0.0788	-0.4788
4c	-	-	-	-0.1030

1c: first harvest in the field; 2c: second harvest in the field; 3c: third harvest in the field; 4c: fourth harvest in the field; 5c: fifth harvest in the field; ** , *: significant by the test "t" at 1% and 5% probability

Table 8. Coefficients of correlation (Spearman) between the distances of five accessions of mentrasto for five harvest periods in the greenhouse

	2cv	3cv	4cv	5cv
1cv	-0.0667	0.5273	-0. 5273	-0.1636
2cv	-	0.7212*	-0.3091	0.1758
3cv	-	-	-0.5030	-0.2364
4cv	-	-	-	-0.5152

1cv: first harvest in the greenhouse; 2cv: second harvest in the greenhouse; 3cv: third harvest in the greenhouse; 4cv: fourth harvest in the greenhouse; 5cv: fifth harvest in the greenhouse; **, *: significant by the test t at 1% and 5% probability

CONCLUSIONS

Variation was observed in the constitution of the accession groups according to the development stage and the environment of estimation. Genetic divergence among mentrasto accessions is therefore related with a particular development stage and the environment in which it is estimated.

The occurrence of significant correlation between the second and third harvest in the field and the greenhouse shows the existence of correlation between the distances of accessions in these periods, regardless of the environment in which it was estimated.

Estabilidade da divergência genética entre cinco acessos de mentrasto em dois ambientes

RESUMO - *A estabilidade da divergência genética entre cinco acessos de mentrasto (*Ageratum conyzoides) *foi estimada em dois ambientes (campo e casa de vegetação) em cinco épocas de colheita. Em cada época de colheita foram avaliadas as seguintes características botânico-agronômicas: biomassa fresca da parte aérea, biomassa seca, área foliar, floração e altura, utilizando três repetições. A análise de agrupamento (método de Tocher) no campo formou dois grupos na primeira, quarta e quinta épocas, e três grupos na segunda e terceira épocas. Na casa de vegetação foram formados dois grupos na primeira e quarta épocas de colheita, e três grupos na segunda, terceira e quinta épocas de colheita. Observou-se variação na constituição dos grupos pelos acessos nas épocas de colheita. A divergência genética estimada entre pares de acessos de mentrasto está relacionada com o estádio de desenvolvimento e com o ambiente no qual foi estimada.*

Palavras-chave: Recursos genéticos vegetais, divergência genética, *Ageratum conyzoides,* métodos

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