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Divergence and estimates of genetic parameters in *Crambe abyssinica*: an oilseed plant for industrial uses¹

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ABSTRACT

Crambe's breeding is still incipient, with few cultivars of interest. The aims of the present study were: i) to evaluate the divergence between 10 genotypes of *crambe*, through morphoagronomic and quality traits, using multivariate analyses; and ii) to estimate genetic parameters related to these traits. The trial was conducted in a greenhouse, and the experimental data were submitted to multivariate analysis and genetic evaluation. The genotypes differed significantly among them for plant height (PH), stem diameter (SD), number of branches per plant (NB), height of the first productive branch (HPB), mass of 1000 grains (M1000), grain yield (YIELD) and oil productivity (OIL). The experimental coefficient of variation revealed good precision (4.29 to 13.81%). The averages of YIELD (1936.94 kg/ha) and OIL (660.10 kg/ha) were high. The traits presented high estimates of broad-sense heritability on a mean genotype basis ($h^2 > 73.65$). The cluster analysis revealed five clusters of genotypes. YIELD showed the greatest contribution (22.31%) to the diversity among the genotypes, followed by HPB, NB and SD. Genotypes 2 and 4 can be used as parents at future crosses, as they are promising in obtaining segregant populations.

Keywords: biofuels; plant breeding; oilseed plant; Brassicaceae.

RESUMO

Divergência e estimativas de parâmetros genéticos em *Crambe abyssinica*: uma planta oleaginosa para usos industriais

O melhoramento do *crambe* ainda é incipiente, com poucas cultivares de interesse. Os objetivos do presente trabalho foram: i) avaliar a divergência entre 10 genótipos de *crambe*, por meio de características morfoagronômicas e de qualidade, utilizando análises multivariadas e; ii) estimar parâmetros genéticos relativos a essas características. O experimento foi conduzido em casa de vegetação, e os dados obtidos foram submetidos a análises multivariadas e de avaliação genética. Os genótipos diferiram significativamente entre si quanto a altura de planta (ALT), diâmetro do caule (DC), número de ramos por planta (NR), altura do primeiro ramo produtivo (ARP), massa de 1000 grãos (M1000), produtividade de grãos (PROD) e de óleo (PO). O coeficiente de variação revelou boa precisão experimental (4,29 a 13,81%). As médias de PROD (1936,94 kg/ha) e de PO (660,10 kg/ha) foram elevadas. As características apresentaram altas estimativas de herdabilidade em nível de médias de progênes ($h^2 > 73,65$). A análise de cluster revelou cinco grupos de genótipos. PROD foi a característica com maior contribuição (22,31%) para a diversidade entre os genótipos, seguida por ARP, NR e DC. Os genótipos 2 e 4 podem ser utilizados como genitores em cruzamentos futuros, pois são promissores na obtenção de populações segregantes.

Palavras-chave: biocombustíveis; melhoramento de plantas; oleaginosas; Brassicaceae.

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INTRODUCTION

Crambe (*Crambe abyssinica* Hochst) is a hexaploid ($2n = 6x = 90$) species and an important Brassicaceae oilseed (Du *et al.*, 2014). This family covers about 350 genus and 3200 species. Other species of this family, such as canola (*Brassica napus*) and various types of mustard (*B. juncea*, *B. campas*, *B. alba*, *B. nigra*) are similar to crambe (Desai *et al.*, 2004). The genus *Crambe* is composed of more than 30 species, being *C. abyssinica* the only cultivated (Desai *et al.*, 2004).

In the past, crambe was used as fodder for soil cover. Currently, the plant presents high potential for oil production (Carlsson, 2009). The oil extracted from crambe seeds has the potential to be used in the manufacture of plastic films, adhesives, nylon, thermal insulation, corrosion inhibitors, synthetic rubber and industrial lubricant (Carlson *et al.*, 1996; Falasca *et al.*, 2010). The crambe seeds oil can be used advantageously for the production of biodiesel (Tavares *et al.*, 2017), as it has a higher resistance to degradation and oxidation (Lalas *et al.*, 2012). However, when refined, this same oil can be used in the production of cosmetics (Falasca *et al.*, 2010) and waxes (Zhu *et al.*, 2016). In addition, crambe bran, a byproduct of oil extraction, presents excellent nutritional quality, with up to 45% crude protein, and can be used as a protein supplement in ruminant feed (Carlson *et al.*, 1996).

The interest in the crambe's crop is increasing and its breeding is in progress. In Brazil, there is already a cultivar adapted to its conditions, the 'FMS Brilhante', obtained by the MS Foundation through the lineages from Mexico in the 1990s. In other countries, such as the USA, there are other cultivars such as Meyer, Indy, Bellan, Prophet and Bellinzian. The main objectives for the breeding in this crop are: increase of grain yield, oil content, erucic acid content in oil, lower seed dormancy, among others (Knights, 2002).

Due to the high productivity, oil and erucic acid content and the possibility of using this crop in the autumn-winter season for oil production and cycle breakdown of several pathogens, researches with crambe has been intensified in the last years. However, the information is still incipient of for this crop, lacking more studies in the genetic improvement.

In breeding programs, studies on genetic divergence to identify potential crossings and to know the genetic basis of genotypes are extremely important. However, efficient methods of quantifying genetic diversity assist in selection success of the best individuals, maximizing gain and the efficiency in development of new cultivars (Farias Neto *et al.*, 2008). The multivariate analysis in the quantification of genetic divergence is an advantageous

method, since it allows identifying sources of variability evaluates the importance of each measured trait and knows the heterotic hybrid combinations, even before the crossings (Sávio *et al.*, 2008).

Another important analysis in breeding programs is the estimation of genetic parameters, since they allow to identify the nature of gene action involved in the control of quantitative traits, as well as to evaluate the efficiency of breeding strategies to obtain genetic gains (Cruz *et al.*, 2014). One of the most important genetic parameters is the heritability coefficient, which measures the proportion of genetic variation in relation to the total variation. However, this coefficient may suffer variations owing to changes in environmental conditions (Falconer & Mackay, 1996).

In this context, the aims of this work were: i) to evaluate the genetic divergence among 10 crambe genotypes, through morphoagronomic and quality traits, using multivariate analyses, and ii) estimate the genetic parameters related to these traits.

MATERIAL AND METHODS

Experimental design and evaluated traits

The trial was conducted in a greenhouse of the experimental field "Diogo Alves de Mello", (20° 45' S, 42° 51' W and 650 m asl), at the Universidade Federal de Viçosa (UFV), in Viçosa-MG, Brazil, from April to July 2016. The experimental design used was a randomized complete block with 10 treatments (genotypes), four replications, being each replication with 5 plants totaling 200 experimental units. The seeds, provided by the Mato Grosso do Sul Foundation (MS Foundation), were treated with the fungicide and planted at a depth of approximately 3 cm, using three seeds per pot. Twenty days after sowing, thinning was performed to conduct one plant per pot.

The following traits were evaluated:

- Plant height (PH, in cm), measured with a measuring tape, as the distance between the soil surface and the apex of the plant;
- Stem diameter (SD, in mm). Measurements performed close to the ground using a pachymeter;
- Number of branches per plant (NB), obtained by counting the branches in each plant;
- Height of the first productive branch (HPB, in cm), corresponding to the measurement of the distance between the soil surface to the first productive branch;
- Mass of 1000 grains (M1000, in g), measured by weighing eight sub-samples of 100 grains per plant. The samples were weighed, correcting the degree of humidity to 9%. M1000 was determined according to the Rules for Seed Analysis (Brasil, 2009);

- Grain yield (YIELD, in kg/ha), evaluated through the harvest of each plant, and the value obtained being transformed to kg/ha, with humidity correction to 9%;

- Oil content (OIL, in %), measured using the Soxhlet extractor. For this, 10 g of crushed seeds were used, which were placed in pre-weighed filter paper cartridges, recording the combined weight. The samples were taken to Soxhlet using hexane extractor at 80 °C and subsequently at 110 °C for 4 hours. After this process, the samples were placed in a drying oven at 70 °C for 24 hours. After this time, the cartridges were weighed again to determine the oil content based on the dry mass. This evaluation was realized at the Biotechnology Laboratory of the Department of Fitotecnia (Plant Science) at UFV. The oil content was estimated using the formula:

$$OIL = \frac{CBE - CAE}{CBE - CWB} * 100$$

wherein:

CBE: cartridge with sample before extraction

CAE: cartridge with sample after extraction

CWB: cartridge without samples before extraction

- Oil productivity (OP in kg/ha), obtained by the expression:

$$OP = \frac{(YELD * OIL)}{100}$$

Statistical analysis

The data were performed to univariate analysis of variance (ANOVA) to evaluate the existence of genetic variability among the genotypes, according to the statistical model: $Y_{ij} = \mu + g_i + b_j + \varepsilon_{ij}$

wherein:

Y_{ij} : value observed for a trait, relative to the i-th genotype, in the j-th block;

μ : general mean;

g_i : effect associated with the i-th genotype, being $i = 1, 2, \dots, g$;

b_j : effect associated with the j-th block, being $j = 1, 2, \dots, b$;

ε_{ij} : effect of experimental error.

The significance of the mean squares was tested by the F test ($p < 0.01$), the means were compared by the Scott-Knott test ($p < 0.05$) and, from ANOVA, the genetic parameters were estimated for each trait, according to Cruz *et al.* (2012):

- Genetic variation coefficient (%): $CV_g = \frac{\sqrt{\hat{\sigma}_g^2}}{\mu} * 100$

- b quotient (CVg/CVe ratio): $\sqrt{\frac{\hat{\sigma}_g^2}{\hat{\sigma}_e^2}}$

- Mean genotypic variance:

- Mean phenotypic variance: $\hat{\sigma}_p^2 = \frac{MSg}{r}$

- Mean environmental variance: $\hat{\sigma}_e^2 = \frac{MSe}{r}$

- Broad sense heritability on a mean genotype basis:

$$h_m^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_p^2} * 100$$

The multivariate analysis for the quantification of divergence was performed using the generalized Mahalanobis distance (D^2) between genotypes, where the distance between the pair of genotypes i and i' were defined by the expression (Cruz *et al.*, 2012):

$$D_{ii}^2 = \delta_{ii} \psi^{-1} \delta_{ii}$$

D_{ii}^2 : generalized Mahalanobis distance between genotypes i and i', being $i = 1, 2, \dots, g$

$\delta_{ii} = [d_1 d_2 \dots d_n]$ being $D_n = Y_{ij} - Y_{ij'}$ for genotypes i and i' and d is the difference between the two genotypes means i and i' for each evaluated trait;

Y_{ij} is the mean of the i-th genotype in relation to the j-th trait, being $j = 1, 2, \dots, p$

ψ^{-1} inverse of the error variance and covariance matrix.

On the generated Mahalanobis distance matrix was processed clustering analysis by UPGMA (Unweighted Paired Group Method using Arithmetic Averages) algorithm. The relative contribution of the traits to the divergence among genotypes was assessed by the method of Singh (1981), using Mahalanobis generalized distance estimates. All the analytical processing was performed in the Genes software (Cruz, 2013).

RESULTS AND DISCUSSION

Analysis of variance (ANOVA)

Univariate ANOVA showed a highly significant ($p < 0.01$) effect of the genotypes for all traits evaluated, indicating the presence of genetic variability among them. Besides, the estimated genetic variation confirms the variability among the genotypes, since all were different from zero (Table 1).

In general, the coefficient of experimental variation was low, varying from 4.29% for YIELD to 13.81% for HPB, which means that the experiment showed good precision. Lara-Fiozeze *et al.* (2016a), in a study with crambe progenies from individual plant selection, observed higher value of CV for number of branches ($> 14.93\%$), but still with good experimental precision.

For grain yield (YIELD), the average of the 10 crambe genotypes evaluated was 1936.94 kg/ha (Table 1). Genotypes

2, 3, 5, 6 and 8 highlighted as the most productive of the experiment, with yields higher than 2010 kg/ha (Table 2). Viana *et al.* (2015), evaluated the yield of the cultivar FMS Brilhante at different sowing densities and obtained average values ranging from 1706.12 to 2118.57 kg/ha. In work with the same cultivar Zoz *et al.* (2018) obtained grain yield ranging from 1176.7 to 1760.7 kg/ha. The values presented by both works corroborate the high productive potential of the crambe genotypes evaluated in this experiment.

For oil productivity (OIL), the average of the 10 crambe genotypes evaluated was 660.1 kg/ha (Table 1). Genotypes 2, 5, 6 and 8 presented yields higher than 690 kg/ha (Table 2). In a study with the cultivar FMS Brilhante, Lunelli *et al.* (2013) reported an oil productivity of 372 kg/ha, much lower than that observed in this study, which shows superiority in the genotypes evaluated by our research to oil

production. It should be noted that these genotypes are present in the group of higher grain yields. In this way, these materials can be incorporated into future crossover breeding blocks in the genetic improvement of crambe.

The height of the first productive branch (HPB) is an important feature when it comes to mechanized harvesting. The low height makes it difficult to harvest mechanized, which can increase the losses due to the cutting platform working close to the soil (Cortez *et al.*, 2008). It was verified that the average height of the first productive branch of the crambe genotypes evaluated was of 11.20 cm (Table 1), with featured on genotype 4, that presented the highest mean for the HPB when compared to the others (Table 2), being possible the use of mechanized harvest for these genotypes, since the crambe crop can use the same machinery harvesting as soybean (Pitol *et al.*, 2010).

Table 1: Summary of ANOVA and estimates of genetic parameters for plant height (PH), stem diameter (SD), height of the first productive branch (HPB), number of branches (NB), mass of 1000 grains (M1000), grain yield (YIELD) and oil productivity (OIL), evaluated in 10 genotypes of crambe

Sources	df	Mean Squares						
		PH	SD	HPB	NB	M1000	YIELD	OIL
Blocks	3	-	-	-	-	-	-	-
Genotypes	9	169.75**	3.06**	20.58**	9.47**	1.04**	6 7679.78**	7668.85**
Error	27	44.72	0.35	2.39	1.38	0.24	6894.55	1341.00
Mean		146.34	11.05	11.20	16.89	8.90	1936.94	660.10
CV (%)		4.57	5.39	13.81	6.95	5.46	4.29	5.55
Minimum		130.00	8.85	6.20	13.40	7.51	1663.48	582.35
Maximum		162.60	12.91	18.40	21.25	10.19	2191.28	808.58
Estimates of Genetic Parameters								
CV_g (%)		3.82	7.45	19.03	8.42	5.04	6.36	6.03
b		0.84	1.38	1.38	1.21	0.92	1.48	1.09
σ_g^2		32.26	0.68	4.55	2.02	0.20	45196.31	1581.96
σ_e^2		44.72	0.35	2.39	1.38	0.24	6894.55	1341.00
h_m^2 (%)		73.65	88.42	88.36	85.44	77.31	89.81	82.51

** $p < 0.01$, F test. CV: coefficient of variation; CV_g : genetic variation coefficient;

b quotient: ratio between the coefficient of genetic and environmental variation;

σ_g^2 : genotypic variance; σ_e^2 : environmental variance; h_m^2 : broad-sense heritability coefficient on a mean genotype basis.

Table 2: Plant height (PH), stem diameter (SD), number of branches (NB), height of the first productive branch (HPB), mass of 1000 grains (M1000), grain yield (YIELD) and oil productivity (OIL) evaluated in 10 genotypes of crambe

Genotypes	PH	SD	NB	HPB	M1000	YIELD	OIL
1	158.27 a	11.58 a	18.67 a	12.20 b	9.14 a	1863.68 b	625.82 b
2	144.97 b	11.51 a	19.95 a	9.03 d	8.44 b	2090.50 a	693.53 a
3	137.67 b	11.56 a	16.38 b	8.59 d	8.84 a	2012.83 a	661.25 b
4	150.87 a	10.08 b	14.40 b	15.30 a	8.96 a	1840.47 b	632.57 b
5	140.67 b	11.30 a	15.85 b	8.07 d	8.75 a	2064.11 a	698.87 a
6	142.60 b	11.74 a	16.84 b	10.40 c	9.16 a	2075.71 a	708.89 a
7	153.80 a	9.21 c	16.70 b	13.30 b	9.06 a	1822.73 b	627.86 b
8	147.20 b	12.09 a	15.85 b	11.80 b	9.86 a	2039.31 a	727.30 a
9	147.20 b	10.76 b	17.05 b	10.87 c	7.88 b	1786.33 b	623.12 b
10	140.11 b	10.70 b	17.20 b	12.48 b	8.89 a	1773.75 b	601.81 b

Means followed by the same letter in the columns belong to the same group by the Scott-Knott test ($P < 0.05$).

In study of the crambe's performance in function of plant spacing and density, Freitas *et al.* (2013) found lower mean values than those observed in our study for the traits plant height (109 cm), mass of 1000 grains (7.95 g), number of branches (12.91) and grain yield (783 kg/ha). Laghetti *et al.* (1995) obtained an average of 7 g for M1000, while Lara-Fiozeze *et al.* (2016a) observed a mean of 7.72 g for the same trait studied in the county of Botucatu-SP and 6.93 g in the county of São Manuel-SP, in 2011, lower values than those found in our research (8.90 g). The broad variability presented in the crambe genotypes studied is important to be explored at future crosses in breeding programs of the species.

Estimates of genetic parameters

Estimates of genotypic and environmental variances, as well as the heritability coefficient (h_m^2) on a mean genotype basis, the coefficient of genetic variation (CVg) and the b quotient (CVg/CVe ratio) are presented in Table 1. The CVg allows to understand the magnitude of the genetic variability that is present in the population for all the evaluated traits, assisting in the selection of superior genotypes. The CVg ranged from 3.82% to 19.03% for PH and HPB, respectively. In the case of selection, the CVg indicates the proportionality of the gain in relation to the mean. The b quotient indicates a situation favorable to the selection of superior genotypes, when it is greater than the unit (>1), since the genetic variation surpasses the environmental one. It is an important parameter to detect the genetic variability in the population (Resende & Duarte, 2007). The b quotient was higher than the unit for SD, HPB, NB, YIELD and OIL, indicating that the genetic variation was superior to environmental variation and favoring selection for these traits.

The coefficient of heritability is one of the most important parameters in genetic breeding. This coefficient shows how much of genetic variation is inheritable to the generation after selection (Resende, 2015). All the evaluated traits showed high heritability ($h_m^2 > 73.65\%$, for PH), even when dealing with environment-influenced traits such as grain yield (89.81%) and mass of 1000 grain (77.31%). This can be explained by the fact that these

materials have varied origins and, consequently, present variations that allow greater success in the selection of superior genotypes. Lara-Fiozeze *et al.* (2016b) also found high heritability coefficients ($> 74.00\%$) for the traits studied in 32 superior self-progenies of the FMS Brilhante, except for a mass of 1000 grains estimated to be 56.00%.

Genetic divergence

The UPGMA dendrogram, based on the Mahalanobis distance matrix, allowed the separation of the genotypes into five clusters. Clusters III, IV and V were formed by only one genotype each (Figure 1). Genotypes 3 and 6 were the most similar pair, with D^2 equal to 3.29 and genotypes 2 and 4 showed greater dissimilarity with D^2 of 57.52. Cluster I presented the second best average grain yield (2047.99 kg/ha) and the highest productivity of oil (699.08 kg/ha) and mass of 1000 grains (9.15 g), which could be considered a promising group present high average of traits desirable to the culture. Clusters II, composed of three genotypes, obtained moderate grain yield (1812.32 kg/ha) and the lowest oil productivity (620.75 kg/ha), as well as smaller stem diameter (9.99 mm) and number of branches (16.1) (Table 3).

Clusters III, IV and V were represented by only one genotype each, with cluster V highlighting the highest grain yield (2090.50 kg/ha), besides high oil productivity (693.52 kg/ha) and number of branches (19.95), being also a potential group due to the high observed average. Cluster III had the lowest grain yield (1786.66 kg/ha) and 1000 grain mass (7.88 g). The IV cluster had the highest plant height (158.27 cm) and high values for stem diameter (11.57 mm), height of the first productive branch (12.2 cm), number of branches (18.67) and 1000 grains mass (9.14 g) (Table 3). These clusters are important for selection of superior genotypes because they show how much of genetic variability is available. In addition, the clustering allows to distinguish genotypes with higher dissimilarity between them that can be used in future crosses. Therefore, the diversity of plant genetic resources allows breeders the opportunity to develop new cultivars with superior characteristics (Govindaraj *et al.*, 2015).

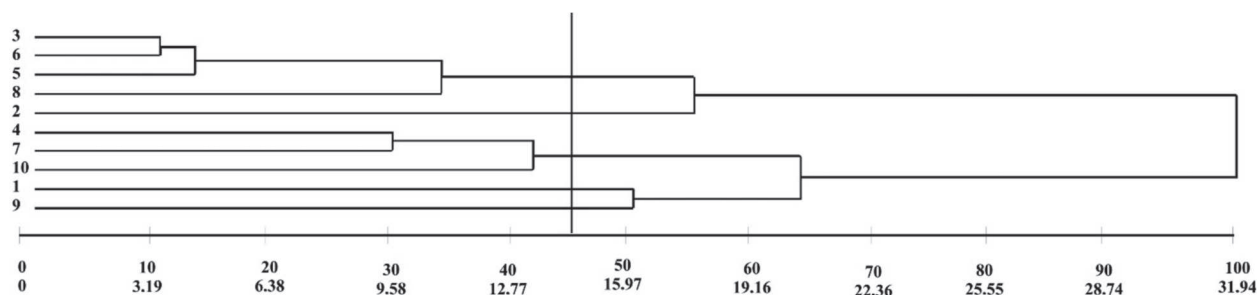


Figure 1: UPGMA dendrogram based on generalized Mahalanobis distance among 10 crambe genotypes.

Table 3: Plant height (PH), stem diameter (SD), height of the first productive branch (HPB), number of branches (NB), mass of 1000 grains (M1000), grain yield (YIELD) and oil productivity (OIL) of the different clusters of crambe formed from UPGMA

Clusters	PH cm	SD mm	HPB cm	NB	M1000 g	YIELD kg/ha	OIL kg/ha
I	142.03	11.67	9.71	16.23	9.15	2047.99	699.08
II	148.26	9.99	13.69	16.10	8.97	1812.32	620.75
III	147.20	10.75	10.87	17.05	7.88	1786.33	623.12
IV	158.27	11.57	12.20	18.67	9.14	1863.68	625.82
V	144.97	11.51	9.03	19.95	8.44	2090.50	693.52

Table 4: Relative contribution (S.j) of traits to divergence, based on the generalized Mahalanobis distance, in crambe genotypes

Traits	Relative contribution	
	S.j	Value (%)
YIELD	242.39	22.31
HPB	210.10	19.3/4
NB	184.60	16.99
SD	155.02	14.27
PH	116.05	10.68
M1000	110.98	10.21
OIL	67.43	6.21

Although some authors (Papathanasiou *et al.*, 1966; Lessman & Meier, 1972) report the lack of variability in *C. abyssinica*, in the present research, in line with that of Lara-Fiores *et al.* (2016a), revealed wide variability for all the agronomic traits evaluated.

Relative importance of traits

Regarding the relative contribution of each trait for genetic divergence, based on Sing's method (1981), was verified that YIELD, HPB, NB and SD contributed with 72.90% (Table 4). It can be affirmed that these traits are important in the genetic divergence study among the genotypes, for presented the most expressive contributions. The oil productivity had the lowest value of S.j (6.21%), being relatively less important for the evaluation of divergence among the genotypes. The traits that are dispensable in the study of genetic divergence are those that relatively do not vary between the individuals studied or are redundant because they are correlated with other traits (Cruz *et al.*, 2012).

CONCLUSIONS

Estimates of genetic parameters indicate the presence of genetic variability among the evaluated genotypes.

The traits of grain yield, height of the first productive branch, number of branches and stem diameter were the ones that contributed most to the genetic diversity.

Genotypes 2 and 4 can be used as parents at future crosses, as they are the most promising for obtaining segregating populations.

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