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Estimates of genetic parameters and path analysis of crambe: An important oil plant for biofuel production

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ABSTRACT. : Crambe is an oilseed plant producing oil that can be used for various industrial purposes, including the production of biofuel. It is a short-cycle, non-food plant that can be grown as a second crop following soybean or corn crops and using the same agricultural machinery. This work aimed to estimate genetic parameters and correlations between traits of genotypes grown for two years in seven locations in two Brazilian states. The cultivar FMS Brilhante and the lines CR 1101, CR 1102, CR 1105, CR 1202, CR 1303, and CR 1304 were used. The traits evaluated were grain yield (YD), plant height (PH), stem diameter (SD), first fruiting branch height (BH), 1,000-seed weight (SW), and number of branches (NB). The correlations between traits were identified from the phenotypic covariance matrix through path analysis. Based on the joint analysis, the interaction of genotypes x years x locations was observed. Expressive genetic variability was verified for all evaluated traits, which allows gains to be made with the selection of genotypes. High broad-sense heritabilities based on plot means were verified for YD, BH, and SW (82, 84 and 77%, respectively), which indicates the possibility of directly selecting them. SW and SD showed the greatest direct effects and strongest correlations with YD.

Keywords: breeding, biofuels, oleaginous plants, brassicaceae.

Introduction

Studies on oilseeds for industrial use, especially for biofuel production, have been gaining importance. The search for vegetable oils with suitable characteristics has been focused mainly on non-food species. Among the species that can potentially fulfil this purpose, crambe (*Crambe abyssinica* Hochst) is an important oilseed plant in the family Brassicaceae (Du, Huang, Guan, Li, & Huang, 2014) that deserves attention.

Oil extracted from crambe seeds is used to make plastic films, adhesives, nylon, corrosion inhibitors, synthetic rubber, industrial lubricants and biodiesel (Carlson, Gardner, Anderson, & Hanzel, 1996). This oil can also be advantageously used for the production of biodiesel (Tavares, Massa, Gonçalves, Silva, & Santos, 2017). Once refined, crambe seed oil can be used to manufacture cosmetics and waxes (Pitol, Broch, & Roscoe, 2010).

According to Falasca, Flores, Lamas, Carballo, and Anschau (2010), crambe has generated great interest from Brazilian farmers due to its short life cycle and potential for cultivation as a second crop following soybean or corn crops using the same agricultural machinery. Other noticeable advantages are its low cost of production and tolerance of drought (Pitol et al., 2010). Thus, crambe became an option for agricultural areas in Midwest Brazil.

As stated by Lara-Fioreze, Pivetta, Zanotto, and Okita (2016), crambe is a plant that presents hermaphroditic flowers and is preferentially autogamous, with a crossing ratio ranging from 9 to 14% depending on the environmental conditions (Vollmann & Ruckenbauer, 1993). The most consistent crambe breeding programme is located at the University of North Dakota, USA, and it aims to increase grain yield, oil content of the grains, and erucic acid content of the oil as well as to promote higher tolerance of various diseases (Knights, 2002).

In Brazil, crambe was introduced in the 1990s by *Fundação Mato Grosso do Sul (Fundação MS)* (Pitol, 2008). In 2007, the first and only crambe cultivar developed in the country was registered as 'FMS Brilhante' (Brasil, 2007), the grain yield of which is considered low, between 1,000 and 1,500 kg ha⁻¹. The cultivar 'FMS Brilhante' also exhibits wide phenotypic variability for agronomic traits of interest, which has resulted in genetic gains by the selection of superior plants within the cultivar (Pitol et al., 2010; Lara-Fioreze et al., 2016).

The success of any breeding programme depends on the existence of high genetic variability and a high average within the population for the trait of interest. This variability is assessed through the estimation of genetic parameters. The most important such parameters to confirm the existence of genetic variability are genetic variance and heritability. Another important parameter is the interaction of genotypes x environments, which can be understood as the differential response of genotypes to different environments. Along with the studies of genetic parameters and genotype x environment interactions, it is extremely important to know the correlation between agronomic traits of interest, especially since such a correlation makes it possible to indirectly select for a main character that has low heritability or is difficult to evaluate.

However, because the cause and effect relationships between the primary and secondary characteristics of yield determinants are not considered in trait selection, one way to expand the efficiency of trait selection is to use path analysis. Such an analysis is a very important tool that can be used to obtain a detailed understanding of the influences of the characters of interest, including revealing the existence of positive

and negative correlations and their low and high magnitudes (Huang et al., 2013; Silva, Moro, Moro, Santos, & Buzinaro, 2016). This analysis is also useful for understanding the changes that the selection pressure on a character can trigger in other inter-correlated characters, which can result in favourable and unfavourable modifications in the population. Path analysis has been useful in determining selection criteria in several crops, such as corn (Matin et al., 2017; Rani, Rao, Ahmad, & Rao, 2017), soybean (Teodoro et al., 2015), and rice (Abreu, Teodoro, Andréa Pantaleão, & Correa, 2016), and its use in the improvement of crambe is indispensable.

The objective of this work was to estimate the genetic parameters, the interaction of genotypes x years x locations, and the correlations between traits of crambe through path analysis in different years and locations of crops.

Material and methods

Six lines (FMS CR 1101, FMS CR 1102, FMS CR 1105, FMS CR 1202, FMS CR 1303, and FMS CR 1304) and the cultivar FMS Brilhante, all of which are from the Programa de Melhoramento de Crambe da Fundação MS, were analysed. Tests were performed in the states of Mato Grosso do Sul and Goiás, Brazil, in the crop years of 2014 and 2015.

The trials were carried out in seven locations: Maracaju, Mato Grosso do Sul State (lat 21° 61' 09.8" S, long 55° 13' 61.2" W, alt 384 m), Rio Verde, Goiás State (lat 17° 49' 58.0" S, long 51° 11' 27.1" W, alt 855 m), IFG Goiânia, Goiás State (lat 16° 70' 56.9" S, long 49° 40' 17.2" W, alt 794 m), São Gabriel do Oeste, Mato Grosso do Sul State (lat 19° 37' 55.9" S, long 54° 57' 57.1" W, alt 658 m), Dourados, Mato Grosso do Sul State (lat 22° 23' 71.1" S, long 54° 90' 71.8" W, alt 430 m), Bom Jesus, Goiás State (lat 17° 57' 52" S, long 49° 56' 33" W, alt 582 m), and Jataí, Goiás State (lat 17° 88' 07.4" S, long 51° 76' 54.4" W, alt 708 m).

The experiment was performed in a randomized block design with four repetitions. Each plot consisted of four 10 m row with 0.50 m between them and a 0.20 m space between plants. Basic fertilization was applied in the crop rows, with 150 kg ha⁻¹ of N-P-K 12-15-15 fertilizer at a depth of 5 cm. The planting was mechanical and carried out directly on top of soybean mulch.

At harvest, ten plants of each of the two central lines were randomly evaluated in each plot, and the following traits were assessed: grain yield (YD), plant height (PH), stem diameter (SD), first fruiting branch height (BH), one thousand seed weight (SW), and number of branches (NB).

Data obtained for the traits were submitted to analysis of variance for the F-test. The genetic parameters were estimated by the following expressions:

Genetic variance:

Environmental variance:

Broad-sense heritability, based on plot means:

Coefficient of genetic variation:

Coefficient of environmental variation:

Ratio (b):

For the path analysis, multicollinearity was assessed. The degree of multicollinearity of the singular matrix $X'X$, which was established based on the condition number (CN), is the relationship between the maximum and the minimum eigenvalues of the matrix. If $CN < 100$, then the multicollinearity is low and does not constitute a problem for the path analysis. If $100 < CN < 1000$, then the multicollinearity is considered moderate to high; and if $CN > 1000$, then it is considered high (Cruz, Regazzi, & Carneiro, 2012). Then, the phenotypic correlations were split into direct and indirect effects of the characters PH, SD, BH, SW, and NB (independent variables) on YD (dependent variable) by using a path analysis model diagram with a single chain, as described in previous literature (Cruz et al., 2012; Wright, 1921); this was accomplished with the system of normal equations $X'X\beta = X'Y$. The diagnosis of multicollinearity and all other analyses were performed with the software Genes (Cruz, 2013).

Results and discussion

Based on the joint analysis of variance (Table 1), the experimental coefficients of variation exhibited values from 3.80 (PH) to 8.63 (BH), which indicates good experimental precision (Dias & Barros, 2009). A significant amount of variation was explained for five out of the six evaluated traits (Table 1), which shows the existence of genetic variability among the studied genotypes and the potential for improvement of the species in Brazil. Lara-Fioze et al. (2016) reported grain productivities ranging from 509 to 1,958 kg ha⁻¹ in tests with progeny of the cultivar FMS Brillhante. Variability in the grain yield for American and Italian cultivars of crambe was also described by Fontana, Lazzeri, Malaguti, and Galletti (1998). For the interaction of genotypes x years x locations, the mean-square values were significant for all the traits analysed, according to the F-tests. The significance of this interaction makes it necessary to use criteria to select genotypes for specific locations and years.

There are reports on the absence of genetic variability in crambe (Lessman & Meyer, 1972), which led these authors to emphasize the importance of crossbreeding between *Crambe abyssinica* and *Crambe hispanica* to promote variability. However, Mastebroek, Wallenburg, and Soest (1996) demonstrated that these two are the same species. In our research, high genetic variability that can be exploited by breeding programmes was verified in the species *C. abyssinica*. The explanation for the presence of this much variability may be the evaluated genotypes. All of these genotypes have advanced homozygosity, even though they are not pure lines.

Table 1

Summary of the joint analysis of variance of grain yield (YD), plant height (PH), stem diameter (SD), first fruiting branch height (BH), one thousand seed weight (SW), and number of branches (NB) evaluated in seven crambe genotypes grown in seven locations in 2014 and 2015.

Sources of Variation	df	Mean squares					
		YD	PH	SD	BH	SW	NB
(B/L)/Y	42	20777.60	39.43	0.63	0.97	0.68	0.63
Genotype (G)	6	627772.10**	2111.96*	12.61*	35.85**	31.02**	20.54
Years (Y)	1	146765.88	491.63	0.34	54.39	0.01	14.02
Locations (L)	6	836378.65**	1269.78	11.09*	78.88	67.20**	8.52
G x Y	6	27860.65	239.57	3.30	1.17	1.77	7.05
G x L	36	114498.53**	548.86**	3.60**	6.22**	6.85**	7.07**
Y x L	6	78018.55**	334.46**	1.51*	32.66**	1.05	10.99**
G x Y x L	36	27861.34**	106.62**	1.51**	1.84**	1.42**	3.05**
Error	252	11844.08	23.59	0.51	0.96	0.68	0.72
Mean		1441.45	127.63	11.91	11.37	10.30	12.08
CV (%)		7.55	3.80	5.99	8.63	8.02	7.06

*and**: significant according to the F-test at 5 and 1% probability, respectively; CV: coefficient of variation; YD in kg ha⁻¹; PH, SD, and BH in cm; SW in g; NB by counting.

For the traits YD, SD, BH, SW, and NB, the environmental variance was greater than the genetic variance (Table 2). Nevertheless, high broad-sense heritabilities, measured based on plot means, were found for YD, BH, and SW (82, 84 and 77%, respectively), indicating the possibility of direct selection of these traits. High heritability for grain yield (78%) was also reported by Lara-Fioze et al. (2016). Lessman and Meyer (1972) also found high heritability values (88%) for the same trait. Heritability allows the genetic study of the character, due to its predictive role, since it expresses the reliability of the phenotypic value as an indication of the genetic value. Thus, if a variable presents significant heritability and a high magnitude, then it can be selected (Resende, 2002).

The ratio *b*, which indicates the genetic variability within a population, was 0.87, 1.04, 0.74, and 0.79 for the traits YD, PH, BH, and SW, respectively, values that are close to or even above 1.00. According to Vencovsky (1978), for traits evaluated in progeny of corn, when *b* is equal to or greater than 1, the situation is favourable for genetic selection. Analogously, the estimates of *b*, together with the estimates of heritability obtained in this study, suggest conditions are favourable for the genetic selection of traits of crambe. It is also possible that the seven genotypes evaluated here are not pure lines, that is, the genetic materials are completely homozygous. This can be verified by the greater values of the environmental variation coefficient than of the genetic variation coefficient observed for four of the five evaluated traits (Table 2).

Table 2

Estimates of genetic parameters for grain yield (YD), plant height (PH), stem diameter (SD), first fruiting branch height (BH), one thousand seed weight (SW), and number of branches (NB) evaluated in seven crambe genotypes grown in seven locations in 2014 and 2015.

Estimates	Traits					
	YD	PH	SD	BH	SW	NB
$\hat{\sigma}_g^2$	9165.61	25.53	0.13	0.54	0.42	0.17
$\hat{\sigma}_e^2$	11844.08	23.59	0.51	0.96	0.68	0.73
\hat{h}_m^2	0.82	0.67	0.57	0.84	0.77	0.46
CV_g	6.64	3.95	3.01	6.46	6.32	3.40
CV_e	7.55	3.80	5.99	8.63	8.02	7.06
Ratio (b)	0.87	1.04	0.50	0.74	0.79	0.48

To verify the direct and indirect effects in path analysis, the matrix $X'X$ must be well conditioned. The presence of multicollinearity in $X'X$ can make it singular and, consequently, make the estimation of least squares unfeasible (Cruz et al., 2012). The diagnosis of multicollinearity in the phenotypic correlation matrix indicated a CN value between 100 and 1000, which indicates moderate to high multicollinearity. Once the character PH was excluded, the problem of multicollinearity in the phenotypic correlation matrix was eliminated, and the path analysis became viable.

According to the path analysis (Table 3), the trait SW presented a strong positive correlation (0.9485) with and a great direct effect (0.5936) on YD, indicating that selection of this trait can provide satisfactory gains in grain yield. A strong correlation between YD and SW (0.821) was found by Huang et al. (2013). For SD, the correlation coefficient was high (0.9132), and the direct effect was 0.5089, but the indirect effect of SW was the one that contributed the most (0.5297). Similar results of a strong positive correlation (0.8728) and a low and negative direct effect (-0.2118) were observed for the NB. However, the indirect effect via SW was strong and positive (0.5189). In cases such as these, the best strategy may be the simultaneous selection of traits by using index selection with an emphasis on the traits of which the indirect effects are stronger and positive, i.e., indirect selection via SW.

For BH, the correlation coefficient was positive and moderate (0.5498), but the direct effect of this trait was weak (0.2608), even though this effect was stronger than the indirect effects. Silva et al. (2014), when studying the correlations among characters in the cultivar FMS Brillhante, also observed a strong correlation between the number of branches and the one thousand seed mass (0.9855). According to Falconer (1987), positive correlations signal the occurrence of pleiotropy or linkage disequilibrium between pairs of traits, leading to the simultaneous selection of two or more characters through the selection of only one of them.

The five studied traits differed in their degree of direct influence on grain yield. Among them, SW was the one that had a positive, relatively strong direct effect, which is indicated by the occurrence of cause and effect, a strong correlation, and a value of b equal to or greater than 1. SW is related to gains in crambe grain yield, i.e., selection to improve seed

weight leads to an increase in yield. In turn, the trait SD can be considered secondary in influencing yield. The occurrence of negative indirect effects of NB on SD and SW shows the difficulties in selecting based only on the behaviour of the main variable. The occurrence of negative indirect effects on NB also shows the difficulties in selecting based only on the behaviour of the main variable. The traits included in the path analysis satisfactorily explain the behaviour of or the variation in crambe grain yield, since the coefficient of determination was high (0.9863), and the residual effect was low (0.1168).

Table 3

Estimates of the direct and indirect effects that involved the main dependent trait, grain yield (YD), and the independent explanatory ones, such as stem diameter (SD), first fruiting branch height (BH), one thousand seed weight (SW), and number of branches (NB).

Traits	Association effect	Estimate ¹
SD	Direct on YD	0.5089
	Indirect via BH	0.0789
	Indirect via SW	0.5297
	Indirect via NB	-0.2043
	Total	0.9132
BH	Direct on YD	0.2608
	Indirect via SD	0.1540
	Indirect via SW	0.1955
	Indirect via NB	-0.0606
	Total	0.5498
SW	Direct on YD	0.5936
	Indirect via SD	0.4540
	Indirect via BH	0.0859
	Indirect via NB	-0.1851
	Total	0.9485
NB	Direct on YD	-0.2118
	Indirect via SD	0.4909
	Indirect via BH	0.0747
	Indirect via SW	0.5189
	Total	0.8728
¹ Coefficient of determination (R ²)		0.9863
Residual effect		0.1168

Conclusion

The cultivar FMS Brilhante and the lines FMS CR 1101, FMS CR 1102, FMS CR 1105, FMS CR 1202, FMS CR 1303, and FMS CR 1304 showed expressive genetic variability, and they offer the possibility of genetic gains in crambe breeding.

The traits SW and SD have a direct effect on the grain yield of crambe, while NB has a negative direct effect on it but a strong indirect effect via SD and SW.

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