

Multivariate analysis as a tool in the initial selections of golden flax (*Linum usitatissimum* L.)

Análise multivariada como ferramenta nas seleções iniciais de linhaça dourada (Linum usitatissimum L.)

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ABSTRACT

The genetic variability in plant populations can be estimated through multivariate analysis, which allows to analyze the genotypes based on a set of traits to identify the traits with the greatest influence for the divergence and the correlation between them. In this sense, the objective of the present work was to estimate the genetic divergence between golden flax lines using multivariate analysis for initial plant selections. For this purpose, 73 lines, in addition to the control, were tested in a randomized complete block design, with three replications, and traits of cycle, stature, and yield were measured. Twelve groups were obtained based on the Mahalanobis distance estimate and Tocher cluster, and the technical length was the most important trait for the dissimilarity. The line of group XI was promising, with early maturation and satisfactory seed productivity. The graphic of dispersion of the canonical variables showed the most divergent lines, and the greatest divergence was observed between groups III and IV. Multivariate analysis was an important tool for the initial choice of superior golden flax.

KEYWORDS: genetic variability; genetic distance; correlation.

RESUMO

A variabilidade genética em populações de plantas pode ser estimada através da análise multivariada, que permite analisar os genótipos com base em um conjunto de características, identificar aquela com maior influência para a divergência e a correlação entre elas. Neste sentido, o objetivo do presente trabalho foi estimar a divergência genética entre linhagens de linhaça dourada, a partir de análises multivariadas, para seleções iniciais de plantas. Para tanto, 73 linhagens, além da testemunha, foram testadas em delineamento de blocos ao acaso, com três repetições, sendo medidas as características de ciclo, estatura e produtividade. Foram obtidos 12 grupos, sendo a característica mais importante para a dissimilaridade o comprimento técnico. O gráfico de dispersão das variáveis canônicas mostrou as progêneses mais divergentes, sendo que a maior divergência foi verificada entre os grupos III e IV. A análise multivariada foi importante ferramenta para a escolha inicial das linhagens superiores de linhaça dourada.

PALAVRAS-CHAVE: variabilidade genética; distância genética; correlação.

INTRODUCTION

Linum usitatissimum L. is an autogamous and diploid species ($2n = 2x = 30$ chromosomes), which has a dual purpose of production: oil and fiber (GILL 1987, ALLABY et al. 2005). The domestication process initially focused on individuals for fiber use, from which further generations were selected for oilseed production (ALLABY et al. 2005). Flax oil is used to manufacture of various biodegradable products such as high-quality drying oil, paints, varnishes, and linoleum floors. It is also used in nutraceuticals and as a functional food for humans and animals due to flax oil is a rich source of omega-3 fatty acids (DIEDERICHSEN & FU 2006).

In order to increase the cultivation of flax in Brazil, it is necessary to develop superior and adapted

genotypes. In Canada, the world's leading flax producer, flax breeding programs began in the early 1900s, with 82 cultivars registered since 1910; among them only 24 cultivars had been introduced from other countries, being the other cultivars developed by Canadian institutions (YOU et al. 2016). In Brazil, there are ten brown flax cultivars registered in the National Register of Cultivars (RNC), although no cultivar or a variety of both brown or golden flax is included as a protected cultivar in the National Service of Protection of Cultivars (SNPC) (BRASIL 2021).

Genetic progress in breeding programs will depend on the range of variability for both superior plant selection and the possibility of grouping alleles into new and favorable combinations through hybridization. In the early steps of selection, it is common that there is not a single trait of interest, but a set of them. Usually, the statistical analysis used is univariate analysis, where it is possible to analyze genetic divergence only from the perspective of each trait, separately. Although very useful, this analysis may lead to the selection of genotype not completely desirable, except in situations of independence between variables (PIMENTEL GOMES 1985), a situation not normally found in practice. Moreover, selections based solely on univariate analysis for many traits are difficult to manipulate when identifying genotypes with better combinations of traits. This makes the procedure virtually unworkable, especially with using a large number of genotypes. Due to that, it is very important to measure several agronomic, morphological, physiological and commercial traits to improve plant selection (CRUZ et al. 2012).

Multivariate analysis is conceptualized by tradition as the statistical study of experiments in which multiple measurements are made on each experimental unit and for which the relationship among multivariate measurements and their structure are important to the experiment's understanding. OLKIN & SAMPSON (2001) present a broader view of the magnitude of genetic variability. In genetic improvement, multivariate statistics has been used to investigate kinship relationships, geographical origin diversity, combining ability and heterosis (CRUZ et al. 2012). In this sense, the objective of the present study was to estimate the genetic divergence between golden flax lines using multivariate analysis, for initial plant selections.

MATERIAL AND METHODS

The experiment was carried out in field conditions at the Federal University of Santa Catarina (UFSC), Curitibanos Campus, SC, Brazil, in the winter season 2018. The experimental farm has an altitude of 1.040 m, soil classified as Haplic Cambisol, and the climate is Cfb, with temperatures below 15 °C in the coldest months and above 25 °C in the warmest months (KOEPPEN 1948).

The performance of 73 golden flax lines and the original population (control) was evaluated in a randomized complete block design with three replicates. The experimental plot consisted of one 2-m length row, 0.34-m spaced from others plots. A plant density of 147 plants m⁻² was used.

Flax lines were selected in Zortéa, SC, Brazil, in a 25 ha commercial crop propagated with seeds of a local variety in 2017. At the capsule maturation stage, plants with both early and uniform maturation and the great visual yield were identified and further harvested individually, generating 73 lines for evaluation in the next harvest. Seeds from plants without selection were used as a control plot (Figure 1).



Figure 1. Implantation and conduction of the test of lines and control of in flax, in the state of Santa Catarina, 2018.

Base fertilization was performed with 300 kg ha⁻¹ of a 4-14-8 fertilizer (N-P-K). At stem expansion stage (5), (FLAX COUNCIL OF CANADA 2015), a top dressing with 30 kg ha⁻¹ of nitrogen was performed. Flax lines were assessed for days for emergence (DFE), when 50% of the plants in the plot emerged; days for flowering (DFF) when 50% of the plants in the plot had flowers; days for maturation (DFM), when 50% of the plants in the plot had straw yellow colored capsules. Ten plants of each flax line were assessed for plant height (PH, cm), and measured were register from the base to the apex. In addition, the technical length (TL, cm), from the base to the insertion of the first branch with capsule; the yield length (YL, cm) from the first branch with capsule to the apex; the number of branches (NB), and the number of branches were also counted; besides primary (PBD, mm) and secondary branch diameter (SBD, mm). Complementary, we also consider the number of capsules per plant (NCP), accounting for the total of capsules harvested from the plot and divided by the number of plants in the plot; the grain yield per plant (GYP, g) weighing the seed mass obtained from the total plot and divided by the number of plants in the plot; and then, the grain yield per area (GYA, kg ha⁻¹), weighing of the seed mass obtained from the plot, transformed to kg ha⁻¹.

Estimates of genetic and environmental variance and heritability coefficients were obtained through the mathematical expectance of the mean squares of the univariate analysis of variance, based on the equations 1 to 3, according to CRUZ et al. (2012), that are described below:

$$\text{Genetic variance: } \sigma_g^2 = \frac{MSG - MSR}{r} \quad (1)$$

$$\text{Environmental variance: } \sigma^2 = MSR \quad (2)$$

$$\text{Heritability coefficient: } h^2 = \frac{\sigma_g^2}{\sigma_f^2} \quad (3)$$

Where, MSG corresponds to the mean square of the lines; MSR corresponds to the average square of the residues; and r is the number of repetitions.

The data were submitted to multivariate analysis. After, the dissimilarity matrix was obtained by Mahalanobis's generalized distance (D2). Then, the average was calculated for each of the selected variables of each line to estimate the distance. The following were established: i) the residual covariance matrix, ii) the transformation of the matrix data, iii) the variance of the transformed variables, iv) the means of the non-correlated variables, and finally, v) the central condensation to solve the dispersion matrix. Tocher's optimization method was also used to cluster the lines from Mahalanobis distance matrix. The relative contribution of the quantitative traits was calculated according to SINGH (1981). Finally, an analysis of canonical variables (ACV) was also carried out, aiming to identify similar groups of individuals in smaller dimensions (CRUZ 1990). The genetic divergence was demonstrated by the dispersion of the scores in graphs, with the axes represented by the first variables. Phenotypic correlations (Pearson) between variables were also estimated. All analyzes were performed using the software Genes® (CRUZ 2016).

RESULTS AND DISCUSSION

Initial data from univariate analysis demonstrate the presence of genetic variability among golden flax lines, especially for the days to flowering, plant height, technical length and grain yield per plant and by area, where the heritability estimates varied from 0.65 a 0.91%. Secondary and primary branch diameter and days for maturation showed values of 0.19 a 0.21% of the heritability (Table 1). The individual estimates suggest genetic gains with the selection of superior flaxseed progenies. However, the present study, being an initial evaluation, provides for the global analysis of the divergence, supported by the results obtained in the multivariate analysis.

The 73 lines and the control formed 12 groups by the Tocher method (Table 2). Range of variability shown by the large number of groups formed indicates the possibility of selecting superior genotypes. Therefore, for the selection of plants for the next stages of the breeding program, it is relevant to analyze the traits of importance in the average of the groups formed.

The number of days required for the lines to emerge (Table 2) were similar to those found in study with other genotypes in other locations (GRUZDEVIENÉ et al. 2006), ranging from the 8 to 9 days. About the number of days to flowering, the clusters varied from 79 to 86 days after sowing (Table 3). Other authors reported shorter periods, such as 51 days (YOU et al. 2016) and 54 days (HASEEB et al. 2016). In spite of don't need vernalization to flowering, the cold stimulus can accelerate the flowering of flax plants (DARAPUNENI et al. 2014). The lines reached the maturation from 143 to 152 days after emergence, longer than that found in other studies, such as from 83 to 126 days (KANWAR et al. 2014), from 82 to 100 days (GRUZDEVIENÉ et al. 2006) 86 to 127 days (HASEEB et al. (2016). Late maturation is related to the length of the reproductive period, which is extensive, also resulting in uneven maturation. Long cycle can be difficult

the flaxseed insertion in some crop systems by the delay in sowing of summer crops. For this reason, the feature has been the aim in our studies for future improvement. Based on the cycle duration, groups III and XI seem to be the most promising (Table 2).

Table 1. Mean values, experimental coefficient of variation (CV%), genetic variance (σ^2_g), environmental variance (σ^2_e), and heritability coefficient (h^2) of the traits in flax lines, in the state of Santa Catarina, 2018.

Trait	p (%)	Mean	Range of variation	σ^2_g	σ^2_e	h^2
Days for emergence	0.0**	8.2	7.0- 10.0	0.08	0.08	0.48
Days for flowering	0.0**	82.7	77.0 - 87.0	2.92	0.30	0.91
Days for maturation	11.6 ^{ns}	146.8	132.0 - 157.0	2.86	10.78	0.21
Plant height (cm)	0.0**	91.0	81.40 - 105.43	14.07	4.41	0.76
Technical length (cm)	0.0**	65.9	58.33 – 77.33.1	8.13	3.04	0.73
Yield length (cm)	0.0**	25.1	15.6 - 40.5	4.59	4.18	0.52
Number of branches	6.6 ^{ns}	3.8	2.5 - 8.7	0.03	0.08	0.26
Primary branch diameter (mm)	5.9 ^{ns}	2.9	1.9 - 4.3	0.01	0.04	0.27
Secondary branch diameter (mm)	14.2 ^{ns}	2.1	0.5 - 4.2	0.02	0.10	0.19
Number of capsules per plant	0.2**	52.9	10.4 - 110.4	61.89	82.90	0.43
Grain yield per plant (g)	0.0**	1.3	0.22 - 4.54	0.16	0.08	0.65
Grain yield per area (kg ha ⁻¹)	0.0**	1615.8	868.3 - 2860.5	60919.64	27621.57	0.69

Table 2. Mean values of the 12 clusters for the days for emergence (DFE), days for flowering (DFF), days for maturation (DFM), plant height (PH, cm), technical length (TL, cm), yield length (YL, cm), number of branches (NB), primary (PBD, mm) and secondary branch diameter (SBD, mm), number of capsules per plant (NCP), grain yield per plant (GYP, g) and grain yield per area (GYA, kg ha⁻¹) in golden flax lines, in the state of Santa Catarina, 2018.

Cluster	Lines	DFE	DFF	DFM	PH	TL	YL	NB	PBD	SBD	NCP	GYP	GYA
I	54+control	8	83	146	90.1	65.6	24.4	3.7	3.9	2.1	52.1	1.2	1,543.8
II	6	8	80	148	90.9	63.4	27.5	3.7	3.1	2.3	57.8	1.6	1,824.9
III	2	8	79	143	103.8	72.2	31.6	4.0	3.3	1.9	49.9	1.4	1,646.7
IV	2	8	85	147	87.9	63.9	24.1	3.5	2.7	2.3	51.5	2.0	2,176.5
V	2	8	86	150	97.0	72.0	25.0	3.9	3.0	2.1	41.3	1.3	1,353.3
VI	1	9	86	148	99.9	77.8	22.2	3.3	3.0	2.6	21.8	0.4	1,059.5
VII	1	9	83	150	97.4	63.6	33.8	4.0	3.3	3.1	81.7	2.7	2,313.8
VIII	1	8	82	151	100.0	71.2	28.8	3.7	3.0	1.9	58.1	2.1	2,181.3
IX	1	8	83	148	90.7	65.7	25.1	3.6	2.7	1.9	40.6	1.1	1,232.0
X	1	9	83	152	96.1	65.7	30.5	4.1	3.5	1.8	66.7	2.1	2,215.1
XI	1	8	80	143	88.2	64.5	23.6	5.4	3.2	2.0	80.8	1.8	1,972.2
XII	1	8	79	147	86.1	59.7	26.4	4.0	3.0	2.2	67.2	3.3	2,385.7

Table 3. Distance of Mahalanobis, mean within a group (diagonal) and between groups for different traits in golden flax lines, in the state of Santa Catarina, 2018.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	12.23	28.11	50.65	23.23	28.09	53.30	27.08	22.65	24.04	30.45	40.14	52.09
II		16.36	30.04	49.16	67.04	97.18	36.34	22.24	38.82	34.94	26.39	28.67
III			10.85	90.61	80.23	103.48	60.84	23.80	52.89	48.42	42.81	59.31
IV				11.31	33.12	56.52	26.56	32.25	45.16	38.26	66.56	63.87
V					14.56	21.21	32.81	37.18	37.40	48.20	86.14	96.21
VI						0.00	54.76	61.13	71.40	73.84	129.67	153.55
VII							0.00	23.55	45.62	26.17	54.78	50.92
VIII								0.00	34.40	21.50	37.37	41.89
IX									0.00	22.64	52.68	55.51
X										0.00	45.29	58.37
XI											0.00	36.48
XII												0.00

Morphological traits must be evaluated among the genotypes for selection, as these can influence the cultivation and crop management techniques. In this study, the plant height varied 17.7 cm among groups (Table 2). Interestingly, other studies have found values from 45.8 to 85.9 cm (HASEEB et al. 2016) and from 33 to 68 cm (KANWAR et al. 2014). In general, flaxseed genotypes used for grain yield have lower heights compared to flax, used for fiber (DIEDERICHSEN & ULRICH 2009). Technical length is an important measure used in flaxseed crop to identify genotypes for fiber production, and productive length refers to the part of the plant where the capsules are inserted. In our study, the technical length ranged 18.1 cm among groups, while the productive length showed variation of 11.6 cm among groups (Table 2). The number of secondary branches varied little among groups (Table 2). The number of branches and the diameter of the primary and secondary branches are also related to the ability for fiber production or grain production. While single-stemmed plants with a larger diameter are preferred for fibers, plants with more branches focus on grain production (SINGH 2016). There are dual-purpose cultivars on the international market, aimed at producing grains and fibers, although they are not the majority. Cluster VI consists of a single line pure that shows potential for use in fiber production since it presented the highest technical length and the lowest value for the number of branches.

The 12 groups formed showed a wide range of variation for the number of capsules per plant, yield grain per plant and area, showing the variability between lines, under the same sowing density. In other studies, under lower sowing density, there was the production of 36.2 (ROSSI et al. 2014) and a variation of 15 to 73 capsules per planta (HASEEB et al. 2016). According to ZAJAC (2004), it is common to observe a high number of capsules per plant when low densities are used, as a form of compensation. The evaluation of the commercial cultivars from England showed a yield per plant of about 2.4 g (100 plants/m²) and 1.2 g (278 plants/m²) (LEITCH & SAHI 1999), while KARIUKI et al. (2014) observed a yield per plant ranging from 0.15 to 3.29 g. In this study, for grain yield per area, the groups ranged from 1,059.5 to 2,385.7 kg ha⁻¹, higher than the national average of 953 kg ha⁻¹, according to data from Food and Agriculture Organization Statistics Division (FAOSTAT 2021). The yield variation of the different lines demonstrates the great genetic variability and the possibility of selecting superior genotypes.

The only line pure of group VII showed the highest grain yield per area, a result associated with a higher number of capsules per plant and higher productivity per plant, although the same showed a longer time for maturation. In this context, to obtain earlier genotypes and with adequate seed productivity, the line of group XI could be evaluated in more field trials (Table 2). Additionally, hybridizations between early line and progenies with high seed production may be relevant for the study of the genetic nature of the traits, as well as the exploration of possible effects of the dominance of the traits.

Genetic diversity studies based on distance estimation and clustering techniques are of great importance for breeding programs and are performed frequently. In *L. usitatissimum*, such studies showed that not always a range of different genotypes, originating or selected in different and distant environments showed great genetic diversity. Other studies have shown the formation of six clusters between the 35 genotypes (KUMAR et al. 2017), ten clusters among 95 lines and three controls (FULKAR et al. 2007), nine

clusters for the 66 genotypes (KUMAR et al. 2018), and nine clusters for the 50 genotypes (KUMAR et al. 2021).

The genetic distance between the groups provides more information about the range of variability found in the flax lines. The shortest distance of Mahalanobis within groups with more than one progeny can be found in group III. Interestingly, group I (with the largest number of lines) showed a smaller distance within the group than other groups with two lines, showing that the group should be composed of very similar or same lines pures, since it is an autogamous species. The greatest distance was found between groups III and VI, and the shortest distance was observed between groups V and VI (Table 3). The results are important for the flaxseed breeding program, since strategies for direct use of progenies or hybridizations for the expansion of genetic variability will be structured based on them.

From the eigenvectors associated with the variables main canonical scores were obtained from 73 flax lines and the control. The graphic dispersion of the scores, in two-dimensional space, of the two main canonical variables, is shown in Figure 2. The canonical variables analysis is used to assess the degree of similarity between genotypes, considering both the sum matrix of squares and residual products as the sum of squares and products between phenotypic means of the evaluated traits (CRUZ 1990). According to canonical variables analysis two canonical variables (VC1 and VC2) accounted for 56.0% of the total variability. The proportions of the total variance attributable to the first and second VC were 39.4% and 16.6%.

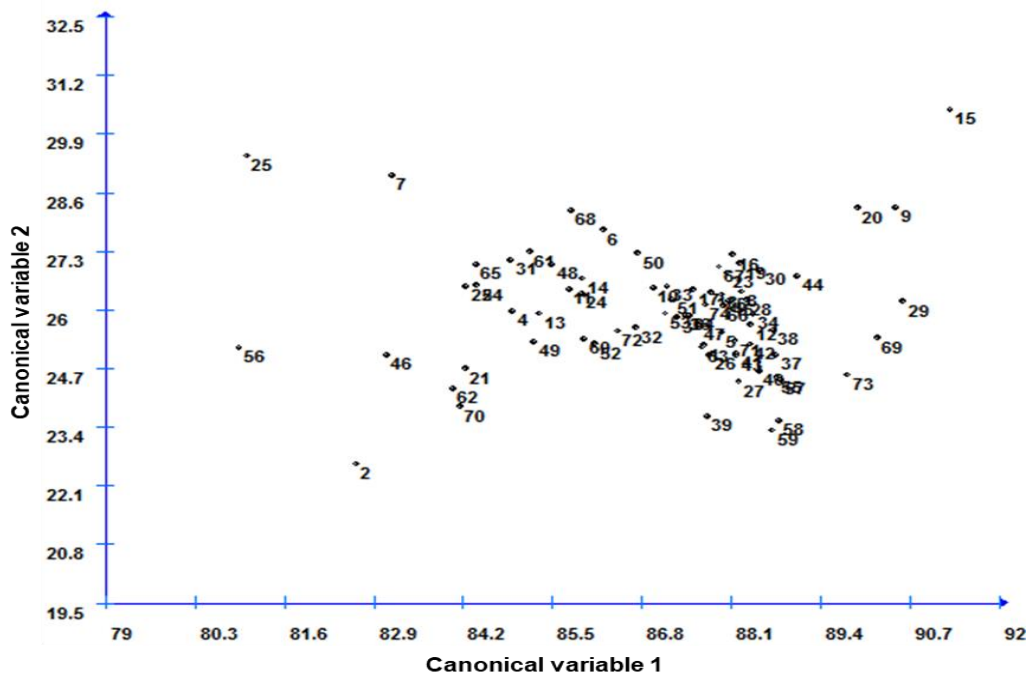


Figure 2. Graphic of dispersion of the golden flax lines based on the scores of the canonical variables, in the state of Santa Catarina, 2018.

The distance between the points on the graph is proportional to the degree of dissimilarity between the lines. The graphic dispersion showed the genetic divergence already observed by the analyzes discussed above. The dissimilarities here represented by the distance between the points may indicate the choice of genotypes for future hybridizations. A group with the great majority of the lines is visible, being able to be pure lines similar or equal genetically (as already mentioned in the Tocher group). Such dispersion suggests that there is a wide variability available for selection in plant populations, which indicates the possibility of gains and genetic improvements for important traits.

The correlation data (Table 4) show negative correlations between days to flowering and the components of seed production. In this study, early flowering would be an important selection factor to increase the production of flax. Positive correlations between days for maturation and the productive length of the plant indicate that plants with greater productive length take longer to mature. Additionally, plants with a longer productive length and a larger number of branches have a higher production of capsules and seeds, verified by positive correlations. Negative correlations were found between technical length and the components of seed production. The greatest number of capsules was, in our experimental conditions, related to greater seed production.

Table 4. Phenotypic correlations between traits evaluated in golden flax lines, in the state of Santa Catarina, 2018.

	Traits	Correlation (r)
Days for flowering	Yield length	-0.40**
	Number of branches	-0.34**
	Primary branch diameter	-0.32**
	Number of capsules per plant	-0.28*
	Grain yield per plant	-0.37**
	Grain yield per area	-0.36**
Days for maturation	Yield length	0.31**
Plant height	Technical length	0.73**
	Yield length	0.63**
	Number of branches	0.26*
	Primary branch diameter	0.48**
Technical length	Grain yield per plant	-0.31**
	Grain yield per area	-0.32**
Yield length	Primary branch diameter	0.56**
	Number of capsules per plant	0.30**
	Grain yield per plant	0.40**
	Grain yield per area	0.41**
Number of branches	Primary branch diameter	0.61**
	Number of capsules per plant	0.26*
	Grain yield per plant	0.28*
	Grain yield per area	0.25*
Primary branch diameter	Number of capsules per plant	0.33**
	Grain yield per plant	0.41**
	Grain yield per area	0.38**
Number of capsules per plant	Grain yield per plant	0.59**
	Grain yield per area	0.58**
Grain yield per plant	Grain yield per area	0.93**

** and *: Significant at 1 and 5% by t test.

The increase in production components in genetic breeding programs is quite common objectives. For this reason, the correlations between production components and other traits are important guidelines for the selection of superior flax genotypes. Additionally, it is important to select genotypes with early and uniform maturation. Other authors have verified the correlation between grain yield and days for maturation as being positive (IQBAL et al. 2015) and other authors as being negative (BINDRA & PAUL 2016). It can be challenging with such plants to increase grain yield and reduce the cycle, which has been the objective of our breeding program. In our experiment, the correlation between days for maturation and production components was not significant. Additionally, developing genotypes with dual purpose, producing grains and fiber would be hampered by such correlations.

The most important trait in the main components was the technical length, showing that it was the one that presented the greatest variation among flax lines, with the possibility of selecting genotypes for fiber production. The yield length and days for flowering were presented (Figure 3). KUMAR et al. (2017) found that the days to 50% flowering, followed by plant height contributed to the genetic diversity among flaxseed genotypes, while FULKAR et al. (2007) reported that the number of capsules per plant, plant height, number of days to flowering and number of days to maturity presented the maximum contribution to the divergence.

Our results show the possibility of selecting flaxseed progenies for fiber production, exploring of the genetic variability available in the population.

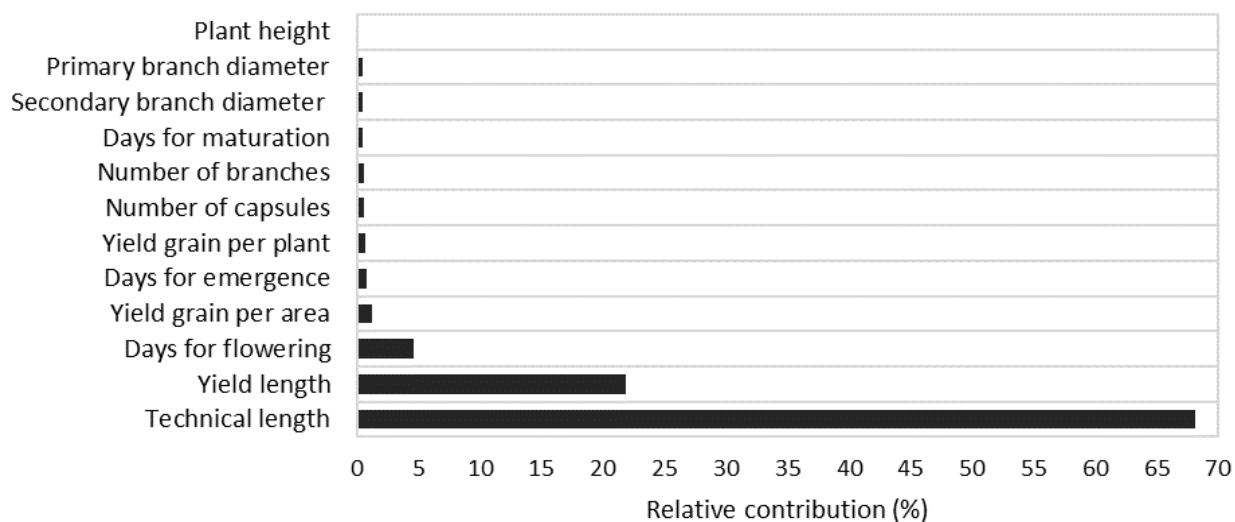


Figure 3. Percentage of relative contribution to 12 traits evaluated in golden flax lines, in the state of Santa Catarina, 2018.

The use of multivariate analysis (Table 2 and 3) in breeding programs is an effective tool and can complement the use of univariate analysis (Table 1). In addition, the possibility of analyzing a set of traits, allows the breeder to choose and select plants with greater potential, since they may present superiority to more than one trait, which is very important in the initial phases of the program improvement (KUMAR et al. 2021).

In this context, our results suggest that the golden flax lines evaluated have genetic variability for the individual traits, and in their global analysis, the genetic divergences presented by the multivariate analysis, can be explored. Furthermore, the groups of lines (Tocher) and the dispersion of the points in the graph of the canonical variables help show the dissimilarity between the lines and are important tools in selecting genotypes for use or future hybridizations.

Such results help in conducting the selection of superior golden flax genotypes in parallel with other field trials in several environments and years of evaluation so that there is efficiency in the future selections of lines in the program.

CONCLUSIONS

The multivariate analysis showed genetic diversity in the golden flax lines, which was proven by the formation of 12 groups with different genetic distance values and by the dispersion of points in the graph of the canonical variables.

The selection of plants with early maturation and with adequate grain yield can be obtained by choosing the group XI. Selection of genotypes from divergent groups may be required for future hybridizations.

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