

Path Analysis under Multicollinearity in Soybean

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ABSTRACT

This study aimed to establish the phenotypic correlations among several soybean traits with grain yield in direct and indirect effects using path analysis, and to compare alternative methods for minimizing the adverse effects of multicollinearity in estimating path coefficients. The experiment was conducted in greenhouse in a randomized complete block design with four replications. Nine soybean genotypes belonging to three seed size categories were used. The correlation studies and the path analysis showed that the seed size was not important for increased yield. The number of nodes and plant height at maturity showed significant correlation with grain yield. Using the least square methodology, the results obtained by path analysis under multicollinearity were not satisfactory. The ridge path analysis and the trait culling were efficient in reducing the adverse effects of multicollinearity. Both methods showed that only the number of nodes at maturity trait had a high direct effect on grain yield per plant.

Key words: genetic breeding, *Glycine max*, grain yield, seed size, root and canopy traits

INTRODUCTION

Grain yield, an extremely complex trait, is the result of the expression and association of several plant growth components. Correlation coefficients, although very useful in quantifying the size and direction of trait associations, can be misleading if the high correlation between two traits is a consequence of the indirect effect of other traits (Dewey and Lu, 1959). Wright (1921) proposed a method called path analysis, which partitions the estimated correlations in direct and indirect effects of traits over a basic one, to better understand the association among traits. This analysis was first carried out on plants by Dewey and Lu (1959) and was later applied to various crops. In soybean, Pandey and Torrie (1973), Wakankar et al. (1974), Ali et al. (1989), Shivashankar and Viswanatha (1989), Akther and Sneller (1996), Board et al.

(1997), Taware et al. (1997), Shukla et al. (1998) and Board et al. (1999) reported its use.

Measuring direct and indirect effects of a trait group on a basic trait requires the estimation of path coefficients, which are obtained by regression equations where the variables have been previously standardized. However, measuring can be adversely affected by the multicollinearity effects among the involved traits. Multicollinearity occurs when the sample observations of the independent variables, or their linear combinations, are correlated. The variances associated to the path coefficient estimators may become too large in the presence of high multicollinearity, making the estimation unreliable (Carvalho, 1995; Carvalho et al., 1999a).

To minimize these adverse effects, selective elimination of variables from the regression model can be performed, or the alternative methodology

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to the least squares proposed by Carvalho et al. (1995) can be used. This method modifies the normal equation system by adding a constant value to the diagonal of the independent variable correlation matrix. Because of its similarity with the ridge regression method proposed by Hoerl and Kennard (1970a), it is called ridge path analysis.

The objectives of this study were: a) to partition the phenotypic correlations among several soybean traits with grain yield in direct and indirect effects using path analysis; and b) compare alternative methods of minimizing the adverse effects of multicollinearity in estimating path coefficients.

MATERIAL AND METHODS

Genetic material and experiment

The experiment was carried out in a greenhouse. Nine soybean genotypes from three seed size categories defined by the weight of one hundred seeds (WHS) were used. The first category included large-seed genotypes with WHS over 34 grams (F825722P, Soja Feira 86-13 and Tamba kurodaisu); the second category consisted of medium size seed genotypes with WHS ranging from 15 to 21 grams (BR 16, BR 36 and a selection from Stewart), and the third category consisted of small-seeded genotypes with WHS ranging from 8 to 10 grams (Lines 603, 626, 629).

The following traits were assessed in individual plants according to the plant developmental stage:

a) at germination (stage VE on the Fehr and Caviness scale, 1977): SD - seedling diameter (mm); b) at the beginning of flowering (R1 stage on the Fehr and Caviness scale, 1977): NDF - number of days to flowering; PHF - plant height (cm); RL - main or pivot root length (cm); RDM - root dry matter (g/plant), assessed after drying the whole root in a chamber; CDM - canopy dry matter (g/plant), assessed after drying the whole canopy in a chamber; c) at maturity, (R8 stage on the Fehr and Caviness scale, 1977): PHM - plant height (cm); SDM - stem diameter (mm); NNM - total number of nodes on the main stem; NP - number of pods with formed seeds; PW - pod width (mm); NSP - number of seeds per pod (mean/plant); PL - pod length (cm); GY - grain yield (g/plant); WHS - weight of one hundred seeds (g). The WHS was obtained from a sample of one hundred seeds from each harvested plant. When the plants did not produce one hundred

seeds, the WHS was estimated by the following equation: $WHS = (GY \times 100)/n_i$, where n_i is the number of seeds per plant.

A randomized complete block design with four replications was used. Each plot consisted of two pots for the traits SD, NDF and PHF. For the other traits, each plot consisted of one pot because of the dry matter assessed (one pot of the plot was harvested at flowering). Seven seeds were sown per pot followed by thinning 25 days after sowing to two plants per pot.

Statistical analysis

Analysis of variance and the phenotypic correlation estimates among traits were obtained as described by Mode and Robinson (1959). The significance of the correlations was tested by the *t* test, with $n-2$ degrees of freedom (Vencovsky and Barriga, 1992). These correlations were partitioned in direct and indirect effects of the traits (independent variables of the regression model) on grain yield (dependent variable or basic) using path analysis (Wright, 1921).

Two procedures were adopted to handle cases of moderate to severe multicollinearity among the independent variables: (i) elimination in the XX correlation matrix of variables that contributed most to the observed multicollinearity until weak multicollinearity was obtained (Carvalho et al., 1999a), and (ii) the use of the alternative least squares methodology proposed by Carvalho et al. (1995) to estimate the path coefficients. According to this method, a constant K is added to the diagonal of the XX matrix, and the path coefficients obtained by solving the following equation:

$$(XX + KI_p)b^* = X'Y \text{ where,}$$

XX is the correlation matrix among the independent variables of the regression model; K is a small amount added to the elements of the diagonal of the XX matrix; I_p is the identity matrix; b^* is the ridge path coefficients vector; and $X'Y$ is the correlation matrix among the dependent variables with each independent variable of the regression model.

The value of the constant K was determined by the ridge trace exam (Hoerl and Kennard, 1970a,b). The ridge trace was obtained by plotting the parameters (path coefficients) in function of the K values in the $0 < K < 1$ interval. The smallest K value capable of stabilizing most of the path

coefficient estimates was used.

The multicollinearity degree of the XX matrix was established based on its condition number (CN - ratio between the largest and smallest eigenvalue of the matrix) (Montgomery and Peck, 1981). If $CN < 100$, the multicollinearity was considered weak and was not a serious problem in the analysis. If $100 > CN > 1000$ the multicollinearity was considered moderate to strong, and if $CN > 1000$ the multicollinearity was considered severe. The analysis of the eigenvalues of the matrix was carried out to identify the approximate nature of the linear dependency between the traits, detecting those that contributed to the presence of multicollinearity (Belsley et al., 1980). The traits that showed the highest elements in the eigenvectors associated to lowest eigenvalues were those that most contributed to this presence. Multicollinearity diagnosis and the other analyses of this study were solved by using the GENES software (Cruz, 1997). The identification of yield components as indirect selection criteria for grain yield was based on Board et al. (1997).

RESULTS AND DISCUSSION

The analysis of variance detected significant differences among lines at the 5% probability level (Table 1) for all the assessed traits. The smallest coefficient of variation was obtained for NDF (1.96%) and the largest for NP (26.01%) and GY (23.6%). Lopes et al. (1997) also reported high coefficients of variation (36.10% for plot yield) in their study. Although these two traits presented high coefficients of variation, the experimental accuracy may be considered satisfactory because of the significance of the effects tested.

The highest grain yield per plant was found in Line 626, although this genotype was significantly different only from BR 16 and BR 36 (Table 2). The GY trait presented significant phenotypic correlations with NNM and PHM (Table 3), indicating that they were the most important traits linked to yield. Similar results were also obtained by Shivashankar and Viswanatha (1989) and Taware et al. (1997) for PHM, and by Akther and Sneller (1996) for NNM. On the other hand, Shukla et al. (1998), obtained non-significant correlations for GY with PHM and NNM.

Chloupek and Rod (1992) reported that, for most crops, the variation in root system size

corresponded to the variation in stem size. Significant correlations were obtained for RDM with PHF, CDM and PHM, which is in line with the quoted authors. The significant correlations of NDF with RDM, CDM and PHF indicated that plant root and canopy growth at flowering were largely controlled by genes that govern NDF. The significant correlation between WHS and SD indicated that seed size affects only seedling development; the larger the seed the higher the seedling diameter.

The highest PHF, PHM, RDM, CDM and NNM were found in Line 626. Plants from small seeds were the highest and had the highest accumulation of canopy and root dry matter (Tables 2 and 3). However, the WHS trait showed no correlation with GY. The non-significant relationship between seed size and grain yield was also observed by Taware et al. (1997), Shukla et al. (1998) and Board et al. (1999). Thus, it was found that soybean could increase or decrease the number of seeds in function of their size in a kind of buffer effect, without significantly varying the yield. The significant negative correlation between WHS and NP also suggested this kind of effect.

Path analysis

The phenotypic correlation matrix of the independent variables showed severe multicollinearity ($CN > 1000$). Using the least squares method ($K = 0$) and carrying out an analysis with all the assessed traits (Table 4), the highest direct effects on GY were obtained with SD (3.915) and PW (2.514). Table 4 did not show the magnitude of indirect effects, but very high values were detected (ranging from -3.385 to 3.818) to be associated with all the traits. This created difficulties in interpreting the results. Furthermore, high residual effect (1.151) (Cruz and Regazzi, 1994) and negative determination coefficients (R^2) were obtained.

High indirect effects associated with all traits, high residual effect and negative R^2 values could be the result of the adverse effects of the multicollinearity in the XX matrix. When this occurred, the least squares methodology ($K = 0$) could not provide safe path coefficient estimates. The vector of these coefficients was an inverse function of the matrix.

Table 1 - Analysis of variance, mean and coefficient of variation for 15 traits assessed in nine soybean genotypes.

Sources of variation	Mean Square								
	df	SD ⁽¹⁾	NDF	PHF	RL	RDM	CDM	PHM	SDM
Blocks	3	0.003	1.296	148.490	50.718	12.623	51.237	289.874	2.028
Genotypes	8	1.103**	317.000**	3494.901**	105.011*	243.015**	7246.964**	4729.155**	2.846*
Residue	24	0.009	0.796	211.574	33.128	5.997	94.641	456.611	0.864
Mean		2.353	45.500	136.747	49.091	13.641	61.127	164.439	7.707
C.V. (%)		4.16	1.96	10.62	11.78	17.95	15.91	12.99	12.06
		NNM	NP	PW	NSP	PL	WHS	GY	
Blocks	3	2.904	323.497	0.073	0.010	0.011	6.166	111.749	
Genotypes	8	27.508**	48678.568**	17.600**	0.148**	2.211**	824.113**	1099.156**	
Residue	24	2.037	1477.020	0.041	0.024	0.018	3.881	312.954	
Mean		15.32	148.320	9.991	2.020	4.249	22.561	74.875	
C.V. (%)		9.31	26.01	2.05	7.80	3.22	8.73	23.62	

* and ** indicate significance at P<0.05 and P<0.01 by F test.

⁽¹⁾ SD - seedling diameter at emergence; NDF - number of days to flowering; PHF - plant height at flowering; RL - main or pivot root length at flowering; RDM - root dry matter; CDM - canopy dry matter; PHM - plant height at maturity; SDM - stem diameter at maturity; NNM - number of nodes on the main stem; NP - number of pods per plant; PW - pod width; NSP - number of seeds per pod; PL - pod length; WHS - weight of one hundred seeds; GY - grain yield per plant.

Table 2 - Means of nine genotypes for 15 soybean traits.

Treatment	SD ⁽¹⁾	NDF	PHF	RL	RDM	CDM	PHM	SDM	NNM	NP	PW	NSP	PL	WHS	GY
Large seeds															
F825722P	2.82 b ⁽²⁾	45.75c	132.55bc	49.95ab	8.67b	32.12c	144.63cd	9.17a	14.12cde	77.25c	13.07a	1.81b	5.11b	40.20b	83.65ab
Soja de Feira 86-13	2.82 b	39.00cd	87.13d	50.95ab	5.97b	22.25c	164.63bcd	6.58b	17.62ab	74.72c	11.70b	1.91b	4.81b	33.87c	86.10ab
Tamba kurodaisu	3.20 a	34.25e	106.15cc	44.95ab	4.47b	27.17c	123.05d	8.21ab	13.12de	38.50c	12.85a	1.83b	5.46a	47.02a	68.00ab

Medium size seeds															
BR16	2.26 d	41.00c	131.40bc	44.37ab	7.67b	28.20c	137.75d	6.80ab	13.25de	65.95c	9.02d	2.44a	4.17c	18.45de	55.35b
BR36	2.55 c	37.00d	134.63bc	49.87ab	9.55b	23.12c	133.75d	7.68ab	11.37e	60.50c	10.27c	1.95b	4.16c	20.70d	50.90b
Seleção de Stewart	2.17 d	49.25b	182.13a	39.12b	20.10a	83.57b	197.25ab	8.01ab	16.25bcd	180.75b	9.27d	2.11ab	4.13c	15.22e	76.05ab

Small seeds															
Line 603	1.79 e	56.00a	152.00at	55.50a	20.60a	100.92b	159.33bcd	7.25ab	15.50bcd	258.00b	7.72e	1.98b	3.40d	9.47f	71.93ab
Line 626	1.79 e	56.00a	172.00a	55.50a	24.60a	127.90a	225.33a	8.49ab	19.82a	347.00a	8.10e	2.05b	3.57d	8.85f	106.20a
Line 629	1.77 e	56.25a	134.25bc	51.62a	21.12a	104.87a	194.25al	7.15ab	16.87abc	232.25b	7.80e	2.11ab	3.42d	9.25f	75.70ab
DMS	0.23	2.14	34.96	13.80	5.88	23.38	51.35	2.37	3.43	87.38	0.49	0.38	0.32	4.73	45.52

⁽¹⁾ SD - seedling diameter at emergence; NDF - number of days to flowering; PHF - plant height at flowering; RL - main or pivot root length at flowering; RDM - root dry matter; CDM - canopy dry matter; PHM - plant height at maturity; SDM - stem diameter at maturity; NNM - number of nodes on the main stem; NP - number of pods per plant; PW - pod width; NSP - number of seeds per pod; PL - pod length; WHS - weight of one hundred seeds; GY - grain yield per plant.

⁽²⁾ Means within a column followed by the same letter are not significantly different (P<0.05 - least significant difference).

If there is perfect multicollinearity among some of the independent variables, the matrix will be singular, and there will be no single inverse matrix (Carvalho et al., 1999b). An infinite number of vectors can thus be established, but none of them will have practical significance. The hypothetical condition of perfect multicollinearity is an extreme

case. However, according to these authors, as the correlation matrix approaches singularity, the corresponding path coefficient estimates become less reliable due to the variance increases associated with these coefficients.

When moderate to severe multicollinearity occurs in the XX matrix, better results can be obtained

using the ridge path analysis or with the trait culling (Carvalho et al., 1999a). In the ridge path analysis, as the K value increases the variances of the path coefficients are reduced. However, the estimates become more biased (Carvalho, 1995). Furthermore, when K increases, the mean quadratic error (variance + square of bias) decreases until a minimum value and then increases again (Hoerl and Kennard, 1970b). The chosen K value should be the smallest possible to reduce the variance of the estimator and to cause only a small bias, so that the mean quadratic error is smaller or equal to that of the least squares estimation. As can be seen in the examination of the ridge line (Fig. 1), the b^* estimates stabilize for a value of $K = 0.05$. This was the K value used for ridge path analysis. For a better visualization, the figure only shows the estimates of the seven traits that contributed most to multicollinearity.

As there is no statistical test to verify whether the mean quadratic error of b^* is smaller than that obtained by the least squares method, it is difficult to decide when the use of the ridge path analysis is most suitable. The results obtained from this analysis, however, were more satisfactory than those from the least squares. When $K = 0.05$, the residual effect decreased considerably (0.206) and

the R^2 value was close to unit. For this K value, SD and PW presented low direct effects. The trait that presented the greatest direct effects on GY (0.646) was NNM, with low indirect effects. In this case, the multivariate analysis ratified the univariate result.

In the analysis with the trait culling, the contribution of each trait to the multicollinearity was estimated. According to this diagnostic, several traits were discarded until a $CN < 100$ was obtained for the XX matrix (Table 4). The results obtained with the trait culling were similar to those of the ridge path analysis. All the traits eliminated had presented low direct effect in the ridge path analysis. Accordingly, the NNM trait also presented high direct effect on GY, a fact that was not observed when the least squares method was used. The PHM trait, which showed negative direct effect (-0.326) in the trait culling method, was the only example of disagreement with the ridge path analysis (0.217). Similar results using these alternative methods were also obtained in sweet peppers by Carvalho et al., (1999a). In spite of the simplicity of the trait culling method, however, Johnston (1972) and Heady and Dillon (1969) reported limitation of this method in regression analysis.

Table 3 - Phenotypic correlation estimates among 15 traits assessed in nine soybean genotypes.

	NDF	PHF	RL	RDM	CDM	PHM	SDM	NNM	NP	PW	NSP	PL	WHS	GY
SD ⁽¹⁾	-0.93**	-0.69*	-0.34	-0.90**	-0.86**	-0.71*	0.21	-0.51	-0.86**	0.96**	-0.53	0.97**	0.96**	-0.23
NDF		0.66*	0.40	0.95**	0.96**	0.80**	-0.04	0.68*	0.95**	-0.83**	0.28	-0.86**	-0.83**	0.48
PHF			-0.09	0.81**	0.71*	0.62	0.37	0.28	0.69*	-0.62	0.32	-0.62	-0.69*	0.22
RL				0.31	0.38	0.26	-0.00	0.40	0.49	-0.25	-0.29	-0.39	-0.26	0.45
RDM					0.97**	0.85**	0.10	0.64	0.96**	-0.82**	0.23	-0.84**	-0.84**	0.47
CDM						0.85**	0.11	0.71*	0.98**	-0.78*	0.19	-0.79*	-0.77*	0.55
PHM							0.08	0.89**	0.86**	-0.61	0.20	-0.63	-0.66*	0.74*
SDM								-0.03	0.08	0.38	-0.50	0.32	0.30	0.36
NNM									0.76*	-0.41	0.04	-0.42	-0.41	0.89**
NP										-0.78*	0.17	-0.80**	-0.78*	0.61
PW											-0.64	0.97**	0.98**	-0.06
NSP												-0.50	-0.59	-0.28
PL													0.98**	-0.11
WHS														-0.09

* and ** indicate significance at $P < 0.05$ and $P < 0.01$ by F test.

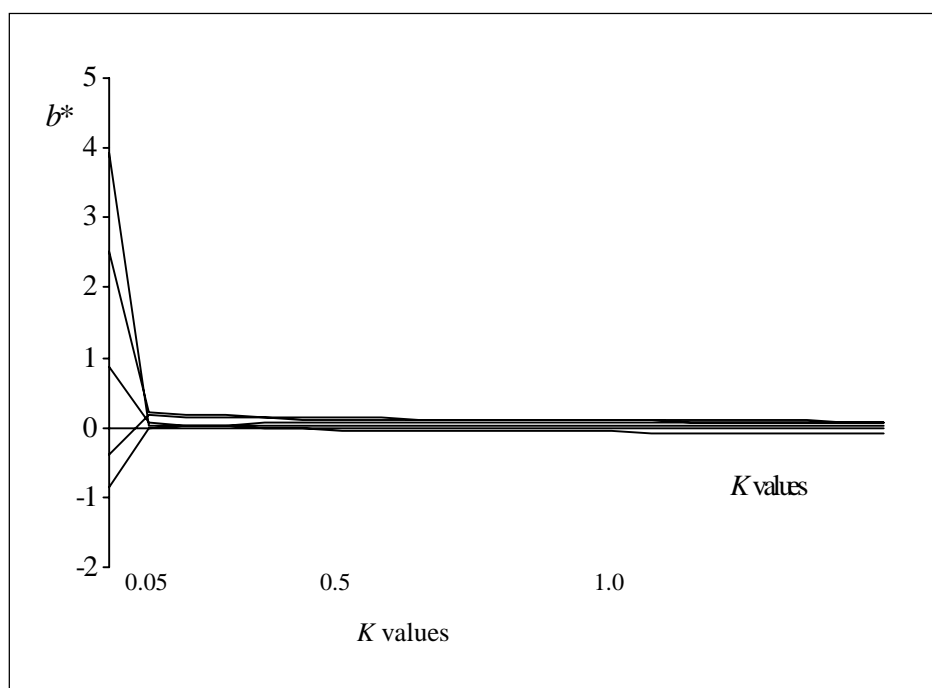
⁽¹⁾ SD - seedling diameter at emergence; NDF - number of days to flowering; PHF - plant height at flowering; RL - main or pivot root length at flowering; RDM - root dry matter; CDM - canopy dry matter; PHM - plant height at maturity; SDM - stem diameter at maturity; NNM - number of nodes on the main stem; NP - number of pods per plant; PW - pod width; NSP - number of seeds per pod; PL - pod length; WHS - weight of one hundred seeds; GY - grain yield per plant.

Table 4 - Estimates of direct effects of yield components on grain yield obtained from nine soybean genotypes.

Yield components ⁽¹⁾	Direct effects on grain yield ⁽²⁾		
	$K = 0$	$K = 0.05$	Trait culling
SD	3.915	0.020	-
NDF	0.060	0.090	-0.151
PHF	0.730	0.088	0.018
RL	0.936	0.222	0.104
RDM	0.508	-0.070	-
CDM	-0.856	-0.010	-
PHM	0.517	0.217	-0.326
SDM	-0.186	0.167	0.425
NNM	0.141	0.646	1.247
NP	0.232	0.169	0.010
PW	2.514	0.197	-
NSP	0.858	0.051	-
PL	0.199	0.178	-
WHS	-0.388	0.161	-
R ²	-0.324	0.958	0.997

⁽¹⁾ SD - seedling diameter at emergence; NDF - number of days to flowering; PHF - plant height at flowering; RL - main or pivot root length at flowering ; RDM - root dry matter; CDM - canopy dry matter; PHM - plant height at maturity; SDM - stem diameter at maturity; NNM - number of nodes on the main stem; NP - number of pods per plant; PW - pod width; NSP - number of seeds per pod; PL - pod length; WHS - weight of one hundred seeds.

⁽²⁾ The path coefficients were estimated using the ridge path analysis ($K = 0$ e $K = 0.05$) and with trait culling.

**Figure 1** - Path coefficient estimates (b^*) as a function of K values.

Similarly to the ridge path analysis, the R^2 for the method with trait culling was close to the unit. This showed that the variation of the basic variable GY was very well explained by the other traits. On the other hand, the $R^2 = -0.324$ obtained in the least squares method could have resulted from the method's poor estimation of the path coefficients.

The different seed size among the genotypes used in this study, measured by the WHS trait, did not greatly influence the grain yield variation. The ridge path analysis indicated that this trait presented low direct effect (0.161) on yield confirming the correlation study data. These results differed from those obtained by Ali et al. (1989), Shivashankar and Viswanatha (1989) and Taware et al. (1997), who found important direct effect of WHS on yield.

In spite of the importance detected in the correlation studies, the PHM trait presented a low direct effect in the path analysis. This showed the contribution of the path analysis in showing the true relationships of cause and effect among the traits assessed. The significant correlation between PHM and GY was the result of the high indirect effect of NNM, through PHM. Shivashankar and Viswanatha (1989), Taware et al. (1997) and Shukla et al. (1998) also found low association between PHM and GY. On the other hand, Ali et al. (1989) reported direct high effects of PHM on GY in soybean cultivated in different periods.

In summary, the results obtained in this study with ridge path analysis and with trait culling highlighted the NNM trait as the most associated with GY. The direct effect was high, as the correlation. NNM also showed minimum negative indirect effects through the other assessed traits. Also using path analysis, similar results were obtained by Akther and Sneller (1996), but highlighting PHM by side of NNM trait. Studies by Board et al. (1997) and Shukla et al. (1998), however, found variable and little influence of NNM on the grain yield, respectively.

CONCLUSIONS

The number of nodes on the main stem trait was highly correlated and has direct effect on soybean grain yield. It could, therefore, help at an indirect selection for yield. The path ridge analysis and the trait culling method were efficient in reducing the adverse effects of multicollinearity in the estimation of the path coefficients.

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RESUMO

Este trabalho teve como objetivos: a) desdobrar as correlações fenotípicas em efeitos diretos e indiretos de várias características sobre a produção de grãos da soja, através da análise de trilha, e b) comparar métodos alternativos de contornar os efeitos adversos da multicolinearidade na estimação dos coeficientes de trilha. O experimento foi conduzido em casa de vegetação, em delineamento de blocos completos casualizados, com quatro repetições. Foram utilizados nove genótipos de soja pertencentes a três categorias de tamanho de sementes. Os estudos de correlações e análise de trilha mostraram que o tamanho das sementes não foi importante no aumento da produção. Número de nós e altura de planta na maturação apresentaram correlações significativas com produção de grãos. Utilizando-se a metodologia dos quadrados mínimos, os resultados obtidos por meio da análise de trilha sob multicolinearidade foram pouco satisfatórios. A análise de trilha em crista e a eliminação de variáveis foram eficientes em reduzir os efeitos adversos de multicolinearidade. Esses dois métodos destacaram apenas o caráter número de nós na maturação como tendo alto efeito direto sobre a produção de grãos por planta.

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