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SELECTION OF PODS IN F4:5 GENERATION OF SOYBEAN TO UNDERSTAND ASSOCIATION BETWEEN YIELD COMPONENTS USING PATH ANALYSIS IN THE GREENHOUSE AND FIELD ENVIRONMENTS

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Abstract: In soybean breeding, several traits should be considered to a new improved variety. Among these traits, the number of seeds per pod has substantial influence, because combined with a large total number of pods per plant raises productivity. However, in plant breeding it is essential to understand the behavior of this trait in subsequent generations. The aim of this study was to analyze the influence of pods containing 1, 2 or 3 seeds of F4 generation in the formation of the next generation and their respective effect on the frequency of the number of seeds per pod, as well as the association of the yield components by path analysis F4:5. The experiment was conducted in the greenhouse and in the field, during the 2013/2014 crop season. Two F4 lines were used (L1 - Line 1 and L2 - Line 2) and a cultivar (M 7908RR). The experimental design was a completely randomized block in a factorial 3 × 3 design, involving three genetic materials (L1, L2 and M 7908 RR) and three different seed numbers per pod (1, 2 and 3) from each genotype. Regardless of the number of seeds in selected pods in F4, the frequency in pods of the next generation (F5) plants was not modified. In both environments studied the pods with 2 and 3 seeds had a direct and positive effect on seed yield. The selection for productivity can be obtained with the selection of plants with greater quantity of pods with two and three seeds.

Keywords: correlation, generation advancement; plant breeding; *Glycine max* (L) Merril; number of seeds per pod.

Introduction

Soybean breeding programs in Brazil aim to create new cultivars, taking into account mainly traits as the adaptation as the cycle cultivate habit and type of growth, juvenile period for flower induction, plant height and insertion the first pod, plant lodging, dehiscence of pods, seed quality, soil fertility, seeding in unconventional times, resistance to insect pests, disease resistance, content and quality of oil, content and quality of protein, flavor, tolerance to herbicides, plant type and productivity (Sediyama et al., 1999).

As the ultimate goal is to increase productivity, it is important to know which traits have the most influence. Several studies have shown that the number of pods is the component that most directly influences soybean yield (Board et al., 1997; Dalchiavon and Carvalho, 2012; Pereiraet al., 2018; Zuffo et al., 2018), since it has the highest correlation with grain production and pods with 1, 2 and 3 seeds may influence the size of seeds that are produced and, consequently, productivity. However, the number of seeds per pod may vary depending on the cultivar. Thus, by increasing the number of pods per plant, it is interesting that these pods contain a larger number of grains.

In this sense, it is fundamental to adopt breeding strategies that increase and maintain a higher number of grains per pod. Among the breeding methods, one of the most frequently used in soybean cultivation is single-pod descent (SPD). In this method, a pod is collected from each plant of the F2 generation, whose seeds will be sown for formation of the next generation, thus maintaining a representative of each F2 plant until homozygosis.

It is known that plants with many pods containing 3 seeds tend to be more productive. Thus, a pod harvested which contains a certain number of grains could influence the frequency of the same number of grains per pod occurring in the next plant generation. Therefore, would become important to harvest only those pods containing 3 grains to form the new generation.

In relation to correlation, because it is only a measure of association, it does not allow conclusions to be drawn about cause and effect or allow inferences about the type of association that governs a pair of characters. To overcome this limitation, Wright (1921) developed a method that allows a partitioning of the correlation coefficients for both direct and indirect effects (path coefficient). Thus, a path coefficient or cause-and-effect analysis can be defined as a standardized regression coefficient, and path analysis is composed of an expansion of multiple regression when complex interrelationships are involved (Cruz and Carneiro, 2003).

Knowledge of the degrees of association between characters makes it possible to identify variables that can be used in the indirect selection of another variable, such as yield, especially when heritability of the main trait is low (Iqbal et al., 2003). The unfolding of correlations between variables, with direct and indirect effects, through path analysis, measures, independently of the other variables, the direct influence of one character on another, following the diagnosis of multicollinearity between the explanatory variables and the independent ones (Cruz et al., 2004). In this way, the results allow identification of selection criteria for soybean yield, as demonstrated by Bizetiet al., (2004), Costa et al., (2004), Arshad et al., (2006), Alcântara Neto et al., (2011) and Santos et al., (2011).

In view of these observations, the objective of this work was to analyze the influence of the occurrence of pods containing 1, 2 or 3 grains on the formation of the next generation and their respective effect on the number of grains per pod, as well as the association of yield components by path analysis.

Material and methods

Local and plants materials

The experiment was carried out in two locations, in a greenhouse and in the field (20º49’23’’ S latitude, and 42º49’59’’ O longitude, and altitude of 804 meters) during the 2013/2014 crop season. Two F4:5 lines and one cultivar (M 7908 RR) were used. The choice of these materials was based on the average frequency of pods containing 1, 2 and 3 seeds, ie genotypes that had pods within the three quantities (1, 2 or 3). This was done by counting and separating the pods harvested in the F4 generation for sowing of the F5 generation. The cultivar M 7908 RR was used as a control for comparison with lineages 1 and 2.

Experimental design and genotypes utilized

The experiment was based on a 3 × 3 factorial scheme, with three genetic materials (L1 - Line 1, L2 - Line 2 and M 7908 RR) and three different seed numbers per pod (1, 2 and 3) from each genotype. The experimental design was a randomized block design, with four replications. Twelve plants (one plant/pot) were used for each treatment for the experiment conducted in the greenhouse. In the field, during the same period, plots each containing 3 rows of 1.5 m length were sown with seeds; the spacing between rows was 0.7 m. For the evaluation, in the greenhouse all 12 plants were evaluated and in the field 12 plants were taken from the central line of the plot. Three plants were used to generate an average value, with four repetitions per treatment.

Traits measured

The following characteristics were evaluated: number of pods with one, two and three seeds, total number of pods/plant, number of grains/ plant, number of grains/pod, 100-seed weight and seed yield/plant.

Statistical analysis

The data were submitted to analysis of variance and comparison of means by the Tukey test. The chi-square test was used to verify if there was difference in the values obtained from the different numbers of grains per pod of each genotype. Using the variances and phenotypic covariates obtained by ANOVA, the correlation matrix between the characters was generated. Path analysis (Wright, 1921) was performed, considering the main variable (yield/plant) as a function of yield components. The analysis was performed using the Genes program (Cruz, 2013). For the path analysis, the values of yield components were transformed to a logarithmic scale, due to the existence of interrelationships between them, due to the multiplicative effect.

Results and discussion

Analysis of variance

Table 1 shows that both in the greenhouse and in the field, there was a significant difference between the genotypes for the characteristics of pods with 1, 2 and 3 grains and total number of grains/plant. This result is expected, since the average number of pods with 1, 2 and 3 seeds, as well as the other yield components vary according to the cultivar. In many cases, there was a very considerable difference in these components. However, the productivity can be the same due to the compensatory effect of the plant.

Table 1. Summary of variance analysis for number of pods with one seed (NP1S), number of pods with two seeds (NP2S), number of pods with three seeds (NP3S) and total number of pods per plant (NP/PL).

Source of Variation	DF	NP1S	NP2S	NP3S	NP/PL
		Medium Squares			
		Greenhouse			
Block	3	12.21	5.13	0.26	30.18
Genotype (GE)	2	313.28**	1023.95**	1736,79**	2961.5**
Number of seeds/pod (NSP)	2	14.33 ns	2.97 ns	34.56 ns	4.35 ns
GE X NSP	4	11.35 ns	17.28 ns	29.87 ns	43.06 ns
Error	24	7.29	22.10	23.64	87.03
General media		**8.81**	**28.69**	**23.94**	**61.61**
CV(%)		**30.65**	**16.38**	**20.30**	**15.14**
		Field			
Block	3	4.00	135.02	58.35	320.0
Genotype (GE)	2	413.90**	2078.2**	1354.70**	10599.0**
Number of seeds/pod (NSP)	2	22.76 ns	75.90 ns	81.65 ns	436.66 ns
GE ×NSP	4	10.52 ns	29.12 ns	88.33 ns	274.18 ns
Error	24	12.72	116.32	71.12	392.84
General media		**10.26**	**28.97**	**27.51**	**66.94**
CV(%)		**34.73**	**37.23**	**30.65**	**29.61**

** Significant at 1% probability by F test
ns = not significant

Regarding the number of grains/pod, there was no significant difference in the number of grains/ pod in the next generation; that is, whether seed originated from a pod containing 1, 2 or 3 seeds of a given genotype, the average yield of pods containing 1, 2 or 3 seeds in the next generation was the same. This result can be better observed in Table 2 where the number of pods produced from each selected pod containing 1, 2 or 3 grains is shown. Using the Chi-square test (Table 2), no significance was detected for any of the genotypes, independent of the pod that the seed was derived from. For the other yield components, there was also only a significant difference between the geno-types, both in the greenhouse and in the field (Table 3).

Table 2. Number of pods containing 1 (P1S), 2 (P2S) or 3 (P3S) seeds for line 1 (L1), line 2 (L2) and M 7908 RR, and result of the Chi-square analysis.

Genotype	Origin	Quantity Produced			DF	χ2 Test
		NP1S	NP2S	NP3S		
L1	P1S	15.50	35.92	20.00	4	2.38 ns
	P2S	16.75	40.33	14.08		
	P3S	11.17	37.83	18.50		
L2	P1S	4.67	30.33	35.08	4	0.52 ns
	P2S	4.26	26.73	36.48		
	P3S	4.67	28.42	41.92		
M 7908 RR	P1S	8.33	18.33	15.29	4	0.33 ns
	P2S	7.17	20.50	17.08		
	P3S	6.83	19.83	17.08		

ns = notsignificant

Table 3. Summary of variance analysis for number of seeds per plant (NS/PL), number of seeds per pod (NS/P), 100-seed weight (100SP) and seed yield per plant (SY/PL).

Source of Variation	DF	NS/PL	NS/P	100SP	SY/PL
	Medium Squares				
	Greenhouse				
Block	3	66.00	0.004	2.48	6.12
Genotype (GE)	2	19787.4**	0.601**	97.98**	84.53**
Number of seeds/pod (NSP)	2	198.16 ns	0.012 ns	1.61 ns	0.75 ns
GE × NSP	4	232.78 ns	0.012 ns	1.17 ns	1.16 ns
Error	24	478.68	0.005	1.34	8.13
General media		**138.66**	**2.25**	**15.40**	**20.22**
CV(%)		**15.78**	**3.36**	**7.53**	**14.40**
	Field				
Block	3	1932.8	0.008	0.204	35.21
Genotype (GE)	2	49711.34**	0.035*	47.91**	651.73**
Number of seeds/pod (NSP)	2	2206.11 ns	0.35 ns	0.89 ns	31.86 ns
GE × NSP	4	1642.34 ns	1.11 ns	1.93 ns	25.22 ns
Error	24	2057.24	0.008	3.97	25.91
General media		**151.5**	**2.28**	**14.79**	**21.12**
CV(%)		**29.94**	**4.09**	**13.47**	**24.09**

* Significant at 5% probability by F test
** Significant at 1% probability by F test
ns = not significant

Comparison of averages

Using the means test (Table 4), it was observed that the number of pods with 1 grain was higher in Line 1 (L1) in the greenhouse, while in the field there was no significant difference between L1 and L2. The same effect occurred with the number of pods containing 2 grains.

In relation to 3 grains per pod, L2 produced large amount of the genotypes in greenhouse and in the field both L1 and L2 were higher than the control cultivar. In the final sum of the total number of pods per plant, it was observed that, in greenhouse and field, Lines 1 and 2 produced a significantly larger number of pods in relation to M 7908 RR. Despite this, the final yield would still depend on the grain mass of each genotype.

Table 4. Average number of pods with 1 seed (NP 1S), 2 seeds (NP 2S), 3 seeds (NP 3S) and number of pods/plant (NP/PL) in three soybean genotypes grown in the greenhouse and the field.

Genotype	NP 1S	NP 2S	NP 3S	NP/PL
	Greenhouse			
L1	14.47 a	38.02 a	17.52 b	70.02 a
L2	4.53 c	28.49 b	37.82 a	71.31 a
M7908 RR	7.44 b	19.55 c	16.48 b	43.48 b
	Field			
L1	15.27 a	39.33 a	36.47 a	91.08 a
L2	11.72 a	33.41 a	30.30 a	75.99 a
M7908 RR	3.80 b	14.16 b	15.77 b	33.75 b

1 Averages followed by the same lowercase letter in the column do not differ from each other at the 5% probability level using the Tukey test.

The final number of seeds per plant is obtained according to the number of pods with 1, 2 and 3 seeds, thus, Seeks out plants that have a higher number of pods with 3 grains, and some genotypes present a certain frequency of 4 seeds per pod. Thus, it was observed that L2 produced a higher number of seeds per plant than L1 and M 7908 RR in the greenhouse, but in the field L1 and L2 did not differ, both being significantly larger than the M 7908 RR. (Table 5). As the cultivar M 7908 RR produced a smaller quantity of pods with 3 seeds than L1 and L2, their total number of seeds was lower in both environments. Regarding the average number of seeds per pod, there was also a difference between the environments; in the greenhouse L2 produced the larger number of the three genotypes, whereas in the field there was little variation between the genotypes.

Table 5. Average number of seeds per plant (S/PL), number of seeds per pod (S/P), 100-seed weight (100SW) and seed yield per plant (SY/PL) in three soybean genotypes grown under greenhouse conditions and in the field.

Genotypes	S/PL	S/P	100SW	SY/PL (g/pl)
	Greenhouse			
L1	143.11 b	2.04 c	15.23 b	21.54 a
L2	176.86 a	2.49 a	12.63 c	21.96 a
M7908 RR	96.01 c	2.21 b	18.34 a	17.17 b
	Field			
L1	203.36 a	2.24 b	14.23 b	28.23 a
L2	171.69 a	2.25 ab	13.14 b	21.63 b
M7908 RR	79.47 b	2.34 a	17.01 a	13.52 c

1 Averages followed by by the same lowercase letter in the column do not differ from each other at the 5% probability level using the Tukey test.

The last important component of the yield is the mass of grains. The ideal would be a plant with many pods containing 3 seeds, and that these seeds would be large. However, it is not easy to combine these traits into a single genotype since soybean can generate compensatory effects, that is, the more the number of seeds per pod is increased, the more the seed mass decreases and vice-versa. Thus, it is observed that L2 which produced the highest number of seeds in the greenhouse showed lower mass; this was also observed in the field for both L1 and L2. In contrast, the cultivar M 79078 RR produced a significantly smaller number of seeds but produced seeds with greater mass. In view of this, it is up to the breeder to consider all the components as a whole in order to obtain greater productivity.

The yield of genotypes studied in the greenhouse was significantly higher in L1 and L2 than in M 7908 RR, while in the field the following order was observed: L1> L2> M 7908 RR.

Path analysis

Table 6 shows the direct and indirect effects of the yield components on seed yield. The determination coefficients (R^2) in the path analysis model were higher than 0.95 with low residual effects. Thus, the explanatory model adopted expressed the cause and effect relationship between the yield components and the seed yield.

Table 6. Estimation of direct and indirect effects of yield components: pod with 1 seed (P1S), pod with 2 seeds (P2S), pod with 3 seeds (P3S), total number of pods per plant (NP/PL), total number of seeds per plant (S/PL), total number of seeds per pod (S/P) and 100-seed weight 100SW) in seed yield/plant (SY/PL) in three genotypes grown in the greenhouse and field by

	Character/Effects	Greenhouse	Field
P1S	Direct on yield	0.1104	0.1396
	Indirect via P2S	0.0948	0.1267
	Indirect via P3S	-0.0185	0.092
	Indirect via NP/PL	0.1086	0.0932
	Indirect via S/PL	0.0644	0.0754
	Indirect via S/P	-0.1355	0.0966
	Indirect via 100SW	-0.1375	0.0766
	TOTAL	**0.0922**	**0.7079**
P2S	Direct on yield	0.3036	0.1838
	Indirect via P1S	0.0344	0.0962
	Indirect via P3S	0.2071	0.1709
	Indirect via NP/PL	0.2235	0.1672
	Indirect via S/PL	0.2352	0.1599
	Indirect via S/P	-0.0244	0.0391
	Indirect via 100SW	-0.2507	0.1722
	TOTAL	**0.7444**	**0.9997**
P3S	Direct on yield	0.3156	0.1746
	Indirect via P1S	-0.0065	0.0736
	Indirect via P2S	0.1993	0.1800
	Indirect via NP/PL	0.1906	0.1699
	Indirect via S/PL	0.2373	0.1664
	Indirect via S/P	0.0653	0.0187
	Indirect via 100SW	-0.166	0.1805
	TOTAL	**0.8518**	**0.9734**
NP/PL	Direct on yield	0.2453	0.1700
	Indirect via P1S	0.0488	0.0766
	Indirect via P2S	0.2767	0.1809
	Indirect via P3S	0.2452	0.1746
	Indirect via S/PL	0.2603	0.1659
	Indirect via S/P	-0.0212	0.0213
	Indirect via 100SW	-0.2728	0.1798
	TOTAL	**0.7948**	**0.9789**

(to be continued)

Table 6. Estimation of direct and indirect effects of yield components: pod with 1 seed (P1S), pod with 2 seeds (P2S), pod with 3 seeds (P3S), total number of pods per plant (NP/PL), total number of seeds per plant (S/PL), total number of seeds per pod (S/P) and 100-seed weight 100SW) in seed yield/plant (SY/PL) in three genotypes grown in the greenhouse and field by (continuation)

	Character/Effects	Greenhouse	Field
S/PL	Direct on yield	0.2684	0.1670
	Indirect via P1S	0.0265	0.0630
	Indirect via P2S	0.2661	0.1761
	Indirect via P3S	0.2791	0.1740
	Indirect via NP/PL	0.2379	0.1689
	Indirect via S/P	0.0193	0.0098
	Indirect via 100SW	-0.2481	0.1817
	TOTAL	**0.8627**	**0.9499**
S/P	Direct on yield	0.1725	-0.1037
	Indirect via P1S	-0.0867	-0.1300
	Indirect via P2S	-0.0430	-0.0693
	Indirect via P3S	0.1195	-0.0315
	Indirect via NP/PL	-0.0302	-0.0349
	Indirect via S/PL	0.0300	-0.0158
	Indirect via 100SW	0.0716	-0.0110
	TOTAL	**0.2419**	**-0.401**
100SW	Direct on yield	0.4834	0.1818
	Indirect via P1S	-0.0314	0.0588
	Indirect via P2S	-0.1575	0.1742
	Indirect via P3S	-0.1084	0.1734
	Indirect via NP/PL	-0.1384	0.1682
	Indirect via S/PL	-0.1377	0.1669
	Indirect via S/P	0.0255	0.0063
	TOTAL	**0.0402**	**0.9388**
	Residual effect	**0.2144**	**0.0920**
	R2	**0.9539**	**0.9901**

The highest values of positive direct effects of the components on yield were observed for NP 2S, NP 3S and 100SW in greenhouse, while in the field NP 2S, NP 3S, NP/PL, S/PL and 100SW showed similar direct effects on yield, ie in the field The formation of productivity was dependent on several components. Negative direct effects were observed only for S/PL in the field, but this effect was low. The greatest difference in the direct effect between the sites was in the 100SW, being much larger in greenhouse (0.48) than in the field (0.18). Perini et al., (2012) worked with cultivars of determinate and indeterminate growth type and concluded that, regardless of the growth type, the number of grains per plant is directly related to the maximum yield in soybean.

Correlations were highly positive for all variables, both in the field and in the greenhouse, except for S/P, with values of 0.24 and -0.40, respectively. The fact that changes in correlations between the same traits in both locals suggests that the selection of plants based on cycle and/or plant traits with grain yield will only result in some gain if it is carried out at the place and sowing season of adaptation of genotype (Nogueira et al., 2012).

Regarding the effect of the variables in relation to pods containing different numbers of grains, in the greenhouse pods with 2 and 3 seeds contributed more to yield, whereas in the field this direct effect of the pods containing 2 and 3 seeds was lower, due to greater indirect effects of other variables on this component. Szareski et al. (2018) concluded that number of three-grain pods is among the components that mostly related to grain yield in both determinate and indeterminate cultivars, regardless of environment. Carpentieri-Pípolo et al. (2005), working with phenotypic correlations between quantitative traits in soybean, observed that for yield values of genotypes, the highest yields were observed in genotypes with higher values of P2S, and not with plants with higher NP/PL values or those with higher P3S values.

For breeding purposes, it is important to identify between high-correlation traits with a main trait and a greater direct effect in favor of selection, so that the correlated response through indirect selection is efficient (Cruz et al., 2004).

Conclusions

Irrespective of the number of seeds (1, 2 or 3) in the pods selected in F4, the frequency of the number of seeds in the pods in the next generation (F5) plants was not modified.

In the two environments studied (greenhouse and field), the pods with 2 and 3 seeds had a direct and positive effect on yield. Thus, the selection for productivity can be obtained with the selection of plants with greater quantity of pods with two and three grains.

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