Grain yield and seed quality of soybean selected for high protein content

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Abstract – The objective of this work was to evaluate the effects of selection for high protein on seed physiological quality and grain yield of soybean. Four populations of BC_1F_4 and four of F_4, each from a cross between a commercial variety and a line bearing high protein seeds, were used. The high protein content selection has a tendency to affect negatively the seed physiological quality. Estimates of correlation coefficients between protein content and grain yield were mostly negative but varied among populations. It is possible to obtain lines with high protein content, keeping the grain yield and the seed physiological quality of their respective recurrent progenitors.

Index terms: *Glycine max*, protein content, progenitors, plant breeding.

Produtividade de grãos e qualidade de sementes de soja selecionada para alto teor de proteína

Resumo – O objetivo deste trabalho foi avaliar a influência da seleção para alto teor protéico na qualidade fisiológica da semente e na produção de grãos de soja. Utilizaram-se quatro populações RC_1F_4 e quatro F_4, originadas, cada uma, do cruzamento de um material com alto teor protéico na semente com uma variedade comercial. Houve tendência de seleção para alto teor protéico influenciar negativamente a qualidade fisiológica das sementes. As estimativas dos coeficientes de correlação entre teor de proteína na semente e produção de grãos foram, na maioria, negativas porém variáveis entre as populações. É possível obter linhagens com teor protéico mais elevado, mantendo as médias de produção de grãos e do potencial fisiológico das sementes de seus respectivos progenitores recorrentes.

Termos para indexação: *Glycine max*, teor em proteína, progenitores, melhoramento de plantas.

Introduction

Soybean is a crop of great world importance due to the widespread applicability of its products and their economic value in the national and international market. It is a consolidated crop in Brazil, which is among the best world producers.

Industry requires soybean grains of high protein content. However this factor is generally inversely related to the grain yield. Many times the most productive cultivars should not be of interest for industry and, as a consequence, there is a demand for cultivars with both improved grain yield and high seed protein content.

Protein and oil are soybean seed components of great economical value. On average, protein content of commercial varieties is around 40%, but it can vary from 30% to 53%. In recent years, soybean breeding programs have been emphasizing the increase of protein content and quality (Sediyama et al., 1996, 1999).

Seeds should possess high germinability and vigor, besides exhibiting intact tegument, uniformity in size and shape, varying from 13 to 15 g/100 seeds, and proper storability under environmental conditions, until the next sowing season. Preference should be given to varieties with at least 80% of germination and high vigor, when harvested even two or three weeks after maturity of 95% of the pods (Sediyama et al., 1999).

Among the available procedures adopted to select seed lots of higher physiological quality, the accelerated aging and the seedling emergence tests have shown consistent results (Sediyama et al., 1999). Differences on speed of water absorption verified in different species would be, according to Carvalho & Nakagawa (2000), mainly related to seed chemical composition; the higher protein content usually corresponds to a faster water uptake by seeds.

Correlation between characters reflects the degree of association between them. Usually in breeding programs, besides looking for the improvement of a main character, others are as well maintained constant or improved together. Therefore, estimating correlation coefficients among the traits becomes particularly important because it makes possible to know how the selection for a certain character influences the expression of another (Piovesan, 2000).

According to Falconer (1987), in genetic studies it is necessary to distinguish two causes of correlation between characters, genetic and environmental. The genetic cause of correlation is chiefly pleiotropy, though linkage is a cause of transient correlation particularly in populations derived from crosses between divergent strains. Environmental correlation among characters occurs when they are influenced by the same differences of environmental conditions.

The objective of this work was to evaluate the effects of selection for high seed protein content on soybean seed physiological quality and grain yield.

Material and Methods

The experiment was carried out at the Universidade Federal de Viçosa (UFV), Viçosa, MG, Brazil, and consisted of two parts. The first stage was conducted in the Campo Experimental “Professor Diogo Alves de Mello”, located at 20°45'55" S, 42º52'5" W and 650 m of altitude. The second stage was carried out in the laboratories of the Departamento de Fitotecnia and of the Instituto de Biotecnologia Aplicada à Agropecuária (Bioagro).

Eight populations, raised up by the Soybean Breeding Program of Bioagro/UFV, were used. The families, which constituted the populations, were part of the fourth generation of self-fertilization either after artificial hybridization (F_4) or after the backcross (BC_1F_4). Each of those families was originated from the cross between a line of high seed protein content (donor ancestor) and a commercial variety (recurrent ancestor) belonging to Cooperativa Central Agropecuária de Desenvolvimento Tecnológico e Econômico Ltda. (Coodetec).

In the F_2 generation, about 300 F_2 seeds were analyzed for protein content by the bicinconinic acid method and the 10% of highest content were selected as progenitors for the F_3 generation. Seeds at F_3 generation were analyzed for protein by the Kjeldahl method. Among the plants with higher seed protein content, four plants genetically closer to the recurrent ancestor of each family were selected. The families were then, successively, self fertilized and harvested in bulks.

The F_4 and BC_1F_4 populations were sown on January 1st, 2001. They were formed by 17, 13, 20 and 11 BC_1F_4 families, identified as OC13BC, CD201BC, CD205BC and CD206BC, respectively and by 23, 22, 20 and 17 F_4 families, identified as OC13CR, CD20CR, CD205CR and CD206CR, respectively. Those families were originated from the four commercial varieties Ocepar 13 (OC13-P), Coodetec 201 (CD201-P), Coodetec 205 (CD205-P) and Coodetec 206 (CD206-P), and were sown on six, six, five and three replications, respectively. Additionally, three other commercial varieties, CAC1 (CAC1-C), Coodetec 203 (CD203-C) and Coodetec 205 (CD205-C), were used as controls in 20 blocks. The donor ancestor for OC13 and CD205 varieties was the IAC12PTN line, for CD201 was BR 80 14887 and for CD206, was the CAC1PTN line. The protein content in the seeds of the three donor ancestors are 51.00%, 47.80% and 54.20%.

Each experimental plot had 20 seeds sown in the field, in a row of 2 m long spaced by 0.5 m. Seeds were treated with benomyl and metalaxyl + mancozeb fungicides and also with *Bradyrhizobium japonicum* inoculant. Weeds were controlled by the pre-emergent herbicide sulfentrazone at 60 g ha^{-1} a.i. Soil fertilization was accomplished during the sowing using 400 kg ha^{-1} of N–P–K (0–30–10). During plant development, the necessary crop management and complementary sprinkling irrigation procedures were provided.

The harvest was carried out ten days after 50% of the plants of the row reached the R8 stadium (Fehr & Caviness, 1977), from April 20 to May 25, 2001, with no rain in the period. Only the seeds produced by the six central plants of each row were collected for grain yield and for the tests described as follows.

Seeds total protein content was evaluated by the modified Kjeldahl method according to Instituto Adolpho Lutz (1985). Three seeds from each of the six plants per plot were aggregated to form a bulk of 18 seeds to be analyzed. The analyses were made with two replications, and in case of differences larger than one percent between them, they were analyzed again. A 6.25 factor was used to convert N into protein (Association of Official Analytical Chemists, 1984).

The seedling emergence test (SET) was accomplished using three replications of 40 seeds per treatment. Washed and sieved sand was treated with methyl bromide and placed in 30x25x5 cm trays. On each tray, six rows of 40 seeds of different treatments were sown. The test was carried out in a greenhouse with relative humidity around 85% and temperature from 21^{o}C to 30^{o}C. Five and eight days after sowing, the seedling emergence was evaluated by scoring the percentage of normal and abnormal seedlings and of the dead seeds.

The accelerated aging test (AAT) was accomplished by the procedure described by Marcos Filho (1999), with modification. Three replications of 50 seeds were used per treatment. Plastic boxes (11.0x11.0x3.0 cm) where adapted with a screen at 1,5 cm height, which was divided into four cells by plastic soda straws, in order to place four 50-seed samples from distinct treatments in each box. Forty milliliters of water was previously placed under the screen to keep the humidity near to 100%. The boxes were then closed with the lids and placed into a BOD-type chamber, where they remained for 48 hours at 41^{o}C. After this period, seeds were germinated on paper towel rolls following the germination test procedure (Brasil, 1992); the substrate was moistened with water amount equivalent to 2.5 times its weight. The paper rolls were placed vertically into a seed germinator, at 25^{o}C and 100% relative humidity. On the fifth day after sowing, the percentage of normal seedlings was recorded.

For both the seedling emergence and the aged seed germination tests, tolerable discrepancy among replications was observed, following Miles (1963), according to Brasil (1992).

The experiments were setup with family rows intercalated by control rows and only one replication of a family was planted due to the small number of seeds available. The completely randomized experimental design was used, so within each population, the family rows were considered replications.

The 20 replications (randomized blocks) of control varieties intercalated with each of the 10 family rows were used to estimate the blocking effect and to evaluate the homogeneity of the environment. Percentage data from the aged seed germination and from seedling emergence tests were transformed to arcsine for statistical analysis and the means were reconverted to percentage for presentation. The analyses of variance and the estimation of means and phenotypic correlation coefficients were accomplished using the SAEG statistical analyses package and the GENES computational program (Cruz, 2001).

Results and Discussion

There was no significant variability among blocks, for the evaluated characters, which indicates that the environmental variation was evenly distributed (Table 1). Thus, it was not necessary to correct the data for variations among blocks. There were significant differences among control varieties, regarding to grain yield per plant and protein content in the seeds, which suggests variability of these characters among the controls.

The precision of the experiment, estimated by means of the coefficient of variation, was at medium to high level, according to Pimentel-Gomes (1984). There was significant variation among populations on all evaluated characters. Comparing the progenitors (P) amongst themselves, as well as with the controls (C) and populations (PP), significant differences of protein content in the seeds (PROT) may be observed.

Table 1. Analysis of variance of soybean grain yield per plant (YIELD), seed protein content (PROT), seedling emergence (SET) and accelerated aging test (AAT).

Source of variation	df	Mean squares			
		YIELD	PROT	SET	AAT
(P+C+PP)	14	256.01**	128.23**	151.25**	541.76**
Error	201	15.25	3.82	39.45	103.03
CV (%)		21.43	4.43	8.28	15.70
Progenitors (P)	3	33.08	11.58**	38.07	232.51
Error	16	17.66	1.91	16.34	113.80
CV (%)		20.04	3.30	5.33	16.01
Blocks of C	19	16.29	1.25	41.46	90.58
Controls (C)	2	63.72**	33.01**	12.10	120.64
Error	38	11.83	1.68	55.48	99.75
CV (%)		17.74	3.29	10.33	15.51
Populations (PP)	7	412.28**	29.40**	266.49**	903.28**
Error	135	15.00	4.82	35.35	99.08
CV (%)		22.95	4.85	7.94	15.86
Among P, C and PP	2	17.46	39.14**	5.59	24.28
Error	12	20.56	1.94	11.05	42.54
CV (%)		23.74	3.23	4.43	10.13

**Significant at 1% probability level, by the F test.

Most populations showed, as expected, higher protein content than their recurrent progenitors (Table 2). The CR and the CD205-BC families showed protein content statistically higher than their recurrent progenitors. Commercial soybean varieties available in the market have, on average, 40% of seed protein content (Sediyama et al., 1996). The average protein content of the studied populations was 45.06%.

The populations OC13-BC, CD205-CR and CD206-BC showed greater seedling emergence scores than population CD206-CR (Table 2), however, all the populations have shown satisfactory seed physiological quality as values was above 80% (Brasil, 1981; Sediyama et al., 1999). According to Egli & Tekrony (1995), a seed lot has high probability to perform high field seedling emergence under varied environmental conditions, if seed germination is higher than 95% and vigor, obtained by the accelerated aging test, above 80%. The use of one or other criterion will depend on the purpose of the seed lot utilization and handling.

Regarding the accelerated aging test, there were no significant differences among most of the populations; only the population CD206-CR showed mean value lower than the means of other populations, which ranged from 71.34% (population OC13-P) to 89.71% (population CD205-P). The results here obtained can be considered as compatible to those obtained by Braccini et al. (1994, 1997) and Oliveira et al. (1999), for seed lots bearing desirable physiological quality, except for population CD206-CR, which despite of presenting emergence rate (SET) above 80%, has shown low vigor (AAT), indicating a poor storage potential.

Table 2. Estimated means of soybean grain yield (YIELD), seed protein content (PROT), seedling emergence (SET) and accelerated aging test (AAT) for each population, progenitor (P), and Controls (C)(1).

Treatments	YIELD (g/plant)	PROT (%)	SET (%)	AAT (%)
OC13-BC	13.01d	45.70abc	94.86a	86.88a
OC13-CR	14.01d	46.08ab	92.54ab	74.71a
CD201-BC	21.47b	43.03de	94.37ab	82.64a
CD201-CR	14.68cd	45.66abc	94.05ab	84.77a
CD205-BC	13.61d	44.81bcd	92.35ab	78.63a
CD205-CR	18.61bc	44.39bcd	96.08a	83.50a
CD206-BC	29.58a	43.50cde	95.95a	86.03a
CD206-CR	17.63bcd	47.26a	83.59b	52.56b
OC13-P	18.20bcd	43.61cde	90.69ab	71.34ab
CD201-P	22.22b	40.97ef	95.11a	88.21a
CD205-P	20.52bc	40.40efg	95.61a	89.71a
CD206-P	24.75ab	42.43de	94.85ab	88.55a
CAC1-C	18.58bc	38.06fg	92.02ab	81.45a
CD203-C	18.16bcd	39.47ef	92.63ab	84.47a
CD20-C	21.44b	40.63g	91.15ab	77.79a
Progenitors	21.43a	41.85b	94.20a	85.07a
Controls	19.39a	39.39b	91.94a	81.32a
Populations	17.82a	45.06a	93.32a	79.39a

(1)Means within a column followed by the same letter are not significantly different at 0.05 probability level, by the Tukey test.

Comparing the population yield means to the means of their respective progenitors, the populations yield were statistically similar to their recurrent progenitors except for populations CD201-CR and CD205-BC.

When the progenitors, controls and populations averages were compared, the populations showed statistically higher protein content, maintaining the grain yield and the seed physiological quality (Table 2).

Correlations between grain yield per plant (YIELD), protein content in the seed (PROT), seedling emergence (SET) and accelerated aging (AAT) are presented for each of the eight populations (Table 3). For all pair of traits, varied results were found for different populations, except for the correlation between SET and AAT that was positive and significant for all the populations.

When averages of the eight populations were analyzed together, there was a negative and significant correlation of PROT with YIELD, SET and AAT (Table 4). The correlations between SET and AAT were positive and significant. Both characters that measure seed physiological quality produced positive correlations, although low and not significant, with YIELD.

The results of correlations between seed protein content and grain yield in soybean, frequently found in the literature, show negative correlation between these

Table 3. Phenotypic correlations between soybean grain yield per plant (YIELD), seed protein content (PROT), seedling emergence (SET) and accelerated aging test (AAT), within each population.

Populations	YIELD/ SET	YIELD/ AAT	SET/ AAT	PROT/ YIELD	PROT/ SET	PROT/ AAT
OC13-BC	0.29	0.21	0.63**	-0.27	-0.18	0.45*
OC13-CR	0.18	-0.16	0.43*	-0.29	-0.19	-0.50**
CD201-BC	0.09	0.03	0.70**	0.35	-0.54*	-0.49*
CD201-CR	-0.07	-0.01	0.71**	-0.41*	-0.13	-0.04
CD205-BC	0.11	0.16	0.58**	-0.49*	-0.34	-0.40*
CD205-CR	-0.28	-0.46*	0.87**	-0.09	0.14	0.09
CD206-BC	-0.58*	-0.18	0.71**	-0.21	0.24	0.00
CD206-CR	-0.34	-0.21	0.55*	-0.29	0.24	-0.02

* and **Significant at 5% and 1% probability levels, respectively, by the t test.

characters (Johnson et al., 1955; Kwon & Torrie, 1964; Wehrmann et al., 1987). Nevertheless, CD201-CR and CD205-BC populations showed, when analyzed individually, significant negative correlations between the referred characters (Table 3). The fact that the families have already been selected for high seed protein content and, at the same time, for genetic proximity to the recurrent progenitor, reduces the correlations among those characters, since the extreme opposites are discarded. The results found by Holbrook et al. (1989) cited by Piovesan (2000), Wilcox & Cavins (1995) and Scott & Kephart (1997) show the possibility of the selection of families bearing high protein content, without losses to the grain yield, or vice-versa. Except for CD201-CR and CD205-BC populations, the correlations between those characters for the populations were not consistent. Positive correlation – for population CD201-BC – between PROT and YIELD was found, so far. When analyzed together, the negative effect of the selection for high seed protein content on grain yield was emphasized, resulting in negative and significant correlation between these characters (Table 4).

The estimates of the correlation coefficients between PROT and the characters that evaluate the seed physiological quality (SET or AAT) were not consistent when each population was analyzed separately (Table 3). It can be inferred that the genetic material has fundamental importance in the establishment of these correlations, since it was the only variant among the populations analyzed.

The negative and significant correlation among those characters, when the populations were analyzed together, indicates the harmful indirect effect of the selection for high seed protein content on the physiological quality of the seeds (Table 4). Nevertheless, as a satisfactory level of seed physiological quality was found (Table 2), it can be pointed out that a breeding program for high protein content in soybean seeds does not seem to reduce seed physiological quality.

Table 4. Phenotypic correlations between soybean grain yield per plant (YIELD), seed protein content (PROT), seedling emergence (SET) and accelerated aging test (AAT), based on the averages of the populations, when pooled together.

Characters	PROT	SET	AAT
YIELD	-0,64*	0.28	0.17
PROT		-0.74*	-0.68*
SET			0.96**

* and **Significant at 5% and 1% probability levels, respectively, by the t test.

Simpson & Wilcox (1983), analyzing soybean seeds in the F_6 and F_7 generations from crosses of high protein and high grain yielding varieties, found negative and significant correlation between protein content and physiological quality of the seeds for three out of four analyzed crosses, and the fourth cross showed very low correlation (0.07) between those characters. Positive and significant correlations between seed protein content and seedling vigor – determined by seedling weight – were found by Ries et al. (1971), cited by Lowe & Ries (1972), working on common bean, and by Lowe & Ries (1972), Lowe et al. (1972) and Ries & Everson (1973), working on wheat. It is worthy to mention that the data from those authors were relative to populations that produced high protein content due to the application of high levels of nitrogen, and not due to genetic breeding, as in the present case.

Conclusion

It is possible to obtain lines with high protein content and keep, simultaneously, the grain yield and the seed physiological quality averages of their respective recurrent progenitors.

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