Genetic variability for traits related to cooking time in soybean

Deonisio Destro*[1]; Henrique Stoco Bizeti[1]; Mario Marega Filho[1]; Lizz Kezzy de Morais[1]; Cláudia Tróia[1] and Ricardo Montalván[2]

[1]Departamento de Agronomia, Universidade Estadual de Londrina, Caixa Postal 6001, CEP 86051-990 Londrina, PR, Brazil; [2] Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Centro Experimental Balsas, Caixa Postal 131, CEP 65800-000, Balsas, MA, Brazil. (* Corresponding Author. E-mail: ddestro@uel.br)

ABSTRACT

Long cooking time for soybean seeds hinders their regular 'in natura' use as a rich source of protein. The objective of this research was to study the genetic variability of cooking time in soybean and its correlation with seed weight and imbibition percent. Pure food-type soybean lines were assessed having been cultivated in a greenhouse (experiment 1) and pure lines derived from crosses between grain type and food type soybean cultivars cropped in the field (experiment 2). In experiment 1, wide variability was detected in all the traits and the cooking time varied from 26 minutes to 170 minutes. In experiment 2 the range of cooking time was less (63 to 124 minutes). The most pronounced correlations were between imbibition percent and cooking time (-0.41*) for experiment 1 and between seed weight before and after imbibition with cooking time (0.42 and 0.41*) for experiment 2. The results showed substantial genetic variability in soybean cooking time. This can be used to obtain cultivars for human consumption with important decreases in cooking time.

KEY WORDS: *Glycine max*, genetic breeding, food-type soybean.

INTRODUCTION

Soybean (*Glycine max.* L. Merrill), a plant of the leguminous family, is of great interest in human nutrition. Its grains are rich in proteins and more recently research has shown important effects in the prevention of diseases such as cancer, arteriosclerosis and diabetes (Carrão-Panizzi and Mandarino, 1998).

Vello (1992) classified soybean in two main types, grain type and food type. Grain type soybean has medium-sized seeds, represented by the weight of a hundred seeds between 10 and 19 grams, and is cultivated mainly to meet the needs of Brazilian and foreign meal and oil industries. Food type soybean presents two categories a) small seeds, with a weight of one hundred seeds of less than 10 grams, for consumption in the form of natto shoots (fermented sprouts); b) large seeds, weight of one hundred seeds equal or greater than 20 grams. Of the latter, ripe grains are consumed as sweet soybean or kuromamme (black tegument), as salad (light colored tegument) and as tofu (curds) and extract (milk) and further green pods are eaten as a vegetable.

In spite of the recognition of the advantages presented by using soybean 'in natura' for human consumption, it is hardly used for this purpose in Brazil. This low acceptance is due to the undesirable flavor and odor characteristics and long cooking time. The unpleasant flavor and odor have been attributed to the lipoxigenase enzyme, that acts on lipid oxygenation. Soybean maceration and cooking are procedures that facilitate the inactivation of the enzyme (Berra, 1974). However Mwandemele et al. (1984) stated that long cooking time impeded the regular use of soybean as a protein source.

Studies have shown that there is genetic variability for quality attributes that influence consumer acceptance such as flavor, cooking time and flatulence factors (Mwandemele, 1986). Turatti et al. (1994) reported significant variation in cooking time for cultivars from different locations and also within the same cropping locations. However, information on the genetic variability of cooking time in soybean is still very limited.

Genetic variability of cooking time was studied and its correlation with seed weight and seed imbibition percent. This information is important for breeding and selecting lines for human consumption.

MATERIAL AND METHODS

Experiment description

Two experiments were carried out to assess cooking time of soybean genotype grains with possibilities for use in human consumption. Thirty pure lines, four

grain type and 26 food type, were used in experiment 1. The seeds of these materials were obtained by harvesting plants cultivated in pots in a greenhouse. Twenty-four pure lines cultivated in the field derived from crosses between food type soybean and grain type soybean were used. The two experiments were harvested manually approximately 15 days after the R_8 harvest maturity stage, according to the Fehr and Caviness scale (1977). A completely randomized block design was used, with four replications for experiment 1 (each plot consisted of one pot with three plants) and three replications for experiment 2 (one plot consisted of one 5m long line). The experiments were carried out in the 1994/95 growing season on the School Farm of the Londrina State University (Londrina, Paraná, Brazil) situated at 23° and 22´LS 51° and 10´long. For experiment 2, the soil was prepared according to conventional practices, with one plowing and two gradings and application of 555 kg/ha fertilizer formula (N-P-K) 00-28-20 at sowing. After sowing, the plants were hoed manually to control weeds. Insecticide was applied to control the defoliating velvet soybean caterpillar (*Anticarsia gemmatalis*) and the soybean stinkbug (*Nezara viridula*) following technical recommendations for soybean cultivation in Paraná state, Brazil (Embrapa Soja, 1995).

The Food and Medicine Technology Laboratory was used to collect the data at the Agricultural Sciences Center at Londrina State University. The seed weight was determined before imbibition and also the weight of seeds after imbibition, imbibition percentage and cooking time. Samples of 25 seeds were removed from each replication from each experiment. The seeds were stored in a cold chamber at 15°C and analyzed two months after harvest.

Water absorption percentage or imbibition percentage

An adaptation of the model proposed by Jackson and Varriano-Marston (1981) was used to determine the water absorption percentage or imbibition percentage. First the dry weight of the samples of 25 seeds from each genotype of each replication and from each experiment was determined. The seeds were later imbibed in distilled water at a proportion of 1:5, and one seed to five parts water. The seeds were left to soak for 18 hours. After imbibition, the seeds were drained and the wet weight determined. The imbibition percentage (CT) was obtained by the following formula:

$$CT = [(WAI - WBI)/WBI]*100$$

Where:

WAI= wet weight after imbibition

WBI = dry weight before imbibition.

Cooking time

The cooking time was measured by a modified Mattson machine (Mattson, 1946), proposed by Jackson and Varriano-Marston (1981). The technique consisted of determining the cooking time by the percentage of grains cooked in the machine (50%). After imbibition the 25 seeds were placed in individual capsules in the modified Mattson machine, that consisted of 25 vertical rods, with a 90 gram weight on each grain, whose points rest on the soybean seed during the test. The machine is then taken to a recipient containing boiling distilled water. With time, the rods fall and perforate the grains. After the fall of the 13th rod the sample is considered cooked. The time taken from immersion of the machine in boiling water until the fall of the 13th rod is considered as the sample cooking time.

Preliminary tests revealed that there was a mistake in the measuring at the start of the cooking time determination by the modified Mattson machine. The cold machine, when placed in the water, took time to heat, making the water bath lose 3 to 4°C. To avoid this inconvenience, the machine was previously heated in a water bath, without the seeds. The water bath was filled up with boiling water whenever necessary because of water loss through evaporation. The water temperature during the measurements remained around 98°C.

Analysis of variance was performed and means were compared by the Tukey test (5%) using the Genes computer program (Cruz, 1997). The phenotypic (s^2_F), genotypic (s^2_G) and environmental variances (s^2_E), the coefficient of genotypic determination (H) and the coefficients of correlation for the traits were assessed. The coefficient of genotypic determination was estimated as indicated (Falconer, 1989; Carbonell and Vello, 2001):

$$H = [\sigma^2_G / \sigma^2_F] = [(\sigma^2_F - \sigma^2_E) / \sigma^2_F].$$

Phenotypic (r_F) and genotypic (r_G) correlations estimates among the 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 (Venkovsky and Barriga, 1992).

RESULTS AND DISCUSSION

Significant differences were detected among treatments for all the traits. The low CV% values (ranging from 1.51 to 6.94) (Table 1) indicated good experimental accuracy and that the methodology used in the assessments was suitable. Furthermore, the high H values showed that the traits studied presented low environmental influence (Table 1). Thus much of the variation detected among treatments is of genetic nature.

The estimated parameters (Table 1) and the minimum significant difference indicated in the Tukey test (Tables 2 and 3) showed that in experiment 1 the differences among treatments for seed weight before (WBI) after (WAI) imbibition and cooking time (CT) were more pronounced than the differences for imbibition percent (IP). In experiment 2, the differences between the treatments were more pronounced only for seed weight before and after imbibition. The cooking time varied more in experiment 1, ranging from 26 minutes for the F828185 genotype to 169.5 minutes for the F825722 genotype. This shows that even food type genotypes presented great variability for this characteristic, which can be exploited in breeding programs for human consumption.

The smaller variation amplitude in experiment 2 for the traits seed weight after imbibition, imbibition percent and mainly cooking time compared to experiment 1 was consequence of the Doko cultivar (not adapted to direct consumption) in the crosses. This resulted in a high degree of kinship between the crosses that originated the genotypes in this experiment. However, the variation amplitude was reduced not only because of the degree of kinship but was also a reflection of the crosses with grain type genotypes (not adopted to direct consumption) that presented long cooking time. Thus, the genotypes studied in experiment 2 did not stand out for low cooking times and the shortest cooking time obtained was 62.67 minutes for FT Monsanto, a much longer time that than obtained in experiment 1. The maximum value for cooking time in experiment 1 (169.5 min) (Table 2) is similar to that obtained by Lam-Sanchez et al. (1982) and Seralathan et al. (1989). On the other hand, Turatti et al. (1994) assessed grains of various soybean cultivars from five different locations in Brazil and reported cooking times ranging from 56 to 290 minutes. The maximum value was much higher than those obtained in the two experiments in the present study and the minimum value (56 minutes) was longer than that obtained in experiment 1. This shows the importance of using food type genotypes as genetic source to decrease cooking time.

Table 1. Mean, range, coefficient of variation (CV%), genotypic (s^2_G) and phenotypic (s^2_F) variances and the coefficient of genotypic determination (H) for the traits assessed in soybean. Londrina, Paraná, Brazil. Agricultural year 1994/1995.

Traits	Mean	Range	CV%	σ^2_G	σ^2_F	H
Experiment 1[2/]						
WBI[1]	7.85	5.04 a 12.27	2.15	4.01	4.02	99.82
WAI	17.02	10.83 a 28.19	2.85	19.43	19.49	99.69
IP	116.80	87.60 a 129.90	3.91	75.81	81.02	93.57
CT	84.14	26.00 a 169.50	6.94	1140.52	1149.05	99.25
Experiment 2[3/]						
WBI	6.43	2.82 a 9.00	1.55	3.54	3.54	99.90
WAI	13.45	5.71 a 18.53	1.51	16.12	16.12	99.91
IP	108.30	101.07 a 116.2	1.62	14.77	15.79	93.53
CT	87.74	62.67 a 124.33	4.25	272.01	276.65	98.32

[1] WBI: dry weight before imbibition (g / 25 seeds); WAI: wet weight after imbibition (g / 25 seeds); IP: imbibition percentage; CT: cooking time (minutes); [2/] Data from 30 pure lines. Seeds obtained by plants cultivated in pots in a greenhouse; [3/] Data from 24 pure lines. Seeds obtained by plants cultivated in the field.

Correlations

Table 4 shows the cooking time associated with other characteristics. Cooking time had significant negative phenotypic correlation (-0.40) with imbibition percentage in experiment 1, indicating an undefined inverse relationship. This shows that imbibition percentage is one of the many variables that influences cooking time. A similar relation, (-0.43), was obtained by Mwandemele et al. (1984). Lam-Sanchez et al. (1982) also reported a negative tendency between the two characteristics with -0.12 correlation. In experiment 2, a relationship between cooking time and imbibition percentage was not detected, probably because most of the pure lines were derived from crossing with Doko, favoring a greater presence of genes from this cultivar.

The correlations of cooking time with seed weight before or after imbibition also varied among the experiments. Cooking time did not correlate with seed weight before and after imbibition in experiment 1; in experiment 2, significant phenotypic correlation was detected for both the traits. In this case, genotypes with larger seeds presented longer cooking time.

In short, substantial genetic variability for the cooking time trait was detected in soybean. This can be used in breeding programs to obtain soybean cultivars for human consumption with shorter cooking time. Cooking time is a complex variable and is not directly correlated with imbibition percentage. It is disassociated in pure lines derived from food type

Table 2. Average seed weight before imbibition (WBI), seed weight after imbibition (WAI), imbibition percentage (IP) and cooking time (CT) in minutes, to the soybean genotypes used in experiment 1. Londrina, Paraná, Brazil. Agricultural year 1994/1995.

Genotype	WBI [1]	WAI [1]	IP [1]	CT [1]
F82-5722-A	8.21 g	17.61 fg	114.5 cdfg	169.5 a
F82-5722-P	11.02 b	20.66 c	87.6 h	145.0 b
F82-5807	7.53 h	16.37 gh	117.3 bcdefg	119.5 c
F825813	7.41 hi	16.34 gh	120.5 abcdefg	118.0 c
BR92-15360	5.04 p	10.83 n	114.9 cdefg	112.0 cd
F83-77843	8.76 ef	19.20 de	119.0 abcdefg	107.75 cde
F82-5782	9.75 cd	20.97 c	115.1 cdefg	107.50 cde
Ivaí	6.89 jk	14.92 ij	116.5 bcdefg	106.50 cde
Easycook	5.33 op	11.69 lmn	124.4 abcde	105.25 cde
F83-7977	10.10 c	22.43 b	122.2 abcdef	99.25 def
Sel. Stwart	5.38 op	11.73 mn	118.2 abcdefg	97.75 def
F83-8207AB	9.32 d	21.04 c	125.7 abc	97.25 def
F82-5769	8.80 e	18.75 de	113.2 cdefg	95.25 ef
PL 1	8.66 efg	18.95 de	118.7 abcdefg	93.25 efg
PI 423909	7.05 ij	14.87 ij	110.9 fg	88.5 fgh
Soja de Feira 86-13	8.30 fg	18.44 ef	122.2 abcdef	86.00 fgh
F83-8017	9.37 d	18.30 ef	95.2 h	85.75 fgh
TMV	7.52 h	16.00 hi	112.9 defg	77.50 ghi
Late Giant	9.65 cd	20.65 c	113.9 cdefg	75.25 hi
Faz. Progresso	9.35 d	19.84 cd	112.3 efg	75.00 hi
BR 16	5.88 mn	12.25 lm	108.6 g	72.75 hij
F83-8192	12.27 a	28.19 a	129.8 a	62.00 ij
BR 36	6.11 lmn	12.82 klm	109.9 fg	58.75 j
BR 92-22106	6.10 lmn	13.92 jk	128.1 ab	42.25 k
Soja de Feira 86-14	6.48 kl	13.97 jk	115.7 bcdefg	41.5 kl
BR 27	6.02 lmn	13.24 kl	120.0 abcdefg	41.25 kl
Araçatuba	5.66 no	12.40 lm	119.2 abcdefg	40.75 kl
F83-8012	6.18 lm	13.9 jk	124.8 abcd	40.00 kl
91K 208-3-1	5.69 no	12.71 klm	123.5 abcde	37.25 kl
F83-8185	11.83 a	27.20 a	129.9 a	26.00 l
DMS (p=0.05)	0.46	1.33	12.52	16.02

[1] Values followed by the same letter are not significantly different in column, Tukey test (P<0.05).

cultivars crossed with the Doko cultivar (grain type). Furthermore, seed weight or size, do not influence cooking time for food type genotypes.

ACKNOWLEDGEMENTS

The authors thank CNPq and CAPES for grants and financial support.

Table 3. Average seed weight before imbibition (WBI), seed weight after imbibition (WAI), imbibition percentage (IP) and cooking time (CT) in minutes, to the soybean genotypes used in experiment 2 (yield). Londrina, Paraná, Brazil. Agricultural year 1994/1995.

Genotype	WBI [1]	WAI [1]	IP [1]	CT [1]
L1 (Hogyoku X Doko)	6.58 i	14.01 ij	113.1 ab	124.33 a
PI 229343 X Doko	7.80 cd	15.74 ef	101.7 g	116.67 ab
Late Giant X Doko	7.99 c	16.37 de	104.7 efg	111.67 bc
Aliança Preta X Doko	8.01 c	17.00 cd	112.3 abc	104.00 cd
Stwart	6.62 i	13.60 jk	105.2 efg	101.00 cde
Japão 2 X Doko	9.00 a	18.53 a	105.8 efg	98.33 def
L2 (Hogyoku X Doko)	6.14 jk	13.07 kl	112.9 ab	97.33 def
L1 (PI80441 X Doko)	7.38 ef	15.62 fg	111.6 abcd	94.33 defg
L3 (Hogyoku X Doko)	7.25 efg	15.41 fg	112.6 ab	93.33 defg
FT 2	6.20 j	12.72 l	105.3 efg	93.00 defg
Tamba X Doko	7.17 fgh	15.03 gh	109.8 bcdef	90.00 efgh
Doko	5.83 k	11.94 m	104.7 efg	89.33 efgh
L1 (PI165676 X Doko)	7.53 de	16.47 d	114.4 ab	88.67 fgh
L2 (PI80441 X Doko)	8.39 b	17.60 bc	106.5 defg	85.33 gh
L1 (Imperial X Doko)	2.97 mn	6.13 op	106.5 defg	83.33 ghi
KS3xAkiyoshi F7-2 X FT 2	6.87 h	14.42 hi	109.8 bcdef	79.67 hij
L3 (PI80441 X Doko)	8.34 b	18.03 ab	116.2 a	73.00 ijk
L2 (Imperial X Doko)	2.82 n	5.71 p	102.2 g	72.67 ijk
L2 (PI165676 X Doko)	7.47 ef	15.70 f	110.0 bcde	72.00 ijk
L3 (Imperial X Doko)	3.25 lm	6.64 no	104.5 fg	70.33 jk
PI80459 X Doko	3.30 l	6.82 n	106.6 defg	69.33 jk
L4 (Imperial X Doko)	3.32 l	6.84 n	105.3 efg	68.00 jk
TMV X Doko	6.95 gh	14.38 i	106.9 cdefg	67.33 k
FT Monsanto	7.17 fgh	15.03 gh	109.7 bcdef	62.67 k
DMS (p=0.05)	0.31	0.64	5.52	11.76

[1] Values followed by the same letter are not significantly different in column, Tukey test (P<0.05).

Table 4. Values for genotypic (r_G) and phenotypic (r_F) correlation found in the tested genotypes. Londrina, Paraná, Brazil. Agricultural year 1994/1995.

Traits	r_G	r_F	Experiment
WBI x IP [1]	-0.09	-0.09	1
	0.42	0.41 [2/]	2
WBI x CT	0.17	0.17	1
	0.43	0.42 [2/]	2
WAI x IP	0.10	0.11	1
	0.49	0.47 [2/]	2
WAI x CT	0.09	0.09	1
	0.42	0.41	2
IP x CT	-0.40	-0.41 [2/]	1
	0.05	0.04	2

[1] WBI: dry weight before imbibition; WAI: wet weight after imbibition; IP: imbibition percentage; CT: cooking time; [2/] significative (P<0.05).

RESUMO

Variabilidade genética para caracteres relacionados com tempo de cozimento em soja

O longo tempo de cozimento das sementes de soja dificulta seu uso regular 'in natura' como rica fonte de proteína. Este trabalho objetivou estudar a variabilidade genética do tempo de cozimento em soja e sua correlação com peso e percentagem de embebição das sementes. Foram avaliadas linhagens puras de soja tipo alimento cultivadas em casa de vegetação (experimento 1) e linhagens puras oriundas de cruzamentos entre soja tipo grão e soja tipo alimento cultivadas no campo (experimento 2). No experimento 1 ampla variabilidade foi encontrada em todos os caracteres e o tempo de cozimento variou de 26 a 170 minutos. No experimento 2 a amplitude do tempo de cozimento foi menor (63 a 124 minutos). As correlações mais pronunciadas foram entre percentagem de embebição e tempo de cozimento (-0,41*), para o experimento 1; e entre peso de sementes antes e após a embebição com tempo de cozimento (0,42* e 0,41*), para o experimento 2. Os resultados mostraram substancial variabilidade genética no tempo de cozimento em soja. Isto pode ser aproveitado para obtenção de cultivares para consumo humano com importante diminuição no tempo de cozimento.

REFERENCES

Berra, R. 1974. Efecto del remojo en algunas propriedades físicas, bioquímicas y organolépticas de la soja. Tecnol. Aliment. 9 (2):76-84.

Carbonell, S.A.M. and Vello, N.A. 2001. Genetic analysis of soybean seed response to mechanical damage. Crop Breeding and Applied Biotechnology.1(1):35-43.

Carrão-Panizzi, M. C. and Mandarino, J. M. G. 1998. Soja: Potencial de uso na dieta brasileira. Documentos, 113. Embrapa-CNPSo, Londrina.

Cruz, C.D. 1997. Programa Genes: aplicativo computacional em genética e estatística. UFV, Viçosa.

Embrapa Soja. 1995. Recomendações Técnicas para a cultura da soja na Região Central do Brasil – 1995/ 1996. Embrapa, Londrina.

Falconer, D.S. 1989. Introduction to Quantitative Genetics. 3.ed. Longman, New York.

Fehr, W.R. and Caviness, C.E. 1977. Stages of soybean development. Special Report, 80. Iowa, Iowa State University.

Jackson, G.M. and Varriano-Marston, E. 1981. Hard-to-cook phenomenon in beans: Effects of accelerated storage on water absorption and cooking time. Journal of Food Science. 46:799-803.

Lam-Sanchez, A.; Durigan, J.F.; Oliveira, J.E.D. and Bressani, R. 1982. Avaliação nutricional e tecnológica de material de soja (*Glycine max* (L.) Merrill) introduzido. Científica. 10(1):87-97.

Mattson, S. 1946. The cookability of yellow peas. Acta Agric. Suec. 1:185.

Mode, J.C. and Robinson, H.F. 1959. Pleiotropism and genetic variance and covariance. Biometrics. 15:518-537.

Mwandemele, O.D.; Mc Whirter, K.S. and Chesterman, C. 1984. Genetic variation in soybean (*Glycine max* (L.) Merrill) for cookability and water absorption during cooking. Euphytica. 33:859-864.

Mwandemele, O.D. 1986. Variability in soybean (*Glycine max* (L.) Merrill) quality caracteristics that influence consumer acceptance. Indian Journal Genetics. 46:394-405.

Seralathan, M.A.; Thirumaran, A.S. and Neelakandan, S. 1989. Screening of soyabean varieties. Indian Journal of Nutrition and Dietetics. 26:84-88.

Turatti, V.M.; Tango,V.S.; Silva, M.T.C., Shirose, I. and Yotsuyanagi, K. 1994. Caracterização dos grãos de cultivares de soja cultivadas em algumas regiões do Brasil. Boletim ITAL. 21(1):73-79.

Vello, N.A. 1992. Ampliação da base genética do germoplasma e melhoramento da soja na ESALQ-USP. p.60-81. In: Anais do Simpósio sobre a Cultura e Produtividade da Soja. Piracicaba.

Venkovsky, R. and Barriga, P. 1992. Genética biométrica no fitomelhoramento. Sociedade Brasileira de Genética, Ribeirão Preto.

Received: December 04, 2002;

Accepted: January 27, 2003.