BREEDING SPECIALTY SOYBEAN CULTIVARS FOR PROCESSING AND VALUE-ADDED UTILIZATION AT EMBRAPA IN BRAZIL.

Mercedes C. Carrão-Panizzi[1], Antonio Eduardo Pípolo[1], José Marcos Gontijo Mandarino[1], Neylson Eustáquio Arantes[1], Antônio Garcia[1], Vera de Toledo Benassi[1], Carlos Arrabal Arias[1], Milton Kaster[1], Marcelo Fernandes de Oliveira [1], Marcelo Álvares Oliveira[1], José Francisco Ferraz de Toledo[1], José Ubirajara Vieira Moreira[1], Geraldo Estevan de Souza Carneiro[1]

1. National Soybean Research Center - Embrapa, Londrina, PR, CEP 86970-001, Brazil (mercedes@cnpso.embrapa.br).

Abstract Through traditional genetic breeding, specialty characters are introduced into soybean, to make it more suitable for differents uses. To attend this special market, Embrapa Soybean released for commercial cultivation the cultivars BRS 213, BRS 257, BRS 216, BRS 267 and BRSMG 790A for production in organic or in conventional systems. The cultivars BRS 213 and BRS 257 are null lipoxygenases enzymes, and can prevent the development of the beany flavor observed in soybean products. These cultivars make available excellent raw materials for soyfood processing. The cultivar BRS 216 has high protein content and small seed size (10g/100 seeds), what makes it suitable for “natto” (Japanese fermented food), and for production of soybean sprouts, as “moyashi”. The cultivar BRS 258, is a conventional cultivar, highly productive that has mild flavor and seeds with yellow hilum. Cultivars BRS 267 and BRSMG 790A presents large seed size (>25g/100seeds), and superior flavor. These cultivars are suitable for “tofu”, flours and soymilk. BRS 267 and BRSMG 790A can also be consumed as green vegetable soybean (edamame), when harvested at R6 stage (developed grains, but immature). Among Brazilian soybean cultivars there are differences for isoflavone content, and to increase concentration of isoflavones it is suggested sowing them in locations where average temperatures are cooler during the seed filling period.

Keywords *Glycine max*, breeding, null lipoxygenase, protein, seed size

Introduction

Soybean consumption should be promoted as human food, not just because of its nutritional advantages and health benefits, but also for the availability of this affordable source of good quality protein. Brazil is the second world soybean producer and for the 2008/2009 growing season, Brazilian soybean production is estimated to reached 58.14 million tons (CONAB, April 2009). Although several high quality soybean products offered in the Brazilian market, the direct use for human consumption represents less than 3,5 % of the Brazilian soybean production. In the past, acceptability of soybean by the consumers was very limited because of its flavor and the lack of proper processing technologies. It was also an exotic legume to Occidentals, which demanded training and education about its nutritional and processing properties. At Embrapa Soybean (National Soybean Research Center) some strategies to promote soybean as human food were established in 1985, starting with a breeding program for special soybean cultivars and a promotional program regarding education and training on soybean processing. Since flavor was the most limiting factor for soybean acceptance, improving soybean flavor was the main objective of the breeding program, by involving in the crosses null lipoxygenases genotypes and vegetable type soybeans. Other quality characters are also observed in the breeding which include: seed size for tofu, natto or sprouts, yellow hilum, high protein, reduced trypsin inhibitor, low linolenic and mid oleic fatty acids. Compounds related to functional foods, such as isoflavones, saponins, protein fractions and tocopherols were also analysed in Brazilian soybean germplasm.

Materials and Methods

Breeding

Traditional breeding methods such as MSSD and genealogic are being used to develop soybean cultivars for human consumption. For special characteristics, genetic sources from Japan, Taiwan and United States were introduced in the crosses with Brazilian soybean varieties adapted to different regions of the Brazilian soybean production area. At the green house, crosses are made during the soybean season, and the F1 generations are advanced, in the winter. In the following summer, the F2 generations are sowed in the field for selections. Lines are harvested at F5 or F6 generations and will be evaluated for adaptation and yield.

The Embrapa´s breeding program is performed in different regions of Brazil, threfore the selected populations and advanced lines are introduced and evaluated nationwide in partnerships with local or state institutions. The lines testing for evaluation of yield, stability and other agronomic attributes are conducted in several locations for 5 years, divided into Preliminary Trials (three years) and Final Trials (two years). These assessments are used to establish the Value of Cultivation and Use (VCU) of a new cultivar for commercial registration and release purposes. Nowadays , in Brazil, early varieties are being prefered to overcome problems with diseases (soybean rust mostly) and insects. In the last two decades, the released cultivars must have resistance to frogeye leaf spot

(*Cercospora sojina*), stem canker (*Diaporthe phaseolorum* f.sp. *meridionalis*), and bacterial pustule (*Xanthomonas campestris* pv. g*lycines*).
Technology transfer and validation actions should aim shortening the time between cultivar generation and adoption by the productive sector, which should also include promotion of the special characteristics for different utilizations of the cultivars.
To identify absence or presence of the lipoxygenases a modified methology of Suda et al. (1995) developed by Kikuchi (2002) is used at Embrapa. This qualitative analysis, based on bleaching carotene allows readily and accurately determination of the isozymes, and it is suitable for routine screening in a breeding program.

Results

The first special soybean variety released by Embrapa through the breeding program for human consumption was BRS 155, that presented reduced Kunitz trysin inhibitor, originated from cross of *Paraná (2) x PI 157.440*. Because of this special characteristic it was considered a good source for animal feeding, by improving protein digestibility. This variety was recommended for cultivation in South Region of Brasil, presented medium size seeds (15 mg/100 seeds) and buff color hilum. However, BRS 155 cultivar was not competible with conventional high yield soybean varieties and was not well accepted by growers. Yield is the main factor for soybean production, but in some cases is not easy to achieve, mainly because in crosses for special characters broadly genetically genotypes are involved, implying in reduced yields. Therefore efforts should be made to promote special cultivars, by informing producers and processors about how special characteristics can improve nutrition or processing technologies of soybean products.

The other variety released in the program was BRS 216 (Carrão-Panizzi et al. 2003), from cross [*(BR79-15807 x Embrapa 4) x IAC 13]*, which has small seed size (10g / 100seeds), yellow hilum, and high protein content (43%). The small seed size of BRS 216 makes it suitable for “natto” and for production of soybean sprouts, as “moyashi”. The lower yield profile of this cultivar made it also not well accepted. However, this cultivar may be useful for the sprouts niche market which is being develop in Brazil.

For better flavor, it was released for commercial production cultivars BRS 213 (Carrão-Panizzi et al. 2002) and BRS 257 (Pípolo et al. 2005. Both cultivars do not present the three lipoxygenases isoenzymes L1, L2 and L3, which were introduced through a null lipoxygenase Japonese genotype (Hajika et al. 1991). These varieties present high yield and are being processed for soy flour and soy milk. Because of the mild flavor of these null lipoxygenase varieties, they can also improve flavor of tofu, favoring its utilization in Ocidental countries. During tofu processing seeds are soaked in cold water, which promotes activation of the lipoxygenases and formation of the beany flavor. The absence of the isoenzymes in seeds of these cultivars prevents bad flavor flavor in soyfoods, and heat treatments are not needed to inactivate the enzymes. With this characterisitic food companies can develop new products with better nutrition qualities, as higher protein solubility.

The cultivar BRS 258 presents mild flavor, large seed size, and yellow hilum. It was originated from cultivar BR 36, which was a variety prefered by organic growers (Pípolo et al. 2005). This conventional cultivar has high yield and a great potential to be utilized for food processing.

In Brazil, availability of soybean varieties with special characteristics to attend the niche markets has been demanded by oriental countries, who import soybean to be manufactured as tofu, natto and other products. At the same time, adding value to the products is also demanded by Brazilian growers. Therefore, other possibilities of soybean production should be considered, and the option of using soybean as vegetable or “edamame” can be an alternative uses for soybean (Carrão-Panizzi 2006a). The cultivar BRS 267 presents large seed size, superior flavor, and yellow hilum, which are suitable characteristics for soyfood processing including tofu, soyflour, and soymilk (Carrão-Panizzi 2006b). When harvested at R6 stage (developed grains, but immature), this cultivar can also be consumed as green vegetable soybean, or ”edamame”, which is a very attractive functional food.

In 2008, it was released the cultivar BRSMG 790A recommended for cultivation in the states of Minas Gerais, Goiás and North of São Paulo. It is an option of special raw material for Central Brazil, since the other cultivars are recommended for the states of Paraná, São Paulo and South of Mato Grosso do Sul. This variety presents yellow hilum seeds and high yield with averages of 3.000kg.ha^{-1} (Neylson et al. 2008), which turned it in great potential cultivar for soybean utilization.

Soybean has been reported as healthy ingredient for functional foods. Isoflavones are the main compounds related to health. Brazilian soybean varieties present genetic variability for isoflavone contents, which are also very affected by the environmental conditions (Carrão-Panizzi et al. 1999). Because of the high variability for isoflavone content in Brazilian Soybean cultivars, genetic breeding is not being conducted for this trait. To increase isoflavone content in soybean varieties, it is suggested to growth soybean in locations where average temperatures are cooler during the seed filling period, favoring higher concentration of isoflavones (Figure 1).

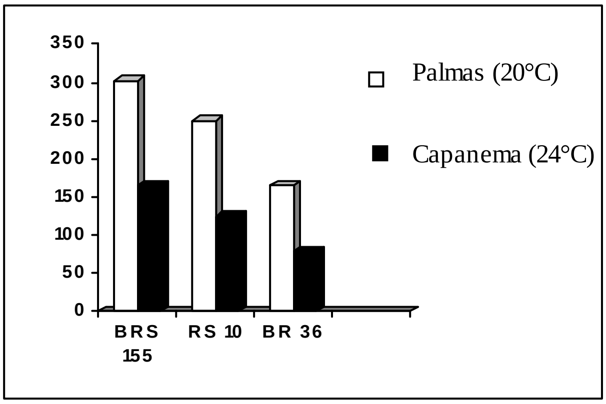

Fig. 1 Total isoflavone content (mg/100g) in soybean cultivars harvested in different locations of Paraná state, Brazil, 2000.

In the 2008/2009 soybean season, breeding lines and segregating populations were carried out in the field for evaluation of the differents traits related to special uses. For modified fatty acid traits (mid oleic and 1% linolenic), Embrapa´s program is conducting F3 and F4 segregating populations.

Although recommendations of the special cultivars for different utilizations, availability of their seeds are limited to small amounts in the Brazilian seed market. Since these cultivars are for small niche markets, seed producers are not interested to attend this niche market. Regarding this situation, in order to reach this small but significative market, some strategies of promotion should be developed in Brazil. Producers of special cultivars should be contracted by processors interested in special products. Organic growers are selling their soybean for about U$40.00 a bag, which is an example of a significant adding value for these special cultivars.

References

Arantes, N, Sá, MEL de,Carrão-Panizzi, MC, Zito, RK, Toledo, JFF de, Kaster, M, Arias, CAA, Zanetti, AL, Dias, WP, Yorinori, JT, Almeida, AMR, Souza, PIM, Monteiro, PMOF, 2008. Cultivar de soja BRSMG 790A. In: XXX Reunião de Pesquisa de Soja da Região Central do Brasil, 2008, Rio Verde. Reunião de Pesquisa de Soja da Região Central Do Brasil, 30., 2008, Rio Verde. Resumos. Londrina: Embrapa Soja, 2008. p.200

Carrão-Panizzi, MC, Beléia, ADP, Keisuke, K and Oliveira, MCN, 1999. Effects of genetics and environment on isoflavone content of soybean from different regions of Brazil. Pesq. Agropec. Bras., 34 (10): 1787-1795

Carrão-Panizzi, MC, Almeida, LA, Miranda, LC, Kiihl, RA de S, Mandarino, JMG, Arias, CAA, Yorinori, JT, Almeida, AMR, Toledo, JFF de, 2002, BRS 213: Nova cultivar de soja para alimentação humana. In: Congresso Brasileiro De Soja, 2. E Mercosoja 2002, Foz do Iguaçu, PR. Resumos... Londrina:Embrapa Soja. p.201. (Embrapa Soja. Documentos 181)

Carrão-Panizzi, MC, Almeida , LA de, Kiihl, RA de S, Miranda, LC, Mandarino, JMG, Domit, L.A and Vieira, OV, 2003. Indicação da cultivar de Soja BRS 216 para o estado de São Paulo. In: Reunião de Pesquisa de Soja da Região Central do Brasil, 25, 2003, Uberaba. Resumos... Londrina: Embrapa Soja: EPAMIG: Fundação Triângulo. p. 51-52. (Embrapa Soja. Documentos 209).

Carrão-Panizzi, MC, 2006a. Edamame ou soja-hortaliça; fácil de consumir e muito saudável. Informe Agropecuário, 27 (230): 59-64

Carrão-Panizzi, MC, Pípolo, AE, Almeida, LA, Mandarino, JMG, Kaster, M, Arias, CAA, Carneiro, GE de S, Toledo, JFF de, Miranda, LC, Yorinori, JT, DIAS, WP, Almeida, AMR, Domit, LA, Benassi, V de T, Arantes, NE, Oliveira, ACB de, Brogin, RL, Lambert, E de S, Bertagnolli, PF, Rangel, MAS, and Souza, PI de M de, 2006b. BRS 267: cultivar de soja para alimentação humana. In: Reunião de Pesquisa de Soja da Região Central do Brasil, 28, 2006, Uberaba. Resumos... Londrina: Embrapa Soja: Fundação Meridional: Fundação Triângulo, 2006. p. 315-317. (Embrapa Soja. Documentos, 272).

Carrão-Panizzi, MC and Pipolo, AE, 2007. Cultivares de sojas especiais para alimentação humana. Londrina, Embrapa Soja, n.01 / 2007. Folder

Hajika, M, Kitamura, K, Igita, K, 1991. Production od soybeans lacking all lypoxigenase isoenzymes induced with gama ray irradiantion, Japan. J. Breed. 40:218-219

Kikuchi, A. 2002. Simple and rapid method for detection of lipoxygenase isozymes in soybean seeds. Japan International Research Center for Agricultural Sciences, Annual report 2001, 47-48

Pípolo, AE, Kiihl, RA de S, Almeida, LA de, Carrão-Panizzi, MC, Miranda, LC, Gomide, F B, Arias, CAA; Kaster, M, Toledo, JFF de, Carneiro, GE de S, Dias, WP, Yorinori, JT, Almeida, AMR, and Domit, LA, 2005. Indicação da cultivar de soja BRS 258 para os Estados de São Paulo, do Paraná e de Santa Catarina. In: Reunião de Pesquisa de Soja da Região Central do Brasil, 27, 2005. Cornélio Procópio. Resumos... Londrina: Embrapa Soja, 2005. p. 375. (Embrapa Soja. Documentos, 257).

Pípolo, AE, Almeida, LA de, Kiihl, RA de S, Carrão-Panizzi, MC, Gomide, FB, Miranda, LC, Arias, C AA, Toledo, JFF de, Kaster, M, Carneiro, GE de S, Yorinori, JT, Domit, LA, Dias, WP, AlmeidA, AMR BRS 257 - nova cultivar para alimentação humana. In: Reunião de Pesquisa de Soja da Região Central do Brasil, 27, 2005. Cornélio Procópio. Resumos... Londrina: Embrapa Soja, 2005. p. 371. (Embrapa Soja. Documentos, 257).

Suda, I.; Hajika, M.; Nishiba, Y.; Furuta, S.; Igita, K., 1995. Simple and Rapid Method for the selective detection of individual lipoxygenase isozymes in soybean seeds. J. Agric. Food Chem., 43: 742-747

Table 1. Characteristics of special soybean cultivars for human consumption in Brazil.

CHARACTERITICS	BRS 216	BRS 213	BRS 257	BRS258	BRS 267
Cultivation region	* PR, SC, SP	PR, SC, SP	PR, SC, SP	PR, SC, SP	PR, SC, SP, Southern of MS
Maturation Group	Semi Early	Early	Early	Semi Early	Medium
Growth habit	Determined	Determined	Determined	Determined	Determined
Pubescence color	Gray	Gray	Gray	Gray	Gray
Flower color	White	White	White	White	Purple
Pod color	Light Gray	Light Gray	Light Gray	Light Gray	Brown
Seedcoat color	Yellow	Yellow	Yellow	Yellow	Yellow
Hilum color	Yellow	Yellow	Light Brown	Light Brow	Yellow
Weight 100 seeds (g)	10,4	16,5	14,5	16,0	25,0
Bacterian pustule	Resistant	Resistant	Resistant	Resistant	Resistant
Frog eye spot	Resistant	Resistant	Resistant	Resistant	Resistant
Soybean mosaic vírus	Susceptile	Moderate Susceptile	Resistant	Resistant	Susceptile
Oidium	Moderate Susceptile	Moderate Resistant	Moderate Susceptile	Moderate Resistant	Moderate Susceptile
Stem canker	Resistant	Resistant	Resistant	Resistant	Resistant
Cyst nematode (Raça 1 e 3)	Suscetível	Susceptile	Susceptile	Susceptile	Susceptile
Root knot Nematode (*M. incognita*)	Moderate Resistante	Resistant	Resistant	Susceptile	Susceptile
Root Knot nematode (*M. javanica*)	Moderate Resistant	Moderate Resistant	Moderate. Resistant	Susceptile	Susceptile
Protein (%)	43,6	39,7	41,3	41,7	40,1
Oil (%)	17,6	19,0	22,6	23,7	20,5
Sowing density São Paulo (45 cm / m)					
Altitude up to 500m	16 a 20	18 a 20	16 a 20	16 a 20	16 a 18
500m to 800m	14 a 18	14 a18	12 a 16	14 a 18	12 a 16
Higher than 800m	10 a 12	12 a 14	10 a 12	10 a 14	10 a 12
Sowing density Paraná (45 cm / m)					
Altitude up to 500m	12 a 16	12 a 16	12 a 16	12 a 16	12 a 16
500 m to 800m	12 a 16	12 a 16	12 a 16	12 a 16	12 a 16
Higher than 800m	12 a 14	12 a 14	10 a 12	10 a 14	10 a 12
Sowing density Santa Catarina (45 cm / m)					
Altitude up to 500m	10 a 12	10 a 12	10 a 12	10 a 12	10 a 12
500 to 800m	10 a 12	10 a 12	10 a 12	10 a 12	10 a 12
Higher than 800m	10 a 12	10 a 12	10 a 12	10 a 12	10 a 12
Maturity (days)					
Altitude to 500m	118	117	115	122	125
500 a 800m	125	124	123	127	135
Higher than 800m	138	138	136	141	147
Plant Heigh (cm)					
Altitude up 500m	60	65	60	67	93
500 to 800m	71	86	67	71	98
Higher than 800m	79	95	89	92	100
Lodging					
Altitude up 500m	R	R	MR	R	R
500 to 800m	MS	MR	MR	MR	R
Higher than 800m	S	MR	MS	MR	R

* Indication of cultivation for Santa Catarina (SC); Paraná (PR), São Paulo and Mato Grosso do Sul (MS) States, (Carrão-Panizzi and Pípolo 2007).