Available online at www.sciencedirect.com

ScienceDirect

Procedia Food Science 3 (2015) 348 – 354

Procedia Food Science

The First International Symposium on Food and Agro-biodiversity (ISFA2014)

Agronomic Characteristic and Nutrient Content from Several Soybean Promising Lines with High Isoflavones

M. Muchlish Adie[a], Ayda Krisnawati[a], and Didik Harnowo[a]

[a]*Indonesian Legumes and Tuber Crop Research Institute (ILETRI) P.O. Box. 66 Malang,*

Abstract

Soybean is a high protein and source of functional food. Ten soybean promising lines were characterized for its agronomic characters and nutrition contents (protein, lipid, and isoflavone) in eight soybean production centers in Indonesia on 2012. Soybean lines of K/IAC100-64-1004-1037, K/IAC100-997-1035, and IAC100/K-60-1092-1141 have total isoflavone 398.50 ppm, 396.69 ppm, and 394.77 ppm, respectively. The seed yield were 2.70 – 2.82 t/ha, protein content from 34.79 – 37.44%, lipid content from 17.34 – 19.18%, seed weight from 11.53 – 15.33 g, and the days to maturity of 83 days. These lines prospective to be released as high yielding and high isoflavone soybean varieties in Indonesia.

Peer-review under responsibility of the organizing committee of Indonesian Food Technologist Community

Keywords: *Glycine max*; promising lines; isoflavone; agronomic character; protein; lipid

Introduction

Soybean is the third most important crops after rice and maize. Soybean has potential role as source for functional food due to its high protein and isoflavone content. The economic value of soybean is very important for the Indonesian society because various processed soy-based foods become their daily consumption. The threshold intake of dietary estrogens necessary to achieve a biological effect in humans appears to be 30–50 mg/d, which is readily attainable by the inclusion of modest amounts of soy protein in the average Western diet [1].

There are two approaches for increasing soybean nutrition as raw material of processed food. First by adding substitution ingredients in processed soybean; and the second is by genetic modification to obtain soybean variety with

* Corresponding author
E-mail address: mm_adie@yahoo.com

Peer-review under responsibility of the organizing committee of Indonesian Food Technologist Community
doi:10.1016/j.profoo.2015.01.038

high nutrition content. The second approach is cheaper in the long term and safe for human and environment. The final product of genetic approach is soybean variety with high nutrition and also high yield, so it is more easily adopted by farmers. Until now, Ministry of Agriculture Republic of Indonesia has released 78 soybean varieties, with range of protein content from 33.06 – 46.00% and oil content from 7.50 to 25.00%; but the data on isoflavone content is not yet measured.

Soybean seed is potential for source of isoflavone, which can be used for prevention and treatment of several chronic diseases. It was reported that there were six isoflavone forms in soybean: daidzin, genistin, glycitin, daidzein, genistein, and glycitein. The three major groups of isoflavones found in soybeans were genistein, daidzein, and glycitein. The isoflavone content and composition were various between seed parts (cotyledon, hypocotyl and integument) [2], and determined by genotype and environment. It was supposed that the environmental factor that influenced the isoflavone content was at seed filling period [3,4,5,6,7]. Lee [7] asses the stability of soybean isoflavone content in South Korea, and revealed that genotype Geomjeong1 have high isoflavone content and stable across locations. It was also reported that there were no major genetically determined negative associations among the isoflavones, except for that between glycitin and malonylgenistin. On the contrary, a strong negative environmental association was observed between genistein (along with glycitein) and daidzin (along with genistin). Both genetically and environmentally, total isoflavone content was most closely associated with malonylgenistin. Study on genetics of soybean isoflavone have been done by several researchers. Gutierrez-Gonzalez et al. [8] reported that a total of thirty-five main-effect genomic regions and many epistatic interactions controlling genistein, daidzein, glycitein and total isoflavone accumulation in seeds. The distribution of isoflavone content was continuous and unimodal. The heritability estimates on a line mean basis were 79% for daidzein, 22% for genistein, and 88% for glycitein. Total isoflavone contents ranged from 427.92 to 965.89 μg per gram of dry seed and the protein content ranged from 45.17 to 34.95% in BARC-8 and IAC-100, respectively [9].

Krisnawati and Adie [10] identified isoflavone content of 127 Indonesian soybean genotypes, and obtained range of daidzein from 37.51 – 98.34 mg/100 g, glycetein range from 8.52 – 19.91 34 mg/100 g, and genistein from 20.45 – 60.25 mg/100 g. Isoflavone total of 127 soybean genotypes ranged from 78.77 – 175.57 mg/100 g, meanwhile the check varieties of G100H, IAC 100 and Wilis were 117.08; 105.40; and 106.86 mg/100g, respectively. Cultivars IAS 5 and FT-Abyara grown at Vacaria Brazillian, RS (28°30' S latitude) with temperature average of 19°C, had the highest isoflavone concentrations (218.7 and 163.8 mg/100 g, respectively) [5].

The research aimed was to characterize the agronomic performance and nutrient content form high isoflavone soybean lines.

Materials and methods

Characterization of agronomic performance of ten soybean promising lines and two check varieties (Wilis and Anjasmoro) were conducted in eight soybean production centers (Bogor, Sleman, Klaten, Bantul, Mojokerto, Blitar, Pasuruan, Probolinggo, Tabanan and West Lombok) in 2012. The experimental design in each location was randomized block design with twelve traits and each was replicated four times. The plot size was 2.4 m x 4.5 m, plant distance of 40 cm x 10 cm, two plants/hill. Fertilizer of 50 kg Urea, 100 kg SP36 and 75 kg KCl per ha were applied after planting. Weeding and drainage were according field condition. Pest and disease were controlled intensively at eight days after planting.

Agronomic characters were measured on flowering time, days to maturity, plant height, number of branches per plant, number of nodes per plant, number of pods per plant, 100 seed weight, and seed yield. Protein content was

measured using micro-Kjeldahl method (AOAC, 2005 No. 12.1.07), lipid content by Soxhlet procedure (SNI 01-2891-1992), and isoflavone content using HPLC following Vyn et al. [11].

Result and discussion

Combined analysis of variance from twelve soybean promising lines which evaluated in eight soybean production centers showed that all agronomic characters and seed yield were significantly affected by location (E), promising lines (G), and their interaction (GEI). The coefficient of variation value range from 1.81 to 22.68% (Table 1). Several promising lines be able to produce high yield in particular environment which was supported by differences of between agronomic characters.

As implication of significant GEI for yield, there was difference in seed yield rank of promising lines across locations. The average of seed yield range from 2.47 – 2.82 t/ha with an average of 2.62 t/ha (Table 2). The three promising lines with highest yield were IAC 100/K-60-1092-1141 (2.82 t/ha), K/IAC 100-997-1035 (2.75 t/ha), and K/IAC 100-64-1004-1037 (2.70 t/ha). Two check varieties of Anjasmoro and Wilis have a similar yield, which were 2.64 t/ha and 2.63 t/ha, respectively. Productivity of location in relation with seed yield is measured by environmental index (Ij), which is average seed yield in each location minus the grand mean. A location with higher Ij value showed greater productive environment in producing yield. Based on Ij values, there were two locations exhibited highest environmental index (productive environments), that were Pasuruan (Ij = 0.40) followed by Klaten (Ij = 0.35). On the contrary, the most unproductive environment was Lombok Barat (Ij = -0.051).

The best three promising lines had a varied range of lowest and highest yield. The yield of IAC 100/K-60-1092-1141 in eight locations ranged from 2.14 – 3.52 t/ha with a difference of 1.38 t/ha. The seed yield range of K/IAC 100-997-1035 was 2.48 – 3.00 t/ha with a difference of 0.62 t/ha, meanwhile the K/IAC 100-64-1004-1037 ranged from 1.79 – 3.25 t/ha. Based on the difference of lowest and highest yield from the best three promising lines, hence the K/IAC 100-997-1035 had the lowest seed yield, and showed relatively stable yield than other lines. The distribution of seed yield each location showed that IAC 100/K-60-1092-1141 had the highest yield in Blitar (3.52 t/ha). The second highest yield was variety of varietas Wilis (3.35 t/ha) in Pasuruan.

The performance of agronomic characters which is consist of days to maturity, plant height, number of branches/plant, number of nodes/plant, number of pods /plant, and 100 g seed weight of twelve promising lines in eight locations were presented in Table 3. Days to maturity ranged from 79 – 83 hari (average of 82 days), plant height from 58.09 – 72.14 cm (average of 66.06 cm), number of branches started from 2.87 – 3.40 branches (average of 3.20 branches), range of nodes number from 12.01 – 14.92 nodes (average of 13.33 nodes), range of number of pods per plant was 44.52 – 52.15 pods (average of 49.35 pods), and 100 seed weight ranged from 11.53 – 15.92 g (average of 12.92 g). The agronomic characters of days to maturity, number of branches, number of nodes, and number of pods were less varied among ten soybean lines, on the contrary, the plant height and 100 seed weight were varied. This was supposed that the genetic background of ten lines was using the similar parent (IAC 100 which was crossed with soybean varieties of Kawi, Burangrang and Gema). Two soybean lines with days to maturity of 79 days was selected from parents of Burangrang and Gema, whereas both of them were early maturing soybean (< 80 days).

Soybean seed isoflavonoids display a broad range of variation, even in genetically stabilized lines that grow in a fixed environment, because their synthesis and accumulation are affected by many biotic and abiotic factors. The total isoflavone of twelve promising lines ranged from 149.71 to 398.50 ppm (average of 271.02). The two check varieties of Wilis and Anjasmoro have isoflavone 342.99 ppm and 177.33 ppm, respectively (Figure 1, Table 4). There were three soybean promising lines with highest isoflavone, i.e. K/IAC 100-64-1004-1037 (398.50 ppm), K/IAC 100-997-1035

(396.69 ppm), and IAC 100/K-60-1092-1141 (394.77 ppm). Those lines have isoflavone content above the grand mean and also higher than the Wilis variety. Eldridge and Kwolek [12] found isoflavone concentrations in soybean to range from 1160 to 3090 µg/g. Wang and Murphy [13] found isoflavone concentration to vary from 1176 to 3309 µg/g within a single cultivar of soybean. Hoeck et al. [6] reported that total isoflavone concentrations ranged from 1212 to 2547 µg/g. Genetic Improvement Program from Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) obtained 14 soybean lines with total isoflavone content varied from 57 to 188mg per 100g of soybeans in crop year of 2003.

Isoflavone content in soybean seed was tend to inversely proportional to protein content, while the lipid content was tend to directly proportional [14]. The protein content of K/IAC 100-64-1004-1037, K/IAC 100-997-1035, and IAC 100/K-60-1092-1141 were 37.44%, 34.79% and 35.53%, respectively; while the lipid content were 19.18%, 17.34%, and 18.94%, respectively (Table 4).

Based on various data above, obtained three high-yielding promising lines, which were K/IAC 100-64-1004-1037, K/IAC 100-997-1035, and IAC 100/K-60-1092-1141, and also have the highest total isoflavone than other lines (Table 4). The promising line of K/IAC 100-64-1004-1037 with highest isoflavone (398.50 ppm) produced yield 2.70 t/ha, days to maturity of 83 days, with medium seed size (11.53 g/100 seeds). The promising line of K/IAC 100-997-1035 with isoflavone 396.69 ppm produced yield 2.75 t/ha, medium maturity (83 days) but have large seed size (15.33 g/100 seeds). The promising line of IAC 100/K-60-1092-1141 have isoflavone 394.77 ppm and produced the highest yield (2.82 t/ha), days to maturity of 83 days, and the seed size was 12.26 g/100 seeds.

Table 1. Combined analysis of variance for agronomic characters of twelve soybean promising lines. 2012.

No	Parameter	Mean Square			CV (%)
		Location (E)	Promising lines (G)	G × E	
1	Flowering time	70. 6543 **	69.3700 **	12.3857 **	1.81
2	Plant height (cm)	3078.8963 **	609.7906 **	77.7899 **	9.46
3	Number of branches	61.2044 **	1.3429 **	0.8386 **	22.68
4	Number of nodes	257.2802 **	26.3469 **	12.5891 **	19.28
5	Pods number	8396.7380 **	235.6786 **	118.6805 *	18.64
6	100 seed weights (g)	39.3238 **	57.7172 **	1.9890 **	8.61
7	Seed yield (t/ha)	4.1326 **	0.4066 **	0.1921 **	12.10

CV = coefficient of variation, * = significant at 1% probability level ($p<0.01$), ** = significant at 5% probability level ($p<0.05$).

Table 2. Seed yield of soybean promising lines. 2012.

No	Genotype	Seed yield (t/ha)								
		Dpk	Klt	Slm	Mjk	Blt	Psr	Tbn	Lbr	Average
1	IAC 100/K-60-1092-1141	2.64	3.22	2.65	2.65	3.52	3.05	2.66	2.14	2.82
2	IAC 100/K-67-1099-1147	2.70	2.83	2.17	2.36	2.75	2.82	3.26	2.36	2.66
3	B/IAC 100-47-678-764	2.47	3.00	2.61	2.07	2.32	2.98	2.35	2.16	2.49
4	IAC 100/SHR-W60-1-252-273	2.46	3.08	2.78	2.15	2.52	3.04	2.30	2.14	2.56
5	K/IAC 100-71-1011-1041	2.29	2.94	2.68	2.27	2.30	2.90	2.51	2.04	2.49
6	IAC 100/K-5-1037-1062	2.63	3.03	2.65	2.44	2.78	3.10	2.91	2.01	2.69
7	K/IAC 100-64-1004-1037	2.86	2.93	2.47	2.51	2.97	3.25	2.83	1.79	2.70
8	IAC 100/K-2-1034-1058	2.50	2.81	2.46	2.41	2.46	3.14	2.53	2.00	2.54
9	K/IAC 100-997-1035	2.82	3.09	2.86	2.48	2.51	3.00	2.56	2.68	2.75
10	IAC 100/SHR-W60-6-257-285	2.49	3.00	2.42	2.16	2.23	2.74	2.59	2.12	2.47
11	Wilis	2.94	2.55	2.49	2.47	2.84	3.35	2.63	1.79	2.63
12	Anjasmoro	2.35	3.16	2.76	2.28	2.55	2.87	3.00	2.11	2.64
Average		2.60	2.97	2.58	2.35	2.65	3.02	2.68	2.11	2.62
LSD 5%		0,30	ns	ns	0,30	0,52	ns	ns	0,37	
Ij		-0.02	0.35	-0.04	-0.27	0.03	0.40	0.06	-0.51	

Dpk = Depok; Klt = Klaten, Slm = Sleman, Mjk = Mojokerto, Blt = Blitar, Psr = Pasuruan, Tbn = Tabanan, Lbr = Lombok Barat. Ij = environmental index, ns = not significant.

Table 3. Average of agronomic characters from twelve soybean promising lines. 2012.

No	Genotype	DTM	PH	NOB	NON	NOP	SW
1	IAC 100/K-60-1092-1141	83	64.86	3.01	13.41	52.15	12.26
2	IAC 100/K-67-1099-1147	82	66.84	3.17	13.79	47.55	12.98
3	B/IAC 100-47-678-764	79	61.21	3.56	12.86	48.44	11.94
4	IAC 100/SHR-W60-1-252-273	82	65.20	3.00	12.78	48.50	12.69
5	K/IAC 100-71-1011-1041	83	64.31	3.18	12.45	44.52	12.94
6	IAC 100/K-5-1037-1062	82	69.49	3.40	14.57	52.69	12.21
7	K/IAC 100-64-1004-1037	83	71.45	3.36	13.99	50.74	11.53
8	IAC 100/K-2-1034-1058	82	70.07	3.38	13.92	50.66	12.57
9	K/IAC 100-997-1035	83	58.09	3.01	12.01	45.55	15.33
10	IAC 100/SHR-W60-6-257-285	79	61.51	3.11	12.69	47.66	12.63
11	Wilis	84	72.14	3.32	14.92	51.64	11.98
12	Anjasmoro	84	67.56	2.87	12.61	52.14	15.92
	Average	82	66.06	3.20	13.33	49.35	12.92
	LSD (5%)	0.7309	3.0779	0.3574	1.2657	4.5288	0.5480

DTM = days to maturity (days), PH = plant height (cm), NOB = number of branches/plant,
NON = number of nodes/plant, NOP= number of pods/plant, SW = 100 seed weight (g).

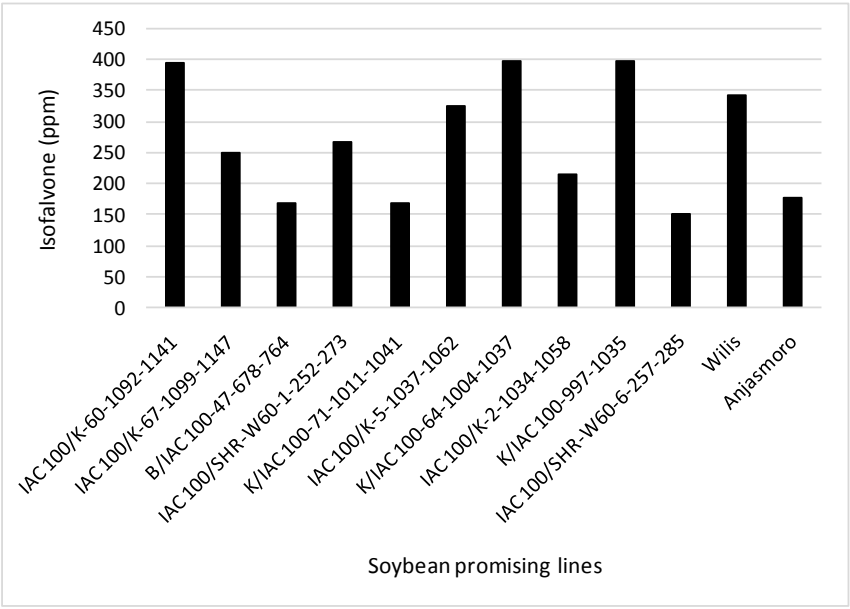

Figure 1. Soybean isoflavone of twelve soybean promising lines. 2012.

Table 4. Soybean isoflavone, protein, lipid, and seed yield of twelve soybean promising lines. 2012.

No	Promising lines	Total isoflavone (ppm)	Protein (% db)*)	Lipid (% db)*)	Seed yield (t/ha)
1	IAC 100/K-60-1092-1141	**394.77**	35.53	18.94	**2.82**
2	IAC 100/K-67-1099-1147	249.17	36.56	19.18	2.66
3	B/IAC 100-47-678-764	168.46	39.10	17.23	2.49
4	IAC 100/SHR-W60-1-252-273	267.09	37.84	19.03	2.56
5	K/IAC 100-71-1011-1041	168.29	40.75	17.87	2.49
6	IAC 100/K-5-1037-1062	324.64	38.28	19.03	2.69
7	K/IAC 100-64-1004-1037	**398.50**	37.44	19.18	**2.70**
8	IAC 100/K-2-1034-1058	214.62	35.97	19.01	2.54
9	K/IAC 100-997-1035	**396.69**	34.79	17.34	**2.75**
10	IAC 100/SHR-W60-6-257-285	149.71	38.94	18.81	2.47
11	Wilis	342.99	36.99	19.32	2.63
12	Anjasmoro	177.33	36.82	18.32	2.64
	Average	271.02	37.42	18.61	2.62
	Standard deviation	92.94	1.61	0.70	0.11

Source: Subandi [14]

Conclusion

Nutrition content, especially soybean isoflavone, could be increased through genetic engineering. The soybean promising lines of K/IAC 100-64-1004-1037, K/IAC 100-997-1035, and IAC 100/K-60-1092-1141 have total isoflavone 398.50, 396.69, and 394.77 ppm, respectively. Soybean isoflavone was tend to negatively correlated with seed protein content. The promising lines of K/IAC 100-64-1004-1037, K/IAC 100-997-1035, and IAC 100/K-60-1092-

1141 produced yield from 2.70 – 2.82 t/ha, 100 seed weight were from 11.53 – 15.33 g, and the days to maturity was 83 days.

References

[1] Setchell KDR. Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. Am J Clin Nutr 1998; 68(suppl): 1333S - 1346S.

[2] Kudou S, Fleury Y, Welti D, Magnolato D, Uchida T, Kiamura K, Okubo K. Malonyl isoflavone glycosides in soybean seeds (*Glycine max* Merrill). Agric Biol Chem. 1991; 55: 2227–2233.

[3] Tsukamoto C, Shimada S, Igita K, Kudou S, Kokubun M, Okubo K, Kitamura K. Factors affecting isoflavone content in soybean seeds: changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. J Agric Food Chem 1995; 43: 1184-1192.

[4] Choi JS, Kwon TW, Kim JS. Isoflavone contents in some varieties of soybean. J Kor Food Biotechnol 1996; 5: 167–169.

[5] Carrao-Panizzi MC, del Pino Beléia K, Kitamura, Oliveira MCN. Effects of genetics and environment on isoflavone content of soybean from different regions of Brazil. Pesq Agropec Bras 1999; 34: 1787-1795.

[6] Hoeck JA, Fehr WR, and Welke GA. Influence of genotype and environment on isoflavone contents of soybean. Crop. Sci 2000; 40: 48-51.

[7] Lee SJ, Yan W, Kuk Ahn JK, Chung IM. Effects of year, site, genotype and their interactions on various soybean isoflavones. Field Crops Res 2002; 4150: 1–12.

[8] Gutierrez-Gonzalez JJ, Wu X, Gillman JD, Lee J, Zhong R, Yu O, Shannon G, Ellersieck M, Nguyen HT, Sleper DA. Intricate environment-modulated genetic networks control isoflavone accumulation in soybean seeds. BMC Plant Biol 2010; 10: 105 – 122.

[9] Chiari L, Piovesan ND, Naoe LK, Chamel Jos IC, Viana JMS, Moreira MA, de Barros EGC. Genetic parameters relating isoflavone and protein content in soybean seeds. Euphytica 2004; 138: 55–60.

[10] Krisnawati A, Adie MM. Agronomic character and isoflavone content of F5 soybean lines (In bahasa Indonesia). Penelitian Pertanian 2009; 28: 23 – 28.

[11] Vyn TJ, Yin X, Bruulsema TW, Jackson C, Rajcan I, Brouder SM. Potassium fertilization effects on isoflavone concentrations in soybean [*Glycine max* (L.) Merr.]. J Agric Food Chem 2002; 50: 3501-3506.

[12] Eldridge AC, Kwolek WF. Soybean isoflavones: effect of environment and variety on composition. J Agric Food Chem 1983; 31: 394-396.

[13] Wang HJ, Murphy PA. Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year, and location. J Agric Food Chem 1994; 42: 1674-1677.

[14] Subandi. Consortium of release acceleration on improved soybean variety and soybean seed production (In bahasa Indonesia). Final Report. ILETRI: 2013.

Presented at ISFA (September 16-17, 2014-Semarang, Indonesia) as paper #92, “Managing Biosafety and Biodiversity of Food from Local to Global Industries”