WFL Publisher
Science and Technology
Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.11 (1): 80-85. 2013 *www.world-food.net*

Stability of linolenic acid in seed oil of soybean accessions with elevated linolenic acid concentration

Krishna Hari Dhakal [1], Jeong-Dong Lee [1]*, Yeon-Shin Jeong [2], Hong-Sig Kim [3], J. Grover Shannon [4] and Young-Hyun Hwang [1]

*[1] School of Applied Biosciences,Kyungpook National University, Daegu, 702-701, Republic of Korea. [2] Department of Farm Management, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 702-701, Republic of Korea. [3] Department of Crop Science, Chungbuk National University, Cheongju Chungbuk 361-763, Republic of Korea. [4] Division of Plant Sciences, University of Missouri-Delta Research Center, Portageville, MO 63873, USA. *e-mail: jdlee@knu.ac.kr*

Received 20 September 2012, accepted 28 January 2013.

Abstract

Increasing linolenic acid composition in soybean [*Glycine max* (L.) Merr.] seed oil is desirable to lower the ratio of linoleic to linolenic acid. Linolenic acid content that varies in soybean oil is affected by temperature of the growing environment during the reproductive growth stage. The objective of this study was to evaluate stability of linolenic acid composition among 18 plant introductions (PIs) with linolenic acid contents ranging from 85 to 155 g kg^{-1} and two checks in 10 environments. Linolenic acid concentration of the commercially grown normal and elevated linolenic acid varieties Taekwang and Daemang averaged 79 and 93 g kg^{-1}, respectively. Linolenic acid composition of each plant introduction and check varieties varied over environments and had a negative relationship with mean maximum temperature during the final 30 days of the reproductive stage. Four stability parameters range, coefficient of variation and two stability coefficients (b_T and b_E) were used to measure the stability of linolenic acid. PI593997, PI153245 and PI587632B were relatively more stable across 10 environments. PI189950 (107 g kg^{-1}) had the highest average linolenic acid composition and showed the lowest (4.8) linoleic to linolenic acid ratio. Thus, linolenic acid content of these elevated PIs and check varieties was too low over the 10 environments to consistently provide soybean oil with a linoleic to linolenic acid ratio of <4 to 1 desired in food or supplements for added health benefits.

Key words: Linolenic acid, stability, soybean.

Introduction

Both linoleic and linolenic acids are essential fatty acids for humans. These two polyunsaturated fatty acids are metabolically and functionally distinct and have different physiological functions [1]. Therefore, consumption of these two fatty acids in proper ratio is important. Linolenic acid in diet can have a benefit in reduction of cardiovascular disease [2, 3]. Linolenic acid favorably affects arterosclerosis, coronary heart disease, inflammatory disease, and perhaps even behavioral disorders [4]. A high intake of very long-chain (omega-3) fatty acids from fish decreases the risk of death from coronary heart disease, and cancer [5-9]. Kim *et al.*[10] reported that ≤4.0 n-6 (linoleic acid)/n-3 (linolenic acid) ratio is associated with a decreasing preneoplastic foci (preceding the development of a tumor) during rat hepatocarcinogenesis while Park *et al.*[11] reported that muscle cholesterol response levels in rat could be minimized with a diet containing of 1.57 n-3/n-6 fatty acids ratio.

Soybean [*Glycine max* (L.) Merr.] is popularly known as a health-enhancing food and is mostly consumed as soymilk, tofu, and fermented products such as miso, temeph and sufu, especially in Asian countries. Many countries use soybean in various forms such as soybean sprouts, paste, soymilk, soybean oil, and tofu as key ingredients in cultural cuisine [12]. In 2010, soybean represented 58% of the worlds oilseeds production [13]. The average fatty acid concentration of commercial soybean seed oil primarily consists of about 120 palmitic, 40 stearic, 230 oleic, 530 linoleic and 80 g kg^{-1} linolenic acids [14]. However, ideal fatty acid composition of soybean oil depends on the specific use for which it is intended. Modifying fatty acid profile in seed would play a vital role for greater use of soybean oil in food and industrial applications. Soybean oil typically has a ratio of 6-7:1 of linoleic (n-6) to linolenic (n-3) acid but a lower linoleic to linolenic acid ratio (<4 to 1) in seed oil is desirable because it has been associated with reduced risk of heart and other human diseases. Thus, for health benefits, elevated linolenic acid genotypes are desirable in food grade soybeans.

Omega-3 fatty acid desaturase genes (*FAD3*) which control seed linolenic acid levels in soybean were identified and characterized. Three versions *FAD3* genes exist in the soybean genome and are *GmFAD3A*, *GmFAD3B* and *GmFAD3C*. *GmFAD3A* was characterized as most highly expressed of the three homologs in developing seeds [15].

The usefulness of specific soybean genes in a breeding program also depends upon the stability of the trait expressed across different environments. Different studies conducted with common cultivars under extreme temperature conditions have indicated that seeds from soybean plants exposed to high daily temperatures have reduced linolenic acid [16]. Mutants with

different fatty acid concentrations exhibited average or higher stability than lines with normal levels of these fatty acids [17]. The higher the average linolenic acid composition of a genotype, the greater the instability it showed across various growing conditions [18]. The lowest linolenic acid content genotype IA 3017 (10 g kg^{-1}) was very stable in linolenic acid composition across 10 growing environments while the highest linolenic acid genotypes were less stable.

Plant Introductions (PIs) with elevated linolenic acid (more than 80 g kg^{-1}) from the USDA soybean germplasm collection in Urbana-Champaign, IL, have been reported in the Germplasm Resources Information Network (GRIN; http://www.ars-grin.gov [verified 26 June 2009]). These genotypes may be useful to develop soybean genotypes with oil containing elevated linolenic acid desired for different soybean food products. The purpose of this study was to determine the stability of linolenic acid concentration across environments for 18 plant introductions from USDA soybean germplasm collection with elevated linolenic acid concentration.

Table 1. Maturity group (MG) and mean fatty acid (g kg^{-1}) profile for each of 20 soybean genotypes over 10 environments evaluated from 2007 to 2010.

Genotype[+]	MG	Linolenic acid*	Fatty acid profile[§]					Linoleic acid: linolenic acid
			Palmatic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	
PI507715B	00	108	111	43	268	500	78	6.4
PI189950	0	114	130	32	222	509	107	4.8
PI238924	0	108	117	39	233	512	99	5.2
PI507715A	0	95	107	37	309	471	76	6.2
PI593997	0	102	111	35	307	467	81	5.8
PI153245	0	105	122	40	236	509	93	5.5
PI532469	I	103	105	31	357	435	72	6.0
PI549067	I	104	104	30	325	463	78	5.9
PI578400	I	104	108	31	330	463	68	6.8
PI603148	I	102	105	30	354	446	65	6.9
PI603361	I	104	105	39	299	476	81	5.9
PI603479	I	108	109	36	346	439	70	6.3
PI593949A	I	112	110	34	262	510	84	6.1
PI603428A	I	104	119	43	291	465	82	5.7
PI593949B	II	85	109	35	244	525	87	6.0
PI549071	II	111	104	28	332	448	88	5.1
[£]TK (CK)	IV	-	96	42	303	480	79	6.1
[£]DK (CK)	IV	-	118	39	221	529	93	5.7
PI200502	VI	159	116	36	216	544	88	6.2
PI587632B	VII	105	109	35	209	558	89	6.3
Mean			111	36	283	487	83	
$LSD_{0.05}$			5	5	48	36	10	

[+] The 20 soybean genotypes include 18 plant introductions with elevated linolenic acid content and two check genotypes.
[§] Mean content averaged over 10 environments. [£] CK=Check varieties (TK = Taekwang and DK = Daemang).
* Linolenic acid concentration of PIs reported in GRIN (Germplasm Resources Information Network -http://www.ars-grin.gov/).

Materials and Methods

Twenty soybean genotypes including 18 with elevated linolenic acid concentration and two checks were used in this study (Table 1). The elevated PIs were selected based on linolenic acid content given in the USDA soybean germplasm collection as reported in GRIN. The 18 genotypes varied in maturity but 16 of the 18 were from maturity group 00 to group II which are considered very early and not well adapted for optimum production in Korea. The two checks (released varieties in Korea in maturity group IV) were Taekwang and Daemang [19] with normal and elevated in linolenic acid concentration, respectively.

The experiment was conducted at two locations which included experimental farm of Kyungpook National University, Gunwi, Republic of Korea and the experimental farm of Chungpook National University, Cheongju, Republic of Korea, from 2007 to 2010, which represent relatively different seasonal growing conditions. There was one planting date in 2007 and 2010 for Gunwi and two planting dates in 2008 and 2009 for both locations. The two planting dates at each location were separated by about 30 days. There were 10 environments with each planting date at each location within various years regarded as an environment. The experimental design was a randomized complete block design with two replications. Each genotype was planted in a hill plot 15 cm apart in 4 m long rows and 60 cm between rows. Two seeds were planted per hill for all environments. Latitude of each location and planting dates in each year are shown in Table 2.

Maturity of each accession was determined from 10 environments and recorded as the date when 95% of the pods of each genotype had reached their mature color [20].

Fatty acid analysis: Fatty acid profiles for each plot were analyzed as described by Wilson *et al.*[21]. Fatty acid (g kg^{-1}) of total oil for each genotype within each environment was determined for each line by randomly sampling five plants and picking three to four pods each from the middle nodes. Ten seeds were randomly selected from each plot sample for fatty acid analysis and ground into powder with a dry mill (Philips, HR2860, KTL SU07129-3004). A portion of the sample powder (0.5 g) was used for fatty acid analysis. Oil was extracted by placing 0.5 g seed powder in 5 ml of chloroform:hexane:methanol (8:5:2, v/v/v) overnight. Derivitization was done by transferring 100 µl of extract to vials and adding 75 µl of methylating reagent (0.25 M methanolic sodium methoxide:petroleum ether:ethyl ether [1:5:2, v/v/v]). Hexane was added to bring samples to approximately 1 ml. An Agilent (Palo Alto, CA) Series 7890 capillary gas chromatograph fitted with a flame ionization detector (275°C) was used with an AT-Silar capillary column (Alltech Associates, Deerfi eld, IL). Standard fatty acid mixtures (Animal and Vegetable Oil Reference Mixture 6, AOACS) were used as calibration reference standards.

Table 2. Latitude and planting date (environments) at each of two locations where studies on soybean were conducted, 2007 to 2010.

Location	Latitude	Planting dates+					
		2007	2008		2009		2010
		Date 1	Date 1	Date 2	Date 1	Date 2	Date 1
Kyungpook National University Research station (Gunwi, Republic of Korea)	36º 14' N	15 June (E1)	1 June (E2)	1 July (E3)	1 June (E6)	1 July (E7)	15 June (E10)
Chungbuk National University Research station (Cheongju, Republic of Korea)	36º 38' N		1 June (E4)	1 July (E5)	1 June (E8)	1 July (E9)	

[+] Letter and number in parenthesis for each planting date in various years in the designation for each environment.

Statistical analysis: To compare the stability of genotypes among environments for linolenic acid in seed oil, range of average linolenic acid concentration, coefficient of variation (CV), and two stability coefficients, b_E and b_T, were used as stability parameters[22]. Stability coefficient (b_E) was calculated from the regression of the mean linolenic acid concentration of a line at an environment on an environmental index. The environmental index was the mean linolenic acid concentration of all lines at an environment minus the mean linolenic acid concentration of all lines averaged across the 10 environments [22]. Mean high temperature during the last 30 days of the reproductive period has previously shown the highest correlation with linolenic acid concentration in soybean seed oil[18]. Therefore, a stability coefficient (b_T) for each genotype was calculated from the regression of mean linolenic acid concentration on mean maximum temperature during the final 30 days of the reproductive period over 10 environments as described by Oliva *et al.* [18]. Mean maximum temperature during the final 30 days of the reproductive period for each accession in each environment was the mean of the daily highs for the last 30 days of seed fill until maturity. Genotypes having stability regression coefficients (b-value) closest to zero with high coefficient of determination (r^2) values were considered as more stable, whereas those that deviate significantly from zero (either positive or negative) were considered less stable to changes across environments. Weather stations at each location provided maximum temperatures for computation of mean maximum temperature during the final 30 days of the reproductive period. PROC REG of SAS (9.1) package was used to calculate the two regression slopes.

Results and Discussion

Linolenic acid concentration in soybean seed was significantly ($P < 0.0001$) different across growing environments. The G × E interaction for linolenic acid concentration was also significant ($P < 0.0001$) (data not shown). Mean and variation of linolenic acid concentration of each of the 20 genotypes across 10 environments are shown in Tables 1 and 3. The mean linolenic acid concentration of 20 genotypes across 10 environments was 83 g kg^{-1}. The mean linolenic acid concentration of two check varieties was 86 g kg^{-1} across 10 environments that ranged from 79 to 93 g kg^{-1}. Among 18 elevated linolenic acid concentration lines evaluated, PI603148 (65 g kg^{-1}) and PI189950 (107 g kg^{-1}) had the lowest and highest mean linolenic acid concentration across 10 environments, respectively. Even though the 18 PIs were selected for elevated linolenic acid content provided in GRIN, six of the 18 PIs averaged lower in linolenic concentration in seed oil than the normal linolenic acid check, Taekwang. In addition only four of the remaining 12 PIs with higher than average linolenic acid content were significantly higher in linolenic concentration than the normal linolenic check Taekwang. Temperature of the growing environment has been shown to influence linolenic acid content [18]. Most of the selected PIs were group 00 to II in maturity and filled seed and matured earlier during hotter periods than either maturity group IV or check which are typically grown in Korea. It has been shown that higher temperatures during the final 30 days of seed filling to maturity is associated with reducing linolenic acid [18]. Therefore, the elevated temperature is the reason these earlier group 00 to II PIs showed reduced linolenic acid content compared to the normal linolenic acid check.

Linolenic acid concentration of each genotype fluctuated across environments (Table 3) and had a negative relationship with mean maximum temperature during the final 30 days of the reproductive stage. For example in 2008, linolenic acid concentration for the check Daemang was 101 g kg^{-1}, at E5 (Cheongju, Republic of Korea, 2008), where average maximum temperature was 25.4ºC, 98 g kg^{-1} at E3 (Gunwi, Republic of Korea, 2008) with a mean maximum temperature of 26ºC; and 86 g kg^{-1} at E2 (Gunwi, Republic of Korea, 2008) which the warmest location with an average maximum temperature of 27.1ºC. Among the Pls, PI189950 produced the highest linolenic acid concentration (107 g kg^{-1}) across the 10 environments. Linolenic acid concentration of PI189950 also changed according to mean maximum temperature during the final 30 days of seed filling to maturity. Linolenic acid concentration was 130 g kg^{-1} at E5 (Cheongju, Republic of Korea, 2008) with a mean maximum temperature of 28.8ºC, 121 g kg^{-1} at E3 (Gunwi, Republic of Korea, 2008) with an average maximum temperature 29.3ºC. The negative relationship of linolenic acid concentration with high mean temperature was in agreement with previous findings [18].

The stability parameters, range, CV, two stability coefficients (b_T and b_E) and coefficient of determination (r^2) for mean linolenic acid concentration of the 18 Pls and two checks are shown in Table 4. Smaller numerical values of range and CV for linolenic acid concentration of a genotype indicate more stable genotypes across environments. Genotypes tested showed different ranges for linolenic acid concentration across environments (Table 4). Among the two checks, Daemang (elevated linolenic acid concentration) had the lowest range (22 g kg^{-1}) in linolenic acid concentration and ranked 1st in stability where as Taekwang (normal linolenic acid concentration) ranked 5th, among all genotypes. PI593949B having 87 g kg^{-1} average linolenic concentration and ranking 2nd among 20 genotypes across environments, was the most stable PI followed by PI593997 (81 g kg^{-1}), PI603148 (65 g kg^{-1}) and PI603361 (81 g kg^{-1}). PI578400 with a mean of 68 g kg^{-1} averaged lower in linolenic acid concentration and had a greater range (55 g kg^{-1}) across the 10 environments indicating it was the least stable among the 20 genotypes.

The CVs for each genotype showed similar pattern to the ranges of the mean linolenic acid concentration (Table 4). Generally, genotypes with relatively high linolenic acid ranges tended to have relatively higher CVs than genotypes with relatively low linolenic acid ranges. PI574800, PI507715A and PI603428A with relatively wide ranges of linolenic acid also showed the high CVs of 22%, 21% and 19%, respectively. Based on high CVs, these PIs were least stable. The CV values of Daemang and PI593949B indicated that these two genotypes were the most stable based on CV among all 20 genotypes. The elevated linolenic acid concentration check Daemang had lowest CV (9%) and ranked first among all 20 genotypes. PI593949B had the lowest CV (9%) and ranked first followed by PI153245 and PI587632B with CVs of 11%, compared to the other genotypes.

Stability coefficients (b_T) for each genotype differed in response to changes in temperature during the final 30 days of the seed filling period to maturity (Table 4). PI603361 was the most stable genotype based on the stability coefficient (b_T). Temperature at the end of seed feeling period had the least effect on the accumulation of linolenic acid in PI603361 ($b_T = -0.68$, $r^2 = 0.3999$,

P < 0.05). Check genotypes were also relatively more stable. Taekwang was the most stable (b_T = -0.63, r^2 = 0.4476, P < 0.03) of the two checks and second in stability based on stability coefficients among all genotypes. The most unstable genotypes for linolenic acid concentration were PI189950, PI593949B, PI238924 and PI593997 that had high b_T values, -0.01 (r^2 = 0.0001, P < 0.98), -0.04 (r^2 = 0.0024, P < 0.89), -0.07 (r^2 = 0.0095, P < 0.79) and -0.07 (r^2 = 0.0156, P < 0.73), respectively. These results indicate that temperature during seed filling periods contributed significantly to linolenic acid accumulation in seed oil for these soybean genotypes. Range values for PI189950, PI238924 also showed that these genotypes fluctuated widely in linolenic acid concentration under various growing environments.

The stability coefficients (b_E) of each genotype ranged from 0.65 to 1.44, which indicated variation among the genotypes for stability across environments (Table 4). PI593997 was the most stable genotype among all followed by PI238924 and PI593949B with the lowest stability coefficient (b_E) values 0.65, 0.75 and 0.76, respectively. Among the checks, Daemang was most stable (b_E = 0.88) and ranked 6th among all genotypes. The most unstable genotypes based on this stability parameter was PI549071 which had the highest stability coefficient value (b_E = 1.44) followed by PI507715B.

Mean rank was determined based on the average value of four stability parameters range, coefficient of variation and both stability coefficients b_E and b_T. Based on mean rank across all stability parameters, Daemang was the most stable across 10 environments between two checks and 2nd overall. Among the 18 PIs, PI593949B (MG II) was the most stable genotype and ranked 1st among all 20 genotypes. PI593997 and PI587632B ranked 4th and 5th, respectively in mean stability. PI507715A ranked last for all stability parameters among all genotypes and was the least stable in linolenic acid concentration. In MG 0, PI593997 and PI153245 were relatively the most stable PIs. The PIs in MG I were not stable based on the mean rank of four

Table 3. Mean linolenic acid content (g kg^{-1}) of 18 plant introductions and two checks (CK) in each of 10 environments+, 2007 to 2010.

Genotype	MG¶	Linolenic acid concentration										Mean ± SE
		E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	
PI507715B	00	70	72	91	77	89	75	83	71	86	86	80±2
PI189950	0	87	115	121	90	130	109	120	93	105	102	107±5
PI238924	0	89	93	97	88	114	108	113	94	108	102	100±3
PI507715A	0	80	70	94	63	87	75	82	65	85	87	79±3
PI593997	0	82	69	84	65	85	86	93	70	79	83	80±3
PI153245	0	92	84	103	82	107	93	105	82	94	101	93±3
PI532469	I	71	62	87	52	81	73	92	58	78	71	72±4
PI549067	I	75	68	86	59	85	91	94	65	86	81	79±4
PI578400	I	103	57	76	55	67	67	75	48	66	67	68±5
PI603148	I	78	57	76	50	66	63	72	54	68	70	65±3
PI603361	I	90	78	87	63	88	73	90	64	90	91	81±4
PI603479	I	85	68	82	59	81	62	83	54	55	74	70±4
PI593949A	I	84	80	91	69	93	81	99	62	89	92	84±4
PI603428A	I	79	94	85	62	88	71	92	68	82	98	82±4
PI593949B	II	98	82	92	73	89	89	86	74	90	94	87±3
PI549071	II	67	96	105	74	98	74	105	63	92	101	88±5
£TK (CK)	IV	98	69	87	65	81	72	88	69	83	81	79±3
£DK (CK)	IV	95	86	98	84	101	87	101	81	98	103	93±3
PI200502	VI	78	80	94	79	90	93	94	78	85	112	88±4
PI587632B	VII	87	81	93	71	89	95	92	83	88	108	89±3
$LSD_{0.05}$		3	12	8	6	8	15	13	4	1	2	

+Environments where soybean were planted: E1 (15 June, Gunwi, Korea, 2007); E2 (1 June, Gunwi, Korea, 2008); E3 (1 July, Gunwi, Korea, 2008); E4 (1 June, Chanju, Korea, 2008); E5 (1 July, Chanju, Korea, 2008); E6 (1 June, Gunwi, Korea, 2009); E7 (1 July, Gunwi, Korea, 2009); E8 (1 June, Chanju, Korea, 2009); E9 (1 July, Chanju, Korea, 2009); E10 (15 June, Gunwi, Korea, 2010). ¶MG, maturity group. £ TK = Taekwang and DK = Daemang.

Table 4. Stability parameters, mean, range, coefficient variation (CV), regression coefficients (b_T and b_E), and coefficient of determination (r^2) for mean linolenic acid (C18:3) content of 18 plant introductions and two checks (CK) calculated from 10 environments.

Genotype	MG¶	Mean (C18:3)		Range (C18:3)		CV		Stability coefficients (b_T)				Stability coefficients (b_E)				Mean* Rank
		(%)	Rank	(%)	Rank	(%)	Rank	b_T+	Rank	P	r^2	b_E§	Rank	P	r^2	
PI507715B	00	78	14	40	9	15	6	-0.27	9	0.42	0.0824	1.36	17	0.0001	0.85	14
PI189950	0	107	1	43	11	13	4	-0.01	15	0.98	0.0001	1.10	13	0.0420	0.42	16
PI238924	0	99	2	45	12	14	5	-0.07	13	0.79	0.0095	0.75	2	0.0375	0.44	10
PI507715A	0	76	16	54	14	21	10	-0.08	12	0.78	0.0106	1.11	14	0.0001	0.86	18
PI593997	0	81	11	28	3	12	3	-0.07	13	0.73	0.0156	0.65	1	0.0226	0.50	4
PI153245	0	93	3	25	2	11	2	-0.08	12	0.77	0.1170	1.06	12	0.0001	0.90	8
PI532469	I	72	17	40	9	17	8	-0.07	13	0.79	0.0095	0.92	8	0.0005	0.80	12
PI549067	I	78	15	35	7	16	7	-0.07	13	0.83	0.0060	1.18	15	0.0014	0.74	15
PI578400	I	68	19	55	15	22	11	-0.51	3	0.18	0.2110	0.99	10	0.0917	0.31	13
PI603148	I	65	20	28	3	14	5	-0.23	11	0.36	0.1047	0.93	9	0.0017	0.73	8
PI603361	I	81	12	28	3	14	5	-0.68	1	0.05	0.3999	1.18	15	0.0002	0.83	6
PI603479	I	70	18	31	4	17	8	-0.31	7	0.47	0.0676	1.06	12	0.0145	0.55	9
PI593949A	I	84	9	37	8	14	5	-0.45	4	0.16	0.2269	1.30	16	0.0001	0.94	11
PI603428A	I	82	10	50	13	19	9	-0.04	14	0.89	0.0024	1.05	11	0.0120	0.57	17
PI593949B	II	87	8	25	2	9	1	-0.34	5	0.21	0.1854	0.76	3	0.0063	0.63	1
PI549071	II	88	6	42	10	19	9	-0.25	10	0.61	0.0340	1.44	18	0.0109	0.58	17
£TK (CK)	IV	79	13	33	5	13	4	-0.63	2	0.03	0.4476	0.90	7	0.1046	0.55	3
£DK (CK)	IV	93	4	22	1	9	1	-0.32	6	0.14	0.2482	0.88	6	0.0001	0.87	2
PI200502	VI	88	7	34	6	12	3	-0.23	11	0.34	0.1134	0.86	5	0.0286	0.47	7
PI587632B	VII	89	5	37	8	11	2	-0.30	8	0.16	0.2314	0.84	4	0.1380	0.55	5

+Stability coefficients were calculated from the regression of mean linolenic acid contents on mean maximum temperature during the final 30 d of the reproductive period over 10 environments. §Stability coefficients for mean linolenic acid content of genotypes at each environments regressed on the environmental index. *Mean rank was ranked based on the average value of four stability parameters. ¶MG, maturity group. £ Check varieties (TK = Taekwang and DK = Daemang).

stability parameters. PI587632B (MG VII) was a relatively stable genotype and ranked 5th among all of the Pls. PI587632B and PI593997 were affected most by temperature during the seed filling period compared to PI593949B which was the least affected PI and did not much fluctuate as much in linoleinc acid concentration as other genotypes across growing environments.

Generally soybean oil has 80 g kg^{-1} linolenic acid [14] which has been identified as an unstable component of soybean oil. One of the most important goals of oil quality breeding in soybean has been to reduce its linolenic acid concentration for oxidative stability and flavor to reduce the need for hydrogenation. In Asian countries soybean is used directly as food rather than as oil. Soybean oil has typically 6-7:1 ratio of linoleic to linolenic acid. The excessive amount of linoleic acid in proportion to linolenic acid (high linoleic to linolenic acid ratio) is found in todays diets. This high linoleic to linolenic acid ratio promotes the pathogenesis of many human diseases, including cardiovascular disease, cancer, inflammatory, and autoimmune diseases. On the other hand, increased levels of linolenic acid (low linoleic to linolenic acid ratio) exert suppressive effects [23] on these diseases. Thus, increasing linolenic acid in soybean oil for food type soybean is an important breeding goal to lower the ratio of linoleic to linolenic acid, which reduces the risk of heart and other chronic diseases. PI soybean genotypes with elevated linolenic acid concentration (85 - 155 g kg^{-1} listed on GRIN) were obtained from the USDA soybean germplasm collection and tested to measure each genotypes stability for linolenic acid over 10 environments. The amount of linolenic acid was highly influenced by temperature and environment. In this study, the average linolenic acid range of Pls across 10 environments averaged lower in linolenic acid concentration than shown values in GRIN (65 - 107 g kg^{-1}). PI189950 (107 g kg^{-1}) had the highest average linolenic acid concentration and showed a 4.8 linoleic to linolenic acid ratio (Table 1). Thus on average, this amount of linolenic acid in *Glycine max* is not enough to reduce linoleic to linolenic acid ratio (4:1 or lower), which is desirable in reducing the risk of many chronic diseases in humans [23]. However, seed oil of PI189950 showed linolenic acid concentrations of 120 to 130 g kg^{-1} in three of the 10 environments and would have met the requirements for a linoleic to linolenic ratio of 4:1.

Another possible way to increase linolenic acid in soybean oil might be by transferring genes from *Glycine soja* with accessions having typically higher linolenic acid concentrations than *Glycine max*. Accessions of *G. soja* with over 200 g kg^{-1} linolenic acid have been reported in USDA soybean germplasm collection [24]. Similarly, other *G. soja* IT183049, IT184172 and IT184256 have linolenic acid concentration 141, 148, and 155 g kg^{-1}, respectively [25] can also be used as sources to increase linolenic acid concentration. Genetic regulation of linolenic acid concentration in wild soybean has suggested that the high-linolenic trait in wild soybean genotypes was determined by a set of desaturase alleles that were different from corresponding alleles in *Glycine max* [26]. Introgression of these alleles from *Glycine soja* to *Glycine max* may lead to the production of high linolenic acid soybean oil for various applications such as omega-3 fatty acid soy-foods and industrial products.

Conclusions

In this study, it is concluded that Pls (*Glycine max)* of high linolenic acid concentration obtained from USDA soybean germplasm collection is not enough to reduce linoleic to linolenic acid ratio (4:1 or lower). So, *Glycine soja* of high linolenic acid concentration from USDA or another source can be used to transfer gene to *Glycine max* to increase linolenic acid production by breeding or biotechnology approach.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRE) funded by the (#2010-0003220).

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