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Stability of elevated α-linolenic acid derived from wild soybean (*Glycine soja* Sieb. & Zucc.) across environments

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Abstract Previous studies reported that omega-3 fatty acid and α-linolenic acid are important compounds that prevent cardiovascular disease and cancer in humans. Soybean [*Glycine max* (L.) Merr.] oil typically contains ~8 % α-linolenic acid (ALA). Elevated (~15 %) ALA content in seed oil is a trait of wild soybeans (*G. soja* Sieb. and Zucc.). Decreasing the ratio of linoleic acid (LA) to ALA to 4:1 or lower (compared to the ratio of 6 or 7:1 found in commercial soybean seed) should have health benefits for humans. This study was conducted to determine the environmental stability of elevated ALA acid recombinant inbred lines (RILs) derived from a cross of PI 483463 (wild soybean with 15 % ALA) and Hutcheson (cultivar with 9 % ALA). The fatty acid profile analysis from nine environments showed that the content of ALA for the RILs 156, 159 and 166 ranged from 10.7 to 15.7, 14.0 to 15.8, and 14.8 to 15.8, and averaged 13.9, 14.9, and 15.2 % respectively. The contents of ALA from these RILs and the wild soybean parent showed consistently higher than cultivated check soybeans. Two of the three RILs with elevated ALA content and ratios of <4:1 LA to ALA were as stable in ALA content as the high and low linolenic acid parents across growing environments. This indicates that lines with elevated ALA content developed from wild soybean PI 483463 are stable in ALA across environments and would be useful in improving soybeans with lower LA to ALA ratios.

Keywords Wild soybean · Fatty acid · Linolenic acid · Omega-6 · Omega-3 · Genotype × environment interaction

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Introduction

Over half of the world's total vegetable oilseed comes from soybean [*Glycine max* (L.) Merr.] in which the seed contains ~20 % oil by weight (Soy stats 2012, www.soystats.com). Soybean oil generally consists of approximately 11 % palmitic, 4 % stearic, 24 % oleic, 54 % linoleic and 7 % linolenic acids (Hui 1996). The functionality of soybean oil for both food and industrial uses is influenced by the fatty acid profile of the oil (Lee et al. 2007). Modification of saturates, oleic

acid, and linolenic acid through breeding and biotechnology are being emphasized to develop desired fatty acid phenotypes. It is impractical to commercially develop all oil phenotypes. Perhaps the most desired phenotype for soybean oil is <7 % saturates (16:0 + 18:0), >55 % 18:1, and <3 % 18:3 because of its multiple uses in many edible and industrial applications (Wilson 2004). Recently, much research has been devoted to increasing the content of ω-9 fatty acids (oleic acid) and reducing ω-3 and ω-6 fatty acids in soybean oil to improve the resistance to oxidation and stability of soybean oil (Pham et al. 2012).

Soybean oil is a source of omega-3 (ω-3) fatty acids such as α-linolenic acid (ALA) which is approximately 7–8 % in the oil. It is an important plant-based source of ω-3 polyunsaturated fatty acid for vegetarians and non-seafood eaters. Omega-3 fatty acids should be included in the human diet because it is essential in growth and development of the brain and retina (particularly in premature infants) (Simopoulos 1991). Omega-3 fatty acids in diets have multiple, positive health benefits including the reduction of cardiovascular diseases, and improved cognitive functions (McCann and Ames 2005; Astorg et al. 2004; Bouwstra et al. 2003). They reduce the risk of colon cancer and other diseases (Messina 1999; Chandalia et al. 2000; Jenkins et al. 2003). Diets high in linoleic acid (LA, ω-6) content may reduce the nutritionally positive effects of the health-beneficial omega-3 fatty acids in tissue (Blasbalg et al. 2011; Clark et al. 1992; Friesen and Innis 2010). A lower ratio of LA to ALA (4:1 or less versus ~6–7:1 as found in commercial soybean seed) or a large ω-3 intake is associated with suppressing illnesses. A ratio of LA to ALA of 4:1 was found to prevent cardiovascular diseases, 2.5:1 for inhibition of colon cancerous cells, 2–3:1 for prevention of rheumatoid and inflammatory arthritis and 5:1 for asthmas (Simopoulos 2008). An ideal diet consists of ω-6:ω-3 fatty acid ratios that range from 1:1 to 4:1 (Renaud 2002; Mattson and Grundy 1985).

In spite of emphasizing the importance of ω-3 fatty acid for human health, there have been few studies which focused on increasing the ALA content in soybean. Cultivated soybeans with over 10 % ALA are available in the USDA Soybean Germplasm Collection (USDA 2011). Recently, Dhakal et al. (2013) reported the stability of elevated ALA for 18 soybean accessions from the USDA Soybean Germplasm Collection varying from 8.5 to 15.5 % in ALA contents. Results showed that 18 *Glycine max* accessions averaged 6.5–10.7 % ALA over 10 environments. These levels of ALA were insufficient to reduce LA to ALA ratio to 4:1 or lower to reduce risks of contacting chronic diseases in humans (Simopoulos 2008).

In contrast, wild soybean (*G. soja* Sieb. and Zucc.) accessions have almost twice the ALA concentration than that of cultivated soybean (Chae et al. 2012). Genetic regulation of ALA concentration in wild soybean suggested that the high ALA trait in wild soybean genotypes was determined by a set of desaturase alleles that were different from corresponding alleles in *Glycine max* (Pantalone et al. 1997). Therefore, wild soybean would be a good genetic resource to increase ALA (ω-3) in cultivated soybean.

The usefulness of soybean with altered fatty acid profiles in a breeding program is dependent upon the stability of each fatty acid across different growth environments. Several studies have investigated the effect of genotype and environments on the fatty acid content of soybean lines (Cherry et al. 1985; Schnebly and Fehr 1993; Oliva et al. 2006). The interaction between genotype and environment results in significant differences in the performance of genotypes when evaluated in different locations (Gauch and Zobel 1997). It is important to test the stability of fatty acid across environments for improving fatty acid production and variety development. However, there were few reports for the stability of high ALA in wild soybean.

The objective of this study was to determine the stability of high ALA content among recombinant inbred lines derived from *G. soja* PI 483463 (~15 %, ALA) × Hutcheson (~9 %, ALA).

Materials and methods

Plant material and growth environments

A recombinant inbred population from a cross of PI 483463 (wild soybean with 15 % ALA) and Hutcheson (with 9 % ALA) was developed by Lee et al. (2009). PI 483463 is a maturity group III wild soybean with salt tolerance gene and Hutcheson is a maturity group V salt susceptible one (Ha et al. 2013). During the evaluation of parents and recombinant inbred lines

Table 1 Latitude, planting dates at each of four locations where studies were conducted, 2008 to 2011

Location	Planting dates[a]				
	2008	2009	2010	2011	
Portageville, MO, USA (Delta Center, Lee Farm)	22 May (E1)	19 June (E2)			
Gunwi, Republic of Korea (Affiliated Experiment and Practice Fields of Kyungpook National University)			14 May (E3)	20 May (E4)	17 June (E5)
Yeosu, Republic of Korea (campus of Chonnam National University)				3 June (E6)	24 June (E7)
Jeongeup, Republic of Korea (Advanced Radiation Technology Institute of Korea Atomic Energy Research Institute)				31 May (E8)	29 June (E9)

[a] Letters and number in parentheses for each planting date in various years is the designation for each environment

(RILs) we found two parents had different fatty acid compositions especially ALA such as PI 483463 had around 15 % and Hutcheson had 9 %.

Three RILs with relatively high ALA (above 14 %), parents and Williams 82 as a check with normal ALA content (Table 1) were used to evaluate the stability of ALA in seed oil. The generation of each RIL was $F_{2:6}$ in 2008, $F_{2:7}$ in 2009, and $F_{2:8}$ in 2010 and 2011. The experiment was conducted in four different locations from 2008 to 2011. The soybeans at Lee Farm Delta Center (Portageville, 36°44′N, MO, USA) were planted in 2008 and 2009. In Korean environments, soybeans were planted at the Affiliated Experiment and Practice Fields of Kyungpook National University (Gunwi, 36°14′N, Republic of Korea); at the Yeosu campus of Chonnam National University (Yeosu, 34.7°127.6′N, Republic of Korea) and at the Advanced Radiation Technology Institute of Korea, Atomic Energy Research Institute (Jeongeup, 35.5°126.8′N, Republic of Korea) in 2010 and 2011. Planting dates for each location are shown in Table 1. The term "environment" was defined as a specific location and planting date combination relative to this experiment (Lee et al. 2012). A randomized complete block design (RCBD) with two replications was used in each environment. Plots were hills with 10 seeds planted in each hill in all environments. In the research conducted at the Lee Farm, Gunwi, Jeongup and Yeosu, soybeans were planted in hills with between row spacings of 76, 85, 100 and 150 cm, respectively. The intra-row spacings for the hill plots were 61, 95, 100 and 150 cm at the Lee Farm, Gunwi, Jeongup and Yeosu, respectively.

Fatty acid analysis

Fatty acid profile (relative percentage of total fatty acid) for each genotype within each environment was determined for each plot. A set of 10 randomly selected seeds from each plot for each environment and genotype were placed in a paper envelope and then manually crushed. Small samples were taken from each envelope and placed in test tubes for oil extraction. The oil was extracted by placing crushed seeds in a 5 mL solution of chloroform:hexane:methanol (8:5:2, v/v/v) overnight. Derivatization was done by transferring 100 μL of extract to vials and adding 75 μL of methylating reagent (0.25 M methanolic sodiummethoxide: 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 (FID) with an AT-Silar capillary column (Alltech Associates, Derfield, IL) was used to analyze fatty acid content. The temperatures of the oven, injector, and detector were set at 210, 250, and 230 °C, respectively. Standard fatty acid mixtures (Animal and Vegetable Oil Reference Mixture 6, AOACS) were used as calibration reference standards. The samples were evaluated for palmitic acid, stearic acid, oleic acid, linoleic acid (LA), and α-linolenic acid (ALA).

Statistical analysis

The analysis of variances (ANOVA) for fatty acids was conducted for environment (E), genotype (G), replication and G × E interactions, and the

significance of mean squares was then estimated by using PROC GLM of SAS (SAS 2005). The data were analyzed in a randomized complete block design with multi-environments. The differences among mean values were determined using least significant difference at $P \leq 0.05$. PROC REG was used to calculate regressions of slopes. The data from some environments (Hutcheson and Williams 82 from E7, RIL 159 for E6 and RIL 166 for E4) were not recorded due to missing plot, and were therefore not included in the analysis of variance.

The range of average ALA content, coefficient of variation (CV) and stability coefficient (b_E) were used as parameters to compare the stability of genotypes among environments for ALA in seed oil (Lee et al. 2012, 2009; Scherder et al. 2008). The b_E was calculated from the regression of the mean ALA content of a line at an environment on an environment index. The environment index was calculated as the mean ALA content of all lines in an environment minus the mean ALA content of all lines averaged across the nine environments (Scherder et al. 2008). The stability regression coefficient (b-value) was calculated for ALA and for each genotype to determine if the altered fatty acid levels were stable across different environments. Genotypes having stability regression coefficients (b-value) closest to zero with a high r^2 values were designated as more stable, whereas those that deviated significantly from zero (either positive or negative) were considered less stable to changes across environments.

Results

Analysis of variance and mean fatty acids profiles

The ANOVA was performed to test the effects of the environments, genotypes, and G × E interactions for fatty acid composition. The results from the ANOVA are shown in Table 2. The replicate effects showed no significant differences for all fatty acids except palmitic acid. This generally indicates that the soybean fields were well managed and fatty acid profiles were consistent across replicates.

The accumulation of fatty acid in seed oil was significantly affected by the growing environments. The genotype was shown to be the most prominent source of variation for all fatty acids in soybean seed oil (Table 2). The significant interaction of G and E was observed for palmitic acid, stearic acid and ALA but, no G × E interaction was observed for oleic acid and LA in this study.

The means and variation of fatty acid profiles of each genotype across nine environments are shown in Table 3. The palmitic, stearic, and oleic acid contents of six soybean genotypes from the nine environments ranged from 10.6 to 11.6 %, 3.2 to 3.8 % and 12.8 to 22.6 % with an average of 11.3, 3.5 and 15.8 %, respectively. Similarly, LA and ALA content ranged from 55.1 to 57.6 % and 8.0 to 15.4 % and with an average of 56.7 and 12.7 %, respectively. The mean ALA composition of six genotypes across nine environments was 12.7 %. Among high ALA RILs, RIL 156 showed the least (13.9 %) and RIL 166 showed the highest (15.2 %) mean ALA composition across environments. These mean values were very similar to the high parent PI 483463 in ALA content.

There were no significant differences in palmitic acid content among three RILs and wild soybean parent PI 483463. Palmitic acid content in cultivated parent Hutcheson was significantly lower than two RILs (RIL 156 and RIL 166) and the wild parent PI 483463. The check variety Williams 82 showed a significantly lower palmitic acid content than the RILs and PI 483463 but the content was not significantly different from that of Hutcheson.

Contents of the other fatty acids varied significantly. Stearic acid content in RILs and PI 483463 were not significantly different but significantly lower than the cultivated parent Hutcheson. In comparison to Williams 82, two RILs (RIL 156 and RIL 166) and PI 483463 had significantly lower stearic acid content. The mean oleic acid composition of RIL 159, RIL 166 and PI 483463 across nine environments was not significantly different but oleic acid composition of RIL 159 and RIL 166 was significantly lower than Hutcheson and Williams 82. The LA contents among RILs (159 and 166) were not significantly different but significantly higher than Hutcheson and Williams 82.

Except for RIL 156 which had a low value (Table 4) in environment 1 (E1), ALA contents of RILs and PI 483463 were similar and significantly higher than Hutcheson and Williams 82. Commodity soybean oil has approximately 6–7:1 ratio of LA to ALA. Because of lower ALA contents, the parent Hutcheson and Williams 82 showed a higher LA to ALA ratio 6.3:1 and 6.9:1, respectively as compared to

Table 2 Combined analysis of variance for fatty acid composition over six genotypes and nine environments

Source of variation		Palmitic acid			Stearic acid			Oleic acid			Linoleic acid			α-Linolenic acid		
	DF	MS	F-value	Pr > F	MS	F-value	Pr > F	MS	F-value	Pr > F	MS	F-value	Pr > F	MS	F-value	Pr > F
Replication (E)	9	0.2	2.2	0.0391	0.1	1.0	0.4322	1.3	0.5	0.8444	1.0	0.6	0.7875	0.2	1.0	0.4315
Environment (E)	8	0.6	7.8	<0.0001	0.3	7.3	<0.0001	14.3	5.8	<0.0001	4.6	2.9	0.0119	9.7	19.4	<0.0001
Genotype (G)	5	2.7	36.9	<0.0001	1.0	21.6	<0.0001	248.3	101.0	<0.0001	13.2	8.1	<0.0001	167.6	336.5	<0.0001
G × E	36	0.3	3.6	<0.0001	0.2	2.4	0.0037	2.0	0.8	0.7313	1.8	1.1	0.3627	1.0	1.9	0.0208
Total	99															

The six soybean genotypes include two parents: PI 483463 and Hutcheson; three RILs with elevated **α**-linolenic acid content and a check variety Williams 82

DF degrees of freedom, *MS* mean square

other genotypes. PI 483463 and all RILs showed lower ratios of LA to ALA with the lowest ratios observed in RIL 166 (3.7:1) and PI 483463. This indicated that genes related to high ALA from wild soybean were essential to reduce the ratios of LA to ALA.

Stability analysis of ALA

The ALA composition of each genotype fluctuated over environments (Table 4). The ALA composition of each RIL, their parents and Williams 82 (check) varied across environments and genotypes. Among the different RILs, RIL 166 showed highest mean ALA composition (15.2 %) across environments and ranged from 14.6 to 15.8 % followed by RIL 159 and RIL 156. The mean ALA composition and range for RIL 159 was 14.9 and 14.0–15.8 %, respectively, and RIL 156 was 13.9 and 10.7–15.7 %, respectively. The wild soybean parent PI 483463 had the highest mean ALA composition (15.4 %) which ranged from 14.0 to 17.1 %. On the other hand, the cultivated genotypes Hutcheson and Williams 82, had lower mean ALA values of 9.2 and 8.0 % across environments and ranged from 8.5 to 9.7 % and 6.9 to 9.1 %, respectively.

The stability parameters, range, CV, b_E and r^2 for mean ALA composition of the three RILs, their parents and the Williams 82 check are shown in Table 5. The smaller numerical values of range and CV for ALA composition of a genotype indicated more stability across environments. The ALA content among genotypes for stability parameters varied across environments (Table 5). Between parents, Hutcheson had lowest range (1.3 %) in ALA concentration with a rank of 2nd followed by PI 483463 (3.1 %) which ranked 5th, among the six genotypes indicating it was the least stable. Among the RILs, RIL 166 had lowest range value (1.2 %) and ranking 1st among all genotypes and was the most stable for ALA. RIL 159 with a range of 1.8 % ranked 3rd and RIL 156 was the least stable genotype with the widest range 5.0 % and ranked 6th (last). Similarly, the check Williams 82 had 2.2 % of range and ranked 4th indicating that it was relatively stable as compared to PI 483463 and RIL 156.

The CV for each genotype showed similar stability patterns compared to the ranges of mean ALA composition (Table 5) across environments. Generally, genotypes with relatively high ALA ranges

Table 3 Mean fatty acid (relative %) profile over nine environments for each of six soybean genotypes and linoleic to α-linolenic acid ratio (LA:ALA ratio), 2008–2011

Genotype	Fatty acid profile[a] (%)					
	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid (LA)	α-Linolenic acid (ALA)	LA:ALA ratio
RIL 156	11.5	3.3	15.0	56.3	13.9	4.1
RIL 159[b]	11.4	3.4	12.8	57.4	14.9	3.9
RIL 166[b]	11.6	3.2	13.1	56.9	15.2	3.7
PI 483463	11.5	3.3	13.0	56.7	15.4	3.7
Hutcheson[b]	11.0	3.8	18.5	57.6	9.1	6.3
Williams 82 (CK)[b]	10.6	3.7	22.6	55.1	8.0	6.9
Mean	11.3	3.5	15.8	56.7	12.7	
$LSD_{0.05}$	0.4	0.3	1.3	1.1	0.8	

The six soybean genotypes include two parents: PI 483463 and Hutcheson; three RILs with elevated α-linolenic acid content and a check variety Williams 82

[a] Mean content averaged over nine environments except for genotype marked

[b] The mean of eight environments for RIL 159, RIL 166, Hutcheson and Williams 82

Table 4 Mean α-linolenic acid (ALA) content (relative %) of five soybean plant genotypes and a check in each nine environments, 2008–2011

Name	ALA content (%)									Mean ± SE
	E1	E2	E3	E4	E5	E6	E7	E8	E9	
RIL 156	10.7	15.7	14.5	14.2	14.6	14.0	14.6	13.0	13.7	13.9 ± 1.4
RIL 159[a]	14.0	15.8	15.8	14.6	14.8	§	15.0	14.8	14.4	14.9 ± 0.6
RIL 166[a]	14.8	15.7	15.3	§	15.4	14.9	15.8	14.8	14.6	15.2 ± 0.5
PI 483463	15.9	17.1	15.7	15.7	15.1	14.0	15.0	15.3	14.8	15.4 ± 0.9
Hutcheson[a]	8.7	9.7	8.9	9.3	9.8	8.5	§	9.3	9.1	9.2 ± 0.5
Williams 82[a] (CK)	6.9	8.0	8.2	7.8	9.1	8.4	§	7.2	8.2	8.0 ± 0.7
$LSD_{0.05}$	1.8	1.1	2.0	0.6	0.6	3.3	2.5	1.0	1.9	

Environments where soybean genotypes were planted: E1 (22 May, Portageville, MO, 2008); E2 (19 June, Portageville, MO, 2009); E3 (14 May, Gunwi, Korea, 2010), E4 (20 May, Gunwi, Korea, 2011); E5 (17 June, Gunwi, Korea, 2011); E6 (3 June, Yeosu, Korea, 2011); E7 (24 June, Yeosu, Korea, 2011); E8 (31 May, Iksan, Korea, 2011); E9 (29 June, Iksan, Korea, 2011)

[a, §] Missing values due to no data and/or poor emergence at this environment

tended to have relatively higher CVs than genotypes with relatively low ALA ranges. RIL 156 and Williams 82 (check) with relatively wide ranges of mean ALA composition also showed the highest CVs of 10.1 and 8.8 %, respectively and were least stable compared to the other genotypes in the study. The lowest CV values of RIL 166 (3.3 %) and RIL 159 (4.0 %) indicate that these genotypes were the most stable among all genotypes.

The stability coefficient (b_E) of each genotype varied and ranged from 0.15 to 0.84, indicating differences among genotypes for stability across environments (Table 5). Among RILs, RIL 159 was the most stable genotype followed by RIL 166 lowest b_E values 0.34 and 0.37, respectively. On the other hand, RIL 156 was the least stable among all six genotypes with a stability coefficient of $b_E = 0.84$. Among all genotypes, wild parent PI 483463 ranked 1st based on the b_E which means it was the most stable ($b_E = 0.15$).

The mean rank was determined based on the average value of the three stability parameters range, CV and b_E. Based on the mean rank across all stability parameters, RIL 166 and RIL 159 ranked 1st and 2nd,

Table 5 Stability parameters, range, coefficient variation (CV), regression coefficient (b_E) and coefficient of determination (r^2) for mean α-linolenic acid (ALA) of five soybean genotypes and one check (CK) calculated from data across nine environments

Genotype	Mean (ALA)		Range (ALA)		CV		Stability coefficient				Mean rank[b]
	(%)	Rank	(%)	Rank	(%)	Rank	b_E^a	Rank	*P*	r^2	
RIL 156	13.9	4	5.0	6	10.1	6	0.84	5	0.080	0.37	6
RIL 159	14.9	3	1.8	3	4.0	2	0.34	2	0.157	0.3	2
RIL 166	15.2	2	1.2	1	3.3	1	0.37	3	0.004	0.77	1
PI 483463	15.4	1	3.1	5	5.8	4	0.15	1	0.633	0.03	3
Hutcheson	9.2	5	1.3	2	5.4	3	0.52	4	0.041	0.53	4
Williams 82 (CK)	8.0	6	2.2	4	8.8	5	0.52	4	0.224	0.23	5

[a] Stability coefficients for mean ALA acid content of genotypes at each environments regressed on the environmental index

[b] Mean rank was ranked based on the average value of three stability parameters

respectively and were the most stable across the nine environments among RILs, parents and the Williams 82. The wild parent PI 483463 was relatively more stable as compared with another parent Hutcheson and check Williams 82. The RIL 156 was the least stable among all genotypes on the mean rank basis.

Discussion

Seed oils are the richest sources of ALA (omega-3 fatty acid), notably those of rapeseed (10 %), walnuts (9 %), flaxseed (55 %), perilla (58 %), chia (64 %), and hemp (20 %) (http://en.wikipedia.org/wiki/Alpha-Linolenic_acid). Omega-3 fatty acids in diets has multiple positive health benefits (McCann and Ames 2005; Astorg et al. 2004; Bouwstra et al. 2003), and reduce the risk of colon cancer and other diseases (Messina 1999; Chandalia et al. 2000; Jenkins et al. 2003).

Many countries use soybean not only for vegetable oil but also directly as food and food ingredients. Therefore, soybeans with higher ALA would be an excellent source of omega-3 with added health benefits. One of the most important goals of oil quality breeding in soybean has been to reduce its linolenic acid content for improving oxidative stability and flavor, and reducing the need for hydrogenation (Lee et al. 2007). On the other hand, in food and food supplements increasing ALA content could be an important soybean breeding goal in the future.

Wild soybean genotypes have shown higher ALA concentrations than cultivated genotypes. Genetic regulation of ALA concentration in wild soybean has suggested that the high-ALA trait in wild soybean genotypes was determined by a set of desaturase alleles that were different from corresponding alleles in *Glycine max* (Pantalone et al. 1997). However, there has been little reported for why wild soybeans accumulate more ALA than cultivated soybean in terms of genetics and biochemistry. ALA content in *G. soja* has been reported in USDA Soybean Germplasm Collection. Similarly, other *G. soja* accessions, IT183049, IT184172 and IT184256 have ALA concentrations of 14.1, 14.8, and 15.5 %, respectively (Lee et al. 2002) and may also be used in addition to PI 483463 as sources to increase ALA concentration.

Little information for the stability of high ALA content in wild soybean has been reported. Therefore, we compared variation in ALA across nine environments among cultivated soybeans, wild soybean and RILs with elevated ALA derived from a cultivated x wild soybean cross. The ANOVA showed that accumulation of ALA was affected by growing environments, genotypes and G × E (Table 2) which has also been reported in previous studies conducted for cultivated soybeans (Hou et al. 2006; Primomo et al. 2002; Bellaloui et al. 2012). The different ALA levels can be attributed to the differences in temperature, which has been shown to be a predominant environmental factor affecting the fatty acid composition (Rennie and Tanner 1989; Dornbos and Mullen 1992; Oliva et al. 2006). Hou et al. (2006) reported that for ALA concentration, environmental effect was the most important source of variation and accounted for over 70 % of the variation. However genotypes only accounted for 15 % of variation based on results conducted over three growing environments for recombinant inbreed lines from RG2 × RG7. Primomo et al. (2002) investigated the

G × E interaction for soybeans with altered fatty acid profiles and found that the genotype by year interaction was significant for all fatty acids, but genotype by location and G × E interaction effects was only significant for oleic, linoleic, and linolenic acids. Bellaloui et al. (2012) reported that genotype, growing environment (shade), and genotype × shade interactions were significant for ALA content. Recently, similar results were also reported by Dhakal et al. (2013) for G × E interactions for accumulation of ALA in cultivated soybean.

Based on previous studies, ALA accumulation in soybean oil is highly affected by genotypes, environments, and G × E. Therefore, finding genotypes with stable target seed components is an important way to accomplish breeding goals. Primomo et al. (2002) and Oliva et al. (2006) reported that genotypes with the lowest ALA content were the most stable across growing environments compared to genotypes with normal ALA concentration. To determine if wild soybean or its derivatives would be good sources to increase ALA content, we compared cultivars with normal ALA with a wild soybean and RIL progenies derived from it with elevated ALA content across nine environments. The results showed that wild soybean and its derivatives had much higher ALA content than the normal ALA parent, Hutcheson, and check cultivar Williams 82 (Table 3). The genotypes were similar in elevated ALA content compared to the wild soybean parent. Two of the three RILs studied showed greater stability than genotypes with lower ALA content (Table 5). These results indicated that lines derived from wild soybean PI 483463 can be selected that are stable for elevated ALA across environments. There is significant variation (Chae et al. 2012) in the range of ALA content in wild soybean accessions (7.3–23.7 % with an average of 15.6 % from 1,806 accessions). To have a greater understanding of stability for ALA content in wild soybean, more experiments comparing lines varying in ranges of ALA should be studied over multi-years and locations, and under different temperature regimes.

Studies showed that lower ratios of ω-6 to ω-3 oil (Simopoulos 2008; Renaud 2002; Mattson and Grundy 1985) and adding more ω-3 to diets (McCann and Ames 2005; Astorg et al. 2004; Bouwstra et al. 2003; Messina 1999; Chandalia et al. 2000; Jenkins et al. 2003) had significant health benefits. The two cultivated soybeans showed ratios of over 6:1 (Table 3) and did not meet the requirement of the 4:1 ratio of LA to ALA. Dhakal et al. (2013) also reported that cultivated soybeans with elevated ALA also had more than 5 times higher ω-6 than ω-3 content. However, the wild soybean parent PI 483463 and its derivative progenies had a 4:1 ratio or less for ω-6 to ω-3 fatty acid (Table 3). This means that genes related elevated ALA content from wild soybean would be useful to increase ω-3 fatty acid to alter ratios between ω-6 and ω-3 fatty acid in cultivated soybean. Wild relatives are one of the important genetic resources to improve cultivated crops (Harlan 1976; Hawkes 1977). Wild soybean (*G. soja*) also has been used as genetic resources to find new genes or improve cultivated soybeans (Concibido et al. 2003; Hwang et al. 2009; Kabelka et al. 2005, 2006; Kang et al. 2011; Lee et al. 2005, 2008). Generally, wild soybean has poor agronomic traits such as low yield, small seed size, viny growth habit, seed shattering at maturity, and so forth. Soybean breeders concerning about such a unique poor agronomic traits linked with important agronomic characters such as yield, protein, and oil.

This study was not designed to test on the agronomic traits, such as yield, maturity, plant height, lodging, weight, seed quality and it is also not known whether these agronomic traits are linked to alter the fatty acid composition of recombinant inbred lines. Therefore, future research will be required through developing backcross populations.

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