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DETERMINATION OF VARIATION IN THE FATTY ACID COMPOSITION OF DIFFERENT FAR EASTERN WILD SOYBEAN ACCESSIONS AND IDENTIFICATION OF SUPERIOR GENOTYPES WITH OLEIC ACID CONTENT AS HIGH OLEIC POTENTIAL

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Abstract

Soybean [Glycine max (L.) Merr.] seeds contain 20% oil, 40% protein, and 12% soluble carbohydrates by dry weight. Because of its seed composition, it is one of the most economically important oil crops globally. Soybean is the largest oilseed crop worldwide with over half of the total oilseed production, followed by rapeseed, sunflower, and peanut. Direct progenitor of cultivated soybean [Glycine max (L.) Merr.], is wild soybean (Glycine soja Sieb. & Zucc.) and it is widely distributed in China, North Eastern Russia, Korea, Japan, and Taiwan. Wild soybean accessions originating from different geographical regions harbour genetic differences. Because of many genetic variations have been lost in the process of domestication of G. max, soybean breeders and geneticists are interested in identifying useful genes in G. Soja to improve different characteristics of cultivated soybeans. Therefore, it is necessary to collect and conserve the wild soybean from different regions. When soybean is consumed directly as food, polyunsaturated fatty acids such as linoleic and linolenic acid (ω-6, ω-3) are essential fatty acids. These fatty acids prevent diseases such as inflammation, cardiovascular, and Alzheimer's, as well as promote fetal development. The consumption rate between ω-6 and ω-3 concentration has an effect on human health. Healthy ratios of ω-6 to ω-3 fatty acids have been reported to range from 1:1 to 4:1. Generally, the ω-6/ω-3 ratio in the seed oils of wild (Glycine soja) and cultivated soybeans (G. max) is 3-4:1 and 6-7:1, respectively. Wild soybeans contain almost twice the α-linolenic acids ALA) in their seed oil. Twenty-four wild soybean accessions from three different countries were investigated under field conditions in 2021. The content of oil, oleic acid, linoleic acid, linolenic acid, palmitic acid and stearic acid ranged between 4.13-12.14%, 11.4-49.74%, 29.3-56.08%, 4.81-14.33%, 10.22-13.85% and 3.3-4.64%, respectively. Also ratios of ω-6 to ω-3 fatty acids varied between 3.368 and 6.091. To conclude, considerable variations in oil content, fatty acid composition, ω6/ω3 ratios were noticed among 24 wild soybean accessions in this study. The Nigata, Japan accession is one of the prominent wild soybean accession in the study with high oleic acid content (49.74%).

***Key words**: wild soybean, fatty acids, ω-6, ω-3, high oleic. molecular biology and biotechnology. CABI, Wallingford, UK. p. 165–168.*

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] seeds contain 20% oil, 40% protein, and 12% soluble carbohydrates by dry weight (Liu, 1997). Because of its seed composition, it is one of the most economically important oil crops globally. Soybean is the largest oilseed crop worldwide with over half of the total oilseed production, followed by rapeseed, sunflower, and peanut. Direct progenitor of cultivated soybean [*Glycine max* (L.) Merr.], is wild soybean (*Glycine soja* Sieb. & Zucc.) and it is widely distributed in China, North Eastern Russia, Korea, Japan, and Taiwan (Hymowitz and Singh 1987, Chung and Singh 2008). Wild soybean accessions originating from different geographical regions harbour genetic differences. Because of many genetic variations have been lost in the process of domestication of *G. max*, soybean breeders and geneticists are interested in identifying useful genes in *G. soja* to improve different characteristics of cultivated soybeans (Hyten et al., 2006). Therefore, it is necessary to collect and conserve the wild soybean from different regions.

Soybean is an important oil crop grown worldwide due to its diverse uses of oil and protein for humans and livestock. Soybean oil accounts for 60.85% of the world's oilseed production; therefore, it has become the most dominant vegetable oil (Abdelghany et al., 2020). Mainly, five major fatty acids are found in soybean oil: palmitic acid (11%), stearic acid (4%,), oleic acid (23%), linoleic acid (55%), and linolenic acid (8%). This profile of soybean oil has been modified depending on the end products of soybean oil to meet different demands of the markets (Fehr, 2007). Recent studies have revealed that soy food such as soy milk, tofu and soy souce has nutritious qualities that have positive effects on human health (Omoni and Aluko, 2005). This may have led to dramatic increases in soy food demand in the global market (Kulkarni et al., 2017). When soybean is consumed directly as food, polyunsaturated fatty acids such as linoleic and linolenic acid (ω-6, ω-3) are essential fatty acids. These fatty acids prevent diseases such as inflammation, cardiovascular, and Alzheimer's, as well as promote fetal development (Serhan et al., 2008; Swanson et al., 2012). Also, it is an important plant-based source of ω-3 polyunsaturated fatty acid for vegetarians and non-seafood eaters. Numerous studies have concluded that a higher intake of ω-3 fatty acid or fatty acids with relatively low ω-6/ω-3 ratios is appropriate from a human health perspective (Abel et al., 2004; Simopoulos, 2008). The consumption rate between ω-6 and ω-3 concentration has an effect on human health. Healthy ratios of ω-6 to ω-3 fatty acids have been reported to range from 1:1 to 4:1 (Mattson and Grundy, 1985; Renaud, 2002). A ratio of ω-6/ω-3 was found 5:1 ratio suppressed symptoms of asthma, for prevention of cardiovascular disease and improvement of blood circulation 4:1, 2:1 to 3:1 for suppression of rheumatoid arthritis; and 2.5:1 for inhibition of colon cancer cell proliferation (Simopoulos, 2008). Generally, the ω-6/ω-3 ratio in the seed oils of wild (*Glycine soja*) and cultivated soybeans (*G. max*) is 3-4: 1 and 6-7: 1, respectively (Asekova et al., 2014; Dhakal et al., 2013). Wild soybeans contain almost twice the α-linolenic acids ALA) in their seed oil (Pantalone et al., 1997). The accumulation of a high concentration of ALA in wild soybean was due to a different set of desaturate alleles controlling ALA in cultivated soybean (Pantalone et al., 1997). Therefore, it is necessary to develop new cultivated soybean lines with high ALA concentration using wild soybean as a genetic resource for increasing ALA lowering ω-6/ ω-3 ratios and concentration by classical breeding.

The functionality of soybean oil for both food and industrial uses is influenced by the fatty acid profile of the oil (Lee et al., 2008). Modification of saturates, oleic acid, and linolenic acid via breeding and biotechnology are being emphasized to develop desired fatty acid phenotypes. It is not practical to commercially develop all oil phenotypes (Asekova et al., 2014). To breed new varieties for desired traits, the gene pool of cultivars must be broadened by introducing wild species, landraces, and exotic germplasm into breeding programs.

Wild soybeans have been reported to contribute new and unique genes for high yield, high protein, resistance soybean cyst nematode, tolerance to salt, and high linolenic acid (Wang et al., 2004; Kabelka et al., 2005; Nichols et al., 2006; Lee et al. 2009; Pantalone et al., 1997; Lee et al., 2010). Therefore, the wild soybeans can be utilized as a genetic resource to develop new varieties with the desired fatty acid composition. The aim of this study was to evaluate the distribution and characterics of the major fatty acids of wild soybean accessions belong to some Far East countries.

MATERIALS AND METHODS

Plant Materials

Twenty-four wild soybean accessions belonging to South Korean, China and Japan wild soybean habitats were used as a plant material (from Chung's Wild Legume Germplasm Collection, Chonnam National University, Yeosu, South Korea) (Table 1).

Accessions were grown at the Experimental Farm of Cukurova University (36°59′N, 35°18′E, and 23 m), in Adana (Turkey) in 2021. In the Adana province, a typical Mediterranean climate prevails and the winters are warm and rainy whereas the summers are hot and dry. Before planting, 200 kg ha^{-1} of DAP (36 kg ha^{-1} N, 92 kg ha^{-1}P) fertilizers were applied. Ammonium nitrate (33%N) at the rate of 200 kg ha^{-1} was applied once before the first irrigation. Additional Ammonium nitrate (33% N) at the rate of 70 kg ha^{-1} was applied once before the first irrigation. (R1 stage). Soybean accessions were sown in the first week of May with distance of 2 m and row-to-row distance of 2 m according to a completely randomized design with three replications.

Table 1. Wild Soybean Material List

1	Jeonnam Shinan Abhaedo, Korea	**13**	Incheon Ganghwa Buleumdo, Korea
2	Jeonnam Shinan Bigeumdo, Korea	**14**	Yeongheungdo, Ansan, Gyeonggi-do, Korea
3	Gyeongnam Tongyeong Yokdo, Korea	**15**	Gyeongbuk Yecheon, Korea
4	Jeonnam Shinan Im Jado, Korea	**16**	Gyeongbuk Andong, Korea
5	Gangwon-do, Korea	**17**	Jilin, China
6	Gangwon Samcheok, Korea	**18**	Wonju, Gangwon, Korea
7	Liaoning, China	**19**	Gyeongbuk Uiseong, Korea
8	Liaoning, China	**20**	Jilin, China
9	Aomori, Japan	**21**	Liaoning, China
10	Akita, Japan	**22**	Ichinoseki, Japan
11	Niigata, Japan	**23**	Yamagata, Japan
12	Chuncheon, Gangwon, Korea	**24**	Japan Kumamoto

Oil extraction and GC analysis

The samples of 24 accessions grown in 2021 were subjected to oil extraction using a Soxhlet apparatus and a gravimetric method.

The experiments were performed three replicates and seeds were bulked (20 g from each replication) and 5 g clean and mature seed samples were taken for oil content and fatty acid analysis.

The oil content was determined by comparing the weights of 5 g seed samples before and after extraction using a Soxhlet apparatus (FOSS) with petroleum ether for 4 hours.

An oil sample of 500 mg was dissolved in 2 ml isooctane followed by 1.5 ml of 0.5 M methanolic NaOH. The tube was then vortexed and held in boiling water for 7 min and allowed to cool to room temperature. Two ml of BF_3 (Boron trifluoride) were added, vortexed, and held in boiling water for 5 min and allowed to come down to room temperature.

The tube was vortexed after adding 5 ml NaCl, centrifuge at 4,000 rpm for 10 min. The supernatant was used for GC analyses (AOAC 1984).

The fatty acid (FA) composition was analyzed using a GC Clarus 500 with auto sampler (Perkin Elmer, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m • 0.32 mm, ID • 0.25 lm, BP20 0.25 UM, USA).

The oven temperature was brought to 140°C for 5 min, then raised to 200°C at a rate of 4°C/min and to 220°C at a rate of 1°C/min, while the injector and the detector temperatures were set at 220°C and 280°C, respectively.

RESULTS AND DISSCUSSIONS

Oil Content and Fatty Acid Profile

The oil content and fatty acid compositions and their range of variation in 24 wild soybean accessions are shown in Table 2. The oil content of the 24 wild soybean accessions varied between 4.13-12.14%, and the average being 7.12%. The highest oil content was obtained from Yeongheungdo, Ansan, Gyeonggi-do, Korea accession, while the lowest value was obtained from Aomori, Japan**.** Different studies have shown that cultivated soybean genotypes have a higher oil content than wild soybean accessions (Kim and Park, 2005; Wee et al., 2017).

Fatty acid composition is one of the most important soybean seed quality traits and affected by genotype, environmental conditions, planting date, fertilization, and the interaction these factors (Kurt, 2018). A significant variation in oil content and fatty acids were found among the 24 wild soybean (*Glycine soja*) accessions as shown in Table 2. Average values for oil content and five major fatty acids components found in wild soybean 7.12, 12.34, 3.71, 18.48, 51.58, 11.58% oil content, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid, respectively. The palmitic and stearic acids fractions are saturated fatty acids and constitute 15% of the soybean oil. The remainder of the oil (about 85%) is made up of unsaturated fatty acids such as oleic, linoleic and linolenic acids (Lee et al., 2007). Since it is preferred that the amount of saturated fat of soybean oil used in food industry is low, the amount of saturated fatty acids of the characterized soybean genotypes was also determined. Palmitic acid is predominant saturated fatty acid in soybean oil and common soybean cultivars contain an average 1%1 palmitic acid. The lowest palmitic acid content was recorded for Nigata, Japan 2 (10.22%) accession, while the highest palmitic acid content was recorded for Gyeongnam Tongyeong Yokdo, Korea (13.85%) with 12.34 % average value. Stearic acid content varied between 3.3-4.64%. The highest value belonged to Gyeongbuk Yecheon, Korea accession, while the Yamagata, Japan accession had the lowest value. The observed stearic acid content in this study was low in comparison with previous studies (Qin et al., 2014; Abdelghany et al., 2020) but higher than reported earlier studies of *G. max* and *G. soja* (Fan et al., 2015; Wee et al., 2017; La et al., 2019). Also, palmitic acid content higher than previous studies of *G. max* and *G. soja* (Qin et al., 2014; Fan et al., 2015; Wee et al., 2017; Abdelghany et al., 2020).

Table 2. Fatty acids composition and ω6/ω3 ratios of wild soybean accessions

Origin	Oil %	Palmitic acid, %	Stearic acid, %	Oleic acid, %	Linoleic acid, %	Linolenic acid, %	ω6/ω3
Jeonnam Shinan Abhaedo, Korea	7.74	13.54	3.81	13.01	53.48	13	4.11
Jeonnam Shinan Bigeumdo, Korea	5.73	12.02	3.96	13.41	54.5	**14.33**	3.80
Gyeongnam Tongyeong Yokdo, Korea	5.41	**13.85**	3.43	**11.4**	54.62	13.7	3.99
Jeonnam Shinan Im Jado, Korea	4.71	13.38	4.01	14.91	52.18	13.78	3.79
Gangwon-do, Korea	5.55	11.46	3.65	13.85	56.94	12.06	4.72
Gangwon Samcheok, Korea	5.13	11.2	4	19.73	50ş79	12.51	4.06
Liaoning, China	5.41	12.42	3.51	13.3	55.8	13.18	4.23
Liaoning, China	7.32	12.05	3.58	12.33	55.26	13.67	4.04
Aomori, Japan	**4.13**	12.7	3.62	13.68	55.37	11.84	4.68
Akita, Japan	5.51	12.63	4.18	18.19	53.69	9.25	5.80
Nigata, Japan	8.23	**10.22**	3.49	**49.74**	**29.3**	**4.81**	**6.09**
Chuncheon, Gangwon, Korea	8.24	10.45	3.01	24.58	51.33	8.76	5.86
Incheon Ganghwa Buleumdo, Korea	11.52	12.97	3.58	19.02	52.3	9.95	5.26
Yeongheungdo, Ansan, Gyeonggi-do, Korea	**12.14**	13.21	3.36	14.24	54.59	11.76	4.64
Gyeongbuk Yecheon, Korea	8.13	13.56	**4.64**	22.91	47.44	9.63	4.93
Gyeongbuk Andong, Korea	7.42	12.75	3.54	16.14	51.92	13.43	3.87
Jilin, China	4.65	12.43	3.73	11.81	**56.08**	13.24	4.24
Wonju, Gangwon, Korea	6.96	13.27	3.68	13.79	51.3	15,23	**3.37**
Gyeongbuk Uiseong, Korea	7.67	10.85	4.18	23.11	52.21	7.886	6.62
Jilin, China	9.28	13.18	3.89	16.29	51.2	13.62	3.76
Liaoning, China	7.16	10.26	3.41	39.91	35.24	7.24	4.87
Ichinoseki, Japan	9.94	13.33	4.01	16.98	53.82	9.49	5.67
Yamagata, Japan	7.63	11.87	**3.3**	15.72	54.13	13.48	4.02
Japan Kumamoto	5.16	12.46	3.54	15.56	54.39	12.19	4.46
Ave.	7.12	12.34	3.71	18.48	51.58	11.58	4.62
Max.	12.14	13.85	4.64	49.74	56.08	14.33	6.09
Min.	4.13	10.22	3.3	11.4	29.3	4.81	3.37

Oleic and linoleic acids were found to be major unsaturated fatty acids in wild soybean accessions. The highest oleic (49.78%) and lowest linoleic acid (29.3%) percentage was recorded for Nigata, Japan 2 accession. Oleic acid content varied from 11.4-49.74% with an average value of 18.48%. Wee et al. (2017) reported that the oleic acid content of 319 wild soybean accessions they collected from different regions of Japan varied between 7.66 and 15.86%. La et al. (2018) reported a range of oleic acid varied between 10.7-16.2% in 80 wild soybean accessions from different origin. The higher concentration of oleic acid in soybean oil is preferred for wider use in food and industrial products, as it is more stable at high cooking temperatures (Lee et al., 2007; Warner and Gupta, 2005; La et al., 2014). Additionally, its high oleic acid content increases the oxidative stability of the oil and reduces the need for hydrogenation, which is negatively associated with heart health in humans (Ascherio and Willett, 1997; Wilson, 2004; La et al., 2014). Linoleic and linolenic acid contents varied between 29.3-56.08% and 4.81-14.33% in the wild soybean accessions. The present study showed a wider range of linoleic acid and linolenic acid content when compare d with previous studies (Wee et al., 2017; La et al., 2018). Abdelghany et al. (2020) reported that linolenic acid content of 633 soybean accessions varied between 3.43-12.76%. In another study performed with wild soybean accessions, Wee et al. (2017) reported that the oleic acid content varied between 12.1 and 25.4%. As the oils of soybean genotypes with high LNA content oxidize rapidly, it is not suitable for producing stable oil, as it induces off-flavor compounds in cooked foods. But, linoleic and linolenic acid is essential for human health and development (La et al., 2019). Furthermore, asthma, heart disease and other syndromes could affect human health due to lack of linoleic and linolenic acid in diets (Simopoulos, 2008; Wang et al., 2012; Abdelghany et al., 2020).

ω-6/ω-3 and PUFA/SFA ratios

Omega-6 (ω-6) and omega-3 (ω-3) fatty acids are essential and the human body, like other mammals, cannot synthesize them and must obtain them in their diets (Asekova et al. 2014). The ω-6/ω-3 ratio is as important as the amount of these fatty acids for human health. The ideal ω-6/ω-3 ratio should be between 3: 1 to 5: 1, as this ratio is associated with a reduction in the risk of cardiovascular and other chronic diseases, such as diabetes, and the improvement of the immune response and brain function (Simopoulos, 2002; Simopoulos, 2008).

The ω-6/ω-3 ratio ranged from 3.37, in “Wonju, Gangwon, Korea” to 6.09 in “Nigata, Japan” with mean a value of 4.62 (Table 2). Of the 24 wild soybean accessions, 18 of them have ω-6/ω-3 ratio of less than 5 and 6 of them have a ratio of less than 4. Lee et al. (2019) reported that the average ω-6/ω-3 ratio was 3.4/1 in their study with 80 wild soybean genotypes. The average value of ω-6/ω-3 ratio was found lower than cultivated soybean reported by Asekova et al. (2014) and Abdelghany et al. (2020). However, the average ω-6/ω-3 ratio in previous studies with wild soybean accessions ranges between 3 and 3.38 (Wee et al., 2001; La et al. 2019). The main reason for the low ω-6/ω-3 ratio of wild soybeans is the higher linolenic ratio than cultivated soybean. Therefore, in order to reduce the-6/ω-3 ratio, it is not only sufficient to lower the linoleic acid content, but also to increase the linolenic acid content. According to the results from the present study, the linoleic acid content of Nigata 2, Japan genotype is 29.3%, while the ratio of-6 / ω-3 is 6.09. However, although the linoleic acid ratio is 51.3% in the Wonju, Gongwon, Korea genotype, the ω-6/ω-3 ratio is 3.37. This is due to the linolenic acid content being 15.23%.

CONCLUSIONS

To conclude, considerable variations in oil content, fatty acid composition, ω6/ω3 and PUFA/SFA ratios were noticed among 24 wild soybean accessions in this study. The Nigata, Japan 2 accession is one of the prominent wild soybean accession in the study with high oleic acid content (49.74%).

Because, high oleic lines have been developed through different ways such as selection breeding, mutation breeding and genetic engineering. However, varieties developed by genetic engineering and mutation breeding have different disadvantages. For example, lines containing FAD2 mutant alleles have increased oleic acid content but decreased yields. In addition, most of the wild soybean accessions in the study had an ω6/ω3 ratio in the human health beneficial range (1-5), unlike those in cultivated soybeans. The identified diversity in oil content and fatty acid composition is useful for the identification of better parents with high oleic acid and linolenic acid contents for developing soybean varieties with traits which are beneficial to consumer health and industrial demands.

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