# Asian Journal of Plant Sciences

ISSN 1682-3974

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OPEN ACCESS
Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2017.52.64

Research Article

# Fatty Acid Composition and Distribution in Wild Soybean (*Glycine soja*) Seeds Collected in Japan

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## Abstract

**Background:** Understanding the distribution of fatty acids in wild soybean accession collected in Japan and the relationship of geographic and environmental factors in the accumulation of fatty acids is fundamental for using the wild accessions as resources for the future development and selection of new soybean varieties with desired traits. **Materials and Methods:** The fatty acid composition of seeds from 319 wild soybean (*Glycine soja*) accessions collected in Japan were evaluated using gas chromatography. **Results:** The distributions of palmitate (16:0), stearate (18:0), oleate (18:1), linoleate (18:2) and α-linolenate (18:3) in seeds were determined for each accession. Significant inverse correlations were observed between the oleate and α-linolenate contents and the linoleate and α-linolenate contents. Moreover, a weak inverse correlation between the stearate and α-linolenate contents was indicated and an inverse correlation between the palmitate and linoleate contents was also found. The total palmitate content and total stearate content of *Glycine soja* collected from regions with high annual temperatures were high and the total α-linolenate content of *Glycine soja* collected from regions with a low annual temperature was high. **Conclusion:** These results provide diverse and wide-ranged fatty acid information of Japanese wild soybean resources according to climatic region for improving of new soybean varieties in global environmental changes.

Key words: *Glycine soja*, seed, fatty acid, palmitate, stearate, oleate, linoleate, α-linolenate, climatic regions

Received: October 25, 2016 Accepted: January 24, 2017 Published: March 15, 2017

Citation: Chi-Do Wee, Masatsugu Hashiguchi, Toyoaki Anai, Akihiro Suzuki and Ryo Akashi, 2017. Fatty acid composition and distribution in wild soybean (*Glycine soja*) seeds collected in Japan. Asian J. Plant Sci., 16: 52-64.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Wild soybean (*Glycine soja*) and cultivated soybean (*Glycine max*) have distinct differences in many morphological and physiological characters, a phenomenon known as the "Domestication syndrome"[1]. In soybean, plant breeding has accelerated genetic gain but narrowed the genetic base[2].

Soybean (*Glycine max*) oil accounts for nearly 60% of the world's oil seed production, making it the most dominant vegetable oil by far. There are five principal fatty acids in soybean seed oil: Palmitate (16:0), stearate (18:0), oleate (18:1), linoleate (18:2) and α-linolenate (18:3). In order to develop modified oils that could better meet the needs of end users than is possible with conventional soybean oil, study on the fatty acid composition of soybean oil has been conducted by soybean breeders for more than 50 years[3].

Soybean research priorities have been set, with guidance from consumers and end users to initially target fatty acid profiles that have the highest probability of expanding the use of edible oils and oils for industrial applications in the USA. Since the preferences for soybean oil are changing, breeding goals to modify the fatty acid composition of soybean oil are needed to improve and expand industrial and food uses and to develop other products[4].

In most Asian countries, soybeans are usually consumed directly as tofu, sprouts, soybean paste and health supplements. As essential fatty acids, linoleate (omega-6) and linolenate (omega-3) acids are desirable components of soybean oil. Both linoleate and α-linolenate are potential precursors of eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6), which in diets can have multiple positive health benefits including reducing cardiovascular disease and improving cognitive function[5-7]. For health benefits genotypes with elevated α-linolenate are more desirable for food-grade soybeans. Also, oils low in saturated fatty acids and high in polyunsaturated fatty acids, such as linoleate and α-linolenate are ideal substitutes for drying oils such as tung and linseed oils in oil-based paints and coatings[4].

On the other hand, wild soybean (*Glycine soja*) is well recognized as the closest wild relative of cultivated soybean (*Glycine max*) and is endemic over a wide range of East Asia including China, Russia, Korea and Japan. A long history of domestication, cultivation and breeding has narrowed the genetic base of cultivated soybean, limiting further improvements in yield and quality.

In addition, wild soybean which inhabits a wide range of geographic regions in East Asia has substantial genetic variability in pest and disease resistance genes and possesses other valuable agricultural and ecological characteristics[8,9]. Thus, wild soybeans have been identified as important genetic resources to be used in cultivated soybean improvement in response to global climatic changes.

Recent advances in sequencing technologies also have highlighted the unique genomic contents of both cultivated and wild soybean and provided an opportunity to use *Glycine soja* to broaden the genetic base of cultivated soybean[10,11]. Furthermore, assessing genomic differences for important traits will provide insights into the process of speciation and domestication and will deepen information about the origin of genes involved in complex traits[12-16].

This study documents the distribution and characteristics of the five major fatty acids in the seeds of over 300 indigenous soybean germplasm accessions from the Japan National BioResource Project[17] that were collected from widely differing environments and defined geographical areas.

## MATERIALS AND METHODS

**Plant materials:** A total of 319 Japanese wild soybean (*Glycine soja*) accessions were used in this study. These strains were obtained from "Legumebase", an online database (http://www.legumebase.brc.miyazaki-u.ac.jp/) supported by the National BioResource Project in Japan. All accessions were evaluated for seed fatty acid composition using gas chromatography. The distribution of fatty acids in each accession was determined and correlated with the fatty acid profiles found in the other accessions.

**Fatty acid analysis:** Flour samples from the wild soybean seeds were directly methylated with acidic methanol and analyzed by gas chromatography as reported previously[18]. Fatty acid methyl esters were extracted with n-hexane and separated under isothermal conditions, 180°C in a Yanaco G6800 series gas chromatograph equipped with a 25×0.25 mm Quadrex 23 bonded fused silica capillary column and a flame ionization detector. Each peak was identified by comparing the retention time with those of fatty acid methyl ester standards.

**Fatty acid distributions according to climatic classifications:** All accessions were classified into the macro and meso-subdivisions of the climatic regions where the accessions were collected[19]. The collection sites of the wild accessions covered a broad geographic range in Japan and are listed in Table 1. For simplicity, the collection sites were divided into geographic areas according to the climatic classifications in Japan (Fig. 1).

Table 1: List of *Glycine soja* accessions and their passport data used in this study

| | | Location | | | Division | | | | Location | | | Division | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Accession No. | Prefecture | City/town | Latitude | Longitude | Macro | Meso | Accession No. | Prefecture | City/town | Latitude | Longitude | Macro | Meso |
| B01001 | | NA | | | | | B02217 | Aomori | Sannai-Maruyama | 40°48'42" | 140°41'44" | IV | 3 |
| B01002 | | NA | | | | | B02219 | Aomori | Sannai-Maruyama | 40°48'42" | 140°41'44" | IV | 3 |
| B01003 | Hokkaido | Shizunai | 42°20'00" | 142°22'00" | III | 5 | B02220 | Aomori | Sannai-Maruyama | 40°48'42" | 140°41'44" | IV | 3 |
| B01027 | Hokkaido | Mitsuishi | 42°15'00" | 142°34'00" | III | 5 | B02222 | Aomori | Sannai-Maruyama | 40°48'42" | 140°41'44" | IV | 3 |
| B01041 | Hokkaido | Hokuto | 41°43'15" | 140°23'07" | IV | 3 | B02223 | Aomori | Sannai-Maruyama | 40°48'42" | 140°41'44" | IV | 3 |
| B01043 | Hokkaido | Shiriuchi | 41°44'23" | 140°13'51" | IV | 3 | B02225 | Aomori | Sannai-Maruyama | 40°48'42" | 140°41'44" | IV | 3 |
| B01055 | Hokkaido | Mitsuishi | 42°15'00" | 142°34'00" | III | 5 | B02227 | Aomori | Sannai-Maruyama | 40°48'42" | 140°41'44" | IV | 3 |
| B01057 | Hokkaido | Shizunai | 42°20'00" | 142°22'00" | III | 5 | B02229 | Akita | Omonogawa | 39°13'05" | 140°26'36" | IV | 3 |
| B01058 | Hokkaido | Niikappu | 42°31'49" | 142°05'09" | III | 5 | B02231 | | NA | | | | |
| B01059 | Hokkaido | Niikappu | 42°22'00" | 142°19'00" | III | 5 | B02232 | | NA | | | | |
| B01064 | Hokkaido | Mukawa | 42°34'00" | 141°56'00" | III | 5 | B02815 | | NA | | | | |
| B01067 | Hokkaido | Hakodate | 41°46'00" | 140°44'00" | IV | 3 | B03016 | Niigata | Gosen | 37°44'41" | 139°14'26" | IV | 2 |
| B01068 | Hokkaido | Kamiiso | 41°49'00" | 140°39'00" | IV | 3 | B03018 | Niigata | Mikawa | 37°42'38" | 139°22'21" | IV | 2 |
| B01069 | | NA | | | | | B03024 | Toyama | Tateyama | 36°39'11" | 137°21'20" | IV | 2 |
| B01070 | Hokkaido | Shiriuchi | 41°36'00" | 140°25'00" | IV | 3 | B03025 | Toyama | Fuchu | 36°35'58" | 137°11'12" | IV | 2 |
| B01071 | Hokkaido | Shizunai | 42°20'00" | 142°22'00" | III | 5 | B03033 | Ishikawa | Nanao | 37°02'30" | 136°57'27" | IV | 2 |
| B01084 | Hokkaido | Niikappu | 42°22'00" | 142°19'00" | III | 5 | B03034 | Ishikawa | Suzu | 37°23'53" | 137°13'52" | IV | 2 |
| B01087 | Hokkaido | Monbetsu | 44°21'00" | 143°21'00" | V | 1 | B03036 | Ishikawa | Wajima | 37°23'42" | 136°53'57" | IV | 2 |
| B01096 | Hokkaido | Biratori | 42°44'20" | 142°24'11" | V | 1 | B03039 | Ishikawa | Kanazawa | 36°37'49" | 136°38'59" | IV | 2 |
| B01098 | Hokkaido | Biratori | 42°46'13" | 142°24'53" | V | 1 | B03044 | Ishikawa | Komatsu | 36°24'29" | 136°29'39" | IV | 2 |
| B01114 | Hokkaido | Mukawa | 42°44'43" | 142°10'08" | III | 5 | B03054 | Niigata | Hamochi | 37°49'47" | 138°18'21" | IV | 2 |
| B01120 | | NA | | | | | B03055 | Niigata | Hamochi | 57°51'01" | 138°18'52" | IV | 2 |
| B01129 | Hokkaido | Hobetsu | 42°46'00" | 142°08'00" | III | 5 | B03056 | Niigata | Mano | 37°58'04" | 138°20'55" | IV | 2 |
| B01133 | Hokkaido | Hokuto | 41°43'04" | 140°23'06" | IV | 3 | B03057 | Niigata | Sawata | 38°00'04" | 138°19'28" | IV | 2 |
| B01136 | Hokkaido | Kamiiso | 41°49'00" | 140°39'00" | IV | 3 | B03058 | Niigata | Kanai | 38°01'00" | 138°22'00" | IV | 2 |
| B01137 | Hokkaido | Kikonai | 41°41'00" | 140°26'00" | IV | 3 | B03059 | Niigata | Kanai | 38°02'57" | 138°22'48" | IV | 2 |
| B01139 | Hokkaido | Kaminokuni | 41°47'48" | 140°07'09" | IV | 3 | B03060 | Niigata | Ryotsu | 38°05'28" | 138°24'36" | IV | 2 |
| B01145 | | NA | | | | | B03061 | Niigata | Ryotsu | 38°03'41" | 138°28'48" | IV | 2 |
| B01148 | | NA | | | | | B03062 | Niigata | Ryotsu | 38°02'38" | 138°26'30" | IV | 2 |
| B01149 | Hokkaido | Kamiiso | 41°49'00" | 140°39'00" | IV | 3 | B03063 | Niigata | Niibo | 38°02'38" | 138°26'30" | IV | 2 |
| B01150 | Hokkaido | Hakodate | 41°46'00" | 140°44'00" | IV | 3 | B03064 | Niigata | Niibo | 38°02'10" | 138°26'59" | IV | 2 |
| B01151 | Hokkaido | Hakodate | 41°52'21" | 140°47'18" | IV | 3 | B03066 | Ishikawa | Kanazawa | 36°53'46" | 136°47'20" | IV | 2 |
| B01152 | Hokkaido | Assabu | 41°55'00" | 140°14'00" | IV | 3 | B04065 | Nagano | Kiso | 35°51'50" | 137°43'20" | IV | 2 |
| B01153 | | NA | | | | | B04096 | Ibaraki | Mito | 36°21'37" | 140°26'27" | III | 3 |
| B01154 | Hokkaido | Assabu | 41°56'08" | 140°20'12" | IV | 3 | B04109 | Chiba | Omigawa | 35°51'33" | 140°37'04" | III | 3 |
| B01156 | Hokkaido | Assabu | 41°55'21" | 140°19'19" | IV | 3 | B04118 | Chiba | Kisarazu | 35°23'36" | 139°57'58" | III | 3 |
| B01159 | | NA | | | | | B04123 | Chiba | Misaki | 35°18'04" | 140°23'39" | III | 3 |
| B01160 | Hokkaido | Kaminokuni | 41°47'01" | 140°05'09" | IV | 3 | B04124 | Tokyo | Akiruno | 35°44'19" | 139°39'01" | III | 3 |
| B01162 | Hokkaido | Kaminokuni | 41°46'25" | 140°05'01" | IV | 3 | B04125 | | NA | | | | |
| B01164 | Hokkaido | Mukawa | 42°46'09" | 142°09'03" | III | 5 | B04126 | Tokyo | Akiruno | 35°44'14" | 139°18'51" | III | 3 |
| B01165 | Hokkaido | Mukawa | 42°46'37" | 142°09'49" | V | 1 | B04129 | Ibaraki | Tsukuba | 36°00'38" | 140°05'48" | III | 3 |
| B01166 | Hokkaido | Abira | 42°50'58" | 141°55'07" | V | 1 | B04130 | Ibaraki | Tsukuba | 36°08'39" | 140°06'43" | III | 3 |
| B01169 | Hokkaido | Ishizaki | 41°45'00" | 140°53'00" | IV | 3 | B04133 | Ibaraki | Ishioka | 36°11'07" | 140°15'24" | III | 3 |
| B02019 | | NA | | | | | B04134 | Yamanashi | Isawa | 35°95'21" | 138°39'04" | III | 3 |

Table 1: Continue

| | | Location | | | Division | | | | Location | | | Division | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Accession No. | Prefecture | City/town | Latitude | Longitude | Macro | Meso | Accession No. | Prefecture | City/town | Latitude | Longitude | Macro | Meso |
| B02110 | Akita | Tenno | 39°52'55" | 139°58'33" | IV | 3 | B04135 | Tokyo | Akiruno | 35°43'15" | 139°18'51" | III | 3 |
| B02118 | Iwate | Miyako | 39°38'29" | 141°55'22" | III | 4 | B04138 | Saitama | Hanno | 35°52'10" | 139°15'52" | III | 3 |
| B02123 | Akita | Nishisenboku | 39°32'00" | 140°21'00" | IV | 3 | B04144 | Tokyo | Kita | 35°47'39" | 139°42'12" | III | 3 |
| B02124 | Akita | Nishisenboku | 39°18'37" | 140°25'24" | IV | 3 | B04146 | Tokyo | Kita | 35°47'39" | 139°42'12" | III | 3 |
| B02134 | Yamagata | Okura | 38°41'07" | 140°14'46" | IV | 3 | B04147 | Chiba | Noda | 35°55'49" | 139°51'46" | III | 3 |
| B02136 | Yamagata | Shirataka | 38°12'51" | 140°05'45" | IV | 3 | B04155 | Tokyo | Katsushika | 35°44'30" | 139°49'21" | III | 3 |
| B02143 | Iwate | Morioka | 39°47'49" | 141°09'55" | III | 4 | B04156 | Tokyo | Katsushika | 35°45'14" | 139°49'52" | III | 3 |
| B02154 | Miyagi | Natori | 38°11'15" | 140°56'22" | III | 4 | B04158 | Tokyo | Edogawa | 35°44'09" | 139°53'06" | III | 3 |
| B02162 | Iwate | Morioka | 39°51'02" | 141°08'31" | III | 4 | B04159 | Kanagawa | Yokohama | 35°30'51" | 139°34'47" | III | 3 |
| B02163 | Iwate | Morioka | 39°39'27" | 141°10'45" | III | 4 | B04160 | Yamanashi | Nagasaka | 35°50'18" | 138°21'23" | III | 3 |
| B02164 | Iwate | Hanamaki | 39°31'39" | 141°29'05" | III | 4 | B04161 | Yamanashi | Nagasaka | 35°49'03" | 138°20'30" | III | 3 |
| B02165 | Iwate | Kanegasaki | 39°11'23" | 141°07'48" | III | 4 | B04162 | Tokyo | Chiyoda | 35°41'02" | 139°44'38" | III | 3 |
| B02166 | Iwate | Hiraizumi | 38°58'47" | 141°07'58" | III | 4 | B04164 | Chiba | Chiba | 35°25'09" | 139°57'22" | III | 3 |
| B02167 | Iwate | Fujisawa | 39°20'05" | 141°08'35" | III | 4 | B04165 | Tokyo | Hachioji | 35°39'57" | 139°20'26" | III | 3 |
| B02168 | Miyagi | Toyoma | 38°40'50" | 141°17'11" | III | 4 | B04171 | | NA | | | | |
| B02169 | Miyagi | Kahoku | 38°31'27" | 141°20'34" | III | 4 | B05023 | Shizuoka | Kanaya | 34°49'30" | 138°08'52" | III | 3 |
| B02170 | Aomori | Aomori | 40°42'06" | 140°31'32" | IV | 3 | B05047 | Gifu | Ena | 35°27'00" | 137°25'00" | III | 3 |
| B02171 | Aomori | Aomori | 40°42'06" | 140°31'32" | IV | 3 | B05048 | Gifu | Nakatsugawa | 35°30'37" | 137°31'03" | III | 3 |
| B02175 | Aomori | Kuroishi | 40°38'00" | 140°36'00" | IV | 3 | B05049 | | NA | | | | |
| B02176 | Aomori | Kuroishi | 40°38'00" | 140°36'00" | IV | 3 | B05050 | Gifu | Nanno | 35°13'00" | 136°36'00" | III | 3 |
| B02178 | Aomori | Aomori | 40°49'09" | 140°42'27" | IV | 3 | B05051 | Mie | Kuwana | 35°03'38" | 136°38'07" | III | 3 |
| B02179 | Aomori | Aomori | 40°49'09" | 140°42'27" | IV | 3 | B05052 | Mie | Ise | 34°31'30" | 136°43'01" | III | 3 |
| B02180 | Aomori | Aomori | 40°49'00" | 140°45'00" | IV | 3 | B05053 | Mie | Matsusaka | 34°33'11" | 136°35'51" | III | 3 |
| B02181 | Aomori | Kuroishi | 40°38'00" | 140°36'00" | IV | 3 | B05054 | Mie | Ureshino | 34°37'29" | 136°28'43" | III | 3 |
| B02182 | Aomori | Kuroishi | 40°38'00" | 140°36'00" | IV | 3 | B05055 | Mie | Nabari | 34°38'36" | 136°05'30" | III | 2 |
| B02183 | Aomori | Namioka | 40°42'00" | 140°35'00" | IV | 3 | B05056 | Mie | Ueno | 34°44'29" | 136°07'27" | III | 2 |
| B02184 | Aomori | Namioka | 40°42'11" | 140°33'54" | IV | 3 | B05057 | Mie | Ueno | 34°44'51" | 136°06'58" | III | 2 |
| B02185 | Aomori | Tokiwa | 40°39'52" | 140°32'31" | IV | 3 | B06021 | | NA | | | | |
| B02186 | Aomori | Itayanagi | 40°42'09" | 140°26'42" | IV | 3 | B06029 | Shiga | Moriyama | 35°06'04" | 135°59'31" | III | 2 |
| B02187 | Aomori | Hirosaki | 40°36'00" | 140°28'00" | IV | 3 | B06034 | Hyogo | Kodera | 34°56'06" | 134°44'36" | III | 2 |
| B02188 | Aomori | Owani | 40°31'00" | 140°34'00" | IV | 3 | B06035 | Kyoto | Ayabe | 35°18'34" | 135°15'34" | IV | 2 |
| B02189 | Aomori | Soma | 40°35'00" | 140°24'00" | IV | 3 | B06037 | Kyoto | Tanba | 35°14'26" | 135°24'57" | IV | 2 |
| B02191 | | NA | | | | | B06038 | Hyogo | Sasayama | 35°04'23" | 135°15'25" | III | 2 |
| B02193 | Aomori | Kuroishi | 40°37'24" | 140°37'42" | IV | 3 | B06039 | Hyogo | Ichijima | 35°12'36" | 135°08'02" | III | 2 |
| B02207 | Akita | Omonogawa | 37°29'55" | 140°20'57" | IV | 3 | B06040 | Kyoto | Sonobe | 35°06'00" | 135°29'00" | III | 2 |
| B06041 | Hyogo | Nishinomiya | 34°45'28" | 135°19'32" | III | 2 | B07139 | Kochi | Tosashimizu | 32°47'00" | 132°57'00" | II | 1 |
| B06042 | Hyogo | Kobe | 34°41'00" | 135°12'00" | III | 2 | B07141 | Kochi | Tosashimizu | 32°51'41" | 132°56'13" | II | 1 |
| B06043 | Shiga | Hikone | 35°14'33" | 136°14'09" | III | 2 | B07142(A) | Kochi | Tosashimizu | 32°51'41" | 132°56'13" | II | 1 |
| B06044 | Shiga | Yasu | 35°02'35" | 136°01'19" | III | 2 | B07142(B) | Kochi | Otsuki | 32°50'48" | 132°42'25" | II | 1 |
| B06045 | Osaka | Shimamoto | 34°53'00" | 135°40'00" | III | 2 | B07143 | Kochi | Tosashimizu | 32°47'00" | 132°57'00" | II | 1 |
| B06046 | Nara | Oyodo | 34°23'08" | 135°48'22" | III | 2 | B07145 | Kochi | Tosashimizu | 32°49'26" | 132°56'57" | II | 1 |
| B06047 | Kyoto | Kyoto | 34°55'26" | 135°45'34" | III | 2 | B07148 | Kochi | Tosashimizu | 32°43'40" | 133°00'30" | II | 1 |
| B06048 | Kyoto | Joyo | 34°50'08" | 135°45'37" | III | 2 | B07149 | Kochi | Tosashimizu | 32°47'06" | 132°56'47" | II | 1 |
| B06049 | Osaka | Osaka | 34°42'27" | 135°28'51" | III | 2 | B07152 | Kochi | Tosashimizu | 32°46'08" | 132°49'35" | II | 1 |

Table 1: Continue

| | | Location | | | Division | | | | Location | | | Division | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Accession No. | Prefecture | City/town | Latitude | Longitude | Macro | Meso | Accession No. | Prefecture | City/town | Latitude | Longitude | Macro | Meso |
| B06050 | | NA | | | | | B07155 | Kochi | Otsuki | 32°50'48" | 132°42'25" | II | 1 |
| B06051 | Wakayama | Arida | 34°04'48" | 135°09'12" | III | 2 | B07156 | | NA | | | | |
| B06052 | Wakayama | Kawabe | 33°54'47" | 135°13'05" | III | 2 | B07157 | Tokushima | Kainan | 33°36'39" | 134°21'22" | II | 1 |
| B06053 | Wakayama | Shirahama | 33°39'07" | 135°24'08" | III | 2 | B07158 | Kochi | Sakawa | 33°29'28" | 133°15'39" | III | 2 |
| B06054 | Wakayama | Shirahama | 33°35'02" | 135°27'42" | III | 2 | B07159 | Kochi | Sakawa | 33°30'30" | 133°16'38" | III | 2 |
| B06055 | | NA | | | | | B07160 | Kochi | Ino | 33°32'35" | 133°25'30" | III | 2 |
| B06056 | Hyogo | Sanda | 34°53'00" | 135°14'00" | III | 2 | B07161 | Kochi | Kochi | 33°34'29" | 133°28'45" | II | 1 |
| B06057 | | NA | | | | | B07162 | Ehime | Komatsu | 33°54'24" | 133°06'23" | III | 2 |
| B06058 | Hyogo | Sasayama | 35°04'33" | 135°11'58" | III | 2 | B07163 | Kochi | Nakatosa | 33°19'56" | 133°13'55" | II | 1 |
| B06059 | Hyogo | Kurodasho | 35°03'48" | 135°00'29" | III | 2 | B08034 | Yamaguchi | Hikari | 33°58'25" | 131°55'51" | III | 2 |
| B06060 | Hyogo | Hikami | 35°10'00" | 135°02'00" | III | 2 | B08037 | Yamaguchi | Ajisu | 34°00'29" | 131°20'52" | III | 2 |
| B06061 | Kyoto | Yakuno | 35°19'00" | 135°00'00" | III | 2 | B08038 | Yamaguchi | Ube | 33°59'10" | 131°13'19" | III | 2 |
| B06062 | Kyoto | Fukuchiyama | 35°18'00" | 135°08'00" | IV | 2 | B08040 | Yamaguchi | Hagi | 34°28'10" | 131°26'46" | III | 1 |
| B06063 | Kyoto | Oe | 35°26'00" | 135°08'00" | IV | 1 | B08041 | Yamaguchi | Tamagawa | 34°37'59" | 131°39'45" | III | 1 |
| B06064 | Kyoto | Maizuru | 35°28'00" | 135°23'00" | IV | 1 | B08050 | Shimane | Izumo | 35°21'01" | 132°43'54" | IV | 1 |
| B06066 | Kyoto | Mineyama | 35°36'23" | 135°04'48" | IV | 1 | B08051 | Shimane | Izumo | 35°21'01" | 132°43'54" | IV | 1 |
| B06067 | Kyoto | Kumihama | 35°38'16" | 134°55'56" | IV | 1 | B08052 | | NA | | | | |
| B06068 | Kyoto | Kumihama | 35°36'22" | 134°54'45" | IV | 1 | B08053 | Shimane | Izumo | 35°21'01" | 132°43'54" | IV | 1 |
| B06069 | | NA | | | | | B08055 | Shimane | Kamo | 35°19'28" | 132°53'07" | IV | 1 |
| B06070 | Hyogo | Izushi | 35°28'15" | 134°51'51" | IV | 1 | B08056 | | NA | | | | |
| B06071 | Hyogo | Toyooka | 35°31'51" | 134°49'45" | IV | 1 | B08057 | Okayama | Soja | 34°42'12" | 133°43'33" | III | 2 |
| B06072 | | NA | | | | | B08060 | | NA | | | | |
| B06073 | Hyogo | Kasumi | 35°38'04" | 134°37'14" | IV | 1 | B08061 | Okayama | Kume | 35°03'20" | 133°54'59" | III | 2 |
| B06074 | Hyogo | Hamasaka | 35°37'28" | 134°28'08" | IV | 1 | B08062 | Okayama | Kume | 35°03'08" | 133°56'27" | III | 2 |
| B06075 | Hyogo | Hamasaka | 35°37'00" | 134°28'00" | IV | 1 | B08063 | Tottori | Yonago | 35°25'00" | 133°22'00" | IV | 1 |
| B06076 | Hyogo | Youka | 35°24'23" | 134°46'11" | III | 2 | B08064 | Hiroshima | Koda | 34°41'40" | 132°45'11" | III | 2 |
| B06077 | | NA | | | | | B08065 | Shimane | Saigo | 36°12'39" | 133°19'25" | IV | 1 |
| B06078 | Hyogo | Wadayama | 35°21'06" | 134°50'23" | III | 2 | B08066 | Shimane | Saigo | 36°13'48" | 133°18'85" | IV | 1 |
| B06079 | Hyogo | Wadayama | 35°20'37" | 134°53'15" | III | 2 | B08067 | Shimane | Saigo | 36°14'42" | 133°17'17" | IV | 1 |
| B06080 | | NA | | | | | B08068 | Shimane | Saigo | 36°10'47" | 133°18'48" | IV | 1 |
| B06082 | Hyogo | Yamasaki | 35°00'00" | 134°32'00" | III | 2 | B08069 | Shimane | Saigo | 36°10'47" | 133°18'48" | IV | 1 |
| B06083 | Shiga | Torahime | 35°25'21" | 136°14'56" | III | 2 | B08070 | Shimane | Saigo | 36°10'47" | 133°18'48" | IV | 1 |
| B06084 | Shiga | Omihachiman | 35°03'00" | 135°59'00" | III | 2 | B08071 | | NA | | | | |
| B06085 | Osaka | Sennan | 34°20'51" | 135°16'30" | III | 2 | B09002 | Kumamoto | Aso | 33°03'35" | 131°12'25" | III | 1 |
| B06086 | Kyoto | Kameoka | 35°01'00" | 135°34'00" | III | 2 | B09014 | Kumamoto | Kamoto | 33°00'06" | 130°44'12" | III | 1 |
| B06087 | Hyogo | Higashiura | 34°32'26" | 134°59'32" | III | 2 | B09055 | Kumamoto | Kumamoto | 32°48'12" | 131°41'11" | III | 1 |
| B06088 | Hyogo | Goshiki | 34°24'31" | 134°47'15" | III | 2 | B09058 | Miyazaki | Kunitomi | 31°59'44" | 131°17'43" | III | 1 |
| B06089 | Hyogo | Seidan | 34°19'00" | 134°44'00" | III | 2 | B09072 | Kumamoto | Tomiai | 32°42'45" | 130°40'48" | III | 1 |
| B06090 | Hyogo | Mihara | 34°17'59" | 134°46'30" | III | 2 | B09073 | Kumamoto | Jonan | 32°44'13" | 130°41'35" | III | 1 |
| B06091 | Hyogo | Nandan | 34°15'00" | 134°43'00" | III | 2 | B09074 | Kumamoto | Kosa | 32°39'04" | 130°50'33" | III | 1 |
| B06092 | Hyogo | Tsuna | 34°27'01" | 134°54'28" | III | 2 | B09077 | Kumamoto | Kikusui | 33°00'47" | 130°36'05" | III | 1 |
| B06093 | Hyogo | Hokudan | 34°31'30" | 134°53'23" | III | 2 | B09080 | Kumamoto | Aso | 32°59'37" | 131°00'09" | III | 1 |
| B06094 | Hyogo | Ichinomiya | 34°28'11" | 134°50'47" | III | 2 | B09081 | Kumamoto | Aso | 32°59'37" | 131°00'09" | III | 1 |
| B06096 | Nara | Yamatotakada | 34°31'27" | 134°43'20" | III | 2 | B09083 | Oita | Kitsuki | 33°26'39" | 131°31'14" | III | 2 |

Table 1: Continue

| | | Location | | | Division | | | | Location | | | Division | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Accession No. | Prefecture | City/town | Latitude | Longitude | Macro | Meso | Accession No. | Prefecture | City/town | Latitude | Longitude | Macro | Meso |
| B06097 | Nara | Yamatotakada | 34°31'00" | 135°44'00" | III | 2 | B09086 | Kagoshima | Akune | 32°00'59" | 130°12'32" | III | 1 |
| B06098 | Osaka | Osaka | 34°43'23" | 135°30'49" | III | 2 | B09087 | | NA | | | | |
| B06099 | Osaka | Moriguchi | 34°45'55" | 135°35'15" | III | 2 | B09088 | Kagoshima | Satsumasendai | 31°49'00" | 130°18'00" | III | 1 |
| B06100 | Osaka | Takatsuki | 34°51'00" | 135°37'00" | III | 2 | B09089 | Oita | Kitsuki | 33°26'39" | 131°31'14" | III | 2 |
| B06101 | Hyogo | Kozuki | 34°59'00" | 134°19'00" | III | 2 | B09091 | Oita | Usa | 33°31'20" | 131°20'43" | III | 2 |
| B06102 | Hyogo | Kamigoori | 34°52'04" | 134°21'34" | III | 2 | B09092 | Fukuoka | Yukuhashi | 33°43'57" | 130°58'25" | III | 2 |
| B06104 | Hyogo | Ako | 34°49'34" | 134°23'01" | III | 2 | B09093 | Fukuoka | Chikugo | 33°10'28" | 130°29'32" | III | 1 |
| B06105 | | NA | | | | | B09095 | Nagasaki | Gonoura | 33°45'05" | 129°42'14" | III | 1 |
| B07043 | Kochi | Nakamura | 32°59'41" | 132°55'27" | II | 1 | B09096 | Nagasaki | Gonoura | 33°45'05" | 129°42'14" | III | 1 |
| B07067 | Kochi | Nakamura | 33°00'14" | 132°55'37" | II | 1 | B09097 | Nagasaki | Gonoura | 33°46'21" | 129°43'03" | III | 1 |
| B07084 | Kochi | Sukumo | 32°57'23" | 132°44'48" | II | 1 | B09098 | Nagasaki | Ashibe | 33°48'37" | 129°44'17" | III | 1 |
| B07086 | Kochi | Sukumo | 32°57'23" | 132°44'48" | II | 1 | B09101 | Nagasaki | Kamiagata | 34°31'56" | 129°20'50" | III | 1 |
| B07107 | Kochi | Otsuki | 32°48'56" | 132°46'08" | II | 1 | B09104 | Saga | Kohoku | 33°12'38" | 130°10'56" | III | 1 |
| B07108 | Kochi | Otsuki | 32°48'56" | 132°46'08" | II | 1 | B09105 | Nagasaki | Higashisonogi | 33°02'30" | 129°55'07" | III | 1 |
| B07113 | Kochi | Mihara | 32°53'34" | 132°51'22" | II | 1 | B09106 | | NA | | | | |
| B07115 | Kochi | Tosashimizu | 32°49'49" | 132°47'57" | II | 1 | B09107 | Saga | Imari | 33°16'57" | 129°51'46" | III | 1 |
| B07117 | Kochi | Nishitosa | 33°08'43" | 132°47'39" | III | 2 | B09108 | Nagasaki | Sasebo | 33°11'28" | 129°39'58" | III | 1 |
| B07118 | Kochi | Nishitosa | 33°06'47" | 132°49'28" | III | 2 | B09109 | Nagasaki | Arikawa | 32°58'49" | 127°07'07" | III | 1 |
| B07119 | | NA | | | | | B09110 | Nagasaki | Fukue | 32°42'00" | 128°50'00" | III | 1 |
| B07127 | Kochi | Oogata | 33°02'13" | 133°04'49" | II | 1 | B09111 | Nagasaki | Kishiku | 32°44'06" | 128°43'48" | III | 1 |
| B07130(A) | Kochi | Oogata | 33°01'00" | 133°01'00" | II | 1 | B09112 | Nagasaki | Kishiku | 32°44'06" | 128°43'48" | III | 1 |
| B07131(A) | Kochi | Oogata | 33°01'00" | 133°01'00" | II | 1 | B09113 | Nagasaki | Kishiku | 32°43'45" | 128°46'01" | III | 1 |
| B07132(A) | Kochi | Oogata | 33°00'14" | 132°55'37" | II | 1 | B09115 | Nagasaki | Fukue | 32°41'49" | 128°50'23" | III | 1 |
| B07133(A) | Kochi | Oogata | 32°58'20" | 132°53'38" | II | 1 | B09116 | Nagasaki | Takaki | 32°54'30" | 130°08'40" | III | 1 |
| B07134(A) | Kochi | Oogata | 32°58'20" | 132°53'38" | II | 1 | B09121 | Saga | Karatsu | 33°31'52" | 129°53'42" | III | 1 |
| B07135(A) | Kochi | Oogata | 33°01'50" | 133°01'09" | II | 1 | B09123 | | NA | | | | |
| B07136(A) | Kochi | Oogata | 32°53'44" | 133°00'12" | II | 1 | B09124 | Saga | Saga | 33°14'15" | 130°14'47" | III | 1 |
| B07137(B) | Kochi | Nakamura | 32°58'20" | 132°53'38" | II | 1 | | | | | | | |

NA: Not available, Macro: Roman numerals and Meso: Arabic numbers scale subdivisions indicate climatic regions of Japan[19]

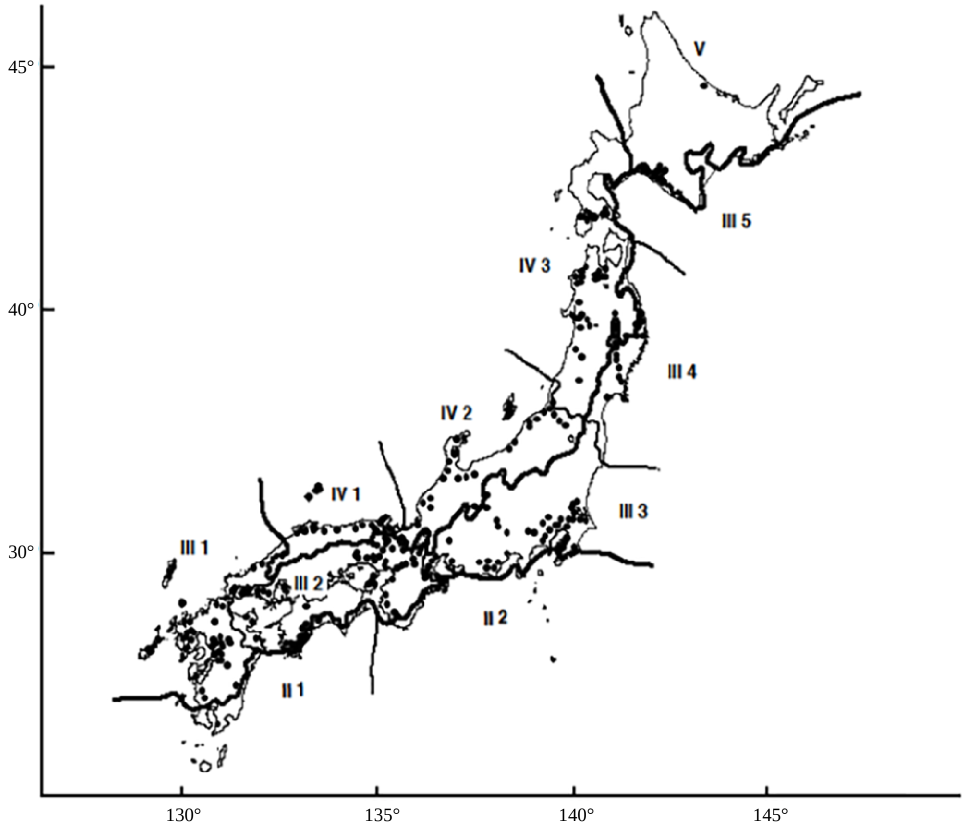

Fig. 1: Collection sites for *Glycine soja* accessions used in this study. Axes indicate latitude and longitude. Macro: Roman numerals and Meso: Arabic numbers scale subdivisions indicate the climatic regions of Japan[19]

## RESULTS

**Fatty acid contents and distributions of wild soybean seeds:** Figure 2 shows the distributions and concentration ranges of the five constituents in the same sample set. The palmitate content ranged from 9.79% (B02170) to 14.06% (B07113) with 70 wild soybean accessions having a palmitate content of 11.5% (Fig. 2a). The stearate content of seeds from wild soybean accessions ranged from 2.29% (B02187) to 4.00% (B06045) and 78 accessions had stearate levels of 3.0% (Fig. 2b). Among the 319 accessions, the oleate content of seeds ranged from 7.66% (B04158) to 15.86% (B02124) and 101 accessions had seed oleate levels of 11.5% (Fig. 2c). Levels of linoleate and α-linolenate in wild soybean seeds ranged from 47.7% (B07131) to 60.6% (B09002) and from 12.1% (B02220) to 25.4% (B07131), respectively. The majority of wild soybean accessions had seed linoleate levels of 56% (Fig. 2d) and seed α-linolenate levels of 19% (Fig. 2e).

**Pairwise correlations of fatty acid types in wild soybean seeds:** Pairwise correlations between the fatty acids analyzed in the Japanese wild soybean accessions are shown in Fig. 3. Significant inverse correlations were evident for the oleate and α-linolenate contents and the linoleate and α-linolenate contents with $R^2$ values of 0.2995 and 0.4298, respectively (Fig. 3i, j); however, there was not a significant correlation between the oleate and linoleate contents in the wild accessions (Fig. 3f). Moreover, there were weak inverse correlations ($R^2 = 0.1084$) between the stearate and α-linolenate contents (Fig. 3h) and the palmitate and linoleate contents ($R^2 = 0.1636$) (Fig. 3d). Furthermore, a very weak correlation was seen between the palmitate and stearate contents ($R^2 = 0.0593$) (Fig. 3a). There were not significant correlations between oleate and palmitate (Fig. 3b), oleate and stearate (Fig. 3c), linoleate and stearate (Fig. 3e), a-linolenate and palmitate contents (Fig. 3g).

**Seed compositions of plants collected from various climatic regions in Japan:** In regions where the annual mean temperature is high in Japan, the total palmitate content of *Glycine soja* accessions was high, especially in regions II-1 (12.79%) and III-1 (12.78%). Moreover, the seed palmitate content of plants collected from regions II-1, III-1 and II-2 were significantly higher than those of

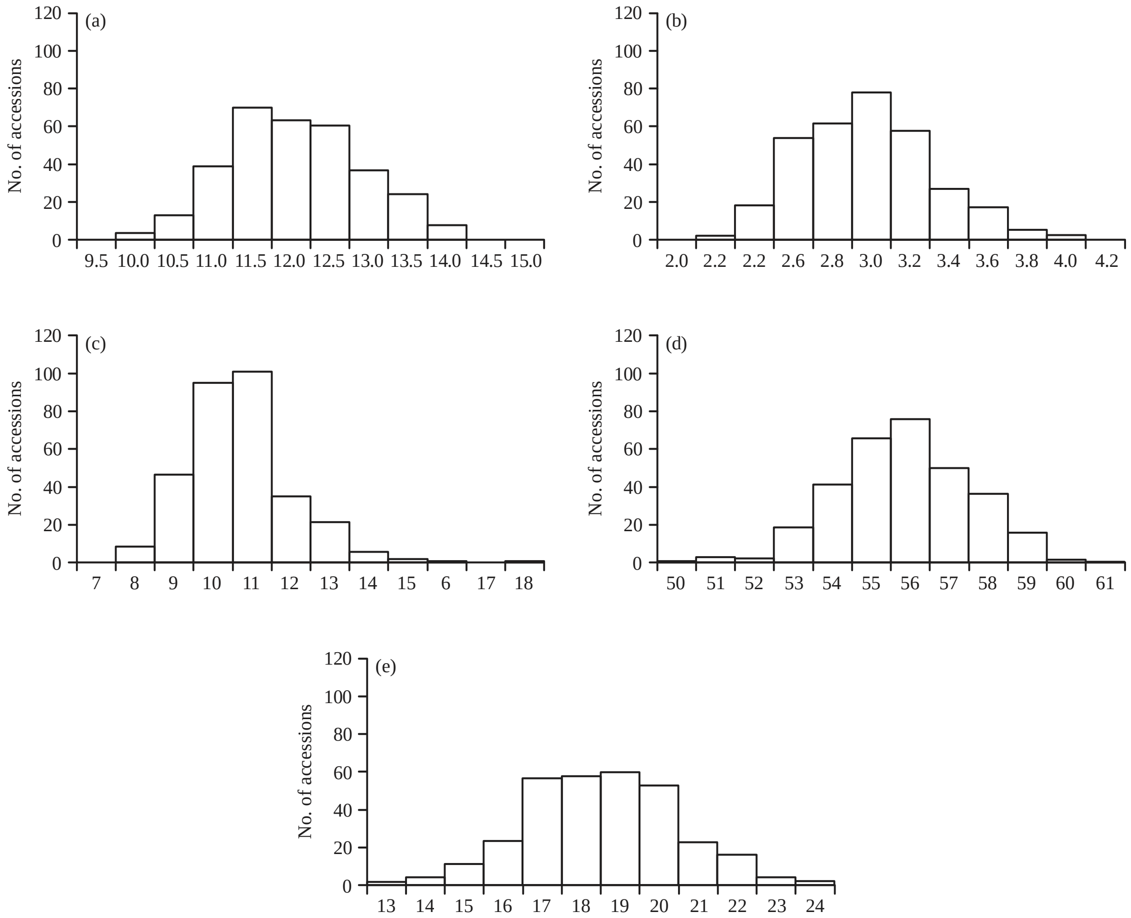

Fig. 2(a-e): Distributions of fatty acid types among *Glycine soja* seeds collected in Japan. (a) Palmitate (%), (b) Strearate (%), (c) Oleate (%), (d) Linoleate (%) and (e) α-linolenate (%)

regions III-4, IV-3 and V-1. Regions with high annual temperatures also produced seeds with high levels of stearate. Notably, the stearate content of accessions collected in region III-2 was higher than that of regions II-1, III-4, III-5, IV-3 and V-1 (Fig. 4a). Moreover, the seed stearate levels of plants collected in regions III-1 and III-2 were significantly higher than those collected in regions II-1, III-5, IV-3 and V-1 (Fig. 4b). In contrast, the total α-linolenate content of *Glycine soja* found in regions with low annual temperatures was high compared with the palmitate and stearate levels in the same samples. The seed α-linolenate content of accessions collected in regions III-5 (19.96%) and V-1 (20.59%) was significantly higher than that found in accessions collected from regions III-1 (17.91%), III-2 (18.02%), III-3 (18.05%) and III-4 (18.22%) (Fig. 4e).

## DISCUSSION

Approximately 20% of the mass of cultivated soybean seeds consists of an oil that contains various glycerolipids, including phospholipids, diacylglycerol and triacylglycerol, the latter being the principal component of soybean oil[4]. Furthermore, the phospholipid composition, primarily phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol have structural roles in plant cell membranes and are metabolically involved in triacylglycerol synthesis[20,21]. Each glycerolipid class is formed by various combinations of the five fatty acids analyzed in our study and polar groups that are esterified at the stereospecific positions of the glycerol molecule.

Average values for the five major fatty acid components found in soybean (*Glycine max*) oil are 12% palmitate (16:0), 4% stearate (18:0), 23% oleate (18:1), 53% linoleate (18:2) and 8% α-linolenate (18:3). The palmitate and stearate fractions are saturated fatty acids and constitute 15% of the soybean oil and the remainder of the oil (85%) is made up of unsaturated fatty acids or oleate, linoleate and α-linolenate[22].

The palmitate content varied from 9.79 to 14.06% with a distribution peak around 11.5% and an average value of 12.05%. Thus, the average value for the palmitate content of

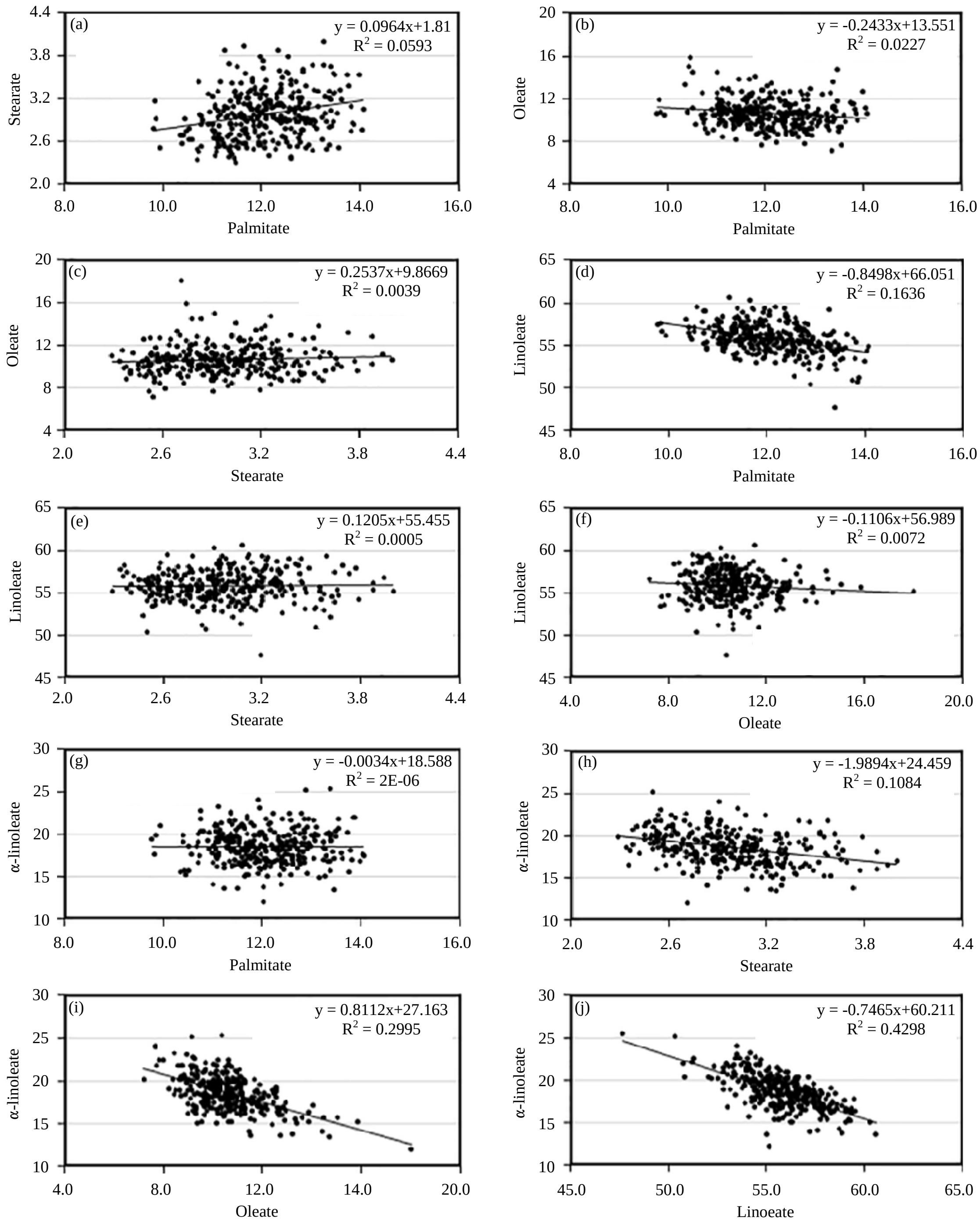

Fig. 3(a-j): Pairwise correlations of the fatty acid content of *Glycine soja* seeds collected in Japan

the wild accessions was nearly identical to that reported for *Glycine max*. The stearate content of the wild accessions varied from 2.29 to 4.00% with an average value of 2.97%. The accumulation of stearate in the wild soybean accessions was approximately 25% lower than that found in past studies of *Glycine max*. Moreover, the oleate content widely varied from 7.66-15.86%, with a distribution peak around 11.5% and an average value of 10.62%. The oleate content of wild soybean seeds was significantly lower than that reported previously. The linoleate and α-linolenate contents varied from 47.7-60.6 and 12.1-25.4% in the wild accessions, respectively. The average values for linoleate and α-linolenate levels were 55.81 and 18.55%, respectively. Thus, higher average levels of linoleate and α-linolenate were found in

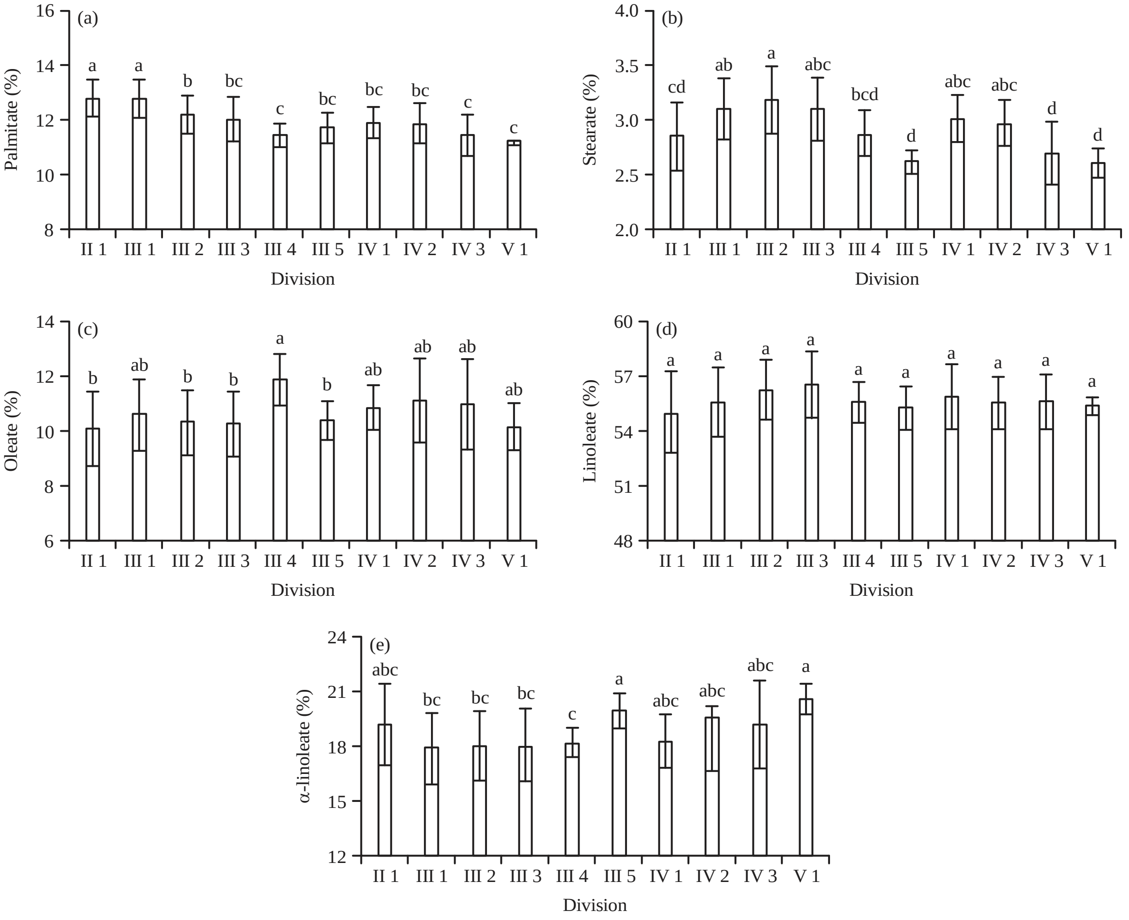

Fig. 4(a-e): Distribution of fatty acid types in *Glycine soja* collected from different climatic regions in Japan. The different letters on the bars indicate a significant difference at p<0.05 by the Steel-Dwass test

*Glycine soja* compared to the levels reported for *Glycine max* in the past. Overall, it was found that *Glycine soja* accessions collected in Japan had higher linoleate and α-linolenate contents and lower stearate and oleate contents than the previously characterized *Glycine max* accessions.

Kim and Park[23] reported the fatty acid contents of 70 Korean wild soybean lines. The palmitate content in Korean accessions was directly and significantly correlated with the stearate and oleate contents and inversely correlated with the linoleate and linolenate contents. Moreover, the stearate and palmitate contents were directly correlated with the oleate content and also inversely correlated with the linoleate and linolenate contents. Furthermore, the oleate content was very significantly and inversely correlated with the linoleate and linolenate contents. That is a high oleate content correlated with low levels of linoleate and α-linolenate accumulation. There was also a very significant and direct correlation between the linoleate and α-linolenate contents of the Korean wild soybean accessions.

In this study, the oleate content of wild soybeans collected in Japan also had a significant inverse correlation with the α-linolenate content, however, the linoleate and α-linolenate contents were inversely correlated, a result quite different than that found for the Korean accessions. On the other hand, there was no significant correlation between the oleate and α-linolenate contents of the Japanese wild accessions, although the oleate and α-linolenate contents of the Korean wild accessions were inversely correlated. Unlike the results reported for wild soybean accessions from Korea in which the palmitate contents were significantly correlated with the stearate, oleate, linoleate and α-linolenate contents, this study did not find a significant correlation of these four fatty acids with the palmitate content of wild soybean accessions collected in Japan.

Genetic resources are very important for improving the content of soybean oil and consumer demand has increased the emphasis to breed soybeans with a high oil content[12-16]. Accessions in the USDA soybean (*Glycine max*) germplasm collection were reported to have a wide range (8-25%) of total oil contents[24]. Moreover, genes influencing oil content and oil biosynthesis can be affected by environmental factors such as temperature and rainfall. Environmental interactions related to oil concentration and fatty acid profiles of soybean oil have been thoroughly investigated[25,26]. Past reports have indicated that temperature plays an important role in the synthesis of oil and fatty acids, however, previous investigations examined the fatty acid content of representative and restricted-use accessions affected by environmental conditions. In contrast, the fatty acid content of publically available and numerous wild soybean accessions collected from a wide range of environmental conditions and geographical locations was measured in Japan.

In this study, we divided the climatic regions as recommended by the standard scale of climates in Japan[19]. The boundaries of these regions are devised as follows. The boundary between regions I and II is defined by the 180°C warmth index line or the 20°C isotherm of the approximate annual temperature. The boundary between regions II and III coincides for the most part with the 0°C isotherm for the daily minimum temperature in January, which is an indicator for frequent frost occurrence. This boundary roughly corresponds with the 16°C isotherm for the annual temperature on the Pacific coast. The boundary between regions III and IV reflects a division between the Pacific side and the Japan sea side climates. The boundary is drawn in accordance with the 50 cm isoline of mean maximum snow depth, which defines a delimiting factor for the distribution of vegetation. The boundary between IV and V is defined by taking into account the regions where the monthly mean temperature is 0°C for 4 months[19].

The total palmitate content of *G. soja* collected from high annual temperature regions was high, especially the palmitate content of accessions collected from regions II-1 and III-1 was significantly higher than those of the other regions. Moreover, the palmitate content of wild soybean seeds from regions II-1, III-1 and III-2 was significantly higher than those of regions III-4, IV-3 and V-1. Similarly, accessions collected from high annual temperature regions had high levels of stearate. For example, the stearate content of region II-2 was higher than those of regions II-1, III-4, III-5, IV-3 and V-1, regions III-1 and III-2 had significantly higher stearate levels than those of regions II-1, III-5, IV-3 and V-1. These results indicate a direct relationship between environmental conditions and seed fatty acid accumulation. Furthermore, the total α-linolenate content of *Glycine soja* accessions collected from low annual temperature regions was higher than the palmitate and stearate contents. Remarkably, the α-linoleate content of accessions collected from regions III-5 and V-1 was significantly higher than those collected from regions III-1, III-2, III-3 and III-4.

Wilson[4] described the oil content of soybean seeds growing in elevated temperatures has been enhanced. In general, soybeans grown under high average temperatures have reduced linoleate and linolenate contents and an increased oleate content; however, the levels of saturated fatty acids are not changed by environmental factors[27-30].

Whereas, previous studies relied on fragmentary composition analysis of identified accessions with only basic environmental effects such as temperature taken into consideration, our investigation has systematically analyzed the fatty acid composition of 319 wild soybean accessions collected throughout numerous environments in Japan and represents the first attempt to model soybean fatty acid composition on the basis of environmental and geographical distribution.

A major objective of this study was to correlate the effects of environmental conditions on wild seed fatty acid composition in order to predict regions and environmental conditions that consistently produce high quality soybeans. As a direct result of this project, it will be feasible to use the wild soybean accessions in the future development and selection of new soybean varieties with desired traits.

## CONCLUSION

We evaluated the fatty acid compositions of seeds from 319 wild soybean (*Glycine soja*) accessions collected in Japan using gas chromatography for using the wild accessions as resources for the future development and selection of new soybean varieties with desired traits. Seventy wild soybean accessions had a palmitate content of 11.5%, 78 accessions had stearate levels of 3.0% and 101 accessions had seed oleate levels of 11.5%. The majority of wild soybean accessions had seed linoleate levels of 56% and seed α-linolenate levels of 19%. Pairwise correlations between the fatty acids had evidently shown significant inverse correlations for the oleate and α-linolenate contents and the linoleate and α-linolenate contents, respectively. Moreover, weak inverse correlations between the stearate and α-linolenate contents and the palmitate and linoleate contents were evident. In regions where the annual mean temperature is high in Japan, the total palmitate content of *Glycine soja* accessions was high and regions with high annual temperatures also produced seeds with high levels of stearate. In contrast, the total α-linolenate content of *Glycine soja* found in regions with low annual temperatures was high compared with the palmitate and stearate levels in the same samples. These results provide diverse and wide-ranged fatty acid information of Japanese wild soybean resources according to climatic region, thus providing a foundation for the future development and selection of new soybean varieties with desired traits in global environmental changes.

## ACKNOWLEDGMENT

This study was supported by the National BioResource Project of the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

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