Chromosome Number, Oil and Fatty Acid Content of Species in the Genus *Glycine* Subgenus *Glycine*

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ABSTRACT

Accessions of wild perennial species in *Glycine* subgenus *Glycine* were analyzed for chromosome number, oil and fatty acid content. The mean oil content appears to be quite similar in seed of those species with $2n=40$. The only species with appreciable variation in oil content was *Glycine clandestina*. The polyploid accessions ($2n=80$) of *G. tabacina* and *G. tomentella* had about the same amount of oil as their diploid counterparts. The fatty acids detected in all seed of the 7 species in the subgenus *Glycine* include palmitic, stearic, oleic, linoleic, linolenic, arachidic and behenic. Among the species, the fatty acid content of *G. clandestina* was the most variable. Within the *G. tabacina* accessions, there was very little difference in fatty acid content between the diploid ($2n=40$) and the tetraploid ($2n=80$) accessions. Within the *G. tomentella* accessions, there were minor differences in fatty acid content between the aneuploid accessions ($2n=38$ or 78) and their complete genome counterparts ($2n=40$ or 80).

INTRODUCTION

The genus *Glycine* Willd. is divided into 2 subgenera, *Glycine* and *Soja* (Moench) F.J. Herm. The cultivated soybean, *G. max* (L.) Merr., and its wild ancestor, *G. soja* Sieb. and Zucc. comprise the subgenus *Soja*. Both species are annuals with $2n=40$. *Glycine soja* is indigenous to China, Taiwan, Japan, Korea and the USSR. The subgenus *Glycine* contains 7 perennial wild species (1,2): *Glycine canescens* F.J. Herm., *G. clandestina* Wendl., *G. falcata* Benth., *G. latifolia* (Benth.) Newell and Hymowitz, and *G. latrobeana* (Meissn.) Benth. are restricted in their distribution to Australia and are diploid ($2n=40$). *Glycine tabacina* (Labill.) Benth. and *G. tomentella* Hayata are primarily tetraploid ($2n=80$). The species range in distribution from Australia north to South China, Taiwan and the Ryukyu Islands and across several islands in the South Pacific west of the Andesite Line. In the past decade, a large number of accessions belonging to the subgenus *Glycine* have been assembled at the University of Illinois and a project devoted to studying intra and interspecific variation has been initiated (2-4).

Although the soybean is a major source of vegetable oil, there are certain flavor problems associated with it. Linolenic acid has been implicated in soybean oil's off-flavor, lack of stability, and odor problems (5). Most of the U.S. Department of Agriculture's germplasm collection of *G. max* and *G. soja* have been screened for genotypes without linolenic acid. Thus far, no such genotypes have been located (6). Linolenic acid ranges from 5 to 18% of the oil in seed of the subgenus *Soja*. Hence, the interest in the seed of the species in the subgenus *Glycine* as potential sources of germplasm without or with reduced levels of linolenic acid.

Investigations on the oil and fatty acid composition of perennial *Glycine* species have been relatively few. Hymowitz et al. (7) reported that 20 accessions, mostly *G. tabacina* and *G. tomentella* had the same 5 major fatty acids as soybean, i.e., palmitic, stearic, oleic, linoleic and linolenic. In addition, the linolenic acid content in seed was about the same as the soybean; however, the total oil content was lower. Similar results were reported for one accession each of *G. canescens* and *G. falcata* (8,9). This investigation was undertaken to determine the chromosome number, total oil and fatty acid contents in a large collection of accessions of species in the subgenus *Glycine* and to establish relationships among them.

MATERIALS AND METHODS

Germplasm

Seed representing 6 species (158 accessions) in the subgenus *Glycine* were collected over a 2 year period from greenhouse-grown plants at the University of Illinois. The seed weighed from 0.5 to 1.5 g/100 seed compared to ca. 17.5 g/100 seed for commercial soybean cultivars. In addition, many plants had low seed productivity when grown under greenhouse conditions. The 2 accessions of *G. latrobeana* were multiplied in Canberra, Australia.

Cytology

Chromosome counts were made primarily during meiosis from acetocarmine smears of developing pollen mother cells. For root tip squashes, scarified seeds were germinated on filter paper in petri dishes at ca. 20 C until the roots were 5-6 mm in length. The temperature was increased to 30 C for 2-3 hr and the root tips harvested. Subsequent preparation followed the technique of Palmer and Heer (10), with the exception that a rubber roller instead of a pellet press was used to flatten the cells. Voucher specimens for all accessions included in this study, except for *G. latrobeana*, are deposited in the herbarium of the Crop Evolution Laboratory (CEL), University of Illinois.

Total Oil

Seeds were dried at 55 C for 7 days to obtain moisture contents of about 3%. Total oil was obtained by a Varian Pat-20 NMR Process Analyzer using a small probe (ca. 1 g seed).

Fatty Acids

For fatty acid analysis of single seed, the following micro-analytical technique was developed. The seed was placed in a Folin-Wu tube (25 × 200 mm), 5 mL of Skelly F petroleum ether (glass-distilled) added and the tube was placed in an ice bath. The seed was ground in Skelly F petroleum ether using a Polytron homogenizer (Brinkman Instruments) at full setting for 10 sec and the tube was returned to the ice bath. The ground sample suspended in Skelly F petroleum ether was filtered (Whatman 50) and transferred to a reaction tube (14 × 100 mm), 2 mL of 1% H_2SO_4 in anhydrous methanol and a Teflon boiling chip were added. The sample was mixed for 1 min using a Vortex mixer. Afterward, the tube was placed in an aluminum heating block set at 90 C for 10 min or until about 0.5 mL of solution was left in the tube. The tube was removed from the heating block and allowed to cool to room temperature. Then 3 mL of glass distilled Skelly F petroleum ether was added to the tube and mixed well for 1 min using a Vortex mixer. A Pasteur capillary tube was used to remove the lower part of the liquid which was mainly alcohol, water

and acid, e.g. About 0.2 g anhydrous Na_2SO_4 was added to the reaction tube, mixed well with the Vortex mixer and centrifuged at 3,000 rpm for 5 min at 4 C. The clear supernatant was transferred into a 1-dram vial, covered with a septum cap, and evaporated to dryness using a stream of nitrogen through a hypodermic needle. Using a syringe, 0.05 mL of spectral grade isooctane was added to the vial which was stored at -20 C until further use. Using 5 soybean cultivars as standards (Clark, Cloud, Cutler, Peking and Wilson-5), the microanalytical fatty acid procedure was compared to the AOAC Soxhlet extraction procedure (11).

A Beckman GC-5 gas chromatograph equipped with a Varian CDS 111 integrator recorder was used to detect and quantify fatty acid content in the seed extracts. A nickel alloy column (1.83 m long, 3.175 mm od) was packed with 10% SP 2330 (cyanosilicone) on Chromosorb W AW (100-200 mesh). About 6 cm of acid-washed glass wool was placed in the end of the column connected to the injection port. The glass wool was replaced after ca. 30 samples.

Fixed temperatures included: inlet 220 C; detector line 250 C; and detector 280 C. Gas flow rates were: air at 300 mL min^{-1}; hydrogen at 45 mL min^{-1}, helium carrier at 20 mL min^{-1}, and helium make-up at 100 mL min^{-1}. Fatty acid standards were purchased from Pierce Chemical Co.

RESULTS AND DISCUSSION

Comparison of the results of the microanalytical fatty acid procedure to the AOAC Soxhlet extraction procedure suggested that the fatty acid values obtained from the 5 soybean cultivars by the microanalytical procedure were comparable to those values obtained by the standard Soxhlet extraction procedure. Therefore, we felt justified in utilizing the microanalytical procedure for the determination of fatty acids in the seeds of the species in the subgenus *Glycine*. Unfortunately, not enough seed was available (ca. 1 g) to analyze all of the accessions for total oil content by NMR. Therefore, total oil content was obtained from only 124 out of the 158 accessions used in this investigation.

Comparison of $2n$ chromosome number to total oil content in seed revealed several interesting points (Table I). The mean oil content appears to be quite similar in the seed of those species with $2n$=40. The only species with appreciable variation in oil content was *G. clandestina*. Second, the polyploid accessions ($2n$=80) of *G. tabacina* and *G. tomentella* had about the same amount of oil as their diploid counterparts. Finally, the missing chromosomes in the *G. tomentella* aneuploids ($2n$=78) do not appear to contain the sites that regulate oil synthesis in the seed. Since chromosome markers are not available, it is impossible to determine whether the same or different chromosomes are missing from each of the aneuploid accessions.

The fatty acids detected in all seed of the species of the subgenus *Glycine* include palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0) and behenic (22:0) (Table II). Palmitoleic (16:1) was found in all seed except those of *G. falcata*. In 2 accessions of *G. tabacina*, traces of lignoceric (24:0) were detected. All of the species had higher percentages of linolenic acid contents than usually found in soybeans. Among the species, the fatty acid content of *G. clandestina* accessions was the most variable, reflecting the high morphological variability also shown by this species (4). The range in percentage fatty acid content among all of the species analyzed was: palmitic (26.5-13.6), stearic (13.4-4.8), oleic (20.9-10.4), linoleic (47.8-30.2) and linolenic (27.2-11.3).

Within the *G. tabacina* accessions there was very little difference in fatty acid content between the diploid ($2n$=40) and the tetraploid ($2n$=80) accessions. Within the *G. tomentella* accessions there were minor differences in fatty acid content between the aneuploid accessions ($2n$=38 or 78) and their complete genome counterparts ($2n$=40 or 80).

The results reported herein do not favor interspecific

TABLE I

Chromosome Number, Mean Oil and Range of Oil Content in Seed of 124 Accessions from 5 Species in the Genus *Glycine* Subgenus *Glycine*

Species	No.	2n	Oil content Mean (%)	Oil content Range (%)
G. canescens	19	40	10.8	9.8-11.8
G. clandestina	37	40	11.0	7.2-15.1
G. latifolia	9	40	11.3	9.9-12.1
G. tabacina	3	40	10.5	9.1-11.9
	29	80	10.3	8.3-11.6
	9	—[a]	9.7	8.2-11.3
G. tomentella	1	40	10.3	
	5	78	9.4	8.4-10.1
	7	80	11.4	8.7-12.3
	5	—[a]	10.1	8.7-11.7

[a]Chromosome number unknown for these accessions.

TABLE II

Chromosome Number and Fatty Acid Content in Seed of 158 Accessions from 7 Species in the Genus *Glycine* Subgenus *Glycine*

Species	No.	2n	Fatty acid content (%) 16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0
G. canescens	19	40	14.7	T	6.0	13.3	39.2	24.4	0.3	1.9
G. clandestina	47	40	16.8	T	6.2	14.5	41.8	18.9	0.5	1.2
G. falcata	3	40	18.2	—	10.0	12.9	41.4	16.4	0.4	0.5
G. latifolia	9	40	20.2	0.2	7.1	21.9	35.5	13.5	0.4	0.7
G. latrobeana	2	40	13.0	0.1	3.0	15.3	41.6	25.0	0.3	1.5
G. tabacina	6	40	20.2	0.3	5.3	14.4	42.9	15.6	0.1	1.1
	31	80	20.9	0.4	5.7	16.2	38.8	16.6	0.1	1.1
	9	—	18.8	0.1	5.1	15.5	42.3	16.1	0.1	1.2
G. tomentella	2	38	15.0	0.2	6.8	9.4	45.8	20.8	1.2	0.6
	1	40	14.2	T	7.8	8.2	45.2	22.9	1.0	0.4
	12	78	16.9	0.1	6.8	8.2	41.7	22.9	2.8	0.9
	11	80	18.9	0.1	9.1	12.0	38.8	18.2	1.9	1.1
	4	—	15.0	T	6.5	10.0	43.7	22.2	2.1	0.4

hybridization attempts between the soybean and the species in the subgenus *Glycine* as a means of producing a low linolenic acid soybean. However, the wild species germplasm might have other chemical or agronomic characteristics of use to the soybean breeder. An understanding of the variability that does exist between and among the wild *Glycine* species is required before they can be utilized in any breeding projects.

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♣ Effect of Ultrasonic Comminution on Liquid Classification of Cottonseed Protein and Gossypol Pigment Glands[1]

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ABSTRACT

Slurries of pin-milled full-fat and flaked, extracted cottonseed were ultrasonically comminuted in hexane and liquid-classified using laboratory differential settling techniques. Sonication of full fat cottonseed slurries increased the liquid classified protein fraction recovery from 25.9% (nonsonicated control) to over 60%, while the protein content of the fraction remained basically constant at 67%, and free gossypol increased slightly from 0.027 to 0.032%. Sonication of flaked, solvent extracted (fat free), slurried cottonseed yielded a 28% classified fraction containing 72% protein and 0.032% free gossypol. Although it was demonstrated on laboratory scale only, ultrasonic comminution may make the price of edible cottonseed protein concentrates produced from glanded seed (via a process such as the liquid cyclone process) competitive with other edible protein products.

INTRODUCTION

Wet disintegration of cottonseed, in conjunction with liquid classification into whole, toxic, gossypol-containing pigment gland and protein meal fractions, was intially investigated by Boatner and Hall in 1946 (1). They disintegrated cottonseed flakes in a specific gravity-adjusted mixture of chlorinated solvents and hexane in a Waring blender, and then removed the floating pigment glands. Vix et al. (2) also disintegrated flakes in a blender but restricted their investigations to a hexane medium in which pigment glands and coarse meal settled to the bottom, and a slow-settling, protein rich, gland free fraction was siphoned from the top. Gastrock et al. (3) capitalized on these distinctive settling characteristics and developed the first continuous wet classification process, the liquid cyclone process (LCP). Gardner et al. (4) and Gastrock (5) improved the LCP, but it has never been fully commercialized.

All past cottonseed classification research used conventional mechanical disintegration methods with blenders and pin and stone mills. Many researchers have used another mechanical disintegration method, sonication, as an aid in extracting vegetable oils (6-8) and protein (9-11), but information about effects of sonication on pigment gland rupture and protein classification is lacking. Ultrasonic disintegration of a slurry is achieved by applying electrical energy to a crystal, which changes shape in tune with the applied electrical field, creating pulsations or ultrasonic waves. The waves, consisting of alternate compressions and rarefactions (a phenomonon known as cavitation), cause formation and collapse of microscopic bubbles that can produce local pressure changes as high as 20,000 atm. These extremely high shearing pressures and shock waves produce the disintegration effect.

EXPERIMENTAL PROCEDURES

Materials

The 1977 crop, ginned, glanded cottonseed was obtained from oil mills located in 4 different, major cottonseed growing areas. The solvent used was Skellysolve B, a commercially available hexane high in *n*-hexane.

Methods

Hulling. All seed samples were hulled in pilot plant Carver equipment to yield whole and cracked meat fractions containing less than 3% hulls.

Flaking. Flakes ca. 0.30 mm (0.012 in.) thickness, were obtained by processing the hulled meat fractions without prior conditioning through Allis Chalmers flaking rolls. In preparing defatted flakes, both raw and dried flakes were batch-extracted 9 times with hexane. Each extraction consisted of portions of hexane equivalent to the flaked sample weight, and lasted 20 min.

[1] Presented at the AOCS Annual Meeting, 1981, New Orleans.