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Seed Oil and Fatty Acid Content in Okra (*Abelmoschus esculentus*) and Related Species

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ABSTRACT: Approximately 1100 genebank accessions of okra (*Abelmoschus esculentus*) and 540 additional accessions that included six of its related species—*A. caillei*, *A. crinitis*, *A. esculentus*, *A. ficulneus*, *A. manihot*, *A. moschatus* and *A. tuberculatus*—were evaluated for seed oil content using time domain NMR (TD-NMR). Oil content in seed of *A. caillei*, *A. esculentus*, *A. ficulneus*, *A. manihot*, *A. moschatus* and *A. tuberculatus* was in the ranges 2.51−13.61%, 12.36−21.56%, 6.62−16.7%, 16.1−22.0%, 10.3−19.8% and 10.8−23.2%, respectively. Accession PI639680 (*A. tuberculatus*) had the highest seed oil content (∼23%). Accessions of *A. esculentus* with high seed oil content included PI nos. PI274350 (21.5%), PI538082 (20.9%) and PI538097 (20.9%). Values for the three accessions of *A. manihot* with the highest seed oil content were PI nos. PI639673 (20.4%), PI639674 (20.9%) and PI639675 (21.9%), all representing var. *tetraphyllus*. Average percent seed oil in materials of *A. esculentus* from Turkey and Sudan (17.35% and 17.36%, respectively) exceeded the averages of materials from other locations. Ninety-eight accessions (total of six species) were also examined for fatty acid composition. Values of linoleic acid ranged from 23.6−50.65% in *A. esculentus*. However, mean linoleic acid concentrations were highest in *A. tuberculatus* and *A. ficulneus*. Concentrations of palmitic acid were significantly higher in *A. esculentus* (range of 10.3−36.35%) when compared to that of other species, and reached a maximum in PI489800 Concentrations of palmitic acid were also high in *A. caillei* (mean = ∼30%). Levels of oleic acid were highest in *A. manihot*, *A. manihot* var. *tetraphyllus* and *A. moschatus*.

KEYWORDS: *Abelmoschus* spp., okra, seed oil content, fatty acids, TD-NMR

INTRODUCTION

Members of the genus *Abelmoschus* Medik. are native to the subtropical and tropical areas of the world, with centers of diversity in the Indian subcontinent and southwestern Africa.[1] The genus was previously placed within *Hibiscus*;[2] however, subsequent taxonomic treatments considered it genetically distinct.[3] Currently, the taxonomic status of *Abelmoschus* is unclear as recent phylogenetic analyses have indicated that this genus is nested within a clade containing both *Hibiscus* and *Malvavisceae*.[4] However, as currently defined, the genus *Abelmoschus* contains nine species, five of which are cultivated: *A. esculentus* (L.) Moench, *A. caillei* (A. Chev.) Stevels, *A. manihot* (L.) Medik., *A. moschatus* Medik. and *A. tuberculatus* Pal & Singh.[1,5]

Abelmoschus esculentus (okra) is widely cultivated in the tropical and subtropical regions of the world.[1] In addition to the use of its immature pods as a vegetable, mature pods are sometimes used as animal feed and as a source of mucilage. Mature seeds are used for oil production and, when ground, as a substitute for coffee.[6] Various plant parts are also used as a thickening or sizing agent in confectionaries. Mays et al.[7] and Anwar et al.[8] have proposed the use of okra (*A. esculentus*) seed oil for fuel/biodiesel production. The crop is generally considered drought resistant and has fewer pests than soybean.[9,10] India currently ranks first in terms of okra production, and the US ranks 15th.[5]

Abelmoschus caillei (A. Chev.) Stevels is grown as a vegetable in the humid regions of Western and Central Africa, sometimes in association with *A. esculentus*.[1] *Abelmoschus manihot* is used as a source of edible foliage in Papua New Guinea and also for its medicinal properties.[11,12] The seeds of *A. moschatus* (musk mallow or ambrette) have a sweet heavy scent, and the oil from these seeds is of use in the perfumery industry, while other plant parts have various culinary, medical and commercial uses.[13,14]

Halverson and Naiman[15] determined the composition of several varieties of okra and concluded that they contained an average of 21.72% crude fat, 31.4% crude fiber and 27% crude protein. These values were similar to those obtained from cotton seed.[9] Trypsin activity has been reported in okra seed[16] as have gossypol-like compounds[17−19] and cyclopropenoid fatty acids.[19] Okra seed is rich in both protein and fat with high lysine content[16,18] and a protein solubility that generally exceeds that of commercial soy products.[20]

References pertaining to the physicochemical properties of okra seed oil and its potential as a supplement to or substitute for other seed-derived oils have occurred periodically. Early reports were made by Jamieson and Baughman,[21] Woodruff,[9] Edwards and Miller,[22] Markley and Dollear[23] and Clopton et al.[24] Okra seed oil is rich in palmitic, oleic, and linoleic acids.[25,26] Miller et al.[27] noted the similarities between okra, cotton, and peanut seed oils. Hussain and Dollear[28] found both hydraulic-pressed and solvent-extracted okra seed oil to be suitable substitutes for cotton seed oil. This led them and others to suggest breeding the crop for higher oil content.

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Although extensive genetic resources of this genus are known to exist[1,5,29] and varietal effects on chemical composition of okra seeds have been reported,[30] the literature contains relatively little information on the range of variation within individual *Abelmoschus* species for seed oil content or fatty acid composition. Oil content from pressed okra seed was reported as 15% by Jamieson and Baughman.[21] Miller[10] reported that the oil content of various okra cultivars and breeding lines averaged ~15% with a high of 19.2% (cv. Charlet). Mangual-Crespo and Martin[31] reported values that ranged from 19.8 to 24.3%. Martin and Rhodes[19] later examined oil content in seed of several lines in each of various *Abelmoschus* species including *A. esculentus*, *A. manihot*, *A. moschatus*, *A. tetraphyllus,* and several unclassified lines using wide-line NMR. These researchers noted that common US varieties of okra had higher oil content than either African varieties of *A. esculentus* or other *Abelmoschus* species, although oil content values for species other than *A. esculentus* were not provided.

In this study we investigated the oil content and fatty acid composition in seed of the five cultivated species of *Abelmoschus*, and also *A. crinitis Wall.* and *A. ficulneus* (L.) Wight & Arn.—wild relatives of the cultivated species—in a collection of *Abelmoschus* germplasm.

MATERIALS AND METHODS

Plant Material. All seeds used in this study were obtained from the USDA/ARS Plant Germplasm Collection in Griffin, GA.[32] Prior to analysis, all seeds (stored at −20 °C in foil pouches) were brought to room temperature for a minimum of 24 h.

Preparation of Oil Standards. Oil standards were prepared from six species including *A. caillei* (PI489936), *A. esculentus* (cv. Clemson Spineless), *A. ficulneus* (PI639668), *A. manihot* (PI379584), *A. moschatus* (PI496932), and *A. tuberculatus* (PI639677). For each of these, 200 g of dried seeds were ground using a coffee bean mill (Black & Decker, CBM205; medium setting) and the powder was transferred to a 1 L round-bottom flask. To the flask was added sufficient hexanes (Acros Organics) to bring the volume of the mixture to ca. 500 mL. The flask was sealed and transferred to a rotary shaker (Thermolyne AROS 160), and the contents were mixed for 24 h. The mixture was then allowed to settle for several hours and then twice vacuum filtered through filter paper (P5, Fisher Scientific, Atlanta, GA). The filtrate was concentrated by rotary evaporation, yielding bright yellow oil. Yields were typically 10−12% oil by seed weight.

TD-NMR Analysis. Seed oil and moisture measurements were carried out by TD-NMR essentially as described by AOCS[33−35] and Krygsman and Barrett,[36] on a Bruker mq10 minispec NMR analyzer operating at a resonance frequency of 9.95 MHz and maintained at 40 °C. For each signal acquisition, spin−echo parameters consisted of a 90° pulse of 10.44 μs and reading at 50 μs followed by a 180° pulse of 21.38 μs (pulse spacing = variable) and reading at 7 ms. A 2 s recycle delay between scans was used, and a total of 16 scans were collected for each sample. Bulk seed measurements were made in a 40 mm glass sample tube, and NMR signals were compared to oil and moisture calibration curves, generated by sample weight. All samples were measured in triplicate, and the results were averaged. Oil standards were generated using extracted oils. For each oil, twelve standards were prepared by oil weight. To prepare a standard tube, shredded paper was added into the tube along with a carefully measured mass of oil. Moisture standards were prepared using okra seeds of known moisture content and calculating the mass of water present in different amounts of seeds. Moisture content was predetermined by measuring the difference in mass of seeds before and after baking at 130 °C for 3 h.

All NMR oil analyses were conducted in triplicate using separate seed samples drawn from the available inventory. Seeds were drawn from the 01 (first regeneration) inventory of each accession, unless noted otherwise. Seed samples were analyzed and oil content was calculated by comparison with a standard curve constructed using oil from the respective species. An exception was *A. crinitis* (PI592390) as insufficient seeds were available for oil extraction and construction of a standard curve of this species. Oil content of PI592390 was calculated using the standard curve for *A. esculentus*.

Isolation and Analysis of Fatty Acids. For isolation of fatty acids, replicate twenty seed samples were ground to a fine powder in a coffee bean mill. Approximately 150 mg of ground powder was transferred into a 16 × 100 mm test tube, and 5.0 mL of *n*-heptane (Fisher Scientific) was added to extract the oil. For conversion of fatty acids to methyl esters, 500 μL of 0.5 M sodium methoxide in methanol solution (Sigma-Aldrich, St. Louis, MO) was added to the test tube and mixed with the sample. The reaction was allowed to proceed for 2 h. Seven milliliters of distilled water was then added to separate the organic layer from the aqueous layer and residue (45 min). An aliquot of the organic layer (1.5 mL) containing the methyl esters was transferred to a 2.0 mL autosampler vial for gas chromatography (GC) analysis.

The fatty acid composition was determined using a Hewlett-Packard (HP) 5890 Series II GC equipped with a flame ionization detector (FID) and an HP-7673 autosampler. A fatty acid methyl ester (FAME) standard mix RM-3 (Sigma-Aldrich) was used to establish peak retention times for myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), gamma-linolenic (18:3γ), alpha-linolenic (18:3α), arachidic (20:0) and behenic (22:0) acids. The peak separation was performed on a DB-225 capillary column (15 m × 0.25 mm i.d. with a 0.25 μm film) from Agilent Technologies. The carrier gas was helium set to a flow rate of ~1.0 mL/min. One microliter of sample was injected onto the column maintained isothermally at 200 °C, with an injection temperature of 280 °C and a detection temperature of 300 °C. The total run time for each sample was 12 min. Fatty acid composition was determined by identifying and calculating relative peak areas.

Statistical Analysis. A Pearson's coefficient analysis was performed to determine significant correlations. An analysis of variance was performed on the data, and means were separated using Tukey's multiple comparison procedure. Data were analyzed using SigmaPlot 11.2 and SAS.

RESULTS AND DISCUSSION

Time domain NMR is routinely used for the analysis of oil content in vegetable seed and also in other materials.[36−40] In a preliminary study (data not shown) we observed small but occasionally significant differences in percent seed oil content when test samples were analyzed against standard curves derived from oil extracted from other *Abelmoschus* species. These differences were generally less than 2% but occasionally exceeded 5%. Differences were always largest when oil of *A. esculentus* was used as the calibration standard against seed samples of other species.

Seed oil content values for all species (other than *A. esculentus*) were highest when using *A. esculentus* as the standard, when compared to values obtained from the use of a standard of the same or another species. Based on the preliminary results, and in keeping with recommendations that NMR oil calibration reference samples be of the same species as the test samples,[36] we adopted this practice (with the exception of *A. crinitis*) in subsequent analyses.

The descriptive statistical data on the seed oil content for the seven *Abelmoschus* species included in this study are presented in Table 1. Values for *A. esculentus* were similar to those previously

Table 1. Descriptive Statistics for Percent Oil Content in Seed of Seven *Abelmoschus* Species

species	*n*	mean	SD	range	min	max	median
A. caillei	156	12.56	1.35	8.15	8.17	16.33	12.68
A. crinitis	1	12.19					
A. esculentus	1100	16.57	1.66	12.36	9.19	21.56	16.70
A. ficulneus	5	14.01	2.86	6.52	10.20	16.72	14.55
A. manihot	310	11.56	1.96	16.14	5.85	21.99	11.52
A. moschatus	25	14.49	2.46	10.31	9.47	19.78	14.94
A. tuberculatus	5	15.70	4.28	10.77	12.40	23.17	14.07

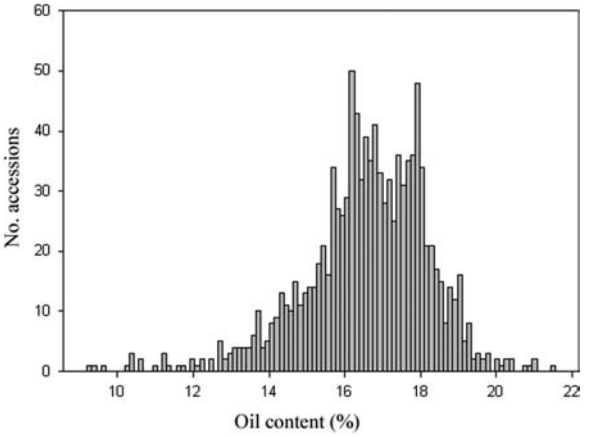

Figure 1. Distribution of seed oil content values in 1100 accessions of *A. esculentus* as determined by TD-NMR.

reported in the literature. Mangual-Crespo and Martin[31] noted that the percent seed oil content of four commercial cultivars grown in Puerto Rico ranged from 19.8 to 24.3%. These values were, on average, higher than those reported subsequently. Martin and Rhodes,[19] using wide-line NMR, examined seed oil content in 77 accessions of *A. esculentus*, one accession each of *A. manihot*, *A. moschatus*, *A. moschatus* var. *tetraphyllus*, and a total of 15 accessions that were unclassified. They noted that the seed of 10 common US okra cultivars had an average oil content of 16.9% and that this was higher than accessions from African countries or other *Abelmoschus* species. Andras et al.[41] reported percent seed oil content values (*A. esculentus*) ranging from 15.9 to 20.7%.

The means for percent seed oil content of *A. esculentus* and *A. tuberculatus* were higher than those of the other *Abelmoschus* species (Table 1). However, sample sizes varied widely and the range of seed oil content for *Abelmoschus* species (including *esculentus*) may well extend beyond those determined in this study. The relatively high oil content in seed of *A. tuberculatus* (PI639680, >23%) suggests that this species may bear further investigation. *Abelmoschus tuberculatus* differs from *A. esculentus* primarily in its shorter capsules (5−7.5 cm long) that are covered with bristly tuberculate hairs.[29] This species is found in the northern and northwestern regions of India[5] and hybridizes with *A. esculentus*.[42] It is believed to be closely related to[5] and/or an ancestral form of[1] *A. esculentus*.[43]

The distributions of the values for percent seed oil content in the analyzed accessions of *A. esculentus* and *A. manihot* are presented in Figures 1 and 2. Neither distribution is normal.

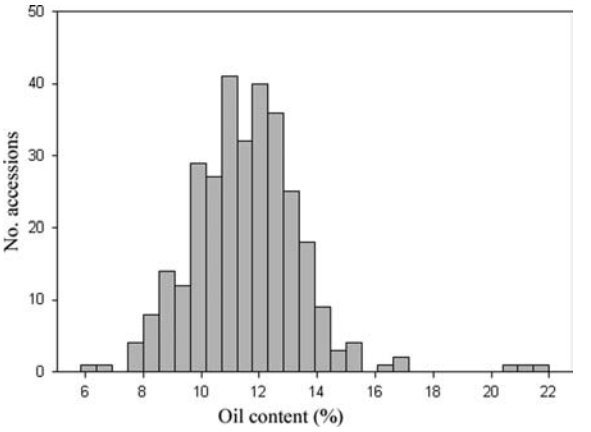

Figure 2. Distribution of seed oil content values in 310 accessions of *A. manihot* as determined by TD-NMR.

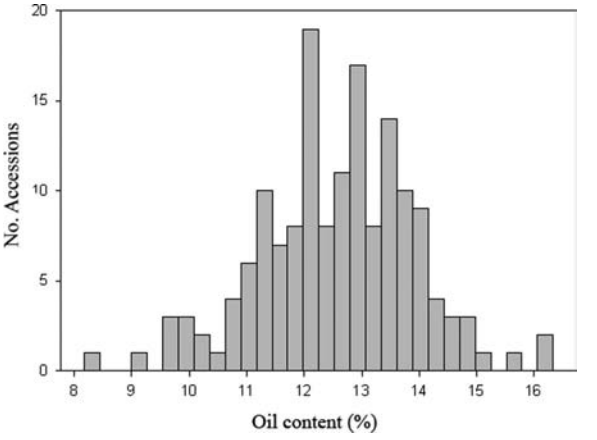

Figure 3. Distribution of seed oil content values in 156 accessions of *A. caillei* as determined by TD-NMR.

Table 2. Statistics of Percent Oil Content in *Abelmoschus esculentus* from Eight African Countries[a]

country	*n*	mean	SD	range	min	max	median
Benin	136	15.85	1.69	8.99	9.36	18.35	16.15
Burkina Faso	116	16.86	1.61	9.91	10.39	20.30	16.85
Côte d'Ivoire	65	15.12	1.87	9.27	9.20	18.47	15.13
Sudan	77	17.36	1.59	6.33	13.86	20.19	17.50
Togo	97	15.97	1.43	7.81	11.22	19.03	16.24
Turkey	110	17.35	0.97	5.06	14.14	19.20	17.47
Zambia	47	16.30	1.16	4.83	13.74	18.57	16.45
Zimbabwe	66	15.58	1.14	4.79	12.47	17.26	15.83

[a] Values represent the mean ± the standard deviation (SD) of three replicate analyses.

Values for the three accessions of *A. esculentus* with the highest percent seed oil content were PI nos. PI274350 (21.5%), PI538082 (20.9%) and PI538097 (20.9%), from Sudan. The accession of *A. manihot* with the highest percent seed oil content was PI497126 (21.9%), from Nigeria. Three of the five accessions of *A. manihot* with the highest percent seed oil content represent examples of *A. manihot* var. *tetraphyllus* (Roxb. ex Hornem.)

Table 3. Fatty Acid Composition of Seed of Six *Abelmoschus* Species as Determined by Gas Chromatography[a]

	fatty acid[b]									
taxon (*Abelmoschus*)	14:0	16:0	16:1	18:0	18:1	18.2	18:3γ	18:3α	20:0	22:0
A. esculentus	0.3115 a	30.42 a	0.3967 b	3.9312 a	21.085 bc	37.782 bc	0 f	0.1775 c	0.4944 a	0.2575 a
A. caillei	0.3617 a	29.97 ab	0.4700 ab	3.7400 a	25.622 ab	31.625 cd	0.2600 e	0.22667 c	0.3800 b	0.2817 a
A. ficulneus	0.1367 b	24.44 cd	0.6167 a	3.2517 ab	18.753 c	48.537 a	0.43 c	0.4333 a	0.3300 bc	0.1633 bc
A. manihot	0.3000 a	27.01 bc	0.5725 a	3.8175 a	31.885 a	27.108 d	0.325 d	0.265 bc	0.4100 ab	0.2775 a
var.*tetraphyllus*	0.1125 b	21.10 e	0.4833 ab	2.7325 b	28.073 a	42.640 ab	0.535 b	0.3800 ab	0.2300 d	0.1525 c
A. moschatus	0.1483 b	19.840 e	0.4917 ab	3.5817 ab	30.37 a	40.188 abc	0.8433 a	0.3867 ab	0.2800 cd	0.2217 ab
A. tuberculatus	0.1100 b	21.72 de	0.4833 ab	2.7617 b	21.205 bc	49.638 a	0.4650 c	0.24167 c	0.2017 d	0.1267 c

[a] Values represent the means of three replicate analyses. Means within columns followed by the same letter are not significantly different from one another as determined by Tukey's multiple comparison procedure ($P < 0.05$). [b] Identification of fatty acids: 14:0, myristic acid; 16:0, palmitic acid; 18:0, stearic acid; 20:0, arachidic acid; 22:0, behenic acid; 16:1, palmitoleic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3γ, gamma-linolenic acid; 18:3α, alpha-linolenic acid.

Borss. Waalk, one of two varieties recognized within *A. manihot*. These were PI nos. PI639673 (20.4%), PI639674 (20.9%) and PI639675 (21.9%) and originated in the Indian states of Gujarat (PI639673), Haryans (PI639674) and Uttar Pradesh (PI639675).

The literature contains little information on variability for seed oil content in *A. manihot*, but the data presented here suggest that var. *tetraphyllus* is clearly separated from other accessions of *A. manihot*, in that respect. *Abelmoschus manihot* var. *tetraphyllus* has been used in the improvement of *A. esculentus*.[44] Velayudhan et al.[45] reported the occurrence of populations of *A. manihot* var. *tetraphyllus* in Karnataka (northeastern India) growing in association with cultivated *A. esculentus*. *A. manihot* var. *tetraphyllus* is morphologically similar to *A. caillei*.[46]

The range of percent seed oil content in the accessions of *A. caillei* is presented in Table 1 and Figure 3. Accessions with the highest percent seed oil content included PI nos. PI489974 (15.7%), PI489975 (16.1%) and PI489977 (16.3%). The distribution of this species is considered limited to western and central Africa.[5] Hamon[47] observed that *A. caillei* shared a number of characteristics with *A. manihot* that has led to the suggestion that this species (*A. caillei*) is a natural amphidiploid of *A. esculentus* and *A. manihot*.[5] All accessions of *A. caillei* included in this study originated in Côte d'Ivoire, within the center of diversity for this species.[1]

As indicated in Table 2, higher mean oil content values were associated with seed from Turkey and Sudan. Higher values for the ranges in seed oil content were associated with seed originating from Burkina Faso and Côte d'Ivoire. Percent seed oil in materials from Turkey and Sudan (17.35% and 17.36%, respectively) exceeded the average of the 10 US okra cultivars reported by Martin and Rhodes.[19] Of note in efforts to identify *Abelmoschus* genetic resources with high oil content seed is the potential of *A. manihot* var. tetraphyllus and *A. tuberculatus*.

Our data indicate, in agreement with previously published reports, that the predominant fatty acid in okra is species dependent (Table 3). Camciuc et al.[48] and Ndangui et al.[49] reported that linoleic acid was the predominant fatty acid in *A. esculentus*. They (Camciuc et al.[48]) reported values of 50.1−51.1% linoleic acid in oil of five varieties of *A. esculentus* whereas Ndangui et al.[49] reported values of 42−43%. Values observed in the present study ranged from 23.6 to 50.65% (mean = 37.7%), indicating that the values reported by Camciuc are likely near the upper limit for *A. esculentus*. Although linoleic acid was the predominant fatty acid in *A. esculentus*, levels of linoleic acid were significantly higher in oil of *A. tuberculatus* (mean = 49.6%) and *A. ficulneus* (mean = 48.5%). Sinha and Osman[50] reported ~10% linoleic acid in a specimen of *A. ficulneus* (referred to as *Hibiscus ficulneus*).

Palmitic acid has commercial value as a raw material for soaps, esters and plasticizers and as a means of improving the quality of soybean oil.[49] Concentrations of palmitic acid in cultivated lines of *A. esculentus*, as reported by Camciuc et al.[48] and Ndangui et al.,[49] averaged ~26%. In the present study (Table 3), levels of palmitic acid were significantly higher in oil of *A. esculentus* (range of 10.3−36.35%) when compared to that of other species, and reached a maximum in PI489800. Concentrations of palmitic acid were also high in samples of A. *caillei* (mean = ~30%). In contrast to levels of palmitic acid, concentrations of oleic acid were significantly higher in samples of *A. manihot*, *A. manihot* var. *tetraphyllus* and *A. moschatus*. Low concentrations of other fatty acids were also present in the samples analyzed (Table 3). No significant correlations were found between percent oil content and levels of specific fatty acids.

Individual studies on *Abelmoschus* seed composition have typically included one to several varieties. The results then presented are assumed to be representative of the crop or species as a whole, when, in fact, they oftentimes are not. A large number of *A. esculentus* lines were examined in the present study, and the values presented may approximate the ranges that occur within this species. However, a smaller number of samples were available for some species (i.e., *A. crinitis*, *A. ficulneus* and *A. tuberculatus*), while others were collected over a limited geographical area. Hence, it is not known to what extent the values presented for them accurately represent the extremes that occur within those taxa for the compounds examined. Knowledge of the range of the individual components in okra seed serves to identify novel sources of variability for agriculturally and economically important characteristics, promotes further investigation into the potential of exploiting naturally existing variation, and is suggestive of the extent to which the concentrations of individual constituents might be enhanced or otherwise manipulated as a prelude to utilization.

Limited or no plant material of many *Abelmoschus* taxa [i.e., *A. angulosus* Wall. ex Wight & Arn., *A. manihot* var. *pungens* (Roxb.) Hochr., and *A. moschatus* var. *tuberosus* (Span.) Borss. Waalk.] was available for this study from any internationally accessible genebank despite repeated calls for additional *Abelmoschus* collection and conservation efforts.[45,46] The information presented

here will inspire greater appreciation for the range of oil content and fatty acid composition within the *Abelmoschus* genepool and will serve to promote the conservation, utilization, and further study of *Abelmoschus* genetic resources.

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