

Survey of the Fatty Acid Composition of Peanut (*Arachis hypogaea*) Germplasm and Characterization of Their Epoxy and Eicosenoic Acids

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ABSTRACT: Peanut (*Arachis hypogaea*) plant introductions (732) were analyzed for fatty acid composition. Palmitate varied from 8.2 to 15.1%, stearate 1.1 to 7.2%, oleate 31.5 to 60.2%, linoleate 19.9 to 45.4%, arachidate 0.8 to 3.2%, eicosenoate 0.6 to 2.6%, behenate 1.8 to 5.4%, and lignocerate 0.5 to 2.5%. The eicosenoate was shown to be *cis*-11-eicosenoate. In addition, epoxy fatty acids were found in many plant introductions in percentages ranging as high as 2.5%. These were tentatively identified as chiefly 9,10-epoxy stearate and coronarate with smaller amounts of vernolate. The percentage of palmitate was shown to be correlated positively with linoleate and negatively with oleate, eicosenoate, and lignocerate. Stearate was highly correlated with arachidate and negatively with eicosenoate and lignocerate. Oleate and linoleate, the two major fatty acids, were negatively correlated. Arachidate was negatively correlated with eicosenoate, and eicosenoate was positively correlated with lignocerate. Behenate and lignocerate were positively correlated. Epoxy esters were positively correlated with palmitate and negatively with oleate. Segregation of the plant introductions by axis flower, growth habit, and pod types showed significant differences that reflected the same fatty acid groupings revealed by the correlations.

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KEY WORDS: *Arachis hypogaea*, *cis*-11-eicosenoic acid, *cis*-9,10-epoxystearic acid, coronaric acid, epoxy fatty acids, fatty acid composition, peanut, vernolic acid.

Peanuts are known to contain significant amounts of C₂₀, C₂₂, and C₂₄ saturated fatty acids (1,2), and it has been suggested that the presence of these fatty acids on the *sn*-3 position of glycerol might account for the unusually high atherogenicity of peanut oil when it is fed along with cholesterol (3–7). The dietary significance of this finding in humans has been questioned by Alderson *et al.* (8), but we wished to explore the possibility of breeding peanuts with reduced concentrations of the long-chain fatty acids. To this end, we analyzed the fatty acid composition of a core group of peanut plant intro-

ductions that had been selected to represent the variance in the peanut germplasm collection (9). Information available in the literature on peanut fatty acid composition has been reviewed by White (1) and Young (2). Treadwell *et al.* (10) analyzed 40 peanut cultivars and found the following percentages: palmitate 7.54–11.90, stearate 1.46–3.26, oleate 39.29–56.56, linoleate 25.95–38.90, arachidate 1.02–1.63, eicosenoate 0.89–1.74, behenate 2.39–4.04, and lignocerate 1.21–2.52. Norden *et al.* (11) reported on percentages in 228 Florida lines: palmitate 6.63–12.87, stearate 1.69–4.88, oleate 36.72–79.91, linoleate 2.14–43.14, arachidate 1.03–2.05, eicosenoate 0.34–1.90, behenate 1.30–4.82, and lignocerate 1.05–2.46. The high-oleate trait found in the Florida collection has been shown to be controlled by two recessive genes, one of which occurs commonly in peanut germplasm (12–14). Fatty acid composition data on seven runner-type cultivars (15) and crosses of a high-oleic variety with five Virginia-type peanuts (14) fall within these ranges.

MATERIALS AND METHODS

Peanut varieties in the core collection described by Holbrook *et al.* (9) were obtained from 1995 production in Griffin, Georgia. This collection contains representatives from the botanical divisions of *Arachis hypogaea* and the United States market classes. The 732 lines in this collection were classified independently for flower, habit, and pod types by the criterion described by Pittman (16). Fatty acid composition was determined by the method outlined by Hammond (17). Samples of five peanuts were cut in half and crushed using a hydraulic press. The crushed peanuts were extracted with hexane for 4 h, which was demonstrated to be sufficient time to give a representative sample of peanut lipid. The lipid was converted to methyl esters with sodium methoxide in methanol, and the methyl esters were analyzed using a Hewlett-Packard 5890 gas chromatograph (Avondale, PA) with a hydrogen flame detector and a 15-M DB-23 column (J&W Scientific, Folsom, CA). The gas chromatographic peak areas were corrected for hydrogen flame detector response. Histograms and correlations were prepared with a Microsoft Excel 5.0 program (Frontline Systems; Incline Vil-

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lage, NV), and analysis of variance and mean comparisons by least significant difference were done with an SAS 6.09 program (SAS Institute, Cary, NC).

Methyl *cis*-11-eicosenoate was purchased from Sigma (St. Louis, MO). To locate the double bond, oil was extracted from five half-peanuts and converted to methyl esters by heating to 55°C with 2% sulfuric acid in methanol overnight. The methyl esters were separated into saturates, monoenoates, and dienoates by chromatography on a 20 × 20-cm thin-layer silver-ion plate produced by spraying a 500- μ m silica gel plate (Absorbosil plus 1; Alltech, Deerfield, IL) with 10 mL of 30% aqueous silver nitrate solution and activating at 80°C. The plate was developed with hexane/diethyl ether 80:20 (vol/vol), and the methyl ester bands were detected by spraying with a 0.1% solution of 2',7'-dichlorofluorescein in methanol and viewing under ultraviolet light. The *cis*-monoenoate band was recovered from the plate and streaked on a 10 × 10 cm 200- μ m C-18 reverse-phase silica gel plate containing a fluorescent indicator (Sigma, St. Louis, MO). The plate was developed with ethanol/water 95:5 (vol/vol), and the area below the methyl oleate band was recovered and eluted. Ozonolysis was carried out according to Hammond (18), and gas chromatography/mass spectroscopy (GC/MS) of the products was done with a 5890 Hewlett-Packard gas chromatograph fitted with a 30-M SE-30 column (J&W Scientific) and a 5970 mass selective detector.

Methyl *cis*- and *trans*-9,10-epoxystearate were purchased from Sigma. To identify epoxy fatty acid methyl esters, oil was extracted from five half-peanuts and converted to methyl esters with sodium methoxide in methanol. The epoxy esters were separated from other esters by streaking the sample on a 500- μ m thin-layer plate of silica gel G and developing with hexane/diethyl ether 85:15, vol/vol. Bands were visualized by spraying with a 0.1% solution of 2',7'-dichlorofluorescein in methanol and eluted with diethyl ether. Methyl vernolate, prepared by transesterification with sodium methoxide/methanol of lipid extracted from *Vernonia galamensis* seed, was used as a standard to identify the epoxy ester band. Epoxy fatty esters were converted to the corresponding dihydroxy derivatives by reaction with glacial acetic acid overnight at ambient temperature and then heated to 75°C for 4 h. The product was dissolved in diethyl ether and washed with water to remove acetic acid; and the residue after evaporation of the ether was treated with excess 1 N methanolic sodium methoxide to deacetylate the product. After 45 min, the reaction mixture was neutralized with concentrated hydrochloric acid, and the precipitated salt was removed. To obtain *tert*-butyldimethylsilyl ethers, the methanol was evaporated and the residue treated with *tert*-butyldimethylsilylimidazole for 10 min at 100°C. GC/MS of the silyl ethers was performed as before.

RESULTS AND DISCUSSION

Figure 1 shows the distribution of the individual fatty acids in the peanut samples. Palmitate (1A) and stearate (1B) show a

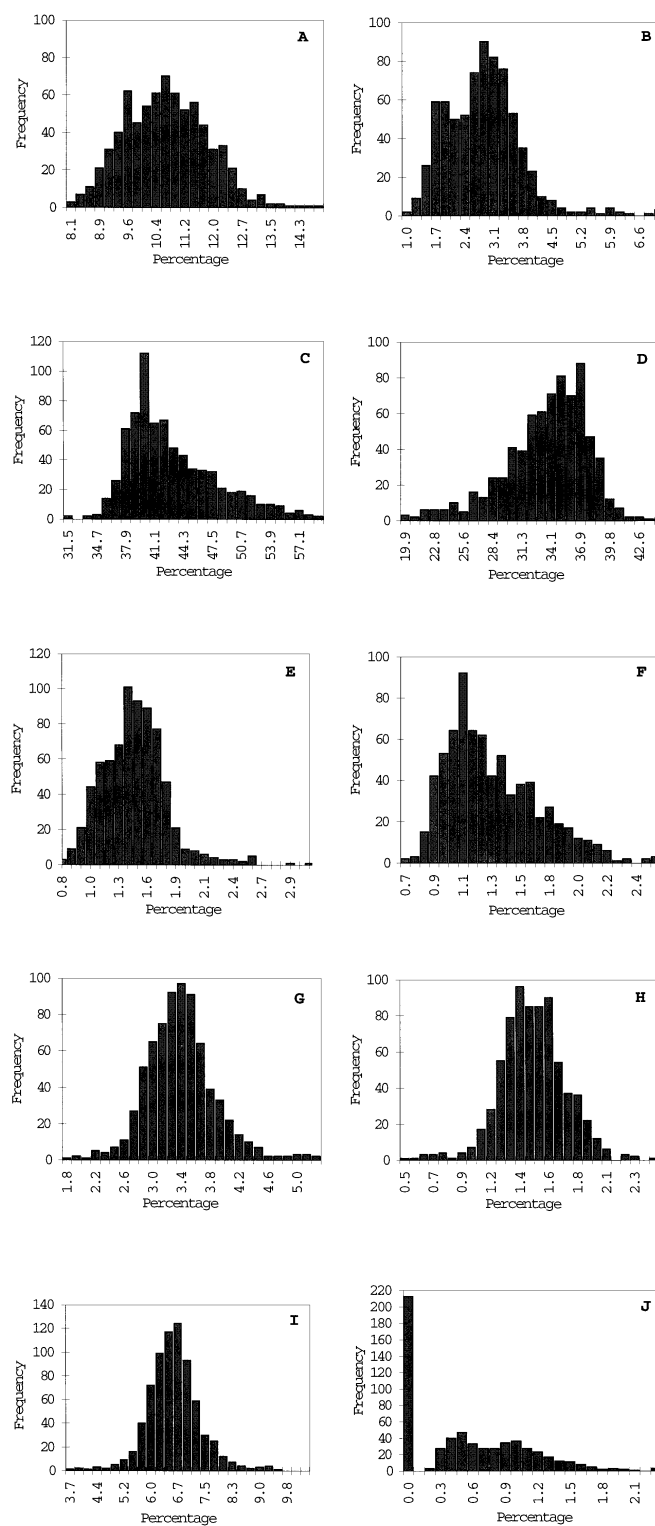


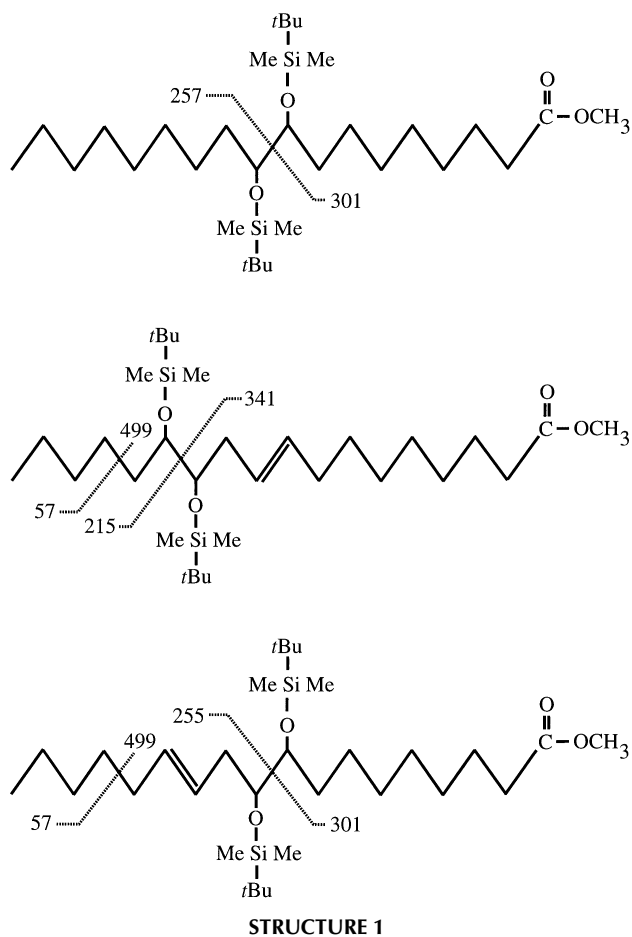
FIG. 1. Frequency distribution of various methyl ester percentages in the core peanut variety collection: A, palmitate; B, stearate; C, oleate; D, linoleate; E, arachidate; F, eicosenoate; G, behenate; H, lignocerate; I, long-chain saturates (20:0 + 22:0 + 24:0); J, epoxy esters.

tendency toward bimodal distributions. Oleate (1C) is skewed toward high values and linoleate (1D) to low values. Arachidate (1E) and eicosenoate (1F) are skewed to high values, but behenate (1G) and lignocerate (1H) are distributed normally. The total long-chain saturate (1I) consisting of arachidate, behenate, and lignocerate is normally distributed and ranged from 3.7 to 10.2% with the mode at 6.5%. These results suggest it should be possible to obtain oils with a range of long-chain acyl group concentrations to verify that the atherogenic effect observed for peanut oil is caused by these acids. The ranges for fatty acid percentages found in the 732 lines included in this study exceeded the ranges reported for smaller sample numbers by previous investigators (10,13–15) except for that of Norden *et al.* (11) where lines with high oleate and low linoleate exceeded the values found in this core collection (9). The lowest values reported for palmitate and eicosenoate by Norden *et al.* (11) also were lower than those found in this study.

The position of the double bond in the eicosenoate of peanuts has not been reported, and some tables of fat composition suggest that it is gadoleate, *cis*-9-eicosenoic acid (for example, see 1) although *cis*-11-eicosenoate, or gondoate, is much more common in plant sources. A concentrate of the *cis*-eicosenoate was isolated by silver-ion chromatography and reverse-phase chromatography from peanut variety PI 158854, which contained about 2.6% of this ester. After ozonolysis of the concentrate and reduction of the ozonides with triphenylphosphine, the scission products were examined by GC/MS. The results showed that the major contaminant was oleate, which yielded nonanal and methyl 9-oxononanoate. Nonanal was the only simple aldehyde present, and methyl 11-oxoundecanoate was also identified by comparing its retention time and mass spectrum with that of authentic methyl 11-oxoundecanoate generated by ozonolysis of an authentic sample of methyl *cis*-11-eicosenoate [mass spectrum 55 (85), 59 (40), 69 (41), 74 (100), 87 (56), 98 (23), 111 (10), 139 (26), 143 (12), 171 (17), 186 (7)]. Thus, the eicosenoate is gondoate, and gadoleate is not present.

In addition, many of the peanut plant introductions contained a component that migrated just before lignocerate on the DB-23 column. A variety, PI 386350 from Argentina, rich in this component was selected for further study. GC/MS with an SE-30 column suggested that the unknown peak contained at least two components, one of which had the mass spectrum of methyl 9,10-epoxy stearate. Methyl *cis*-9,10-epoxy stearate was shown to have the same retention time on DB-23 and SE-30 columns. Isolation of the methyl epoxy esters, conversion of the epoxy group to dihydroxy, and formation of the *tert*-butyldimethylsilyl ethers gave mass spectral data that allowed for better identification of the epoxy esters. Major ions were obtained by cleavage between the adjacent silyl ester groups (Structure 1).

Ions of mass 301 and 257 confirmed the presence of methyl 9,10-epoxy stearate. A component emerging from the SE-30 column just before methyl 9,10-epoxy stearate had masses of 301 and 255, suggesting that it was a 9,10-epoxy



octadecenoate with a double bond in the terminal half of the molecule. This component was tentatively identified as methyl coronarate, methyl *cis*-9,10-epoxy octadec-12-enoate. A component emerging just after methyl 9,10-epoxy stearate had major ions at 341 and 215, consistent with a 12,13-epoxy octadecenoate having a double bond in the ester end of the molecule. This component was tentatively identified as methyl vernolate, methyl *cis*-12,13-epoxy octadec-9-enoate. The ratio of coronarate to epoxy stearate to vernolate was estimated at 44:46:10 on the basis of the mass spectral data. The nutritional significance of the epoxy fatty acids in some peanut varieties is not clear. Maity and Mandal (19) attributed the poor growth response of rats fed *Acacia arabica* oil to its content of epoxy- and hydroxy-fatty acids.

Figure 1J shows the distribution of epoxy fatty acids in the peanut varieties. Many of the peanut introductions had less than about 0.1% epoxy ester and failed to integrate in our GC program. Relatively few of the chromatograph traces showed no ripple at the retention time of epoxy ester. Some varieties, particularly South American varieties, had up to 2.5% epoxy ester. Epoxy fatty acids also have been reported in sunflower (20) and soybean (21) varieties. It has been unclear from earlier work whether variation in epoxy acids arose from autoxidation of oleate and linoleate, genetic variation, or environmental influences. Because our samples were all grown in the

TABLE 1
Methyl Ester Percentages of Peanut Varieties with Extreme Compositions^a

| Plant introduction number | 16:0 | 18:0 | 18:1 | 18:2 | 20:0 | 20:1 | 22:0 | 24:0 | Epoxy |
|---------------------------|-------------|------------|-------------|-------------|------------|------------|------------|------------|------------|
| 269067 | 8.2 | 2.4 | 54.1 | 28.0 | 1.2 | 1.7 | 2.8 | 1.7 | — |
| 280688 ^b | 15.1 | 2.4 | 36.5 | 39.2 | 1.2 | 1.1 | 2.9 | 1.3 | 0.3 |
| 501272 ^c | 11.8 | 1.1 | 31.5 | 45.4 | 0.8 | 2.6 | 3.7 | 2.3 | 0.9 |
| 259639 | 9.6 | 7.2 | 33.8 | 36.9 | 3.2 | 0.9 | 5.3 | 2.0 | 1.0 |
| 288178 | 9.3 | 3.0 | 60.3 | 19.9 | 1.5 | 1.3 | 2.8 | 1.6 | 0.4 |
| 313171 | 11.3 | 6.0 | 39.5 | 35.6 | 2.3 | 0.7 | 3.6 | 1.1 | — |
| 268975 | 9.3 | 1.5 | 42.4 | 37.0 | 1.0 | 2.7 | 4.0 | 2.2 | — |
| 371521 | 9.6 | 3.8 | 58.5 | 23.1 | 1.4 | 1.1 | 1.8 | 0.7 | — |
| 494795 | 9.7 | 3.3 | 43.3 | 33.1 | 1.7 | 1.8 | 5.4 | 1.8 | — |
| 268714 | 14.4 | 3.4 | 42.3 | 35.4 | 1.2 | 0.9 | 2.0 | 0.5 | — |
| 339973 | 9.7 | 2.7 | 43.0 | 33.8 | 1.6 | 1.9 | 4.8 | 2.5 | — |
| 386350 | 13.5 | 3.2 | 38.4 | 34.9 | 1.6 | 1.1 | 3.6 | 1.3 | 2.5 |

^aExtreme values for the peanut plant introductions are in boldface.

^bVariety *hirsuta*.

^cVariety *hypogaea*.

same environment and were relatively fresh, we believe that the variation in epoxy fatty acids arises from genetic variation. Like most fatty acids, the amounts undoubtedly would be affected by growth environment (10,11).

Table 1 shows the fatty acid composition of peanut introductions that are extremely high or low in one or more fatty acyl group. It is not practical to publish the fatty acid composition of all 732 plant introductions examined, but the authors will make these data available on request.

Table 2 shows correlations among the fatty acyl groups. These correlations may reflect precursor–product relations in some instances but probably also reflect genetic linkages of various enzymes involved in the conversions. The strong negative correlation between oleate and linoleate results from their being the chief acyl groups in the oil so that one cannot increase much without a decrease in the other. All the saturated acyl groups are correlated with the saturated acyl group containing two more carbons, reflecting a precursor–product relation, but it is not clear why the correlation between stearate and arachidate is stronger than the others. Lignocerate is negatively correlated with palmitate, stearate and arachidate, whereas behenate is positively correlated with arachidate and lignocerate. Also oleate is negatively correlated with all the saturated acyl groups except lignocerate. Linoleate, on the other hand, is positively correlated with palmitate and behenate. The epoxy esters are positively correlated with palmitate and linoleate and negatively with oleate

and eicosenoate.

Many of the plant introductions in the core peanut collection have been classified for the presence or absence of flowers on the main axis, plant growth habit, and pod type. Classification for each trait was independent of the other classifications. These traits are illustrated in *United States Peanut Descriptors* published by the Agricultural Research Service (15). Where possible, the plant introductions were separated into these classes, their fatty acid compositions were compared, and the results are shown in Table 3. Many of these groupings had fatty acid compositions that were significantly different, and in general they showed the same relations among fatty acids noted in the correlations of Table 2. For example, there is a tendency for plants with erect habits to have greater saturate (except lignocerate), linoleate, and epoxy percentages and less oleate and eicosenoate than those with spreading habits. Similar differences occur when plants with *vulgaris* pod types are compared with those with *hypogaea* pod types. This classification also partially accounts for the bimodal distribution of palmitate and stearate seen in Figures 1A and 1B. The lower mode for these acyl groups was dominant in lines with class 2 growth habits (spreading) and pod class 4 (*hypogaea*).

TABLE 2
Correlations Among the Fatty Acids of 732 Peanut Plant Introductions^a

| | 16:0 | 18:0 | 18:1 | 18:2 | 20:0 | 20:1 | 22:0 | 24:0 |
|-------|-------|-------|-------|-------|-------|-------|------|-------|
| 18:0 | 0.19 | — | | | | | | |
| 18:1 | -0.58 | -0.18 | — | | | | | |
| 18:2 | 0.40 | -0.06 | -0.93 | — | | | | |
| 20:0 | 0.13 | 0.94 | -0.21 | -0.04 | — | | | |
| 20:1 | -0.49 | -0.79 | 0.19 | 0.01 | -0.71 | — | | |
| 22:0 | 0.06 | 0.22 | -0.37 | 0.20 | 0.39 | 0.10 | — | |
| 24:0 | -0.45 | -0.45 | 0.07 | -0.01 | -0.30 | 0.73 | 0.41 | — |
| Epoxy | 0.34 | 0.07 | -0.37 | 0.21 | 0.10 | -0.16 | 0.10 | -0.04 |

^aCorrelation coefficients >0.1 are significantly different from zero.

TABLE 3
Comparison of the Fatty Acid Composition of Peanut Plant Introductions (PI) Segregated According to Plant Growth Traits

| Main traits | Class | Number | | | | | | | | | | |
|---------------------|-------|--------|--------------------|-------|-------|-------|------|-------|-------|-------|-------|--|
| | | of PI | 16:0 | 18:0 | 18:1 | 18:2 | 20:0 | 20:1 | 22:0 | 24:0 | Epoxy | |
| Axis flowers | | | | | | | | | | | | |
| No | 1 | 479 | 10.9B ^a | 3.0A | 43.5A | 34.1B | 1.5A | 1.4A | 3.5B | 1.5A | 0.7A | |
| Yes | 2 | 117 | 11.2A | 3.0A | 41.7A | 35.5A | 1.5A | 1.3A | 3.6A | 1.5A | 0.7A | |
| LSD | | | 0.2 | 0.2 | 1.0 | 0.8 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | |
| Habit | | | | | | | | | | | | |
| Spreading | 2 | 105 | 10.5B | 2.6B | 46.6 | 32.1C | 1.4B | 1.5A | 3.4B | 1.6A | 0.4B | |
| Spreading and bunch | 3 | 231 | 11.0A | 3.0A | 42.8B | 34.7B | 1.5A | 1.4B | 3.5A | 1.6A | 0.7A | |
| Bunch | 4 | 238 | 11.0A | 3.1A | 43.4B | 34.1B | 1.6A | 1.3BC | 3.4AB | 1.5B | 0.6A | |
| Erect | 5 | 80 | 11.1A | 3.1A | 41.6C | 35.7A | 1.6A | 1.3C | 3.5A | 1.5B | 0.7A | |
| LSD | | | 0.3 | 0.2 | 1.1 | 1.03 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | |
| Pod type | | | | | | | | | | | | |
| Vulgaris | 1 | 158 | 11.8A | 3.5A | 41.4A | 35.0A | 1.7A | 1.1D | 3.6A | 1.4C | 0.6AB | |
| Fastigiata | 2 | 150 | 11.1B | 3.4AB | 41.9C | 35.0A | 1.6A | 1.3C | 3.6A | 1.5B | 0.7A | |
| Hypogaea | 4 | 255 | 10.3C | 2.6C | 47.1A | 31.8B | 1.4C | 1.5A | 3.3B | 1.6A | 0.3C | |
| Hirsuta | 5 | 28 | 11.1B | 3.1B | 44.5B | 32.9B | 1.5B | 1.4B | 3.6A | 1.5AB | 0.4BC | |
| LSD | | | 0.3 | 0.3 | 1.4 | 1.2 | 0.1 | 0.1 | 0.2 | 0.1 | 0.2 | |

^aGroups of means in a column with the same letter are not significantly different; LSD, least significant difference.

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