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Significant genetic variation for lauric acid {12:0} and capric acid {10:0} composition and seed weight was measured within lauric acid-rich, self-pollinating germplasm accessions of *Cuphea wrightii, C. tolucana,* **and** *C. lutea.* **Means and ranges of individual plant progenies for 12:0 content of** *C. wrightii* **accessions was** $60.5 \pm .63\%$ **{49.8-65.8%}, 10:0 content was 23.7 ___ .54% {18.6-33.0%}, and 1000-seed weight was 1.50 • .03 g {1.20-2.47 g}. Pro**genies of single plant selections carried to the S₂ genera**tion exhibited reduced variability within selections, but significant variation among selections for 12:0, 10:0 and 1000-seed weight. Variation among single plant selections of** *C. tolucana* **was less than that of** *C. wrightii* **and attributed to a restricted germplasm base. Means and ranges for 12:0 content were 61.6** \pm **.47% (59.2-69.9%), 10:0** $was 22.3 \pm .62\%$ (11.7-25.3%), and 1000-seed weight was $1.40 \pm .05$ g (0.90–1.69 g). *Cuphea lutea* has a significantly **different 12:0--10:0 profile than the other lauric acid-rich** species. Means and ranges for $12:0$ were $36.8 \pm .14\%$ $(33.7-40.8\%)$, 10:0 was $21.8 \pm .08\%$ (16.4-23.9%), 1000-seed weight was $2.26 \pm .02$ g $(1.82 - 2.72$ g). The 1000-seed weight **was highly positively correlated with 8:0, 10:0, 18:1 and 18:2 contents and highly negatively correlated with 12:0, 14:0 and 16:0 in both C.** *wrightii and C. tolucana.* **No such relationship was found for** *C. lutea.* **A highly significant negative correlation was also measured for 12:0 and 10:0 contents in** *C. wrightii* **and** *C. tolucana.*

KEY WORDS: Breeding, *Cuphea lutea, Cuphea tolucana, Cuphea wrightii,* **genetic diversity, lauric acid, medium chain fatty acids, seed weight, selection.**

The U.S. chemical industry is heavily dependent upon imported coconut and palm kernel oils as the primary source of lauric (12:0} acid and other medium-chain fatty acids. Current importation is about 450,000 metric tons, and essentially equal quantities of petrochemicals are also converted and utilized annually to meet domestic demand {1-3}. Recent research has stimulated interest in the utilization of capric {10:0} and caprylic {8:0} acids for nutritional and medical purposes {4-6}, which may increase future demands. Species of *Cuphea* have emerged as promising candidates for providing new domestic sources of medium-chain fatty acids {2,3,7,8}, and unparalleled genetic diversity of fatty acid patterns within *Cuphea has* been demonstrated, with $12:0$, $10:0$ and $8:0$ acids predominating {9-12). However, the extensive data reported by Graham *et aL* (10} on variations in mean fatty acid contents of *Cuphea* species gave no information on the variation within or among accessions of the species.

Agronomic studies including breeding and selection of adapted species and new cultivars were initiated in the mid-1970's at the University of Göttingen in West Germany. A major cooperative research program was initiated in the United States in 1983, involving ARS/ USDA, Oregon State University, and member companies of the Soap and Detergent Association {2,3,7,8,13,14}. Information on agronomic potential, seed composition and morphological descriptions of various species evaluated was published during the early phase of this program {13,14}. Thompson and Kleiman (3} studied the effect of seed maturity on seed oil, fatty acid and crude protein content of eight *Cuphea* species, and concluded that variations in seed maturity does not present major constraints to commercialization.

Although significant variation in fatty acid content has been reported among the large number of *Cuphea* species evaluated, very little information is available on plantto-plant variation within the various species. The amount of genetic variability within a population of plants is partly conditioned by the mode of pollination and fertilization. In general, plants within populations that are normally cross-pollinated exhibit greater genetic variability than those normally self-pollinated, since they have a much greater frequency of genetic recombination and segregation. Of the four lauric acid-rich *Cuphea* species that have received research attention, only *C. laminuligera* is a cross-pollinator, while *C. lutea, C. tolucana, and C. wrightii are* self-pollinators {13}. The objective of this research was to determine the extent of variability in fatty acid content and seed weight both within and among all available accessions of the three self-pollinating species of *Cuphea.* Such information is highly essential in order to accurately assess the probability of successful selection and enhancement of germplasm, and development of high yielding cultivars.

EXPERIMENTAL PROCEDURES

Six ARS/USDA accessions of *Cuphea wrightii,* one accession of *C. tolucana,* and two accessions of *C. lutea* were available for determination of plant-to-plant variation of fatty acid content and 1000-seed weight, both within and among accessions. The source and relationships among these accessions are detailed in Table 1.

Populations of all accessions were grown to maturity as single plants in pots in a greenhouse at Phoenix, Arizona, from 1984 to 1985. Plant numbers within populations varied depending upon availability of viable seeds. Quantities of seeds collected from individual, self-pollinated plants varied considerably. The seeds produced on each self-pollinating plant (designated S_0) are designated as the $S₁$ generation. For two accessions of *C. wrightii* (A255 and A261} and one accession of C. *tolucana* (A262), a series of S_2 populations were generated by self-pollinating and collecting seed from individual S_1 plants. No remnant S_1 seeds were available for A255, but sufficient S_1 seeds of A261 and A262 were available for analysis and comparison with that of their respective S_2 populations. In total, 14, 11, and 4 S_2 populations produced by individual S_1 plants within

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TABLE 1

Source and Relationships Among Laurie Acid-Rich *Cuphea Species* **Evaluated for Fatty Acid Content and Seed Weight**

aThese two populations are thought to be related, and represent advanced generations successively grown in Davis, CA in 1982 and Corvallis, OR in 1983.

A255, A261 and A262, respectively, were available for analysis. Seeds were collected and analyzed from five plants of each of these S_2 populations. The variance within each of these five-plant populations was used as a sampling error to test the variance among their respective S_2 's for 1000-seed weight, 12:0 and 10:0 fatty acid content.

Small quantities of seeds of each plant were weighed, placed in seed packets and sent to the ARS/USDA Northern Regional Research Center (NRRC), Peoria, Illinois, for fatty acid analysis in 1987-88. The quantities of seeds for individual lots varied from about 0.2 to 2.0 g. Seed weight determinations were made, and a small quantity of seeds of each lot was retained in Arizona as a remnant. Due to the relatively small quantities of seed available for analysis of single plant progenies, gravimetric oil determinations were not made. Fatty acid analyses were conducted utilizing previously reported methods (3). Data for seed weight, seed oil and fatty acid contents were analyzed utilizing conventional statistical methods and tests of significance.

RESULTS AND DISCUSSION

The original data on the fatty acid analyses reported by Graham *et al.* (10) were made available by Dr. Frank Hirsinger. From these data, standard errors, ranges and coefficients of variation for 12:0 and 10:0 fatty acids were calculated and summarized in Table 2. Only one accession of *C. wrightii* (Graham collection #651) and one accession of *C. lutea* (Graham collection #662) are related to the accessions listed in Table 1. These data give some indication of the range of variability that can be expected within the four species. There appears to be significant variation among the *nine C. wrightii* accessions for both 12:0 and 10:0 content. The range of variability for 12:0 content for *C. tolucana and C. laminuligera* is similar and both have significantly higher content of 12:0 than C. *wrightii. Cuphea lutea* is unique in that the 12:0 content is much lower than that of any of the other lauric acidrich species.

Means and standard errors (S.E.) for seed weight and fatty acid percentages within and among the $S₁$ populations of five accessions of *C. wrightii are* summarized in Table 3. The overall means \pm S.E., ranges and coefficients of variation (CV) for the populations are also presented for comparison. Ranges of about 16% were measured in the 12:0 means of individual plant progenies within the five populations, and the 10:0 range was about 14%. The range in 1000-seed weight of individual plant progenies was also substantial.

Seeds of four of the *C. wrightii* accessions (A77, A84, A158 and A243) were made available for research at Oregon State University in 1985. Small $S₁$ generation populations were developed from self-pollinated plants within each accession in a greenhouse at Corvallis, Oregon in 1986. Fatty acid determinations were made on these $S₁$ populations (unpublished results). The 12:0 and 10:0 contents of these S_1 populations were comparable to those of the Arizona grown material (Table 3), and fully substantiate the conclusion that rather large differences are evident among the accessions that may be amenable to selection.

The statistical test (t-test, Table 3) comparing the mean difference between the two S_2 populations of A255 and A261 of *C. wrightii* generally indicates that the two accessions are distinct for seed size and fatty acid content. However, the difference of 0.89% in 12:0 was only significant at the 5% level, and no difference was measured for 14:0. Because records of the source of A255 are not complete nor available, there is some question regarding the commonality of origin. However, it is reasonably certain that they both came from the original Graham $#651$

TABLE 2

Variation of Lanric and Capric Acid **Content Among Accessions** of Four Lauric Acid-Rich *Cuphea* Species Originally Reported in 1981 (10)

VARIATION IN LAURIC ACID RICH *CUPHEA* **SPECIES**

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collection. Although the differences between the two populations are relatively small, these data indicate that some genetic variability exists that is amenable to selection even within closely related populations.

There appears to be some difference in the S₁ and S₂ **populations of A262,** *C. tolucana,* **especially in the 10:0 and 12:0 contents {Table 3). Most of these differences may be attributed to environmental factors, since the two populations were grown in the greenhouse at different times. However, when one compares the differences be**tween the S_1 and S_2 generations of A261 (C. *wrightii*), **which were also grown at different times {Table 3), the differences in the means for 10:0 and 12:0 were less than 1%, whereas those for A262 were about 20% and 6%, respectively. A possible explanation for this difference is that** *C. wrightii* **is undoubtedly of an allopolyploid origin with an n chromosome number of 22.** *C. tolucana* **is a** diploid species (n=12) that closely resembles and is pro**posed to have been one of the parents in the formation of C.** *wrightii* **(8}. Polyploidy usually exerts a buffering effect on gene flow and may be a contributing factor to lower genetic variability in this instance.**

The two accessions of *C. lutea,* **A144 and A370, which were collected in different locations in Mexico, appear to be distinct in all respects except for 10:0 content. In this** instance both of the $S₁$ populations were grown at the **same time in the same greenhouse, so that environmental variation is greatly minimized.** *Cuphea lutea* **is most probably of polyploid origin (8), which may partially account for the low variability in seed weight and fatty acid contents within each accession. In addition, the amount of original seed of A144 was very limited and may have come from a very small number of plants, thus placing a restriction on the genetic variability found within this accession. The apparent genetic variability between the two accessions can be accounted for by their geographic isolation and lack of relationship. The difference of 3% in 12:0 content indicates that even greater variation and higher lauric acid content may be found within the species if a larger collection of accessions were available for chemical evaluation. It would appear to be highly desirable if more germplasm of this species could be collected throughout its natural range in Mexico.**

An analysis of variance was run on the variation in 12:0, 10:0 and 1000-seed weight among the five-plant S₂ generation progenies of S₁ plant selections from two ac**cessions of** *C. wrightii* **(A255 and A261)** *and C. tolucana* **(A262). The variance within the five-plant progenies was used as a sampling error to test the variation among the plant means. In all instances for the two** *C. wrightii* **accessions, the F ratios were highly significant (P=0.01). With** *C. tolucana,* **the F ratios for 12:0 and 10:0 were not significant, and the F ratio for 1000-seed weight was only significant at the 0.05 level.**

The arrays of plant means of the S₂ generation pro**genies were further evaluated utilizing Duncan's Multiple Range Test {Table 4). It is clear that there was little genetic variability within the limited number of selections of** *C. tolucana.* **The significant variation in the ranges of the means in 12:0 and 10:0 contents, as well as for seed weight for the single plant selections within both C.** *wrightii* **accessions, indicates that concurrent selection for both higher 12;0 content and seed weight is feasible. Although high 12:0 content and smaller seed weight are**

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TABLE 5

Linear Correlation Coefficients Among 1000-Seed Weight and Percent Fatty Acids for Plants of *Cuphea wrightii, C. tolucana,* **and** *C. lutea*

*******Significantly different from zero at the 0.05, 0.01, and 0.001 probability levels.

associated, there are some instances where this negative relationship is not strong. Selections 255-14 and 261-05 are good examples of favorable combinations of high 12:0 and heavier seed weight.

Comparisons of mean CV's among the various populations of *C. wrightii are* of interest. In general, the lowest CV's were measured for the S_2 generation progenies of A255 and A261; 1.4%, 3.2% and 3.8% $(\bar{x} = 2.8\%)$ for 12:0, 10:0 and 1000-seed weight, respectively {Table 4}. In comparison, the respective mean CV's for the A255 and A261 S_2 's (Table 3) were 4.2%, 7.8% and 5.7% ($\bar{x} = 5.9$ %). The CV's for the various S_1 populations (Table 3) were much higher-8.8%, 19.1% and 18.7% ($\bar{x} = 15.5$ %). In contrast, 19 seed lots of *C. wrightii,* which were either from or closely related to A255 and A261 and grown at nine field locations throughout the United States between 1982- 1985, had means of $54.0 \pm .54\%$, $34.7 \pm .46\%$, and $1.68 \pm .7\%$.05 g, and CVs of 4.4%, 5.7% and 12.9% ($\bar{x} = 7.7\%$) for 12:0, 10:0 and 1000-seed weight, respectively (3). These data indicate that some genetic variation exists within the population of material that descended from the original Graham collection #651, and the variation was reduced by selection and inbreeding within the population. However, the much higher array of variation for 12:0, 10:0 and 1000-seed weight {Table 3) among the accessions that trace back to five different original *C. wrightii*

germplasm collections leads to the conclusion that selection within the broader germplasm base for higher 12:0 yields should be feasible.

Linear correlation coefficients were calculated to determine the relationships among 1000-seed weight and fatty acids contents for each of the three lauric acid-rich species {Table 5}. Highly significant correlations were measured between seed weight and essentially all of the fatty acids. However, there is marked difference in the response of the three species. Both *C. wrightii and C. tolucana,* which are thought to be taxonomically related (8), performed similarly in that seed weight is positively correlated with 8:0, 10:0, 18:1 and 18:2 and negatively correlated with 12:0, 14:0 and 16:0. The relationships among seed weight and 8:0, 10:0 and 12:0 for *C. lutea are* essentially opposite to that of the other two species. However, the correlation of .853 for 1000-seed weight and 12:0 is spurious. The apparent relationship derives from the combined correlation analysis of the two relatively discrete populations. The nonsignificant correlation coefficients of 1000-seed weight and 12:0 calculated for each population was only $-.161$ $(n=81)$ and .134 ($n=66$), respectively, for A144 and A370.

The relationships among the various fatty acids again point to the similarity of C. *wrightii and C. tolucana* and their difference from *C. lutea.* Of most practical significance is the highly negative correlations for the

FIG. 1. Regression of capric acid (10:0) on lauric acid (12:0) contents within lauric acid-rich species. A, *Cuphea wrightii; B, C. tolucana; and C, C. lutea.*

FIG. 2. Regression of 1000-seed weight on laurie acid (12:0) contents within lauric acid-rich species. A, *Cuphea wrightii;* B, *C. tolucana;* and C, *C. lutea.*

association of 12:0 and 10:0 contents. This association accounts for over 87% of the variability (mean $r^2 = .879$) within the three populations. In contrast, no such relationship was detected for *C. lutea*. These data strongly confirm the highly significant correlation $(-.848)$ between 12:0 and 10:0 in another population of field grown *C. wrightii* previously reported by Thompson and Kleiman (3).

Linear regressions were calculated for the important relationships of $12:0$ and $10:0$ (Fig. 1), and $12:0$ and 1000-seed weight {Fig. 2) for populations of each of the three species. The regression of $10:0$ on $12:0$ (Figs. 1A and 1B) clearly shows the negative relationship that exists between these two fatty acids. The high r^2 values indicate that about 90% of the variability in 12:0 is accounted for by this association, which agrees closely to that previously reported by Thompson and Kleiman (3) for *C. wrightii.* Although no relationship exists for 12:0 and 10:0 within *C. lutea,* the means are plotted for sake of comparison {Fig. 1C). The plot clearly shows the marked difference in 12:0 and the similarity in 10:0 contents of the two *C. lutea* accessions previously characterized in Table 3.

The regression of 1000-seed weight on 12:0 shows the negative relationship that exists between seed weight and 12:0 (Figs. 2A and 2B) for *C. wrightii* and *C. tolucana*. The r^2 values indicate that from about 50-60% of the variability in 12:0 is accounted for by this association. As indicated previously, the apparent high positive association of $12:0$ and 1000 -seed weight in \overline{C} . *lutea* (Fig. 2C) is spurious due to the pooled correlation analysis of two relatively discrete populations.

It is concluded that genetic variability for fatty acid content and seed weight exists within the currently available germplasm of the three self-pollinating lauric acid-rich species of *Cuphea.* Also, some increase in 12:0 content may be possible by selection within these and other self-pollinating populations. It is suggested that even greater gains in yield may be realized by intermating high 12:0 selections, and following up with a recurrent selection program for favorable combinations of high 12:0 and increased seed size. Additional progress should be obtained by the more extensive collection and chemical evaluation of new germplasm that could be integrated into the existing and rather limited germplasm pool of these species.

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REFERENCES

- 1. Arkcoll, D., *Econ. Bot.* 42:195 (1988}.
- 2. Arndt, S., *J. Am. Oil Chem. Soc. 62:6* {1985).
- 3. Thompson, A.E., and R. Kleiman, *Ibid.* 65:139 (1988}.
- 4. Babayan, V.K., *Ibid.* 58:49A (1981}.
- 5. Babayan, V.K., *Lipids* 22:417 {1987}.
- 6. Bach, A.C., and V.K. Babayan, *Am. J. Clin. Nutr. 36:950* (1982).
7. Thompson, A.E., *HortScience 19:352* (1984).
- 7. Thompson, A.E., *HortScience* 19:352 {1984}.
- 8. Graham, S.A., *Crit. Rev. Food Sci. Nutr. 28*:139 (1989).
9. Miller. R.W., F.R. Earle, I.A. Wolff. and Q. Jones. *J. At*
- 9. Miller, R.W., F.R. Earle, I.A. Wolff, and Q. Jones, J. *Am. Oil Chem. Soc.* 41:279 (1964).
- 10. Graham, S.A., F. Hirsinger and G. RSbbelen, *Am. J. Bot.* 68:908 11981}.
- 11. Wolf, R.B., S.A. Graham and R. Kleiman, J. *Am. OilChem. Soc.* 60:27 (1983}.
- 12. Graham, S.A., and R. Kleiman, *Ibid.* 62:81 {1985}.
- 13. Hirsinger. F., and P.F. Knowles, *Ecor~ Bot.* 38:439 (1984).
- 14. Hirsinger, F., J. *Am. Oil Chem. Soc.* 62:76 (1985).

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