Oil Content and Fatty Acid Composition of Seed Oil from Guayule Plants with Different Chromosome Numbers

A. Estilai
Department of Botany and Plant Sciences, University of California, Riverside, California 92521

Guayule, a perennial desert plant, is being developed for domestic production of natural rubber, a strategic commodity for which the United States presently depends totally on foreign sources. At present, rubber alone is not sufficient to make guayule a commercial crop, and additional revenues are being sought from by-products. Because guayule flowers profusely during several years of growth before it is harvested for rubber, seed may also contribute to the economics of guayule production. Seed from 120 plants, including 20 genotypes with 36, 37, 54 and 72 chromosomes, were analyzed for oil content and fatty acid composition. Oil content ranged from 17.1 to 30.5%. On average, seed from diploid and aneuploid plants (with 36 and 37 chromosomes) contained 40.4% more oil than the seed from polyploid plants. The oil consisted of four fatty acids—palmitic (8.7–11.5%), stearic (3.7–6.2%), oleic (6.5–13.9%) and linoleic (69.1–80.2%)—at all ploidy levels. Guayule seed oil was similar to the seed oil from high-linoleic safflower varieties. The use of genetic variation to increase seed yield and seed oil will depend on the absence of negative correlation between oil and rubber production.

KEY WORDS: Apomixis, fatty acid composition, linoleic acid, natural rubber, oleic acid, palmitic acid, *Parthenium argentatum*, resins, stearic acid, vegetable oils.

Guayule (*Parthenium argentatum* A. Gray) is a promising source of natural rubber for semiarid and arid zones of the world. During the last decade, the United States Department of Agriculture (USDA) and the land-grant universities of the Southwest have conducted extensive research to improve rubber yield (1–3). The ultimate goal is domestic production of some of the nation's needed natural rubber, a strategic commodity of vital importance.

To generate additional revenues from the rest of the plant after rubber is extracted, efforts are also being made to find viable markets for guayule by-products such as resins, bagasse, leaves, waxes and whole-plant oils (4,5). Because guayule flowers profusely under adequate light, moisture and temperature conditions, it has been suggested that the seed may also contribute favorably to the economics of guayule production (6).

The majority of guayule plants found in natural populations in Texas and Mexico and the selections developed by plant breeders are polyploids with 54 and 72 chromosomes (2,3,7). Polyploid guayule produces seed by facultative apomixis, the simultaneous occurrence of sexual and asexual reproduction by seed (8). Apomictically produced seed is a clone of the plant from which it was collected and has the same genotype. Diploid plants with 36 chromosomes have also been found (9). Diploids have a limited range of distribution and have been collected primarily from several populations near Mapimi in Durango, Mexico (3,7). Seed production in diploid guayule is by sexual means. Sexual reproduction generates genetic recombination, a prerequisite for continued crop improvement through plant breeding methodologies.

Information on guayule seed oil content at different ploidy levels is scarce. In a study of 113 plant families, Earle and Jones (10) reported that guayule seed contains 24% oil and 34.9% crude protein. More recently, Banigan *et al.* (4) reported that a sample of seed obtained from mixed guayule varieties contained 26.9% oil and 38.9% crude protein. The present investigation was undertaken to determine oil content and fatty acid composition of seed oil obtained from guayule genotypes with different chromosome numbers.

EXPERIMENTAL PROCEDURES

Materials. One-hundred and twenty guayule plants from two separate experiments were used in this study. The first experiment provided seed from sixteen 3-year-old polyploid genotypes (nine USDA varieties: A48118, N396, N565, N565II, N575, N596, 4265XF, 11591 and 12231; the standard variety 593, which was planted extensively during World War II; and six California selections: C249, C250, C251, C254, C255 and C355). Five plants from each genotype were used. The second experiment provided seed from four 2-year-old genotypes with 36, 37, 54 and 72 chromosomes. Each genotype was represented by ten individual plants. Heads (inflorescences) were hand-collected from May through October in both experiments. After threshing and cleaning the seed, two 2-g seed (achene) samples from each plant were used for chemical analyses. No chromosome counts were made to determine triploid or tetraploid nature of the polyploid genotypes in the first experiment. Chromosome counts were made for every plant in the second experiment by previously described procedures (11).

Chemical analyses. Oil contents were obtained by grinding two 2-g seed samples per plant in a Wiley mill, extracting the oil with petroleum ether in a Soxhlet extracting chamber, drying and weighing the residue and determining the weight of the oil by difference. Fatty acid composition was determined by gas-liquid chromatography of methyl esters of fatty acids prepared by acid methanolysis (12) on a Varian Model 3700 gas chromatograph (Varian Associates, Palo Alto, CA), equipped with a flame-ionization detector.

Statistical analysis. The data were analyzed by means of Analysis of Variance, and Duncan's Multiple Range Test was used to compare means of guayule genotypes at a 0.05 level of significance.

RESULTS

Table 1 presents oil content and fatty acid composition of the seed oil from 16 polyploid guayule genotypes used in the first experiment. The oil content ranged from 17.1 (genotype N565) to 26.9% (genotype 4265XF). The oil consisted of two saturated fatty acids (palmitic acid, 16:0, ranging from 8.7 to 11.5%, and stearic acid, 18:0, ranging from 3.7 to 6.2%); one monounsaturated fatty acid (oleic acid, 18:1, ranging from 6.5 to 13.9%); and one polyunsaturated fatty acid (linoleic acid 18:2, ranging from 69.1 to 80.2%).

TABLE 1

Oil Content and Fatty Acid Composition of Guayule Seed from 16 Polyploid Genotypes[a]

Genotypes	Oil content (%)	Relative fatty acid composition			
		Palmitic	Stearic	Oleic	Linoleic
A48118	20.7	10.7	4.2	10.8	74.3
N396	20.1	11.5	3.7	13.9	70.9
N565	17.1	10.3	4.9	11.2	73.6
N565 II	23.6	10.4	4.2	10.4	75.0
N575	22.3	10.7	4.7	10.8	73.8
N595	20.8	10.5	4.8	10.1	74.6
4265XF	26.9	10.6	5.2	10.3	73.9
11591	25.5	10.4	3.7	9.1	76.8
12231	19.5	10.4	5.1	9.7	74.8
593	23.1	11.2	6.2	13.5	69.1
C249	26.1	9.5	4.1	8.3	78.1
C250	20.6	9.5	3.7	8.9	77.9
C251	26.6	9.9	4.4	7.8	77.9
C254	25.6	9.5	4.3	6.5	79.7
C255	20.1	8.7	4.1	7.0	80.2
C355	26.6	9.5	4.0	6.8	79.7
$LSD_{(0.05)}$	2.64	1.52	0.81	1.91	2.68

[a]Values represent the average of duplicate runs of five replicates. LSD, least significant difference.

TABLE 2

Oil Content and Fatty Acid Composition of Guayule Seed from Genotypes with 36, 37, 54 and 72 Chromosomes[a]

Genotypes[b]	Oil content (%)	Relative fatty acid composition			
		Palmitic	Stearic	Oleic	Linoleic
Diploid (36)	30.5	11.3	4.1	12.2	72.4
Aneuploid (37)	30.3	11.1	4.3	12.5	72.1
Triploid (54)	21.3	10.6	4.9	10.4	74.1
Tetraploid (72)	22.0	10.7	5.0	12.1	72.2
$LSD_{(0.05)}$	1.93	1.32	0.61	1.17	2.16

[a]Values represent the average of duplicate runs of ten replicates. LSD, least significant difference.
[b]Chromosome number in parentheses.

Table 2 summarizes the results from the second experiment. The oil content averaged 30.4% for the diploid and aneuploid genotypes (plants with 36 and 37 chromosomes) and 21.6% for the two polyploid genotypes (plants with 54 and 72 chromosomes). On average, diploid and aneuploid seed contained 40.4% more oil than polyploid seed, a statistically significant difference. The oil from triploid seed had significantly less oleic acid than the oil obtained from diploid and tetraploid seed. Compared to triploid and tetraploid, diploid seed was significantly lower in stearic acid.

DISCUSSION

Guayule seed oil is not unique. The fatty acids found in guayule seed oil are the common fatty acids present in a number of vegetable oils. Knowles (13) reported that standard high-linoleic safflower (variety US-10) has 7.9% palmitic acid, 2.4% stearic acid, 17.9% oleic and 71.8% linoleic acid. Averaged over all plants from all ploidy levels, guayule seed oil contains 10.4% palmitic acid, 4.5% stearic acid, 10.1% oleic acid and 75.0% linoleic acid. Guayule and safflower oils share the same kinds of fatty acids but they are different, to some extent, in the proportions of those fatty acids. Guayule oil contains more palmitic, stearic and linoleic, and less oleic acid than safflower oil.

Diploid seed had considerably higher oil content than the seed obtained from triploid and tetraploid plants. This may suggest that diploid genotypes produce more oil per unit area than polyploid genotypes. Oil yield per hectare, however, is also a function of seed yield. At present, no information on seed yield of diploid and polyploid genotypes is available. In one study (3), it was observed that the mass of diploid seed was 20–34% lower than that of polyploid seed. The presence of an extra chromosome at the diploid level (aneuploid plants with 37 chromosomes) did not alter oil content or fatty acid composition of the seed. This suggests that the extra chromosome does not carry genes that affect oil production. Additional aneuploids involving other chromosomes must be tested before a conclusion can be drawn regarding the effect of extra chromosomes on seed oil content and oil composition.

The observed genetic variation for oil content and relative amounts of fatty acids is encouraging as far as

TABLE 3

Rubber Content of 12 Guayule Genotypes

Genotype	Rubber content (%)
A48118	3.32 (14)[a]
N396	6.44 (14)
N565	4.11 (14)
N565II	15.2 (15)
N575	6.85 (14)
11591	4.98 (14)
12231	5.14 (14)
593	5.22 (14)
C250	7.72 (16)
C254	7.18 (16)
C255	6.58 (16)
C355	5.20 (16)

[a]The number in parentheses gives the reference in which the rubber content was reported.

breeding for improved oil content is concerned. However, the effects that the increase in seed yield and seed oil content may have on rubber and resin production of the plant (compounds of primary interest) are not yet known. Rubber yield of a guayule plant may be independent of, positively correlated with or negatively correlated with the seed yield and seed oil content of the same plant. Table 3 was constructed from published data (14–16) to show the rubber content for 12 of the guayule genotypes used in the present investigation. The correlation coefficient between the rubber content (data of Table 3) and the seed oil of the same genotypes (Table 1) was found to be small ($r = 0.22$) and statistically nonsignificant. The data suggest that oil content and rubber content are independent traits, and breeding for increased oil content, without adversely affecting the rubber content, is possible. This conclusion, however, needs to be verified by additional experiments in which rubber content, seed yield and seed oil content are measured simultaneously on the same plants growing in the same environment.

Experimental data on seed yield of guayule per hectare is not available. Estimates ranging from 5 to 200 kg seed per hectare per year are cited in the literature (7,17). With such levels of productivity, guayule can hardly be considered a competitive oilseed crop, especially when it is realized that a similar oil may be obtained from more productive crops, such as safflower and sunflower. However, in commercial rubber production, thousands and thousands of hectares are expected to be occupied by guayule plants for several years. Seed production from those fields will not include the cost of raising the crop, but only the costs associated with harvesting, threshing and cleaning the seed. If the price of seed could offset the cost of seed production and leave a small profit for growers, one may expect a substantial revenue when the total area under cultivation is taken into consideration.

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REFERENCES

1. Estilai, A., B. Ehdaie, H.H. Naqvi, D.A. Dierig, D.T. Ray and A.E. Thompson, *Agron. J. 84:*420 (1992).
2. Thompson, A.E., and D.T. Ray, *Plant Breeding Reviews 6:*93 (1989).
3. Estilai, A., and D.T. Ray, *Guayule Natural Rubber,* edited by J.W. Whitworth, and E. Whitehead, GAMC and USDA Cooperative Research Service, Tucson, 1991, pp. 47–91.
4. Banigan, T.F., A.J. Verbiscar and C.W. Weber, *J. Agric. Food Chem. 30:*427 (1982).
5. Buchanan, R.A., F.H. Otey, C.R. Russell and I.M. Cull, *J. Am. Oil Chem. Soc. 55:*657 (1978).
6. *Guayule: An Alternative Source of Natural Rubber,* National Academy of Sciences, Washington, D.C., 1977.
7. Hammond, B.L., and L.G. Polhamus, *Research on Guayule (Parthenium argentatum):*1942-55, USDA Tech. Bull. 1327, U.S. Government Printing Office, Washington, D.C., 1965.
8. Esau, K., *Proc. Natl. Acad. Sci. USA 30:*352 (1944).
9. Bergner, A.D., *Science 99:*224 (1944).
10. Earle, F.R., and Q. Jones, *Econ. Bot. 16:*221 (1962).
11. Estilai, A., A. Hashemi and V.B. Youngner, *Am J. Bot. 72:*1522 (1985).
12. Hitchcock, C., and E.W. Hammond, *Development in Food Analysis Techniques,* Vol. 2, edited by R.D. King, Applied Science Publishers, London, 1978, pp. 185–223.
13. Knowles, P.F., *J. Am. Oil Chem. Soc. 49:*27 (1972).
14. Naqvi, H.H., *Bull. Torrey Bot. Club 112:*196 (1985).
15. Mills, D., A. Benzioni and M. Forti, *Econ. Bot. 43:*387 (1988).
16. Estilai, A., *Bioresource Technol. 35:*119 (1991).
17. Tipton, J.L., J.L. Craver and J. Blackwell, *HortScience 16:*296 (1981).

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