Reprinted from the AMERICAN JOURNAL OF BOTANY, Vol. 54, No. 9, October, 1967
Printed in U.S.A.

Amer. J. Bot. 54(9): 1158–1162. 1967.

THE TUBERS, SAPOGENINS, AND VIRUS RESISTANCE OF DIOSCOREA SPECIES HYBRIDS[1]

FRANKLIN W. MARTIN AND NORMAN E. DELFEL

Federal Experiment Station, Crops Research Division, Agricultural Research Service,
U. S. Department of Agriculture, Mayaguez, Puerto Rico

ABSTRACT

Dioscorea species differ in sapogenin content, tuber size and shape, and virus resistance. The amount and type of sapogenins in tubers of F_1 hybrids among four sapogenin-bearing and one other *Dioscorea* species were determined chromatographically. Characteristics of the tuber and resistance to a virus were observed and compared to those of the parents. The presence of particular sapogenins appeared to be a dominant trait modified by minor genes from either parent. Other traits appeared to be determined by many genes. Heterosis was expressed in some hybrids by superior yields and excellent virus tolerance. Some of the hybrids could be used as clonal varieties, but none contained the desired keto sapogenins except in mixtures with diosgenin.

THE PRINCIPAL *Dioscorea* species yielding sapogenins used in steroidal synthesis differ in types of sapogenin in the tuber. *Dioscorea composita* Hemsl., *D. floribunda* Mart. & Gal., and *D. friedrichsthalii* R. Knuth contain principally diosgenin or a mixture of diosgenin and yamogenin (Wall et al., 1957; Martin, Delfel, and Cruzado, 1963). In addition to these substances, *D. spiculiflora* Hemsl. contains correllogenin and gentrogenin, which are of particular interest because of their more complex chemical structure (Wall et al., 1957). The commercial cultivation of varieties containing only one sapogenin would simplify the extraction and purification process and thus increase the value of the raw materials. Varieties containing only correllogenin or gentrogenin would be especially valuable.

At the present time commercial plantings of *Dioscorea* consist of geographical races of the species in question. These do not represent varieties in the better usage of the word, and evidence is lacking to show that any one type is definitely superior to another. As part of a series of studies on the breeding and improvement of *Dioscorea*, we have produced all of the 12 hybrid combinations among the four principal sapogenin-bearing species. In addition several hybrids were produced between a sapogenin-bearing and a non-sapogenin-bearing species. The vegetative and fertility characteristics of such hybrids were previously described (Martin and Cabanillas, 1966). In this paper the sapogenin contents of hybrids are compared to those of the parents. Because the tubers of the species differ in certain readily recognized features, some of which have agronomic implications, the tubers are also described and compared. Finally, degree of resistance to a virus disease is estimated.

[1] Received for publication 13 December 1966.

MATERIALS AND METHODS—Methods of production and identification of hybrids were previously described (Martin and Cabanillas, 1966). Hybrids were grown in pots in the greenhouse for 6 months to 1 year and then planted in the field. Harvests were made after one to three seasons of field growth during the dry season when the plants were not growing. At the time of harvest tubers were weighed, their color, shape, and position in the soil were noted, and drawings and color photos were made. The tubers were then cut into small pieces which were mixed, and a sample was taken for sapogenin analysis by the method of Morris, Roark, and Cancel (1958). The dry weight, percentage sapogenin, and total sapogenin were determined. After analysis, the total extracts of the acid-hydrolyzed tubers were reconstituted in preparation for further tests.

The crude sapogenin extracts from the hybrids and the parent species were dissolved in 95% ethyl alcohol (1 mg/ml). A 1-μliter aliquot of each sample was applied to 0.25-mm silica gel G plates for thin-layer chromatography. The plates were developed with cyclohexane-ethyl acetate-water 1000:1000:3 (Bennett and Heftman, 1962) or other solvent systems (Blunden and Hardman, 1964), dried until free of solvent odor, sprayed with either 50% sulfuric acid (aq.) or chlorosulfuric acid: acetic acid (1:3), and heated for 15 min at 120 C. Spots corresponding to diosgenin and yamogenin, and to correllogenin and gentrogenin were identified by their characteristic colors under ultraviolet light (greenish-yellow and rusty orange, respectively) and by comparison of their R_F values in several solvent systems to those of the corresponding known sapogenins. An unidentified compound was noted on the chromatograms of certain tuber extracts. This material fluoresced blue under shortwave UV after treatment with either spray reagent above; it formed a precipitate with digitonin; and it had the R_F value of a monohydroxy-, or possibly a hydroxyketo-, sapogenin. These facts suggest that it is a steroid, and we refer to it as "steroid X" in this article. The small amounts present precluded further identification. An estimate of the relative amounts of the various steroids present permitted comparison of the hybrids. Additional sapogenins in trace amounts appeared on the chromatograms, particularly at the origin. Since there was no correlation between the presence or amounts of the trace steroids and the genetic background of the hybrid, they are not recorded here.

RESULTS—Hybrids were produced over a period of 3 years and analyzed over 2 years. Consequently, the tubers were of different ages at the times of analysis. The 1-, and 2-, and 3-year ages represent very young, young and mature stages in the life cycle. During the years of harvest, a virus disease infested the hybrid plots and some plants were badly retarded. The results obtained herein thus probably represent minimum yields obtainable under normal conditions.

Characteristics of the tubers—The tubers of *D. floribunda* are yellow, whereas those of the other three species are white. Tuber color in the hybrids varied from white through cream, shades of yellow and orange (Table 1). Occasionally these colors were modified by irregular deposits of anthocyanin. Whereas color was a distinguishing

TABLE 1. *Comparison of the tuber characteristics of four Dioscorea species and their hybrids*

Species combination	Number of hybrids	Color of tuber[a]	Length of neck	Disposition in soil	Tuber size	Shape of branches
D. composita	—	White	Long	Vertical	Large	Long, thin
D. floribunda	—	Yellow	Short	Horizontal	Medium	Short-thick
D. friedrichsthalii	—	White	"	Intermediate	"	" "
D. spiculiflora	—	"	"	"	Small	" "
D. composita × *D. floribunda*	2	White, cream	Long	"	Large	Long, thick
D. composita × *D. friedrichsthalii*	2	White	"	"	Medium	" "
D. composita × *D. spiculiflora*	5	"	Medium	"	Large	Short-thick
D. floribunda × *D. composita*	12	White, cream, yellow, orange	Long	"	"	Long, thick
D. floribunda × *D. friedrichsthalii*	5	Cream, yellow,	Short	Vertical	Small	Short, thick
D. floribunda × *D. spiculiflora*	6	White, cream	"	Intermediate	Medium	" "
D. friedrichsthalii × *D. composita*	2	" "	Long	Vertical	"	Long, thin
D. friedrichsthalii × *D. floribunda*	4	", yellow orange	"	"	Small	" "
D. friedrichsthalii × *D. spiculiflora*	5	White	"	"	"	" "
D. spiculiflora × *D. composita*	5	White, cream	"	"	Medium	Long, thick
D. spiculiflora × *D. floribunda*	7	Yellow	"	"	"	Short, thick
D. spiculiflora × *D. friedrichsthalii*	5	White	Thick	"	Small	Long, thin

[a] Anthocyanin distribution was too irregular within combinations, and even within tubers to note here.

feature of the species, the colors of the hybrids were too variable to deduce any rule on their inheritance, except that several genes must be involved and that the parent materials must have been heterozygous for some of the controlling genes.

A prominent neck sets tubers of *D. composita* apart from the other species. This trait was present in all hybrids with a *D. composita* parent. However, certain other species combinations not including *D. composita* also produced tubers with long necks, or with long, thin trunks not readily distinguished from a neck. The tuber of *D. floribunda* is distinguished from those of the other species by its more or less horizontal habit of growth, a desirable trait in that the tubers can be readily plowed from the ground. The tubers of all hybrids were more vertical in growth than those of *D. floribunda*. Tubers tended to be intermediate in size to those of parents, but larger-than-parental tubers in many of the hybrids of *D. floribunda* × *D. composita* and of *D. composita* × *D. spiculiflora* were probably manifestations of hybrid vigor. We have observed considerable variation in tuber size and shape even among clonal offspring grown from cuttings, and we hesitate to emphasize hybrid differences based on observations of single plants. Nevertheless, these morphological variations are of considerable importance in an improvement program.

Disease resistance—During the 12 years in which this station has conducted *Dioscorea* research, we have seen only one serious disease, that caused by a virus (Ruppel, Delpin, and Martin, 1966). This disease spread rapidly through all of the experimental plots in the area. Most of the hybrid plants were infected during their second year of growth. Thus symptomology could be observed and relative resistance evaluated over the second and third years of field growth. Without doubt both second- and third-year yields were reduced, especially in the more susceptible hybrid combinations. The chief symptom of the disease (interveinal lightening of the leaf tissue) and preliminary studies of transmission have been reported (Ruppel et al., 1966).

All parent species were infected by the virus, but the degree of debilitation varied (Table 2). *D. friedrichsthalii* and *D. floribunda* are both particularly susceptible, and many plants were eventually killed during the course of the disease. On the other hand, *D. spiculiflora* may live with the disease for several years, showing only mild symptoms. *D. composita* is intermediate in reaction.

Apparently, all hybrids became infected with the virus, but as in the case of parents some hybrid combinations were more debilitated by the disease than others (Table 2). Hybrids of *D. spiculiflora* with *D. composita* were least affected. Some plants showed no disease symptoms during a 3-year period, but others grew vigorously in spite of definite infection. Nevertheless, total yields of all plants were depressed in comparison to the yields of the parent species (Table 3). Hybrids between *D. spiculiflora* and *D. floribunda* were highly susceptible to virus damage, and many plants were killed especially during the third year of growth. Although all plants of a given hybrid combination reacted uniformly with respect to resistance or susceptibility to virus infection, resistance of the hybrid could not be predicted from parental responses. Thus hybrids of *D. spiculiflora* × *D. friedrichsthalii* were disease resistant, although those of *D. spiculiflora* × *D. floribunda* were not.

TABLE 2. *Relative resistance of Dioscorea species and hybrids to green-banding virus*

Species or combination	Resistance or susceptibility
D. composita	Partially resistant
D. floribunda	Susceptible
D. friedrichsthalii	Highly susceptible
D. spiculiflora	Highly resistant
D. composita × *D. friedrichsthalii*	Susceptible
D. composita × *D. spiculiflora*	Highly resistant
D. floribunda × *D. composita*	Susceptible
D. floribunda × *D. friedrichsthalii*	Highly susceptible
D. floribunda × *D. spiculiflora*	Highly susceptible
D. friedrichsthalii × *D. composita*	Susceptible
D. friedrichsthalii × *D. floribunda*	Susceptible
D. friedrichsthalii × *D. spiculiflora*	Partially resistant
D. spiculiflora × *D. composita*	Highly resistant
D. spiculiflora × *D. floribunda*	Highly susceptible
D. spiculiflora × *D. friedrichsthalii*	Partially resistant

Yields of hybrids—Hybrids showed the same pattern of tuber growth and sapogenin accumulation as did parents (Cruzado, Delpin, and Roark, 1965). Tubers were small and percentages of sapogenin were low the first year, but these increased rapidly the second, and particularly the third year (Table 3). Mean yields of the hybrids were roughly comparable to those of the parent species. However, yields of sapogenin per plant of several hybrids were outstanding during the third year and surpassed those of either parent species (*D. floribunda* × *D. composita; D. spiculiflora* × *D. floribunda*). This occurred in spite of virus infection. The hybrids themselves were highly variable within each combination; thus outstanding performance was actually masked by calculation of means. In all combinations tested at 3 years some plants yielded more than 50 g sapogenin per plant. Thus hybrid vigor, manifest in vigorous vegetative growth (Martin and Cabanillas, 1966), was also manifest in increased tuber size and sapogenin yield even under adverse conditions.

Types of sapogenins—Thin-layer chromato-

TABLE 3. *Mean yields of 1- to 3-yr-old hybrids of Dioscorea species as compared to those of parents*

Species or combinations	Age	Number of hybrids tested	Yield per plant (g)			Percentage sapogenin
			Fresh	Dry	Sapogenin	
D. composita	1	—	186	57	1.04	1.80
" "	2	—	929	272	9.00	3.33
" "	3	—	2104	615	24.22	3.92
D. floribunda	1	—	183	52	2.34	4.45
" "	2	—	649	177	8.41	4.81
" "	3	—	1221	314	16.91	5.43
D. friedrichsthalii	1	—	a[a]	a	a	a
" "	2	—	a	163.6	2.58	1.58
" "	3	—	a	164.0	6.18	3.77
D. spiculiflora	1	—	a	a	a	a
" "	2	—	435.44	136.07	5.03	3.7
" "	3	—	1605.7	412.76	26.41	6.4
D. composita × *D. friedrichsthalii*	2	4	277.0	93.8	2.52	2.69
D. composita × *D. spiculiflora*	2	5	489.6	115.3	2.59	2.25
D. composita × *D. spiculiflora*	3	7	1775.0	647.0	23.20	3.58
D. floribunda × *D. composita*	2	3	397.0	111.9	3.74	3.12
D. floribunda × *D. composita*	3	5	2905.4	1020.2	42.95	4.21
D. floribunda × *D. friedrichsthalii*	2	5	169.8	52.5	0.97	1.85
D. floribunda × *D. spiculiflora*	2	6	1060.6	101.54	3.22	3.18
D. friedrichsthalii × *D. composita*	1	1	37.0	11.5	0.06	0.57
D. friedrichsthalii × *D. floribunda*	1	4	38.0	13.2	0.13	1.00
D. friedrichsthalii × *D. floribunda*	2	1	253.0	52.5	3.13	5.98
D. friedrichsthalii × *D. spiculiflora*	1	5	57.9	24.4	0.21	0.87
D. friedrichsthalii × *D. polygonoides*	1	3	69.5	26.1	0.26	1.02
D. spiculiflora × *D. composita*	2	5	501.2	144.2	5.06	3.51
D. spiculiflora × *D. floribunda*	2	7	549.8	134.7	5.28	3.92
D. spiculiflora × *D. floribunda*	3	5	2088.2	724.2	36.21	5.00
D. spiculiflora × *D. friedrichsthalii*	1	2	38.0	12.5	0.01	0.10
D. spiculiflora × *D. friedrichsthalii*	2	3	897.6	131.8	4.05	3.08

[a] Symbol a indicates data not available.

graphic tests separated total sapogenin contents into three major groups, diosgenin-yamogenin, correllogenin-gentrogenin, and an unknown steroid (X). This steroid occurs between diosgenin and gentrogenin when chromatographed by either the Bennett and Heftman (1962) or Blunden and Hardman (1964) solvent systems. From sizes and intensities of the spots and a study of effects of dilution, rough estimates of the amount of sapogenin in each spot were possible.

All hybrids, including those of *D. friedrichsthalii* × *D. polygonoides*, contained diosgenin and/or yamogenin as the principal steroidal components. These components always made up the bulk (about 90% or more) of the steroidal content of the tubers. In addition many hybrids contained small amounts (about 10% or less) of keto steroids or of an unknown (steroid X) compound (Table 4). Trace amounts of other materials, contaminants or small quantities of other sapogenins, were sometimes seen, but the test probably did not reveal those contaminants making up less than one percent of the total amount of material tested. Thus the hybrids contained the same types of sapogenins as the four parents.

In general, a minor steroidal component of a particular parent was found also in its hybrids. Thus hybrids with *D. spiculiflora* contained keto sapogenins. Five plants of *D. spiculiflora* × *D. freidrichsthalii* and one of *D. floribunda* × *D. spiculiflora* were exceptions and did not contain either of the minor steroidal components. The species themselves varied with respect to the presence and amount of steroid X, and it is not surprising that its presence in the hybrids was variable and unpredictable. Small amounts of keto sapogenin occurred unexpectedly in one plant each of *D. composita* × *D. friedrichsthalii* and *D. floribunda* × *D. friderichsthalii*. Keto sapogenin and steroid X did not occur together in the same plant in any of the parents or hybrids tested.

DISCUSSION—The hybrids of the sapogenin-bearing *Dioscorea* species constitute a unique group of plant materials, of interest not only for the insights they afford of speciation in the genus (Martin and Cabanillas, 1966), but also as possible clonal varieties for sapogenin production and as starting materials for a breeding and improvement program. Although most of the materials were lost because of virus infection, the hybrids can easily be redeveloped from the

TABLE 4. *Types and amounts*[a] *of sapogenin (in addition to diosgenin-yamogenin) in Dioscorea species and their hybrids*

Species or combinations	Number of plants tested	Amount of keto sapogenin	Amount of X sapogenin	Notes on variability among hybrids
D. composita	4	none	2 plants, WE-ME	Variable
D. floribunda	4	none	WE-ME	Uniform
D. friedrichsthalii	4	none	1 plant ME	Variable
D. spiculiflora	4	ME-ST	None	Uniform
D. polygonoides[b]	4	none	None	Uniform
D. composita × *D. floribunda*	No hybrids available for these tests			—
D. composita × *D. friedrichsthalii*	4	1 plant WE	1 plant ME	Extremely variable
D. composita × *D. spiculiflora*	5	WE-ST	None	Uniform
D. floribunda × *D. composita*	5	none	WE-ME	Uniform
D. floribunda × *D. friedrichsthalii*	5	none	3 plants TR-WE	Variable
D. floribunda × *D. spiculiflora*	5	4 plants WE-ST	None	Variable
D. friedrichsthalii × *D. composita*	1	TR	None	—
D. friedrichsthalii × *D. floribunda*	4	1 plant WE	None	Variable
D. friedrichsthalii × *D. spiculiflora*	1	ME	None	—
D. friedrichsthalii × *D. polygonoides*	3	None	None	Uniform
D. spiculiflora × *D. composita*	5	WE-ST	None	Uniform
D. spiculiflora × *D. floribunda*	7	TR-ST	None	Uniform
D. spiculiflora × *D. friedrichsthalii*	5	None	None	Uniform

[a] TR = trace, WE = weak, ME = medium, ST = strong (no more than 10 per cent of total sapogenin content).
[b] *D. polygonoides* did not produce any sapogenins in detectable amounts.

parental species. The actual usage of these materials will probably depend on the development of *Dioscorea* as a commercial farm crop and the continued demand for sapogenins as steroidal starting materials.

In addition to the interest in yields, types, and purity of sapogenins the plant breeder would be interested in virus resistance and tuber shape and size. As demonstrated herein, these characteristics are not under simple genetic control, for their expression in the hybrids cannot be predicted from parental performance. Because the sapogenin-bearing *Dioscorea* species are tetraploids (Martin and Ortiz, 1963), the inheritance and transfer of all characteristics is complicated. Nevertheless, the hybrid vigor and disease resistance of some hybrid combinations, especially *D. composita* × *D. spiculiflora*, encourages examination of the F_2 generation.

Separation of sapogenins of the species and hybrids showed that all plants except *D. polygonoides* produce diosgenin or yamogenin, and presumably they have a common genetic system in this respect. This character appeared to be dominant to the absence of sapogenin as in *D. polygonoides*. Although the presence of other sapogenins also appears to be a dominant character, considerable variation in the parents and in the hybrids and the unexpected occurrence of keto sapogenins in hybrids other than those of *D. spiculiflora* suggest that modifying genes are also present. These genes of minor effect may be recessive or dominant with respect to the presence or absence of particular sapogenins, and without doubt the hybrids are more variable in such genes than are the parent species. The accurate assessment of these modifiers would require several generations more of breeding. However, the F_1 generation would not suggest the possibility of obtaining plants in later generations with keto sapogenins but without simpler types.

LITERATURE CITED

BENNETT, R. D., AND E. HEFTMANN. 1962. Thin-layer chromatography of steroidal sapogenins. J. Chromatogr. 9: 353–358.

BLUNDEN, C., AND R. HARDMAN. 1964. Thin-layer chromatography of *Dioscorea* sapogenins. J. Chromatogr. 15: 273–276.

CRUZADO, H. J., H. DELPIN, AND B. A. ROARK. 1965. Sapogenin production in relation to age of tuber in two *Dioscorea* species. Turrialba 15: 25–28.

MARTIN, F. W., AND E. CABANILLAS. 1966. The F_1 hybrids of some sapogenin-bearing *Dioscorea* species. Amer. J. Bot. 53: 350–358.

———, N. E. DELFEL, AND H. J. CRUZADO. 1963. *Dioscorea friedrichsthalii*, another sapogenin-bearing species. Turrialba 13: 159–163.

———, AND S. ORTIZ. 1963. Chromosome numbers and behavior in some species of *Dioscorea*. Cytologia 28: 96–101.

MORRIS, M. P., B. A. ROARK, AND B. CANCEL. 1958. Simple procedure for the routine assay of *Dioscorea* tubers. J. Agric. Food Chem. 6: 856–858.

RUPPEL, E. G., H. DELPIN, AND F. W. MARTIN. 1966. Preliminary studies on a virus disease of a sapogenin-producing *Dioscorea* species in Puerto Rico. J. Agric. Univ. Puerto Rico 50: 151–157.

WALL, M. E., J. J. WILLAMAN, T. PERLSTEIN, D. S. CORRELL, AND H. H. GENTRY. 1957. Steroidal sapogenins. XXIX. Occurrence and isolation of gentrogenin from *Dioscorea spiculiflora*. J. Amer. Pharm. Assn. Sci. Ed. 46: 155–159.