REGENERATIVE ABILITY TRIALS ON TUBER PIECES OF *DIOSCOREA COMPOSITA* IN MEXICO

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Rhizomes of *Dioscorea composita* Hemsl., a sapogenin bearing yam, have been used during the last 15 to 20 years as the raw material for the synthesis of cortisone and steroidal hormones medically useful for different diseases and as ovulatory regulators in man and other animals. Up to now Mexico has been the main source of this raw material. It is only in the last few years that India and China have produced steroidal hormones isolated from *Dioscorea deltoidea* and several *Solanum* species.

Other sapogenin-bearing species of the genus *Dioscorea* have been used or are in use at present in Mexico: *D. floribunda, D. spiculiflora, D. mexicano* named in order of importance.

Abundance or sapogenin content of these species is lower than that of *D. composita;* herein after we will refer exclusively to this species.

D. composita, whose comon name is "barbasco", has its main area of distribution and abundance in the lowlands of the Gulf Coast of Mexico in areas with a hot-humid climate (the A type of climate in Koeppen's climatic classification). The best soils for the growth of *D. composita* are red, yellow or brown, deep latosolic or lateritic with a fairly good drainage, formed from volcanic materials and with pH values ranging from 5 to 6. The native vegetation in these conditions is in most of the cases a high evergreen forest with dominant *taxa* such as *Terminalia amazonia, Callophyllum brasiliense, Dialium guianense, Guatteria anomala, Sweetia panamensis,* and similar species.

The productivity of *D. composita* in natural conditions ranges from 200 to 500 kg/Ha to 4 to 6 Tons/Ha. Average yields range from 1 to 2.5 Tons/Ha. The amount of Diosgenin present in the rhizome, which is the product that gives value to the tuber, is affected quite drastically by environmental factors. Water content of the soil is one of the main factors that produces variability in the Diosgenin content. Genetic variation could exert influence also on the tuber's sapogenin content. Percentages of Diosgenin in tubers of *D. composita* averages, in natural conditions, between 4.5 and 5% of the fresh weight. The average production per plant is close to 2.5 to 3.4 kg. The highest yield found for a single rhizome was a bit more than 250 kg.

Barbasco is exploited as a wild plant in Mexico, and hence is considered as a natural renewable resource rather than a crop. In regard to its value, barbasco occupies the second place, after timber, among the nation's forest products. The total amount extracted in 1966 was 15,000 metric tons of fresh rhizome. The value of a metric ton of rhizome, (fresh weight), with an average content of sapogenin between 4.5 and 5.0% is approximately the equivalent of $30.00 U.S.

Soon after the initiation of exploitation in Mexico, the Mexican Government through its National Institute of Forestry Research, established a Commission for the study of ecology of this plant.

The ecological studies have involved the careful analysis, description and characterization of the main plant communities in the Gulf coastal tropical regions of Mexico, the productivity of *D. composita* within each plant community and each geographical area, and the effect of the anthropogeneic activities upon such productivity.

A general picture of the ecological studies concerning *D. composita* was presented by Gomez Pompa *et al,* (1964). Sarukhan, (1967), elaborated a thorough analysis and discussion of the method employed in those studies.

The basis of all the ecological field work lies on the definition and delimitation of units in a given geographical area. These have been designated as *ecological units* (Sarukhan, 1967) These ecological units are separated by means of both the physiognomy and the floristic composition of the communities and always correspond with a *vegetational type.* (Mirands and Hernandez X., 1964).

Vegetation maps are made delimiting these ecological units showing the geographical distribution of the communities that include or exclude the presence of *D. composita.* These ecological units include primary as well as secondary vegetation produced by the anthropological activities.

Once the vegetation maps for different areas have been elaborated, intensive random samplings are made to determine the productivity of *D. composita.* Productivity for both primary and secondary stages of vegetation and hence for the vegetation type to which those stages belong is calculated. The addition of the productivities of different vegetation types, gives the approximate productivity potential of a given georgraphical area.

In theory, one would expect that higher productivity levels should occur in the primary forest. But in our conditions, highest productivity levels are founded in secondary stages some 15 years of age.

This is the case when peasants fell the original forest or high vegetation for the cultivation of his crop. While ploughing the field, a frequent fragmentation of many of the *Dioscorea* tubers present in the ground occurs. This is a very efficient way of stimulating vegetative propagation. With this increase of individuals of *D. composita* one needs only to allow time to get a very high production in that abandoned area. It is necessary to say, however, that if shifting agriculture permits and even stimulates the productivity of *D. composita,* permanent agriculture, on the other hand eliminates its presence quite drastically.

It is our feeling that *D. composita* occupies at present a privileged position in relation to most root crops or other crops, since this is one of the few species in which a good deal of its environmental requirements are known in the wild and its relationships with the physical and biotic components are fairly well established before it becomes a widely distributed cultivar.

Most of the problem of cultivation of *D. composita* have been studied and solved (Martin et al, 1966). Its cultivation in a commercial scale is possible.

Whether or not *D. composita* will become a very important economic crop, is a matter that falls beyond the scope of this paper. However, a point that we would like to stress here is that the basic knowledge of the ecological relationships and requirements of any species at the wild level would help considerably in any attempt of domestication of such a species.

REGENERATION STUDIES

Although *D. composita* has a fairly good index of self-regeneration both by tuber pieces left in the ground or by seeds, we became interested in understanding as much as possible the dynamics of its regeneration. We were looking for a method as natural as possible to induce the repopulation of this species. Our main experiences concern the ability of regeneration of different parts of the tuber in experimental conditions, the characteristics of seed dispersal and the study of viability of seeds in natural conditions.

The following trials have been carried out in an area with deep, red lateritic loamy-clay soils, of alluvial origin, with a fairly good drainage (Red Lateritic Soils, Semimaturm; Cuanalo, 1965).

A. — *Experimental objectives.*

The trials conducted had the following objectives:

a) to establish the effects of disturbance of the vegetation upon the regeneration and the growth of barbasco tubers;

b) to determine a quick and effective method of regeneration;

c) to know which tuber pieces sprout first and/or regenerate best;

d) to determine the best season for the initiation of regeneration practives and the optimum depth of planting; and

e) to obtain information concerning increase in tuber weight.

B. — *Materials and Methods*

Tubers of plants from an area contiguous to the experiment, were collected at random for the trials, one or two days before the planting of the experiment. Digged rhizomes were stored in a fresh, shaded place.

Three parts of the rhizome were used in all trials; the area of emergence of the vines or crown: middle portions of the tuber and terminal or tip pieces. Two sizes of each of the three portions were assayed: 5cm. (2 in.) and 10 cm. (4 in.). Treatments were as follows:

T 1 — Crown piece 5 cm. (CP 5)
T 2 — Crown piece 10 cm. (CP 10)
T 3 — Middle piece 5 cm. (MP 5)
T 4 — Middle piece 10 cm. (MP 10)
T 5 — Terminal piece 5 cm. (TP 5)
T 6 — Terminal piece 10 cm. (TP 10)

The experimental design used was random plots, with 20 tuber pieces per plot 1 m., (40 in.) apart from each other. Plots were separated 2m (80 in.) on all sides. Total number of propagules in a experiment was 480.

Tuber pieces were cut to the appropriate size (5 or 10 cm.) with a machete only a few hours before planting. Holes were made and the tuber pieces covered with a shovel-like instrument ("coa") at a depth of 10 — 15 cm. (4-6 in). No fungicide or any other preservative was used.

Four different trials were established to determine the influence of the disturbance of the vegetation and the different season of planting upon the regeneration of tuber :

Experiment 1. — *Planting in a denuded area, during the dry season.*

Experiment 2. — *Planting in secondary vegetation 3-5 m. high (2-3 years old), during the dry season.*

Experiment 3. — *Planting in secondary vegetation 15-20 m. high (15 years old), during the dry season.*

Experiment 4. — *Planting in secondary vegetation 3-5 m. high during the rainy season.*

The first three experiments had exactly the same treatments and the same experimental design of random plots. The fourth had different treatments in random plots.

RESULTS

Experiment 1. — *Planting in a denuded area.*

An area with secondary vegetation 2—3 years old with dominant species such as *Waltheria brevipes, Heliocarpus donnell-smithii and Bixa orellana,* was selected and cut to obtain a bared area, but natural vegetation was allowed to grow after planting. The experiment was planted in December 1960 and harvested December 1961. Seven observations were made between these two dates. Results of Experiment 1, are presented in Tables 1, 2 and 3.

Table 1. Number of live plants at harvest, planted in a denuded area. Plants 1 year old originating from different tuber pieces. Original number of propagules per plot: 20.

Treatments°	Replicates I	II	III	IV	Total of Treatments
CP5	5	2	8	4	19
CP10	9	13	6	5	33
MP5	7	5	7	7	26
MP10	7	9	7	5	28
TP5	11	12	9	13	45
TP10	14	13	5	9	41
Total of Replicates	53	54	42	43	192

°See text for explanation of treatments.
C (correction of mean): 1536.
Sum of squares of treatments: 118
Sum of squares of replicates: 20. 33
Total sum of squares: 246.

Table 2. Analysis of Variance of data in Table 1.

Source of Variation	Sum of Squares	Degrees of Freedom	Variance	F. Calc.	F. Tables 0.05	Significance
Treatments	118.00	5	23.600	3.2878	2.90	+
Replicates	20.33	3	6.776	0.9439	3.29	
Error	107.67	15	7.178			
Total	246.00	23				

+ Significant difference between treatments.

Table 3. Least significant differences of Experiment 1.

L.S.D. at 0.05 level: 16.146

Treatment	Totals of treat.	Diff.	% Sprouting	Significance°
TP5	45	–	56.25	a
TP10	41	4	51.25	ab
CP10	33	8	41.25	abc
MP10	28	5	35.00	bc
MP5	26	2	32.50	bc
CP5	19	7	23.75	c

°Treatments that do not have letters in common are significantly different at p:0. 05, according to Duncan's method, (1955).

Treatments that sprouted first were CP5 and CP10, (Crown pieces, 5 and 10 cm. size); however, crowns of 5 cm. presented the lowest number of living plants at harvest, probably due to the amount of reserves present in that part of the rhizome. 211 out of 480 propagules were lost by rotting. Highest decay was found in middle pieces.

Experiment 2. — *Planting in secondary vegetation 3-5 m. high (2-3 years old).*

The area selected for this experiment was contiguous to that of Experiment 1, with similar soil properties but different vegetation coverage, with dominant *taxa* such as *Heliocarpus donnell-smithii, Apeiba tibourbou, Cochlospermum vitifolium, Bixa orellana, Waltheria brevipes, Clibadium arboreum* and*Luehea speciosa.*

Treatments and experimental design used was the same of that used in Experiment 1, but each tuber piece was weighed.

The experiment was planted in February, 1961 and harvested December, 1965. Six observations were made between these two dates consisting in counting the number of sprouted propagules. Results from this experiment are presented in Tables 4, 5, 6 and 7.

Table 4. — Number of live plants at harvest planted in a secondary vegetation 3-5 m. high Plants 46 months old originating from different tuber pieces. Original number of propagules per plot :20.

Treatments°	Replicates				Total of Treatments
	I	II	III	IV	
CP5	2	3	3		8
CP10	2	1	4		7
MP5	–	5	1	3	9
MP10	1	3	1	3	8
TP5	4	5	11	3	23
TP10	9	2	4	2	17
Total of replicates	18	19	24	11	72

Table 5. Analysis of Variance of data in Table 4.

Source of Variation	Sum of Squares	Degrees of Freedom	Variance	F. Calc.	(F. Tables) 0.05	Significance
Treatments	53.00	5	10.60	1.79	2.9	+
Replicates	14.33	3	4.77	0.80		+
Error	90.67	15	5.91			
Total	158.00	23				

+ No significant difference between treatments nor between replicates.

Results obtained at almost four years of growth contrast with those obtained at 1 year. One of the observations between planting and harvesting was analyzed in an attempt to locate the cause of this difference Table VI shows the analysis of variance of data obtained in an observation two years after the planting.

Table 6. Analysis of Variance of data obtained in an observation of number of sprouted propagules two years after planting in secondary vegetation, 3-5 m. high.

Source of Variation	Sum of Squares	Degrees of Freedom	Variance	F. Calc.	F. Tables 0.05	Signifcance.
Treatments	120.00	5	24.00	6.93	2.90	+
Replicates	4.50	3	1.50	0.44	3.29	
Error	52.00	15	3.46			
Total	176.50	23				

+ Significant difference between treatments.

Table 7. Least significant differences for the Analysis of Variance in Table 6.

L.S.D. at 0.05 level: 11.203

Treatments	Total of treat.	Diff.	% Sprouting	Significance+
TP5	34	–	42.50	a
TP10	28	6	35.00	ab
MP10	17	9	21.25	bc
CP5	14	3	17.50	cd
MP5	13	1	16.25	cd
CP10	8	5	10.00	cd

+ Treatments that do not have letters in common are significantly different.

Best treatments at 2 years after planting were again terminal parts of both sizes. Crown parts showed a consistent tendency of prompt sprouting just after planting but a poor survival. Only 15% of the original propagules planted remained alive until the fourth year. Highest losses were found in treatments involving middle and crown tuber pieces.

Experiment 3. — *Planting in secondary vegetation 15-20 m. high (15 years old).*

A site with an advanced secondary vegetation was selected located 25 m. from preceeding experiments. The site had the same soil properties and topography as the sites of Experiment 1 and 2. Dominant species here were *Terminalia amazonia, Apeiba tiborbou, Luehea speciosa* and *Cordia alliodora.* Trees were 15-20 m. tall and the surface of the soil was almost completely shaded and covered with a thick layer of humus. Vegetation was not distributed for the establishment of the experiment.

The date of planting was February 1961 and of harvesting December 1965. Between planting and harvesting 5 counting of sprouted propagules were made. The same treatments and experimental design as in the preceeding experiments were used. Tables 8, 9, 10 and 11 show results and statistical analysis.

Table 8. — Number of live plants of D. composita at harvest. Propagules planted in an secondary vegetation, 15-20m. high. Plants 46 months old originating from different tuber pieces. Original number of propagules per plot: 20.

Treatments°	Replicates I	II	III	IV	Total of treatments
CP5	2	–	–	3	5
CP10	8	1	1	1	11
MP5	–	2	–	1	3
MP10	1	2	3	–	6
TP5	7	1	5	5	18
TP10	3	2	1	3	9
Total of replicates	21	8	10	13	52

°See text for explanation of treatments.

C (correction of mean): 112.66
Sum of squares of treatments: 36.34
Sum of squares of replicates: 16.34
Total sum of squares: 109.34

Table 9. — Analysis of Variance of data in Table 8.

Source of Variation	Sum of Squares	Degrees of Freedom	Variance	F. Calc.	F. Tables 0.05	Signif.,
Treatments	36.34	5	7.26	1.92	2.90	+
Replicates	16.34	3	5.44	1.43	3.29	+
Error	56.66	15	3.78			
Total	109.34	23				

+ No significant difference between treatments nor replicates.

As in the case of Experiment 2, no difference was founded of regenerative ability among the different parts of tubers of *D. composita* almost 4 years after planting. Table X shows the analysis of Variance of data obtained in an observation two years after planting.

Table 10. Analysis of Variance of data observed on number of sprouted propagules of D. composita two years after planting in secondary vegetation, 15-20 m. high.

Source of Variation	Sum of Squares	Degrees of Freedom	Variance	F. Calc.	F. Tables 0.05	Signif.,
Treatments	233.84	5	46.76	4.84	2.90	+
Replicates	23.17	3	7.72	0.80	3.29	
Error	144.83	15	9.65			
Total	401.84	23				

+ Significant difference between treatments.

Table 11. — Least significant differences for the Analysis of Variance in Table 10

L.S.D. at 0.05 level: 18.71

Treatments	Total of treat.	Diff.	% Sprouting	Significance+
TP10	44	—	55.00	a
TP5	33	11	41.25	ab
MP10	29	4	36.25	ab
CP10	21	8	26.25	bc
MP5	10	11	12.50	cd
CP5	9	1	11.25	cd

+ Treatments that do not have letters in common are significantly different.

Terminal pieces 10 cm. size again showed the higher regenerative ability. Middle pieces showed a similar regenerative ability as terminal pieces 5 cm. in length. The middle and crown pieces of the tuber gave the worst response. In Experiment 3 only 10.8 percent of the total initial population survived. Almost 95 and 90% respectively of the propagules of treatments with middle and crown pieces, decayed at fourth year.

Experiment 4. — *Planting in secondary vegetation, 3-5 m. high during the rainy season.*

A fourth Experiment was planned to observe the periodical increase in weight of tubers. Treatments with terminal and crown pieces only 10 cm. in length of *D. composita* were programmed to be harvested 6, 12, 18 and 24 months after planting. A new variant was introduced in this experiment: to plant the propagules in the middle of the rainy season. The high moisture of the soil caused an almsot complete lost of propagules due to decay. No analysis of results was made.

Analysis of weight increase of D. composita tuber pieces.

The individual weight at planting and harvest of each propagule used in Experiments 2 and 3 was analyzed. However, all treatments except two showed a loss in weight at harvest. Almost all the surviving plants had considerable gains in weight and increase in tuber length was quite noticeable.

Difference in weight (gain or lost) of tuber at harvest are presented in Table 12.

Table 12. — Differences in weight at harvest of propagules of D. composita planted in secondary vegetation (3-4 years old). Age of plants at harvest : 46 months.

Treatment	Difference in weight (neg. values)	Diff. bet. treatments	Significance +
TP5	1.528	—	a
TP10	4.225	2.697	ab
CP5	4.245	0.020	ab
MP5	7.090	2.845	ab
CP10	9.605	2.515	b
MP10	19.360	9.755	c

+ Treatments that do not have letters in common are significantly different.

Table 13. — Differences in weight at harvest of propagules of D. composita planted in a secondary vegetation (15-20 years old). Age of plants at harvest: 46 months.

Treatment	Difference in weight (negative values)	Diff. bet. treatments	Significance +
TP5	0.030	—	a
CP5	3.765	3.735	b
TP10	4.100	0.335	b
MP5	7.620	3.250	c
CP10	9.775	2.155	c
MP10	15.610	5.835	d

+ Treatments that do not have letters in common are significantly different. Middle detrimental values are found in terminal pieces both of 5 and 10 cm. in length. Highest detriments were found in treatments with middle pieces. This difference could be due in part to the wide difference in weight between the light terminal parts and the bulky middle pieces.

Discussion

A definite behavioral pattern was found in the tests of regeneration ability of tuber pieces of *D. composita*. Terminal pieces, where a great amount of meristemal tissue is found, presented a consistent high regenerative capacity from the first year of growth. Although crown pieces sprout very promptly, they do not have the capacity to survive probably due to the lack of reserves. Middle pieces, although containing a good amount of reserves are very susceptible to decay due to their relative lack of meristemal tissues and the large area exposed to fungus and nematode invasion.

A marked difference in the number of surviving plants was found among the three different experiments at the end of the first year; number of surviving plants decreased as the age of secondary vegetation increased, e.g. the denuded area gave a higher number of survival than the young and advanced secondary vegetation. The young (3) 4 m. high) secondary vegetation had more live plants at harvest than the site with advanced secondary vegetation (15-20 m. high).

Obviously a strong competitive factor produced by the native vegetation plays a very important role in the regenerative ability of *D. composita* tubers. This phenomena was observed also by Cruzado, Delpin and Roars, (1964) who found that all live supports sharply reduced *Dioscorea* yields. *Melinis minutiflora* Beauv., the molasses grass," showed a very interesting kind of root inhibition with *D. composita* propagules. Tuber pieces growing under an area heavily covered by this grass remained alive with the same original size and weight during the almost four years of the experiment.

An abundant wild rodent, the "tuza", (*Geomyces sp.*) affected to some degree the planted population of *D. composita* propagules. However, highest loss was produced by decay of the tuber on the ground. The rainy season was very unfavourable for the planting of any *D. composita* tuber fragment.

The results of the experiments conducted show that reduced regeneration practices with *D. composita* tuber pieces are not favourable. Natural regeneration from abandoned terminal pieces of tuber seems to be more successful. Observations on the influence of the dept of planting of propagules and on the periodical gain in weight are needed to obtain a more complete picture of the regenerative ability of *D. composita* tuber segments.

SUMMARY

Tuber fragments of *Dioscorea composita* were used to observe their regenerative ability. Crown, middle and terminal pieces of the tuber were used and two sizes of each part tested. To determine the effect of the vegetation on establishment, — propagules were planted in three stages of secondary vegetation. Duration of experiments was from 1 to 4 years. At four years no significant difference among the treatments was found. However, at the end of the first and second year, terminal parts presented the highest regenerative ability. Crown pieces sprout promptly but do not survive after few months. Middle pieces are very susceptible to decay. Competition by the native vegetation showed to be an important limiting factor — for practices aimed at increasing population. A sketch is — presented of *D. Composita* in its natural environment in Mexico, derived from the ecological studies conducted to date.

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