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EXPERIMENTAL CULTIVATION OF REBAUDI'S STEVIA IN CALIFORNIA

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The leaves of Rebaudi's stevia (Stevia rebaudiana Bertoni) contain stevioside and other compounds about 250 times as sweet as table sugar (1). Preliminary trials at Davis have shown that stevioside production could be equivalent to the sweetening power of 28 tons/acre of sucrose sugar. However, much needs to be learned about production problems before the plant could be grown for commercial production.

Stevia rebaudiana belongs to the eupatory tribe of the composite family and is native to northeastern Paraguay. Stevia rebaudiana is related to other members of the genus found in the US and south into Central and South America (2,3).

History and Potential

Stevia has been used historically by the peoples of Paraguay as a sweetener and herbal remedy. The compounds of interest are the chemicals stevioside (Figure 1), rebaudioside A, and at least 6 other sweet compounds which have glucoside groups attached to a three-carbon-ring central structure (4).

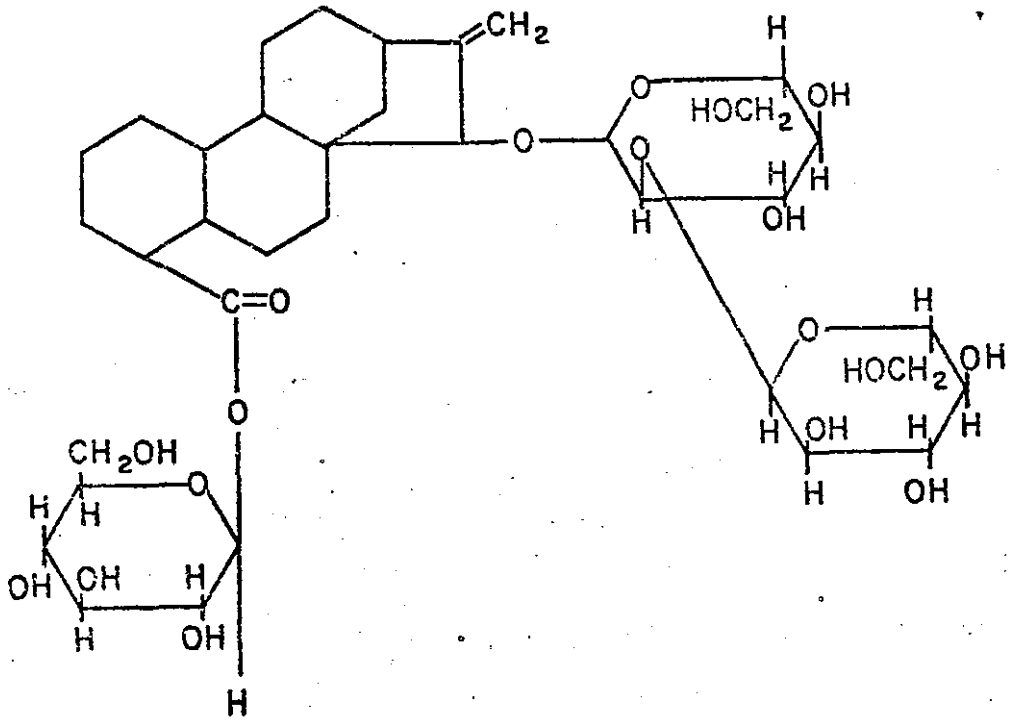

Figure 1. The chemical structure of stevioside.

Stevioside concentrations usually range between three and ten percent of the leaf dry weight (5) while rebaudioside A is less concentrated ranging from one to three percent (6).

Stevioside has been of interest not only as a sweetening agent, but also as a source of gibberellins, plant growth homones, most of which are not available commercially. The glucoside groups are removed from stevioside to yield steviol. Modified or normal steviol has been placed in the growth medium of Gibberella fujikuroi mutants to produce several gibberellic acids (7). Also gibberellic acid GA is known to occur naturally in stevia leaves but not in commercial quantities (8).

Health risks from human consumption of the various compounds in Stevia rebaudiana are not well defined and are being studied by others.

Climate and growth habit in area of origin

Stevia occurs naturally in northeastern Paraguay in the Departmento del Amambay. Populations have been found in the watershed of the Ypane River at about 650 feet elevation (Figure 2). Stevia has been heavily grazed, harvested, and transplanted to cultivated areas reducing its natural occurrence. Plants occur on the edges of marshes or in grassland communities on soils with shallow water tables. All sites are continuously moist but not subject to prolonged inundation. The soils are typically infertile, acid sands or muck. The climate can be characterized as semihumid subtropical with temperature extremes from 110 to 21°F. Average annual temperature is 75°F and rainfall about 55 inches per year.

In competition with natural vegetation, stevia is a slender perennial herb growing to 24 inches tall. The plant remains vegetative in the spring through early summer and flowers in the late summer and fall as a short day plant. The shoots usually die after maturing or are frosted off, with new growth coming from tillering at the plant base. In the wild, it reproduces by seed, crown division, or the rooting of branches that lodge or are trampled by cattle. Viable seed production is erratic. Most of the plants alive in the wild are survivors from plants present the previous year.

Cultivation

When stevia is placed under cultivation at or near its natural habitat, it is much more vigorous than in natural populations, reaching one meter or more in height. Branching and tillering are also much more profuse. It does require frequent irrigation and is a poor competitor with weeds. Currently the plant is under experimental cultivation not only in Paraguay but also in Brazil, Japan, Korea, Taiwan, and Southeast Asia.

Little is known about the cultural practices that would allow efficient commercial cultivation of this crop in California. The partial information available from other areas is useful. Also some of the physiological requirements and agronomic potential can be deduced from the plant's origin.

The occurrence of stevia on acid, infertile sandy or muck soils with ample supplies of water is consistent with observations of plant performance under cultivation. Frequent shallow irrigations are apparently indicated, and the plant has poor tolerance to water stress. The plant has little salt tolerance so it should not be grown in saline soils or with poor quality water. Most agricultural soils are of better fertility than the soils at the plant's origin so fertilization requirements are expected to be minimal. Experiments in Korea resulted in leaf yield increases with moderate applications of nitrogen, phosphorus, and potassium fertilizers (9).

Poor germination in cold weather, slow initial seedling growth rates and greater yields by early planting have combined to lead producers in Korea and other temperate regions to start plants indoors and then transplant outside during the early spring. Seeds germinate better when given light and warm temperatures (10), but plants are more productive when seedlings or rooted cuttings are set out as early as possible in the spring (11), Plants will overwinter in Davis, California, and may be grown as a perennial.

Short days promote flowering. In its native habitat at 21-22^{o}S latitude plants start flowering from January to March equivalent to July and September in the northern hemisphere. Subsequent flowerings occur in rapid succession as regrowth from the plant crown grows shorter each time until winter in July.

Long days favor leaf yields and leaf stevioside contents (5). Consequently the growth of plants in temperate areas with long summer days would be ideal for high stevioside yields, but seed production would be difficult.

In its region of adaptation stevia competes with other small plants adapted to infertile soil. Under cultivation weeds grow much faster than well-established stevia plants. Weed control practices in stevia plantings remain a problem.

Experimental cultivation in California

Seeds and live plants were collected by the author in the wild along the tributaries of the Ypane river in Paraguay. Seeds were planted in the greenhouse and in the field at the University of California, Davis to monitor suvival. Germination occurred mostly on the sixth day and was very poor. In order to find stevia plants that would overwinter, about 200 plants were set out in the field in 1979. Eighteen lines survived the moderate winters of 1979-80 and 1980-81. Plant tops died back completely after light frosts except for line 18 which retained green branches.

After overwintering, sprouting began in March, but significant growth did not occur until April. Survival of resprouting plants in the spring was threatened by slugs. Flowering occurred in October but frost or cold weather terminated the reproductive cycle in November before seed development was completed.

The 18 lines were clonally propagated to compare the productivity of the surviving lines and collect information on how the plants could be harvested for leaf production. Cuttings were taken from different parts of the plant to determine the best parts for vegetative propagation. Cuttings were made from the growing tips of branches, the second 3 inch segment down the branch or other parts of the plant top. These cuttings were kept separate by clone and plant part, and the success of rooting and vigor of the rooted cuttings was measured.

Cuttings 3 inches long were treated with rooting hormone, Rootone F, and planted in a fertilized mixture of peat and sand on a 2 x 2 inch spacing. The fertilizer consisted of 7.5 lb dolomitic lime, 2.5 lb hydrated lime, 2.5 lb single superphosphate, 1.5 lb urea, 3/8 lb of potassium sulfate and 1/4 lb of 12-12-12 per cubic yard of mixed sand and peat. Cuttings were maintained in a mist chamber at about 70°F for four weeks with 10 seconds of mist every 10 minutes.

All plant parts from all clones rooted successfully under mist. Cuttings from the growing tips of branches rooted most quickly and grew into the largest plants (Table 1). Plant dry weights after 100 days reflect the difference in initial vigor even though these differences were minimized by competition by other cuttings in the dense stand.

A greenhouse experiment was designed to test the relative productivity of 17 of the surviving clones of stevia. Each clone was planted, two plants per pot, with three replicates. These plants were repeatedly clipped to 4 inches height to measure their relative productivity. Given the high variability of yields between the replicates, the data show only a few significant differences in dry matter yield among clones (Table 3). The clones 1, 3, 5, 7, 12, 15, and 16 were the most productive. It appears that line 12 is very stemmy and lines 6 and 14 are subject to fungal attack. Stevioside content of each clone has not been determined.

A field clipping trial was planted in 1981 with plants of one clone, line 1 (Figure 3). The objective was to obtain data on stevia productivity as a function of plant density and harvesting strategy. Plants were established at densities of 8, 16, and 32 plants per square yard, and 225 lbs/acre of ammonium sulfate fertilizer was applied to supply nitrogen. Each plot consisted of 2 rows of plants. The rows were 12 inches apart with 28 inches separating plots laterally. Plants were harvested one or three times during the growing season, and only the central part of each plot was weighed. Clipping heights were 2, 4, and 6 inches, with 3 replicates.

When the plants were allowed to grow throughout the growing season dry matter yield averaged 920 g/m^2. A yield of 200 g/m^2 occurred where plants were clipped 3 times at 2 inch height (Table 3).

The plants clipped at 2 inches had a very low survival rate after the first clipping, and dry matter yields dropped off precipitously (Figure 3). In contrast, clipping at 6 inches resulted in greater plant survival and a different yield distribution pattern. Clipping at 6 in seemed to be superior to close clipping due to the enhanced vigor and survival of the plants.

Plants with densities of 32 plants/yd^2 had high initial mortality rates. There appeared to be no advantage in planting stevia at densities of greater than 16 plants/yd^2.

Stevia grown at Davis gave the greatest yields when harvested only once at the end of the growing season. Yields of 900 g dry matter per m^2 appear to be possible. If this yield consists of 40% leaf and if the leaf contains 7% stevioside, there would be 25 g of stevioside per m^2, the sweetening equivalent of 6.3 kg/m^2 of sucrose. On a per acre basis this would translate into 220 pounds of stevioside per acre with a sweetening power equvalent of approximately 28 tons/acre of sucrose sugar.

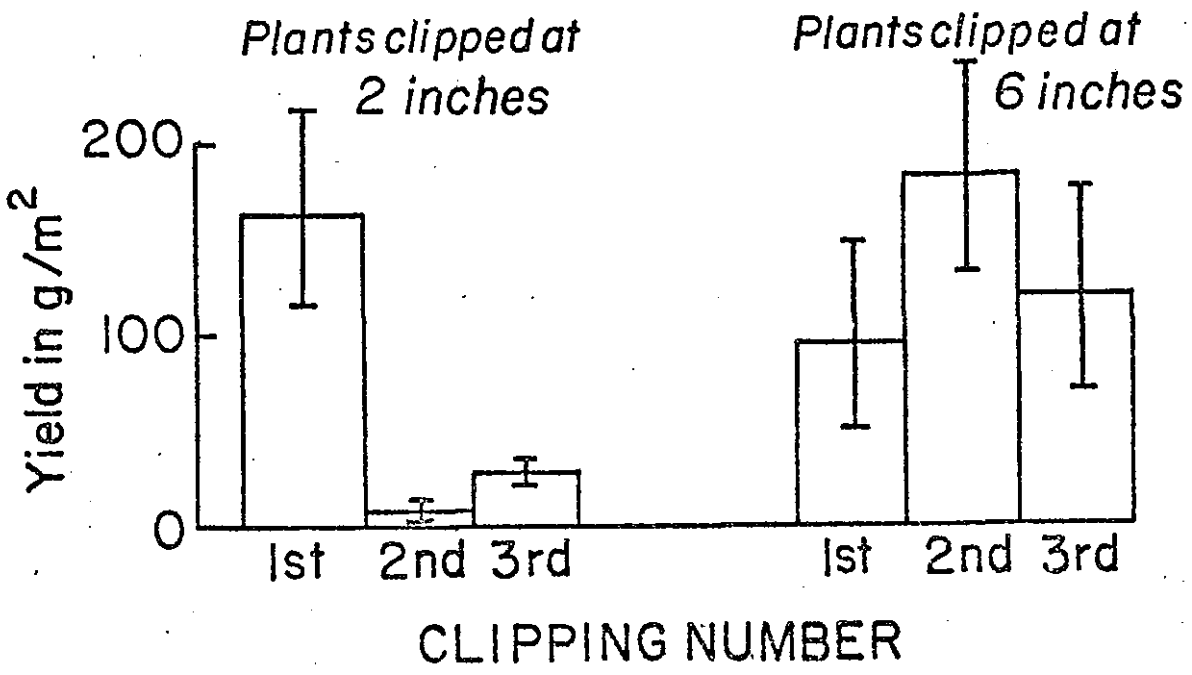

Figure 3. Comparison of the pattern of dry matter yield of Stevia rebaudiana clipped at 2 or 6 inches.

Possible sites for experimental plantings

Stevia apparently will produce best where there is a long growing season, minimal frost, high light intensities and warm temperatures. The plant is not adapted to water stress or saline conditions. Stevia occurs naturally on acid soils of pH 4-5 but will grow well on soils with a pH of 6.5-7.5, so the plant is apparently not demanding of acid soils. Although a wide range of sites might be tried throughout the state, southern coastal valleys away from the immediate influence of coastal fog would appear to be the most appropriate. Any production should be attempted with caution appropriate for a new crop with untested potential and problems.

Research needs

Stevia is still a plant of very recent domestication. It is believed that yield improvements can be achieved by selection and breeding for stevioside content, leaf to stem ratios, and plant response to fertilization. Other aspects needing investigation include methods of propagation, weed control, and water management. Plant materials with high production of viable seed need to be found. Optimal fertilizer formulations for California need to be determined. Research in these areas might result in a new crop plant for California agriculture.

No seed is available for distribution at this time. A limited number of rooted cuttings are available.

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Table 1. Stevia rebaudiana cuttings treated with rooting hormone rooted readily under mist in the greenhouse. Cuttings from the stem apex grew most rapidly and had significantly higher dry matter after 100 days averaging 2.9 g versus 0.9 g for the plants derived from other cuttings.

Clone	Plant part	Cuttings planted	Cuttings rooted	Dry weight of plant tops per plant
		No.	No.	g
1	Top	52	52	3.67
	2nd	12	12	0.95
	Lower	17	17	1.11
9	Top	13	13	3.02
	2nd	4	4	0.63
	Lower	3	3	1.07
14	Top	56	54	2.95
	2nd	20	18	1.49
	Lower	17	14	1.25
17	Top	29	29	3.50
	2nd	8	8	0.91
	Lower	9	9	0.72
18	Top	33	33	1.50
	2nd	22	22	0.66
	Lower	32	31	0.66

Table 3. Dry weight yields in the greenhouse of 17 clonal lines of Stevia rebaudiana after three harvests.

Clone	Dry weight g per pot
16	24.0
7	21.7
12	20.9
15	20.3
3	20.3
5	19.9
1	19.3
4	18.2
8	18.1
2	17.7
10	16.8
13	16.5
17	16.3
6	14.6
11	14.6
9	14.2
14	11.6

LSD (0.05) = 5.4 g

Table 2. Biomass yields of *Stevia rebaudiana* of clone line 1 given in the field different planting densities and harvesting strategies.

Treatment	Total yield leaves and stems		Plants surviving/yd^2 after clipping		
			1	2	3
	g DW/m^2	lb/acre equivalent			
1. 16 plants/yd^2 clipped once at 6 inches	920	8300	8[1]	8[1]	8
2. 16 plants/yd^2 clipped 3 times at 6 inches	390	3600	13	13	10
3. 16 plants/yd^2 clipped 3 times at 4 inches	360	3200	14	11	8
4. 16 plants/yd^2 clipped 3 times at 2 inches	200	1800	12	3	3
5. 32 plants/yd^2 clipped 3 times at 4 inches	300	2700	11	10	7
6. 8 plants/yd^2 clipped 3 times at 4 inches	160	1400	5	3	3
	LSD (.05)= 320				

[1]Not clipped on the 1st and 2nd harvest dates.

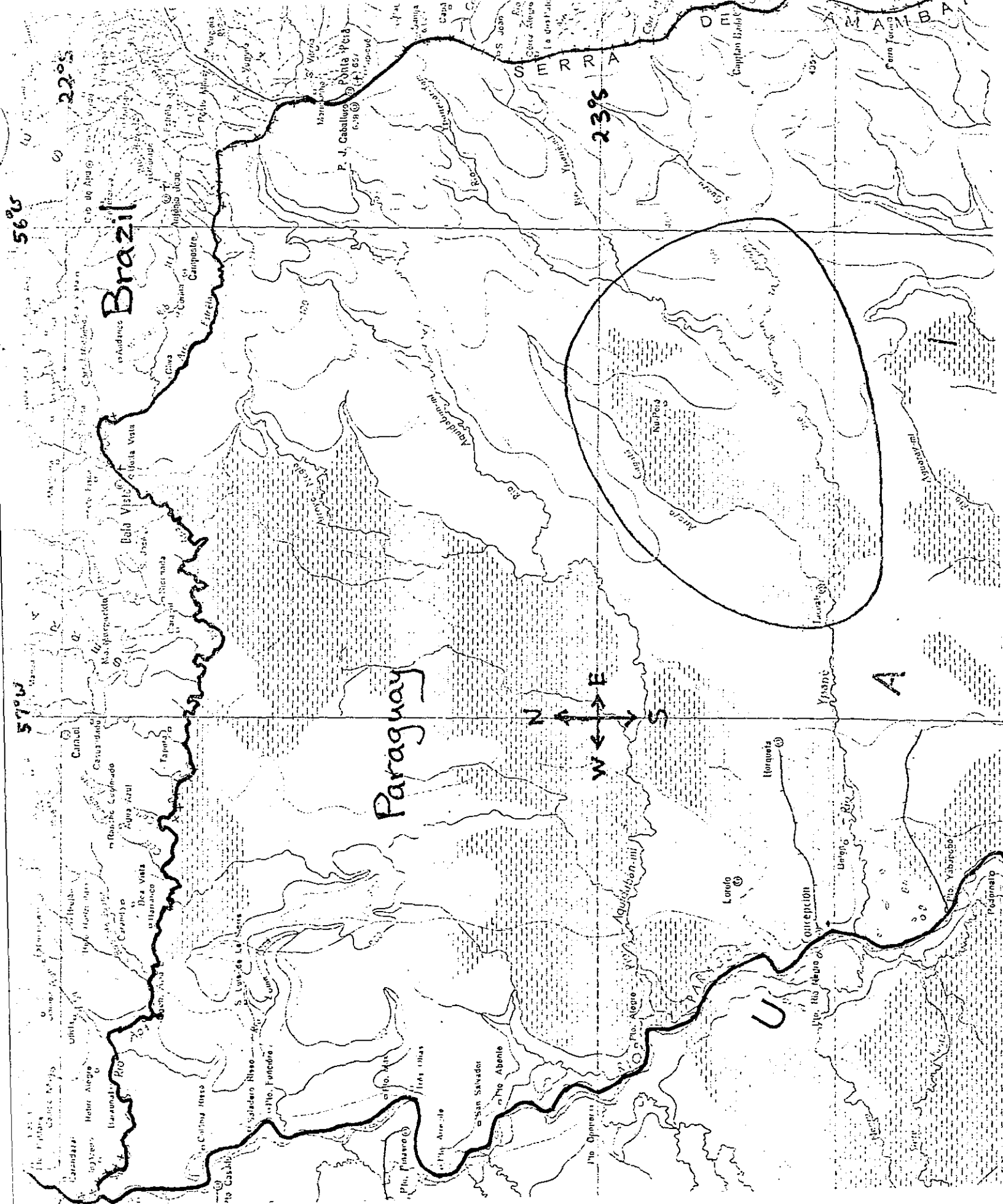

Figure 2. Collections of Stevia rebaudiana were made from natural populations near the tributaries of the Ypane River in the Departamento del Amambagy, Paraguay.