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New Marker Genes Found in Cassava

Clair H. Hershey and
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Marker genes control the expression of traits which can be easily classified into distinct states of the plant's development. Each state is controlled by a different allele whose expression is little influenced by the environment. Such genes can be a tool for studying linkage groups and mating systems; and sometimes they are directly important in determinating important economic characteristics.

In some well-studied crops like maize and peas, several hundred morphological marker genes are known. In cassava, only three have been reported: broad (recessive) versus narrow (dominant) leaf lobe shape (Graner 1942); light (recessive) versus dark (dominant) root surface color (Graner, 1942; Jos and Hrishi 1976); and male sterility (recessive) versus fertility (dominant) (Jos and Nair 1984). This report describes five new marker genes for cassava. ➪

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Materials and Methods

During 1987 and 1988 the breeding section of CIAT's Cassava Program studied several other traits to determine whether segregation patterns were consistent with single-gene inheritance. Those showing two distinct states, and with little environmental influence, were preselected for more detailed genetic studies. The traits studied and the two contrasting states of each were: chlorophyll production (normal versus albino), growth habit of the stem (straight versus zigzag), stem collenchyma color (light versus dark green), root parenchyma color (white versus yellow), and leaf lobe shape (pandurate versus smooth) (Figure 1). In all cases, the plants studied were the first generation progeny (F_1) from highly heterozygous clones.

Hypotheses for the inheritance of the individual traits were developed on the basis of empirical evidence and then crosses chosen which would test the hypotheses. Statistical analysis of the results was by chi^2 to determine whether segregation ratios differed significantly from those hypothesized from a single-gene inheritance pattern.

Results and Discussion

Chorophyll production

The clone CG 165-7, when selfed, produced progeny in the ratio of 3:1 (normal:albino). All other crosses resulted in all normal seedlings. It is concluded that albinism is the result of a single recessive gene. The authors propose that the name of the locus be "A". Thus, albino plants (homozygous recessive) are denoted "aa", and normal plants are either "Aa" or "AA". The clone CG 165-7 is a heterozygous carrier of the allele for albinism (Table 1).

Stem growth habit

In CIAT's germplasm collection, only three clones are recorded as having zigzag stem growth habit. Of these, only MVen 217 has produced sufficient seed for genetic studies. The selfed progeny of this clone were all zigzag, while all other crosses produced straight stems (Table 2). There is strong evidence to suggest that zigzag stem is controlled by a single recessive gene. The name proposed for this locus is "Z". Thus, the genotype of MVen 217 is "zz", and the other clones crossed onto MVen 217 were all "ZZ".

This character is especially interesting as a marker gene in cassava since it is the only nonlethal, single-gene-seedling trait so far identified. One practical use of this gene would be to study outcrossing versus selfing rates in a given open pollination situation.

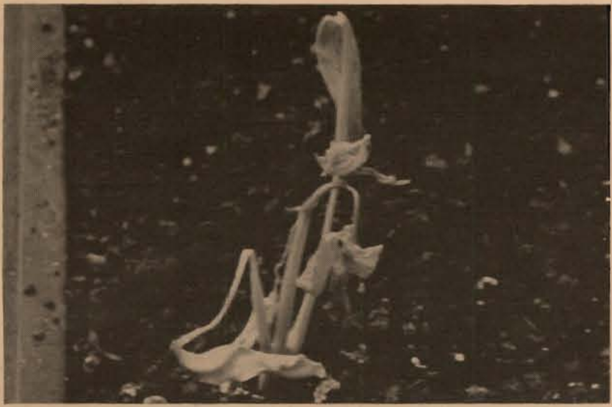
Stem collenchyma color

The stem collenchyma can be observed easily by scraping off the outer epidermis of a mature stem. The most common external stem colors in cassava are the result of the four possible combinations of light or dark brown epidermis, and light or dark green collenchyma.

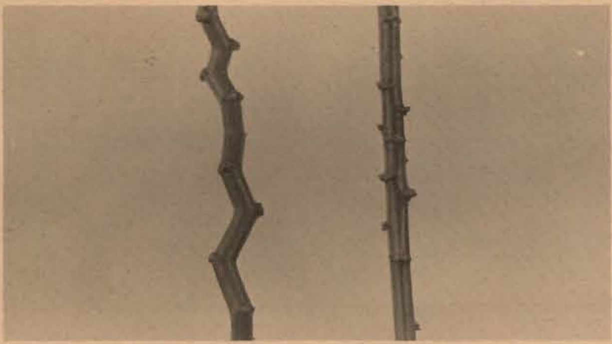
From preliminary observations of the data from several crosses, the authors developed the hypothesis that stem collenchyma color is controlled by a single gene, with light green dominant to dark green. The (chi)^2 analyses in Table 3 confirm the hypothesis. The authors propose to name this locus "G". Plants with light green collenchyma may be "Gg", or "GG", and those with dark green are "gg".

Root parenchyma color

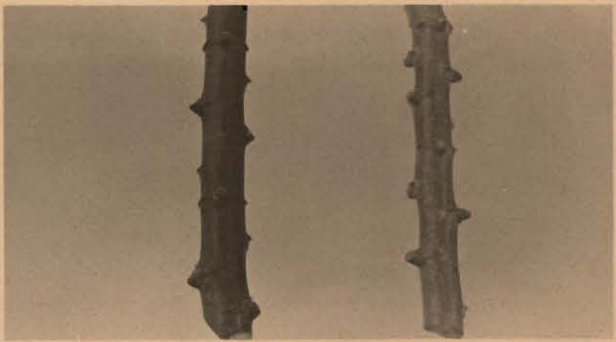
In the initial phase of this study on genetic markers, the observations on root parenchyma color were divided into just two classes: white and yellow. Data from several crosses were used to hypothesize single gene control for the trait, with yellow dominant to white (Table 4). ⇨



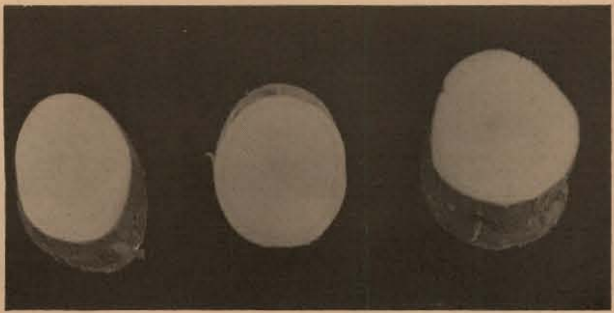
Chlorophyll production: Albino plant (left) versus normal plant.



Stem growth habit: Zigzag stem growth habit (left) versus straight stem.



Stem collenchyma color: Dark green (left) versus light green.



Root parenchyma color (left to right) white, light yellow, intense yellow.

Leaf lobe shape: Normal lobe (left) versus pandurate lobe.

Figure 1. *Constrasting phenotypes of the genetic markers studied.*

Table 1. Crosses used to study inheritance of seedling albinism in cassava.

Cross	Parent phenotype		Parent genotype		Progeny (no.)		Expected	
	♀	♂	♀	♂	Normal	Albino	ratio	$(chi)^2$
CG 165-7 Ⓧ	Normal	Normal	Aa	Aa	55	21	3:1	0.28
CM 523-7 Ⓧ	Normal	Normal	AA	AA	22	0	1:0	0.00
CG 165-7 x CM 523-7	Normal	Normal	Aa	AA	60	0	1:0	0.00
CM 523-7 x CG 165-7	Normal	Normal	AA	Aa	31	0	1:0	0.00

Table 2. Crosses used to study inheritance of stem growth habit.

Cross	Parent phenotype ♀	Parent phenotype ♂	Parent genotype ♀	Parent genotype ♂	Progeny (no.) Straight	Progeny (no.) Zigzag	Expected ratio	$(chi)^2$
Mbra 12 ⓧ	Straight	Straight	ZZ	ZZ	4	0	1:0	0.00
MVen 217 ⓧ	Zigzag	Zigzag	zz	zz	0	17	0:1	0.00
Mbra 12 x MVen 217	Straight	Zigzag	ZZ	zz	18	0	1:0	0.00
MVen 217 (O.P.)	Zigzag	?	zz	?	71	6	?	—

Table 3. Sample of crosses (from total of 67) used to study inheritance of stem collenchyma color.

Cross	Parent phenotype ♀	Parent phenotype ♂	Parent genotype ♀	Parent genotype ♂	Progeny (no.) Light	Progeny (no.) Dark	Expected ratio	$(chi)^2$
MCol 948C ⓧ	Light	Light	Gg	Gg	43	15	3:1	0.02
MCol 948C x CM 847-11	Light	Light	Gg	GG	45	0	1:0	0.00
CG 165-7 ⓧ	Dark	Dark	gg	gg	0	26	0:1	0.00
CG 165-7 x CM 523-7	Dark	Light	gg	Gg	30	25	1:1	0.45
CM 847-11 x CM 922-2	Light	Dark	GG	gg	36	0	1:0	0.00

Table 4. Sample of crosses (from total of 68) used to study inheritance of root parenchyma color.

Cross	Parent phenotype ♀	Parent phenotype ♂	Parent genotype ♀	Parent genotype ♂	Progeny (no.) Yellow	Progeny (no.) White	Expected ratio	$(chi)^2$
MCol 948C x CM 847-11	White	Yellow	yy	Yy	22	23	1:1	0.02
CM 1585-13 ⓧ	Yellow	Yellow	Yy	Yy	12	7	3:1	1.42
CM 1999-5 ⓧ	Yellow	Yellow	Yy	Yy	10	3	3:1	0.03
CM 1999-5 x CM 1585-13	Yellow	Yellow	Yy	Yy	33	8	3:1	0.66
MBra 12 x CG 165-7	White	White	yy	yy	0	33	0:1	0.00

However, in some of the segregating progeny, it was observed that there was an apparent segregation into three distinct classes: white, light yellow, and intense yellow. Therefore, some of the crosses which were still available in the field near the close of the study were reevaluated, and plants classified into three rather than two groups. The segregation patterns suggest that the gene may show a dosage effect, where the dominant homozygote shows an intense yellow, the heterozygote a light yellow, and the recessive homozygote is white (Table 5).

The authors propose "Y" as the name of this locus. Thus, "YY", "Yy", and "yy" are the intense yellow, light yellow, and white phenotypes, respectively.

Of the markers studied here, root parenchyma color is the one with the most obvious agronomic importance. In some markets, yellow fleshed roots are preferred. The yellow component of the roots is carotene, a precursor of Vitamin A, a nutrient that claims increasing attention among nutritionists because of deficiencies in large sectors of some populations. Knowing the genetics of the trait can greatly aid the plant breeder in selecting appropriate parents.

Leaf lobe shape

Of the clones used as parents, only CG 406-1 displayed the pandurate leaf character. All others had broad leaves, presumably homozygous recessive for leaf width (Table 6).

Segregation follows classical Mendelian patterns in some but not in all

➪

Table 5. Crosses used to test hypothesis of dosage effect of Y gene for root parenchyma color.

Cross	Parent phenotype		Parent genotype		Progeny (no.)			Expected ratio	$(chi)^2$
	♀	♂	♀	♂	Intense yellow	Light yellow	White		
CM 507-37 x CM 523-7	Yellow	White	Yy	yy	0	14	18	0:1:1	0.50
CM 430-37 x CM 996-6	Yellow	Yellow	Yy	Yy	14	24	14	1:2:1	0.46
CM 1585-13 x CM 1999-5	Yellow	Yellow	Yy	Yy	16	32	10	1:2:1	1.87

Table 6. Sample of crosses (from total of 22) used to study inheritance of pandurate leaf shape.

Cross	Parent phenotype		Parent genotype		Progeny (no.)		Expected ratio	$(chi)^2$
	♀	♂	♀	♂	Pandurate	Normal		
CG 401-6 x MCol 2016	Pandurate	Entire	Pp	pp	38	37	1:1	0.01
CG 403-18 ⓧ	Entire	Entire	Pp	Pp	18	164	3:1	411.50
CG 403-18 x CG 354-12	Entire	Entire	Pp	pp	4	38	1:1	27.52
CG 401-6 x Cg 501-1	Pandurate	Entire	Pp	pp	64	60	1:1	0.13
MCol 1488 x MCol 2016	Entire	Entire	pp	pp	0	70	0:1	0.00

crosses. Excluding the crosses in which CG 403-18 is one of the parents, one can hypothesize a single dominant gene controlling pandurate leaf shape. If this is the case, then, the clone CG 403-18 could be suspected of having the dominant gene, but its expression masked by one or more nonallelic genes, which also serve to mask expression of the pandurate gene in some proportion of the progeny.

Conclusions

The studies reported here have resulted in the description of five new marker genes in cassava controlling the following traits: chlorophyll production in seedlings, stem growth habit, stem collenchyma color, root parenchyma color, and leaf lobe shape. Names are proposed for each loci, and dominant and recessive phenotypes dêscribed. In the case of parenchyma color, the dominant gene appears to have a dosage effect, resulting in more intense color in the homozygote as compared to the heterozygote. Pandurate leaf shape presents some unclear segregation patterns, suggesting epistatic effects of other genes.

Recriprocal crosses studied for segregation of seedling albinism, stem collenchyma color, and root parenchyma color show identical ratios regardless of which parent was male or female, indicating that inheritance is not influenced by cytoplasmic effects.

While CIAT has done some preliminary studies on linkages among the newly identified genes, more crosses need to be analyzed. It will also be important in the future to study possible linkages to multigenically controlled traits of economic importance.

CIAT has begun to establish a marker gene collection, consisting of known genotypes of all the described marker genes with the possible genotipic combinations for these markers. These will be available for study by any interested scientist.

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Performance of Cassava in Multitier Cropping Systems

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The southern state of Kerala, India, has led the country in area cultivated and production of cassava since its introduction in the 16th century. Nevertheless, there has been a steady decline in area cultivated as profitable rubber and other plantation crops have moved into the area to take advantage of desirable humid tropical conditions. This has necessitated a thorough analysis of existing cassava production systems in the state.

The use of multitier cropping with crops having differing canopies is seen as a realistic approach to sustaining cassava production in the region.

Layout

Field experiments were conducted on fairly large plots (700-800 m^2) on sloping land (8-9%) during 1983-86, at the Central Tuber Crops Research Institute (CTCRI) farm. Four perennial species—coconut, banana, *Eucalyptus*, and *Leucaena*—constituted the first-tier crops, while cassava occupied the second tier, and seasonals such as groundnut or vegetable cowpea for fresh consumption constituted the ground tier. Single stands of perennials and cassava and intercropped stands of cassava with groundnut and cassava with vegetable cowpea were also evaluated.

Performance of Perennials

Aerial growth

Intercropping with cassava and other seasonal crops promoted the growth of *Eucalyptus*, the effect being conspicuous at the six-month stage and gradually narrowing toward the 30-month stage. *Leucaena* growth was adversely affected by cassava during the first 12 months and flowering and fruiting in banana took place earlier in the pure stands. Total leaf production and girth at the collar region of the young coconut plants were greater in single stands at 30 months.

Root spread

Cassava intercropping restricted the spread of lateral roots of both *Eucalyptus* and *Leucaena* without affecting the number of first-order roots. In *Eucalyptus*, compared with the maximum lateral spread of 4.85 m in a pure stand at the 32nd month, the spread was only 2.68 m in plants raised in association with cassava. In banana plots, the interrow space (5 m) was not entirely covered by the banana roots even at the 32nd month. Apparently the central row of intercropped cassava did not encounter the roots of the banana plants. Cassava intercropping carried out for up to three years had no apparent effect on the maximum spread and mean length of the lateral roots of coconut, since basins were maintained at 60 cm below ground level.

Shade effect

The shade of *Eucalyptus* plants increased from 15.0% to 52.6% over a period of three years. The shade effect of other perennials could be observed only on the adjacent cassava rows, which did not change much in the case of banana and *Leucaena*. In the case of coconut, there was a gradual increase in shade from 5.5% to 18.2% in three years (Table 1).

Performance of Cassava

Growth

Cassava growth in association with banana was more vigorous when compared with other combinations in all three seasons. The result was more conspicuous, however, during the first-year crop cycle of cassava with banana. Cassava growth in association with *Eucalyptus* was very much reduced from the second year onward. Inclusion of the seasonal intercrop (groundnut) reduced the growth of cassava, though reductions were not marked in the case of vegetable cowpea.

Table 1. Shade effect (%) of perennials on cassava.

Year	Perennials			
	Banana	*Eucalyptus*	Coconut	*Leucaena*
1983-84	13.6	15.0	5.5	25.0
1984-85	11.2	22.5	9.7	20.5
1985-86	15.7	52.6	18.2	21.5

Leaf area index (LAI)

The mean LAI of cassava in association with banana was 2.34, 1.64, and 1.85 during the first, second, and third year, respectively, compared with 1.55, 1.37, and 1.34 recorded in the pure cassava stand. During the first-year crop cycle there was no significant effect of *Eucalyptus* and *Leucaena* on the LAI of cassava. From the second year onward, however, there was a significant reduction in the LAI of cassava, both under *Eucalyptus* (11% to 38%) and *Leucaena* (15% to 44%).

LAI of cassava was not affected until the third year when cassava was grown in association with coconut. Coconuts were planted in pits, where root spread was confined in initial years and, therefore, there was no effect on LAI. Groundnut and cowpea influenced the mean LAI of cassava and the effects were more pronounced in intercropped stands of cassava with groundnut and cassava with cowpea when compared with three-tier cropping in association with perennials. Seasoned intercrops being shallow rooted resulted in competition, influencing the LAI of cassava.

Light-transmission ratio (LTR)

Light penetration to the lower layers of cassava canopy was less in banana when compared with other perennials as well as with pure stand cassava. The reduction in LTR of cassava under banana was more pronounced during the first year because of higher leaf area indices. There was no shade effect on cassava because of banana except on adjacent plants, which was only 11% to 15%. In other treatments, light penetration to the lower canopy was 64% to 79% during the first-year crop cycle. During the second- and third-year crop cycle, the mean LTR of cassava was 64% to 90% and maximum values were recorded under *Eucalyptus*, as the LAI of cassava was low.

Tuber yield

Analysis of tuber yield data during the first four years (Table 2) reveals that:

1. Except in the first year, when first-tier perennials were mostly in early establishment stages, cassava yield was generally higher in pure stands.

2. Fast growing tree species such as *Eucalyptus* and *Leucaena* adversely affected yield of cassava. Over the years, reduction in tuber yield was heavy in *Eucalyptus* + cassava plots, registering about 33%, 80%, 67%, and 36% in successive years (between 1983 and 1987), as compared to sole crop yields of cassava.

3. Replanting of banana after three years and coppicing of *Eucalyptus* at ground level after three crops of cassava (33-month-old *Eucalyptus*) significantly improved cassava yield in the fourth year.

4. Of the two ground-tier crops, cowpea appeared to be better and imparted less adverse effects on cassava yield. In a drought year (1984-1985), all crops adversely affected cassava yield.

Performance of Seasonals

The dry pod yield of groundnut was highest (1.07 t/ha) when intercropped with cassava. Growing of groundnuts as the third-tier crop under perennials and cassava resulted in considerable reduction in pod yield: lowest in association with *Eucalyptus* (0.41 t/ha) and highest in association with banana (0.78 t/ha). The green pod yield of vegetable cowpea was maximum in the intercropped stands of cassava + vegetable cowpea (4.81 t/ha). As a third-tier intercrop, vegetable cowpea performed better with banana + cassava (4.72 t/ha) and produced satisfactory yield with *Eucalyptus* + cassava (1.94 t/ha) as well. ➪

Table 2. Tuber yield of cassava in association with perennials and seasonals.

Crop combinations	Tuber yield (t/ha)				
	1983-84	1984-85	1985-86	1986-87	Mean
Cassava	29.4	17.7	28.1	30.0	26.3
Cassava + groundnut	32.2	13.6	19.6	23.9	22.3
Cassava + cowpea	36.4	17.2	22.0	24.3	25.0
Banana + cassava	47.8	12.6	21.3	32.1	28.4
Banana + cassava + groundnut	44.1	8.1	14.4	21.0	21.9
Banana + cassava + cowpea	43.7	11.4	15.4	20.4	22.7
Leucaena + cassava	23.8	5.4	11.1	14.0	13.6
Leucaena + cassava + groundnut	34.0	8.3	6.4	8.4	14.3
Leucaena + cassava + cowpea	32.2	7.8	9.1	7.9	14.3
Eucalyptus + cassava	20.0	3.6	9.3	19.3	13.3
Eucalyptus + cassava + groundnut	18.6	5.6	7.1	13.0	11.3
Eucalyptus + cassava + cowpea	20.6	7.0	6.1	16.7	12.6
Coconut + cassava	26.0	9.5	20.7	22.2	19.6
Coconut + cassava + groundnut	18.3	5.7	12.8	16.2	13.3
Coconut + cassava + cowpea	20.5	10.2	14.2	22.7	16.9
CD (5%)	5.97	2.99	4.78	6.11	—

Soil fertility and nutrient up-take

The organic carbon content of the soil increased when cassava was grown as a pure crop or along with perennials; it tended to decline when perennials were grown alone. There were more available nutrients in pure cassava plots when compared with cassava-perennial combinations, especially *Eucalyptus* plots. During the first year, nutrient removal by cassava was significantly higher when grown in association with banana. From the second year onward, however, nutrient uptake by cassava was significantly higher in the pure stand, followed by cassava intercropped with banana. Nutrient uptake by groundnut and cowpea was also higher when grown in association with banana + cassava, when compared with other perennial combinations.

Runoff and soil loss

Maximum water and soil losses were recorded from the bare fallow plot (19% to 25% and 4.7 to 11.9 t/ha, respectively), followed by cassava alone (10.5% to 18.9% and 1.78 to 7.3 t/ha, respectively). Pure stands of *Eucalyptus* and *Leucaena* were found to be almost on a par with single stands of cassava in checking soil erosion. Intercropping cassava with perennial trees was found to reduce soil loss 84% over a bare fallow plot. Soil loss was generally low in coconut plots, probably because of deposition of displaced soils in big coconut basins.

Pests and diseases

The incidence of red spider mites, the major pest of cassava, was high when grown in association with *Eucalyptus*, but low in banana plots. Cross infestation by red spider mites, as well as other species of mites, was observed on banana. Similarly, cassava thrips (*Retithrips syriacus*) were found infesting *Leucaena*.

The population density of the insect vector *Bemisia* of cassava mosaic disease (CMD) was maximum when cassava was grown in association with coconut and minimum in *Eucalyptus* + cassava. Although seasonal intercrops did not have any influence on vector population in the first three years, during the fourth year, the ground-tier crop (vegetable cowpea) showed 20% more vectors.

Economic Analysis

Calculation of net returns from different combinations through December 1986 indicated that cassava as a pure crop and in combination with seasonal intercrops provided the highest net returns. Cowpea as a ground-tier crop was more profitable when compared with groundnut. The negative net returns in banana and *Leucaena* pure stands result partly from low population density (800 plants/ha). In the case of *Eucalyptus*, however, even with similar low density of plant populations, the pure crop gave modest returns. Of the various crop combinations with *Eucalyptus*, *Eucalyptus* + cassava + cowpea provided maximum returns (Rs. 20,000/ha) through December 1986. ★

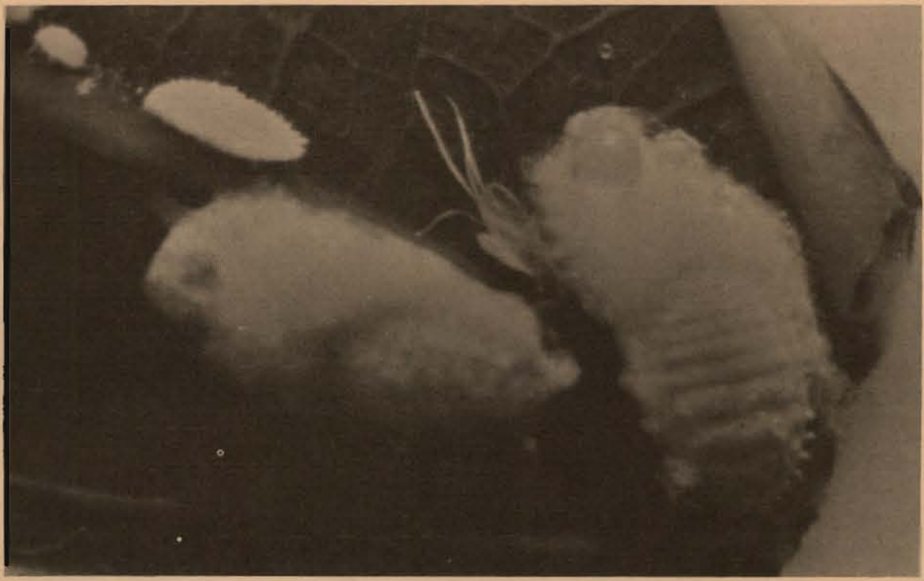
Calcium Extraction by *Phenacoccus herreni*: Symptoms and Effects on Cassava Photosynthesis

Octavio Vargas H., Anthony C. Bellotti, Mabrouk El-Sharkawy, and Ana del Pilar Hernández

Calcium plays a vital role in several aspects of growth and metabolism of the cassava plant, such as cell division, expansion of cell walls, ion exchange, and transportation of sugars. It is a chief compensating cation in the balancing of organic acid and cell pH control. The various compartments within the plant's cells contain different amounts of calcium. Cell integrity and form is maintained through a cell wall containing a large amount of calcium linked to pectic substances (Demarty et al, 1984, cited by Hughes, W.A.). This element is commonly known as a mitosis regulator. ➪

The bug *Phenacoccus herreni* causes cabbaging of buds and curling of the leaves of cassava.

The mealybug *Phenacoccus herreni* is an economically important pest in the Americas, while *P. manihotae* is important for Africa where it causes yield losses higher than 80% in cassava crops (Vargas and Bellotti, 1984; and Nwanze, 1982). The damage in both species is quite similar: a curling of the plant's buds, which gives them a cabbage-like look.



Female with ovisac and winged male of the cassava mealybug *Phenacoccus herreni.*

Lack of Calcium and Curling

Several experiments have been made in order to establish the reason why *P. herreni* causes curling. Before this study was undertaken, it was thought that curling was caused by a systemic toxin that had a localized effect.

In one of these experiments, 1, 5, 10, 20, and 25 first-instar nymphs were placed for 10 days at the plant's lower third 30 days after germination, in order to monitor if the buds curled. This did not happen. Another experiment placed 1, 5, 10, 15 and 20 nymphs and adult females in the plant's buds, and the pest's typical symptoms appeared. Thereupon, it was thought to be a localized toxin, that is, a toxin that cannot change location (CIAT Annual Report, 1984).

In order to elucidate whether it was indeed a toxin or some other factor, several tissue analyses were performed. The plant's Ca, P, K, and Mg levels were measured. Fifteen plants/treatment of variety MCol 1468 were used in pots containing 10 kg of soil. The treatments monitored were: 1) with bug, without extraction; 2) with bug, extracted at the moment of tissue analysis; 3) without bug, control.

The plants were infested with an ovisac (250-300 eggs) 30 days after stake germination. A 30-day attack was allowed on the pests, after which the elements mentioned above were measured.

In the first treatment, the bugs were withdrawn one by one for laboratory analysis. The percentage obtained was 0.33%, which indicates that *P. herreni* is extracting Ca from the plant; tissue analysis for this treatment yielded a 0.77% Ca level. By adding these two figures, we get 1.10%, a figure similar to the treatment without bug or the control one (1.14% Ca).

Longitudinal cuts were made both of infested leaves and of non infested ones, in order to monitor them at the microscope. Cuts from infested leaves showed that their lamella (calcium pectate) did not present inter-cell continuity. During the mitosis process, at the onset of the telophase, formation of lamella begins, which possibly suffers a dillution process through an enzime action caused during insect feeding. This is reflected in an inter-cell stiffness and becomes apparent by an undulation of the leaf film edges with a subsequent, more serious, curling (Figure 1).

The tissue analyses showed that the proportion of calcium presented the greatest differences (32.5%) between the treatments with or without the bug (Table 1).

Measuring Photosynthesis

In order to measure the damage made by the mealybug a photosynthesis experiment was carried out with two cassava varieties: SM 301-3 with 0.54% Ca and CM 2087-101 with 0.78% Ca. The varieties were planted in 30-kg pots of soil ⇨

Table 1. Tissue analysis for establishing Ca, P, K, and Mg withdrawn by *Phenacoccus herreni* in cassava.

Element	Without Bug (%)	Difference (%)	Removed Bug (%)	With Bug (%)
Ca	1.14	32.5	0.77	1.10
P	0.33	8.3	0.36	0.44
K	2.26	4.4	2.16	1.96
Mg	0.46	6.5	0.43	0.48

and were infested in natural conditions 30 days later with an ovisac/plant. The measurements or records of photosynthesis in the laboratory were made 33 days after infestation, during 10 consecutive days. Laboratory conditions for these measurements were as follows: ambient temperature: 30.65 ± 2.2°C; temperature of the water that regulates the measurement chambers: 23.02 ± 1.64°C; light intensity of the HPLR-type, 400-watt lamps: 1255 ± 111 UEM^{2}s-1; atmospheric pressure: 907 MB, and humidity: 60%.

Photosynthesis measurements showed that, as an effect of *P. herreni* infestation, a noticeable decrease of the photosynthetic rate, transpiration, water use efficiency, and conductance, both foliar and in the mesophyle, was observed for both varieties, as well as a moderate increase of the vapor pressure deficit, VPD, internal CO_2, and leaf temperature.

Photosynthesis decrease (the most important parameter for this experiment) was 54.1% for variety SM 301-3, and 45.4% for CM 2087-101 (Table 2).

The decrease in the photosynthetic rate is caused by the closure of stomata, as it is shown by the reduction in leaf water vapor conductance, that is, the gas exchange capacity between the leaf and the air layers next to it. When conductance is reduced, transpiration is also reduced, and this explains the increase in temperature, because transpiration helps, partially, to regulate leaf temperature. This explains, too, the increase in VPD. The calcium ion is now acknowledged as the greatest inter-cell regulator in numerous biochemical and physiological processes of the plants.

The lack of calcium and the reduction in photosynthesis contribute to increase, in one way or another, yield losses in cassava cultivars.

The studies described here, particularly that relating to calcium, help in the screening of cassava at the germplasm bank. They also help identify those varieties with a high content of calcium and select them for subsequent studies, with the ultimate objective of determining yield losses caused by *P. herreni*'s attacks.

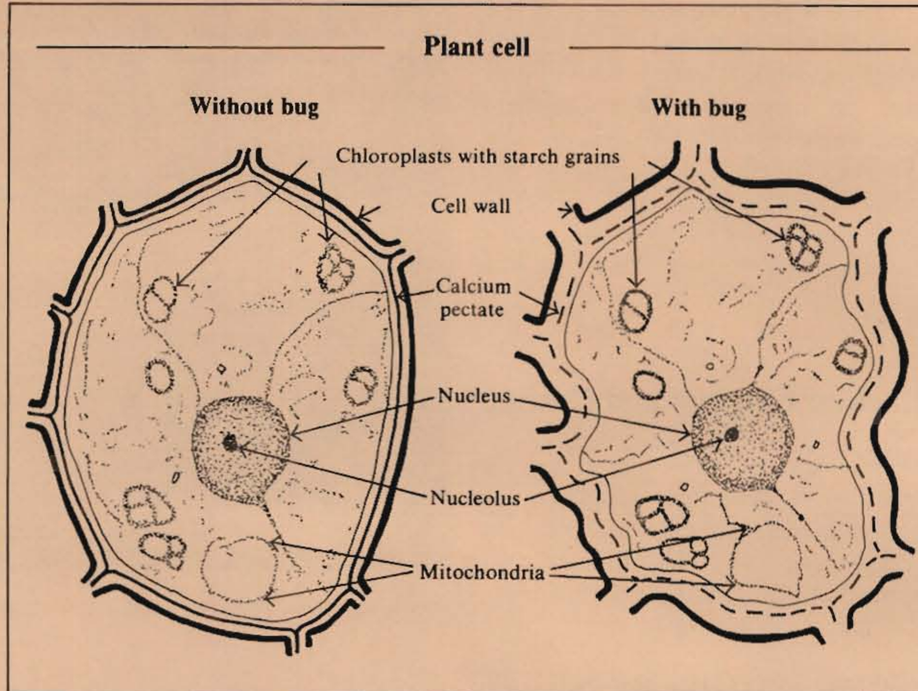


Figure 1. *Dissolution of Calcium pectate for the enzimatic action of the insect. Normal cell (left), affected cell (right).*

Table 2. Physiological parameters affected by the *Phenacoccus herreni* attack on two cassava cultivars.

	Clones					
	SM301-3 (%)			CM 2087-101 (%)		
Parameters	W/O Bug	W/Bug	Difference	W/O Bug	W/ Bug	Difference
Photosynthesis μmol Co_2 $m^{-2}S^{-1}$	19.6	9.0	54.1	15.2	8.3	45.4
Transpiration μmol H_2O $m^{-2}S^{-1}$	2.9	2.0	31.0	2.2	1.7	22.7
Efic. H_2O Utiliz. μmol CO_2 $mmol^{-1}H_2O$	7.2	4.8	33.3	7.3	4.5	38.4

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Producing *Saka Saka* from Cassava Leaves in the Ivory Coast

Aboua Firmin

Introduction

Saka saka, a traditional African food made from cassava leaves, is eaten by many people in Africa. In the Ivory Coast it is only consumed in the northwestern part of the country and it cannot be considered an important food by Ivorians. Consequently, farmers, after harvesting cassava roots, throw away the cassava leaves and lose part of their revenue from this crop. Failure to use cassava leaves as food creates a nutritional problem for Ivorians because cassava leaves are nutritional. They are a good source of protein (30% on dry weight basis), vitamins B^1, B^2, C, and minerals.

The review "Exportateur Ivorien" in 1983 reported that cassava leaves proteins are characterized by a methionine deficit but they can be regarded as nutritionally balanced. Other researchers have noted that the other essential amino acids do not present a deficit above 40%. Lysine content averages 7%. The amino acid content of cassava leaves of three Nigerian varieties averages 6% lysine, 2% methionine, 11% aspartic acid, 6% valine, 5.5% arginine, and 2.2% tryptophan. Nigerian varieties averages 14,7% protein, 8.4% ether extract, and 16% total ash. Cassava leaf meal is nearly equivalent to alfalfa meal in feed value.

Cassava leaf consumption can improve the nutritional quality of Ivorians foods commonly made from cassava roots, yam tubers and plantain. Another problem is that *Saka saka* is a very perishable product because of its high water content (92.4%). With a view for increasing its contribution to the food supply in the Ivory Coast, research was looked into the possibility of producing a product which has a long storage life and to develop a simple technique that does not require additional equipment to that commonly used by other food processors in the country, a high capital investment or expensive energy or labor.

This technique could be easily used in the rural areas of the country. Likewise, another objective is to introduce the use of saka saka and to promote its production and commercialization.

Materials and Methods

Young leaves from two local varieties of sweet cassava (Bononua, Bouaga) are used in *Saka saka* processing, these types of cassava grown everywhere in the Ivory Coast.

The preparation process consists essentially of harvesting fresh cassava leaves without leaf stalks, washing and boiling them and pounding them into a mash. This is either eaten as spinach or used for the stew processing.

A simple technique was developed for increasing the shelf-life of this traditional *saka saka.* The cassava leaves without leaf stalks are cut in pieces about 0.5-1 cm in length with scissors in order to partly reduce cyanogenic glucosides which on tissue damage are hydrolysed via cyanohydrins to HCN. The pieces of leaves are spread on a rush matting for one hour. They are washed three times in plastic pans and boiled in large cooking pots over a wood fire for 45-60 minutes. The boiled leaves are ground in wooden mortars with pestles into a mash. This is then canned, closed with a crimper and then heated at 120°C for 20 minutes in a sterilizer under atmospheric pressure. The sterilized cans are cooled in the water at room temperature for two hours and then stored for three or four days in order to eliminate defective products. The good cans are washed, wiped off and then check marked. They are put in the caboards in groups of thirty. Each can weighs 320 g (crude weight), 266 g (net weight).

Before eating saka sakaí, it should be heated adding meat, fish, red pepper and onion for 10 minutes and served hot as vegetable. It can also be crushed and used for sauce processing.

Saka saka is a green and acid food (pH = 5.1). The process developed is simple but preserves the "fresh" characteristics of the raw food. Through 15 months storage, more of the original flavor, color and taste are retained in canned food that by the comparable conventional *saka saka.* No quality changes in canned food are observed in any of the product. This has been possible because the canned *saka saka* is very sterilized. The process developed is limited by using the mortar for grinding. This gives a low yield, but it can be improved by mechanizing the grinding.

Organoleptic Tests

Some cans of *saka saka* were used for stew making. Stew was prepared and served as fresh traditional *saka saka* for organoleptic evaluation. The panel considered color, taste and flavor and rated them on the following scale: 5 = very agreeable; 4 = moderately agreeable; 2 = not agreeable; 1 = no opinion, (Table 1).

In the organoleptic test panel results, expressed in percentage of testers, the highest color evaluation (24%) was attributed to the green *saka saka* color. Regarding the taste evaluation, the highest appreciation (25%) was obtained with *saka saka* having a slightly acid taste. For the flavor, most of the individuals (23%) participating in the panel showed their preference for the very strong cassava leaves flavor. This represents a good acceptance of *saka saka* with similar organoleptic charac-

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Table 1. Panel evaluation of *saka saka* from cassava leaves expressed by percentage according to the scale in the text.

I Color					
Scale:	5	4	3	2	1
Green	24	5	4	1	0.22
Dark-green	3	26	1	2	1
Pale-green	1	4	7	20	1
II Taste					
Scale:	5	4	3	2	1
Slightly acid	25	7	3	1	0.44
Moderately acid	6	11	7	7	1
Strongly acid	2	5	7.44	17	2
III Flavor					
Scale:	5	4	3	2	1
Strong	23	5	4	3	2
High	7	4	22	1	1
None	1	3	6	20	0.44

Table 2. Proximate chemical composition of *saka saka* expressed by percentage.

Moisture	92.4
Dry matter xx	7.6
Ash x	10.2
Cellulose x	18.3
Protein x	27.6
Fat x	11.9
Starch x	3.65
Sugar x	1.44
Calcium mg/100g	960
Phosphorus mg/100g	250
Potassium mg/100g	620
Sodium mg/100g	2140
Magnesium mg/100g	3100
Iron mg/100g	36
Copper mg/100g	0.88
Zinc mg/100g	7.80
Manganese mg/100g	7.90
Hydrogen cyanide mg/100g	1.5

x. Expressed in percentage of dry weight.
xx. Expressed in percentage of wet weight.

teristics of the traditional *saka saka*. Proximate chemical compositions and minerals are listed in Table 2.

Saka saka is a moist food having a water content above 90%. Tests show that *saka saka* is rich in ash, cellulose, protein (10.2%, 18.3%, 27.6%) and mineral constituents on a dry-weight basis. The sugar and starch levels are low (1.4%, 3.7%). The high fat content (11.9%) is due to the result of red palm oil in the cooking pots. Cooking greatly reduces the cyanide content in *saka saka*. The residual level of cyanide is 15 mg/kg. Boiling, reducing a large fraction of the original cyanogen, gives a product which is safe to eat.

In conclusion, *saka saka* processing is simple and does not need sophisticated equipments with high capital investment and high energy consumption. *Saka saka* can contribute to improve the nutritional quality of Ivorian foods. It can be also an important commercial product for the farmers in the Ivory Coast. ★

James Cock Leaves CIAT Cassava Program Leadership

James Cock, Ph.D. in physiology, and a scientist with CIAT's Cassava Program since 1971, retired from the center to become a senior agronomist at the World Bank, Washington, D.C., in its Latin American and the Caribbeann agricultural projects.

At the time of his retirement, Cock was the leader of the Cassava Program. In this capacity he undertook work in physiology looking initially for ideal plants and researching their growth and development. He was part of the team that studied the interaction between the plant and the environment—the temperature effects, water stress, photoperiod, and other factors that led to the elucidation of why cassava has such high tolerance to drought. This research made it possible to identify cassava as a crop with intermediate characteristics between a C3 and a C4.

During the first years of his leadership, the CIAT's Cassava Program concentrated on the search for new varieties with a wide range of adaptation, following the green revolution trends set for rice and wheat. Data showed, however, that the cassava crop could not follow this trend, and the focus was switched to work in various ecosystems. Small-farmer problems were studied, and product postharvest and marketing were identified as the main concerns. The Program then began to work in areas of utilization and economics. Likewise, the idea that integrated projects linking the development needs of third world countries would be successful was reinforced.

In November 1988 Cock received the Donald Plucknett award given by the International Society for Tropical Root Crops (ISTRC) for research contributions to tropical root crops. He is also the author of "Cassava, New Potential for a Neglected Crop", published in 1985 and recently translated into Spanish. ★

Cassava Research Projects in Cuba

Sergio Rodríguez

A cassava research program has been conducted in the Republic of Cuba for more than two years aimed at supplying the market with this staple throughout the year.

With respect to plant breeding, it has been possible to make available clones with a greater production potential than the local types. In order to obtain genotypes adapted to specific conditions, zonal ecological trials are being conducted which serve as a foundation for simultaneously localizing and selecting clones in various ecosystems.

The program is studying two new, root-attacking, pests (*Cyrtomenus bergii* and *Pachneus* spp.). This has led to the establishment of a trials network aimed at solving this new problem for Cuban cassava producers. Currently, studies are carried out to ascertain when the pest's most prevalent and when it causes economic losses.

One important problem that had to be dealt with was root rotting caused by soil pathogens caused by the excessive moisture due to continuous rains. This was frequently present in soils with a deficient drainage. The solution was to establish the crop on ridges 30-40 cms high with a plant distance of 1.20 - 1.40 x 0.70 - 0.80 m according to the phenological characteristics of the clone. By using ridges, root rotting is minimized and the semimechanized harvest of the product is favored. This is important in Cuba because of the scarcity of labor.

Seed Production

The establishment of a program to produce reproductive material is one of the activities that has had a great influence on increasing crop yields. The program comprises everything from the production of original seed by research institutions to its certification by the producers.

Organization in the production of planting material and studies to identify the best stakes have helped to guarantee high percentages of sprouting, thus eliminating, in most cases, the need for replanting. The stakes have the following characteristics:

- They come from primary stems (the section included between the soil and the first branching point).
- Length of 20-25 cm.
- More than seven buds.
- Straight cut (when the plantations are extensive, the cut is made by sawing).
- Free from pests and diseases.
- Treated with fungicides and insecticides.
- Treated with zinc (when the plantation is going to be established in a field where a zinc deficiency is traditionally present).

Biotechnology has made it possible to exchange germplasm through in vitro cultures and to eliminate the risks occurring when this is done by stakes. Training technicians through postgraduate courses and producers by agricultural extension meetings and workshops in production areas have contributed notably to establish strong links between science, technology, and the producers.

At this time, conditions are appropriate to exploit the uses of cassava for animal feed.

A production program has been projected with the following objectives:

1. Continue looking for new genotypes with greater yield potential than the present clones; with resistance or tolerance to the main pests and diseases and good cooking quality.
2. Develop technologies for postharvest conservation.
3. Continue earliness studies in order to obtain genotypes with shorter harvest cycles.
4. Obtain clones with a disposition and length of tuber roots appropriate for mechanized harvest.
5. Identify Cuba's nutritional system for cassava. ★

A Mechanical Cassava Chipper

V. V. Sreenarayanan, R. Visvanathan, and K. R. Swaminathan

Cassava (*Manihot esculenta*), a root crop, is grown in the tropics to produce starch and allied products, such as animal feed. It is also eaten by humans. In the latter case, the shelf life of the tuber is very short; deterioration commences a few days after harvest.

For starch and feed production, freshly harvested tubers are used. The tuber is first cut into chips of desired thickness and dried for storage until required. Manual slicing, however, is a very time-consuming and inefficient procedure.

A vertical feed-type, power-operated cassava chipping machine has been developed at Tamil Nadu Agricultural University, Coimbatore. The various subassemblies of the chipper are the frame assembly, the power transmission system, the chipping disc and knives, feed hopper, guides and outlet (Figure 1).

The 37.5 mm x 37.5 mm x 3 mm motor frame is joined to a 285 mm x 360 mm x 400 mm shute. The bearing blocks, hopper assembly, primemover and outlet are mounted inside the smaller frame. A 0.5 h.p., vertically

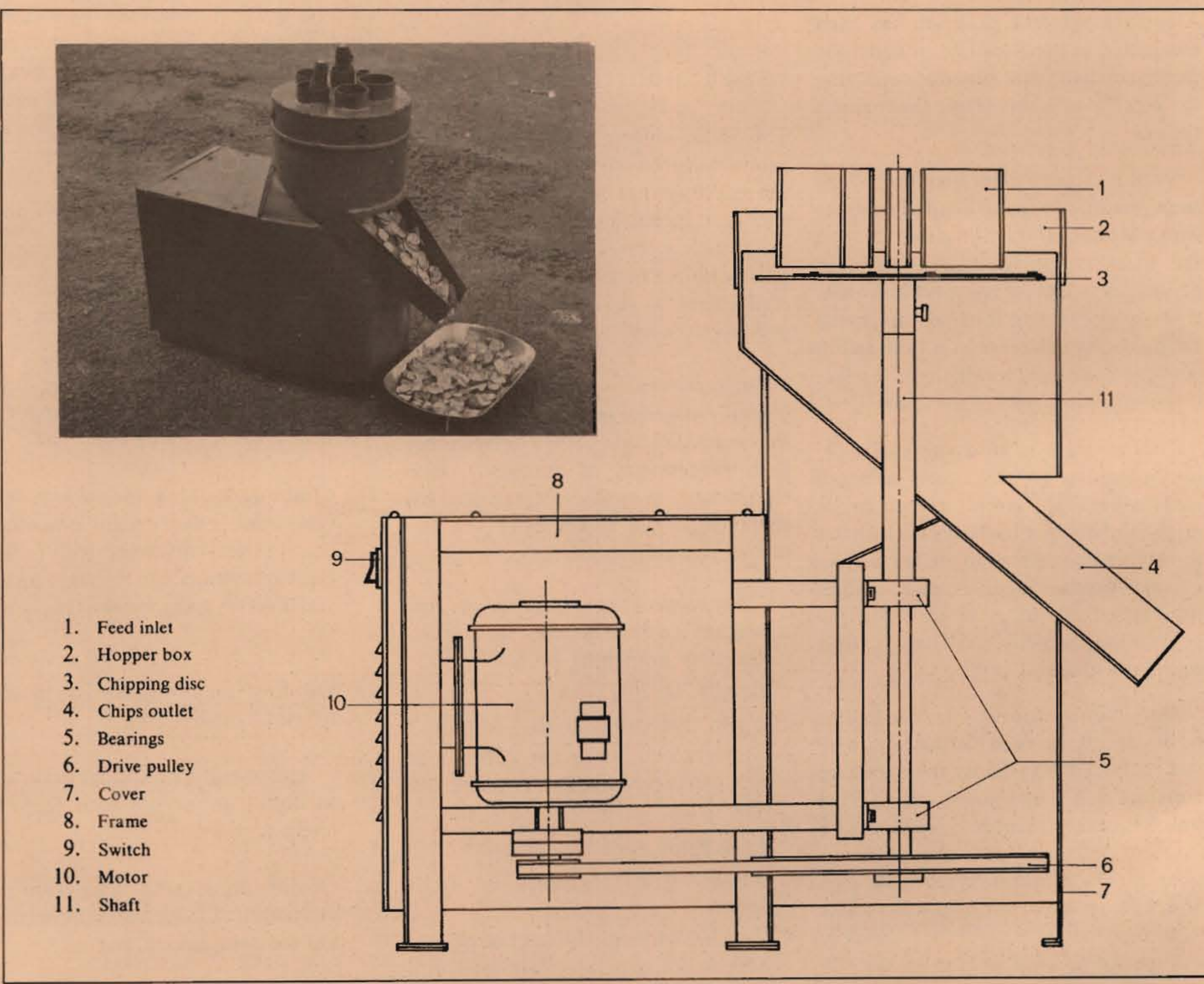

Figure 1. *Mechanical cassava chipper developed in India. Side view.*

mounted single-phase electric motor is used to rotate (by means of a V-belt drive) the chipping disc at 295 rpm. The 300 mm wide x 5mm thick chipping disc is made of soft steel. Three 110 mm x 35 mm slots are radially bored in the disc at equal angles. Three types of 110 mm x 35 mm high strength steel blades with 30 bevel edges are fixed to the slots into the chipping disc.

The assembly is mounted vertically on the two ball bearings at the top of a soft steel shaft (25 mm in diameter x 620 mm long). The 330 mm diameter feed hopper is fixed above the chipping disc and provided with guides varying in diameter from 80 to 25 mm through a space of 125 mm to accomodate various size tubers. The chip outlet has a 41° inclination for easy collection. A 3 mm thick, soft steel cover surrounds the unit.

Cassava tubers of varying diameter are fed through the guides to the rotating chipping disc. The slices are discharged through the inclined outlet and collected. Any desired thickness of chips, varying from 1 mm to 40 mm, can be obtained by adjusting the clearance between the chipping disc and knives. The capacity of the unit is 270 kg of cassava per hour. The chipper can be used to slice other tuber crops, like radish, carrot, potato, etc. ★

Keeping in Touch

Brazil to Benefit from Kellogg Foundation Grant

The W. K. Kellogg Foundation has approved a grant of US$961,000 to the International Center of Tropical Agriculture (CIAT), Cali, Colombia, to introduce cassava production and processing technologies in Brazil during the next three years. The objective of the project—which will be conducted in the states of Ceará, Bahía, Pernanbuco, and Paraíba—is to develop an alternative market for cassava by using it as a source of energy in animal feed. These states supply 40% of the total cassava production in Brazil. Brazil and Thailand are the world's largest producers of cassava.

The tropical root has a wide range of uses, including consumption as a fresh food, as an energy component in animal feed, as a partial wheat substitute in bread and pasta, as a starch in industrial and food products, and as fuel when converted into alcohol.

Project planners expect to replicate in Brazil the successful rural development projects based on cassava production and marketing that operate in Colombia, Ecuador, and Mexico.

When the project is launched, 25 agro-industries are expected to be created with small groups of farmers. Demonstration and technology validation units will also be used, and there will be multiplication plots for planting material.

Brazilian Ministry of Agriculture agencies and CIAT will participate in the project. ★

Cassava Residues Working Group Organized in Brazil

In April, technicians, professors, and researchers from various states in Brazil gathered at the Ponta Grossa State University, Parana State, to discuss the present status of the use and treatment of cassava residues. The problems pertaining to residue quantity and quality generation were highlighted. Besides polluting the environment with their high organic content, they are toxic to most organisms because they release cyanide. In spite of this, they have great potential for utilization. Three research areas were reviewed: direct use of residual water in agriculture (the fertilization/herbicide/nematocide/insecticide effect), treatment by anaerobic digestion, and production of microbial biomass. Some of these areas already have generated research results in pilot projects and field trials.

The need to evaluate the impact of these residues on soil microorganisms was also analyzed. Technicians, part of that multidisciplinary and multiinstitutional group, expressed their desire to interact with other technicians and other agencies interested in this area. Contact can be made through the coordinator, Magali Pascali Cereda, at the Agricultural Products Technology Department, Faculty of Agronomic Science, Campos de Botucatu, UNESP, Fazzenda Experimental Lageado, Cx P. 237, CEP 18600, Botucatu, SP. Brazil, Telex 0142107. ★