Effects of *Bauhinia monandra* (Kurz) Seed Extracts on Yield, Morphology and Anatomy of Cassava Plants[1]

*Z. Russom**

ABSTRACT

Cassava stem cuttings of TMS 30572 were partially soaked in extract of *B. monandra* seeds for 70 hours, planted in the field in April 1988 and harvested in April 1989. Second and third experiments were carried out during April 1989 - May 1990 and during May 1990 - May 1991. Stem cuttings used in Experiment 2 were taken from Experiment 1 and those used in Experiment 3 were taken from Experiment 2. Extracts were applied only in April 1988. Yield and morphological characters were recorded every year. In a fourth experiment, cotyledon extract of *B. monandra* was applied to tubers of 3-month-old cassava plants, and the control plant was left untreated. These plants were harvested a year later, and then planted again without re-treating. Morphological and anatomical characters were recorded from these second-generation plants. The *B. monandra* extract consistently increased the following characters: tuber yield, tuber number per plant, number of primary stems per plant, stem girth, height of stem from ground level to the first branching point, number of lateral branches per stem, number of branches at the first, second and third branching points, leaf petiole length and number of leaf lobes per leaf. Furthermore, plants treated with the cotyledon extract had different anatomical structures than the controls in the primary growth of the root, petiole and stem. Trichomes were observed only in the stems of the treated plants. Stomata and calcium oxalate crystals were observed in the leaf and stem of both treated and control plants. The morphological and anatomical changes observed in the extract treated plants appear to be stable.

RESUMEN

Estacas de yuca TMS30572 fueron remojadas parcialmente en extractos de semillas *B. monandra* por 70 h y, luego, sembradas en el campo en abril de 1988 y cosechadas en abril de 1989. Se llevaron a cabo un segundo y un tercer experimento en abril de 1989 y mayo de 1990 y durante mayo de 1990-1991, respectivamente. Se utilizaron estacas del Experimento 1 para el Experimento 2 y estacas del Experimento 2 para el Experimento 3. Se aplicaron los extractos solamente en abril de 1988 y se registraron el rendimiento y las características morfológicas cada año. En el Experimento 4, se aplicó extracto de cotiledón de *B. monandra* a tubérculos de yuca de tres meses, mientras que no se trató la planta-testigo. Estas plantas fueron cosechados un año después y luego resembradas, sin ser tratadas de nuevo. Se registraron las características morfológicas y anatómicas de estas plantas de segunda generación. Los extractos aumentaron consistentemente las siguientes características: rendimiento del tubérculo, número de tubérculos por planta, número de tallos primarios por planta, circunferencia del tallo, altura del tallo desde el nivel del suelo hasta el primer punto de ramificación, número de ramas laterales por tallo, número de ramas al primer, segundo y tercer punto de ramificación, largo del peciolo foliar, y número de lóbulos foliares por hoja. Además, las plantas tratadas con el extracto del cotiledón presentaron estructuras anatómicas diferentes a las de los testigos en cuanto al crecimiento primario de la raíz, el peciolo y la estaca. Se observaron tricomas sólo en los tallos de las plantas tratadas; y estomas y cristales de oxalato de calcio en la hoja y el tallo tanto de las plantas tratadas como en los testigos. Los cambios morfológicas y anatómicas observados en las plantas tratadas con el extracto parecen ser estables.

INTRODUCTION

Extracts from the seeds of *B. monandra* increased leaf size and grain number in maize (Russom 1987), pod number and seed size in ground nut (Russom 1990) and tuber yield of cassava plants (Russom s.f.). All these results were obtained after the extracts were directly applied either on

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* Abubakar Tafawa Balewa University, PMB 0248, Bauchi, Bauchi State, Nigeria, West Africa

seeds, stem cutting or on intact plants. The consequence of re-planting either seeds or cuttings taken from plants previously treated with extract is unknown.

The objective of this experiment was to study the performance of cassava stem cuttings taken from plants previously treated with extract. This was carried out by propagating cuttings from extract-treated plants for three generations. It is difficult to carry out genetic testing of regenerants, but it is generally accepted that transmission of off-type traits through at least two successive clonal field generations provides reasonable certainty of a true genetic base for the variation (Karp and Bright 1985; Scowcroft 1984?). This is why this study was carried out for three generations. Also, extract from the whole seed of *B. monandra* was used on cassava for the first time in this experiment.

MATERIALS AND METHODS

Experiment 1 (April 1988 - April 1989)

Sterile distilled water was used as an extraction medium. Seeds of *B. monandra* were removed from their pods and each seed was separated into its cotyledon and seed coat. The following extracts were then prepared using the seeds and water: 1) Two cotyledons (seed coats removed) were soaked in 100 ml of sterile distilled water; 2) seed coats of two seeds were soaked in 100 ml of sterile distilled water; 3) seed coat and cotyledon of two seeds were soaked in 100 ml of sterile distilled water.

After a week, the extracts were distilled using a Nylon cloth. Each of the extracts was then diluted by adding a liter of distilled water. These three extracts and the control were the four treatments used in this experiment.

Cassava stem cuttings (30 cm) of TMS30572 were partially soaked in each of the extracts for a period of 70 h and then sown in the field April 21, 1988, and harvested in April 1989. Stem cuttings for the control were soaked in distilled water.

A randomized completed block design with four replications was used. There were 20 plants per plot and the spacing between plants was 1 x 1 m. Spacing between plots was 2 m. Data collected were: fresh tuber yield (t/ha) and tuber number per plant.

Experiment 2 (April 1989 - May 1990)

Stem cuttings were taken from Experiment 1. The cuttings were not treated again with the extracts. They were planted in April 1989 and harvested in May 1990. Experimental design, spacing and size of cuttings were the same as in Experiment 1, but there were 10 replications.

All characters considered in Experiment 1 were recorded. Additional characters recorded included: number of primary stems per plant; stem girth (measured at 25 cm above ground level); length of each primary stem from ground level to the first branching point; number of branches at the first, second and third branching points; and number of lateral branches per plant (recorded only to the third branching point).

Experiment 3 (May 1990 - May 1991)

Stem cuttings were taken from Experiment 2. The cuttings were not treated with the extracts. They were planted in April 1990 and harvested in May 1991. The type of experimental design used, spacing, size of cuttings and type of data recorded were the same as in Experiment 2, except that there were five replications. Additionally, the stem cuttings were planted on ridges

Experiment 4 (October 1989 - November 1990 and November 1990 October 1991)

The main objective of Experiment 4 was to determine whether the extracts from the cotyledon of *B. monandra* had induced anatomical changes in the primary growth of the root, petiole and stem of the vegetatively grown second generation cassava plants.

A single 60 cm stem was cut from a plant of TMS30572, and was cut again into two pieces each measuring 30 cm. These two stem pieces were planted in identical soil in October 23, 1989. Three months later, 2 ml of the cotyledon extract was introduced into the young developing tubers using a hypodermic syringe. This application was continued for three months, applying the extract once a month. The control plant was similarly treated, but using only distilled water.

The extract used in Experiment 4 was prepared using the same procedure as in Experiment 1a, except that it was not diluted with one liter of distilled water.

The two plants were harvested a year later, on November 2, 1990. Each plant was separately cut in 18 pieces (30 cm long) per treatment, and they were planted on the same day on ridges, using 1 x 1 m spacing. The soil used to make the ridges was thoroughly mixed before use.

To study the anatomy of the root, petiole and stem, two samples from each plant were taken when the plants were eight months old. The anatomical study was carried out using the procedure described by Cutler (Cutler 1978). Root cross sections were made from young roots and cross sections of petiole were prepared from the middle part. Cross sections of young stems were prepared from the center of an internode. Sections were cut to 15 μ using a Sledge microtome.

White nail polish was used to study the stomata. It was smeared on the surface of leaf or stem and allowed to dry for about 1 hour. It was then peeled of from the surface of leaf or stem and the impression on the peel was directly studied under the microscope. The following characters were also recorded from every plant of the two treatments: number of leaf lobes per leaf; length of leaf petiole; and length of primary stem from ground level to first branching point. The first two characters were recorded from those fully expanded leaves that were formed between ground level and the first branching point of every stem.

RESULTS AND DISCUSSION

Effect of the extracts on fresh tuber yield

Cassava stem cuttings that were soaked in the extracts gave significantly higher tuber yield ($P < 0.01$) than the control plants (Table 1).

In both Experiments 2 (second generation) and 3 (third generation), cassava plants that originated from the extract-treated plant continued giving higher tuber yield than the control plants (Table 1). On the whole, results in Table 1 confirmed earlier findings that extracts from the seeds of *B. monandra* do increase tuber yield of cassava plants (Russom s.f.).

Furthermore, the extracts significantly increased number of tubers per plant (Table 1). This partially explains why the extract-treated plants had higher tuber yield than the control plants. This view was supported by the highly significant correlation coefficient between tuber yield and tuber number per plant. The correlation coefficient values in control, coat, cotyledon and coat + cotyledon treatments of Experiment 2 were $r = 0.80$, $P < 0.001$; $r = 0.72$, $P < 0.001$; $r = 0.65$, $P < 0.01$; and $r = 0.80$, $P < 0.001$, respectively. In Experiment 3, the range for number of tubers per plant in control, coat, cotyledon, and coat + cotyledon was 1-26, 1-41, 2-27 and 2-31, respectively. These ranges appear to be high because the number of tubers per plant in cassava varies between five and 10 (Onwueme 1982; Purseglove 1974).

Effect of the extracts on the morphology of cassava plants

When a piece of cassava stem cutting is planted, primary stems grow form the nodes the cuttings. The extracts changed the morphology of these primary stems by increasing: number of primary stems per plant (Table 2); stem girth (Table 2); height of stem from ground level to first branching point (Table 2); number of branches at the first, second and third branching points (Table 3); number of lateral branches per stem; leaf petiole length; and number of leaf lobes per leaf.

Table 1. Effect of *B. monandra* seed extracts on tuber yield and tuber number per plant of cassava plants grown in 1988-1989, 1989-1990 and 1990-1991.

Treatment	Experiment 1 (1988-89)		Experiment 2 (1989-1990)		Experiment 3 (1990-1991)	
	yield (t/ha)	tuber no.	yield (t/ha)	tuber (no.)	yield (t/ha)	tuber (no.)
Control	21.6	5.1	25.5	6.5	30.7	7.7
Coat	32.3	8.6	32.0	9.9	50.4	16.7
Cotyledon	37.4	8.2	34.4	8.8	40.0	11.5
Coat + cotyledon	32.2	8.5	34.9	10.2	45.9	15.8
LSD (P = 0.05)	7.87	1.36	5.24	2.01	8.21	2.81

Table 2. Effect of *B. monandra* seed extracts on number of primary stems per plant. Height to the first branching point and stem girth of cassava plants grown in 1989-1990 and in 1990-1991.

Treatment	Experiment 2 (1980-1990)			Experiment 3 (1990-1991)		
	Stem no. per plant	Height to 1st branch (cm)	Stem no. girth (cm)	Stem no. per plant (cm)	Height to 1st branch (cm)	Stem girth (cm)
Control	1 8	58 6	2 2	2 1	49 6	2 4
Coat	2.0	86.7	2 3	2.9	102 1	3.4
Cotyledon	2 5	85 0	2 1	2.9	93 9	2.6
Coat + cotyledon	2.1	98 1	2.4	3.1	103 6	3.1
LSD (P < 0.01)	0 39	24 27	N S	0 46	18 10	0 35

Table 3. Effect of *B. monandra* seed extracts on number of branches at the first, second and third branching points: Data presented are percentage of plants with 2, 3, 4, 5 and 6 branches at the first, second and third branching points and in each of the treatments. 1989-1990 Experiment.

Branches (no.)	First branching point Treatments			
	Control	Coat	Cotyledon	Coat + Cotyledon
2	53 7	29 8	32 2	26 3
3	42 9	53 9	51 0	58 2
4	3 4	12.7	14.7	14 1
5	0.0	2.6	1.8	1.1
6	0.0	1 0	0.3	0 3
	Second branching point			
2	30.2	19 0	33.0	27 4
3	60 2	61.2	54 4	66 3
4	9.2	8 8	11.2	6 0
5	0 3	8 8	0.0	0.0
6	0 0	0 2	0.0	0.0
	Third branching point			
2	50 8	49.5	46.2	50.8
3	43.1	43.3	47.0	43.1
4	5 2	6.2	6 0	5.2
5	0 9	1 0	0.8	0 9
6	0 0	0.0	0.0	0.0

Some cassava varieties are known to develop one primary stem per cutting because they have strong apical dominance (CIAT 1983). It is not known whether the extracts increased the number of primary stems per cutting by reducing the effect of the apical dominance. Although the extracts increased stem girth, significant differences were observed only in Experiment 3.

The most significant ($P < 0.001$) effect of the extracts was to increase the height at which the first branching point occurs (Table 2). There were two other results, in addition to those results presented in Table 2, for the height at which the first branching point occurs.

In April 1990, a local farmer planted untreated and treated (stems that were treated with the three extracts were bulked together) stem cuttings from Experiment 2. After a year, the height of each primary stem from ground level to the first branching point was recorded in both treated and control plants. The mean height of the control plants was 49.6 cm ± SE = 1.82, n = 294, and the mean height of the extract treated plants was 146.6 cm ± SE = 3.37, n = 141. The difference between these two means was highly significant ($P < 0.001$).

The other result was obtained from Experiment 4. The control had a mean height of 70.0 cm ± SE = 9.71, n = 48, while the extract-treated stems had a mean height of 176.6 cm ± SE = 10.2, n = 54. The difference between these two means was also highly significant ($P < 0.001$).

The above results clearly suggest that the extracts changed the branching pattern of TMS 30572 because that variety branches early and very close to the ground (Arthur *et al.* 1987; Ezumah 1988). Furthermore, the height at which the first branch occurred in the extract-treated plants appear to be stable because the change persisted for three generations. Such change may have a genetic basis because branching near the base or high up is a distinguishing characteristic of cassava varieties (Onwueme 1982; Purseglove 1974). The height at which the first branch occurs is also a strongly inherited character.

Once the first branching point is formed, this can divide dichotomously, trichotomously and tetrachotomously (CIAT 1983). This agreed with the results of the control plants, but not with the results of the extract-treated plants (Table 3). Some of the extract-treated plants had five and even six branches at the first branching point (Table 3). In addition, plants in control, coat, cotyledon, and coat + cotyledon treatments had 46.3, 70.2, 67.8 and 73.8%, respectively, of their stems with three or more branches at the first branching point (Table 3). Even at the second and third branching points, all extract-treated plants except those treated with cotyledon had slightly more branches than the controls. In the extract-treated plants, the pattern of branching at the first, second and third branching points was three, three and two, respectively, while in the control plants it was two, three and two, respectively (Table 3).

Cassava stems also branch laterally, but this is sporadic (CIAT 1983). Among the control plants, there was no stem that had lateral branches. But in Experiment 3, plants treated with coat, cotyledon, and coat + cotyledon had 0.9, 2.0 and 4.4% of their stems, respectively, with at least one lateral branch per stem.

In experiment 4, the mean leaf petiole length of the control plants was 22.9 cm ± SE = 0.88, n = 1726; and the mean leaf petiole length of the extract-treated plants was 32.9 cm ± SE = 0.14, n = 1303. The difference between these two means was highly significant ($P < 0.001$).

The cotyledon extract increased significantly the level of potassium in both leaves and tubers of cassava plants (Russom s.f.). Plants in Experiment 4, which were treated with the extract, may have had higher amount of potassium than the controls. In turn, the potassium may have increased petiole length because highly significant and positive relationships between length of petiole and level of potassium have been reported (Nair and Aiyer 1985). In fact, short petioles in cassava plants are said to be symptoms of potassium deficiency (Nair and Aiyer 1985).

The mean number of lobes per leaf in the control and in the extract-treated plants of Experiment 4 were: 6.6 ± SE = 0.024, n = 1726; and 7.6 ± SE = 0.038, n = 1303, respectively. The difference between these two means was also highly significant ($P < 0.001$). Among the control plants, only 0.7% of the 1726 leaves had eight lobes per leaf, and no control plants had leaves with nine lobes. By contrast, 20% of the 1303 leaves of the extract-treated plants had eight lobes per leaf and 21% had nine lobes per leaf. The number of lobes per leaf is a varietal characteristic (CIAT 1983).

The morphological characters described above could have important implications in cassava cultural practices. Low-branching cassava varieties may have an advantage of reducing weeds, but most of the shoots tend to lie close to or on the ground, so that weeding and harvesting are made more difficult (Onwueme 1982). The best type of cassava would be one in which the initial branches are a reasonable distance from the ground, so that in the field the crop stands relatively open beneath the canopy of leaves. Such a canopy allows room for work between the rows (Onwueme 1982). This description fits the branching pattern of the extract-treated plants well. What is more, the extract-treated plants closed the canopy early and reduced the weeds. They did this by increasing the number of primary stems per plant (Table 2), which in turn increased the number of leaves per plant. They also increased the petiole length, enabling the neighboring plants to touch each other. An increase in the number of lobes per leaf and number of branches at the first branching point also helped to close the canopy early. Upright and late-branching types of cassava are preferable for both intercropping and monoculture systems (Leihner 1983).

Root morphology of the extract-treated and control plants was also different. The extract-treated plants had very numerous rootlet branches which appear to be VA mycorrhizae; the roots of the controls had few such rootlets. Spores of the fungus were similar to those reported by Rhodes (1980:277, Fig. 5b).

Another interesting aspect of the extract-treated plants was that their tubers did not rot quickly. For example, tubers stored in August 1991 are still fresh after nine months.

Effect of the extracts on cassava plant anatomy

The *B. monandra* cotyledon extract induced changes in the anatomy of the cassava root (Fig. 1), leaf petiole (Fig. 2) and stem (Fig. 3). The control plants had one very large metaxylem element in the center of the root; this was absent in the root of the extract-treated plants (Fig. 1). The center of root of the extract-treated plants had a pith consisting of parenchyma cells. A closer look at the controls' root center showed that the protoxylem was away from the center. This implies that the development of the xylem was centripetal, or the xylem is said to be exarch (external origin). The development of xylem in the roots of extract-treated plants was an endarch, or inner origin. Differentiation of xylem is part of overall genetic control (Cutler 1978), but there are also evidences of hormonal controls (Esau 1977). The shape of the vascular cylinder (stele) was circular in the roots of the control plants, but pentagonal in the roots of the extract-treated plants. There were five vascular bundler in both the control and treated plant roots, but some roots of the control plants had only four vascular bundles. A cross section of the petiole in the control plants had nine vascular bundles, while that of the extract-treated-plants had 10 vascular bundles (Fig. 2).

Cross sections of the stem in the extract-treated plants had five ridges and five furrows, a shape similar to that of the root. of the stem cross sections for the control plants were circular, again similar to the root (Fig. 3).

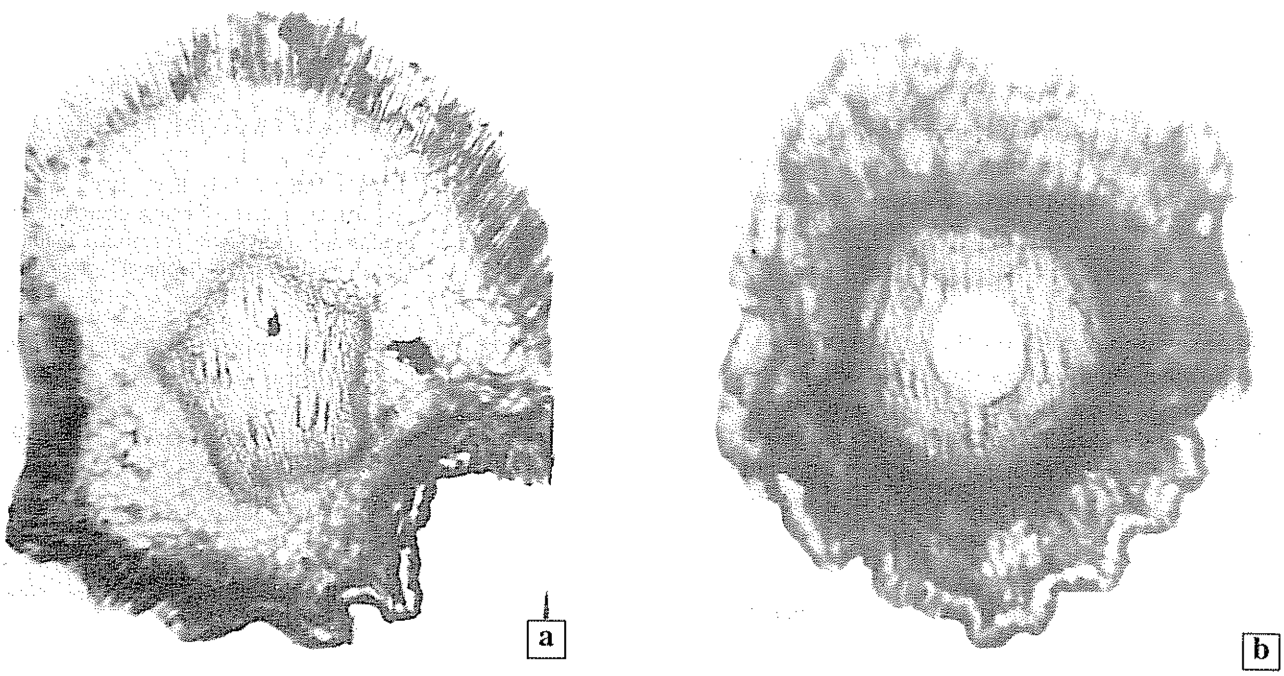

Fig. 1. Cross-sections of roots in treated (a) and in control (b) cassava plants.

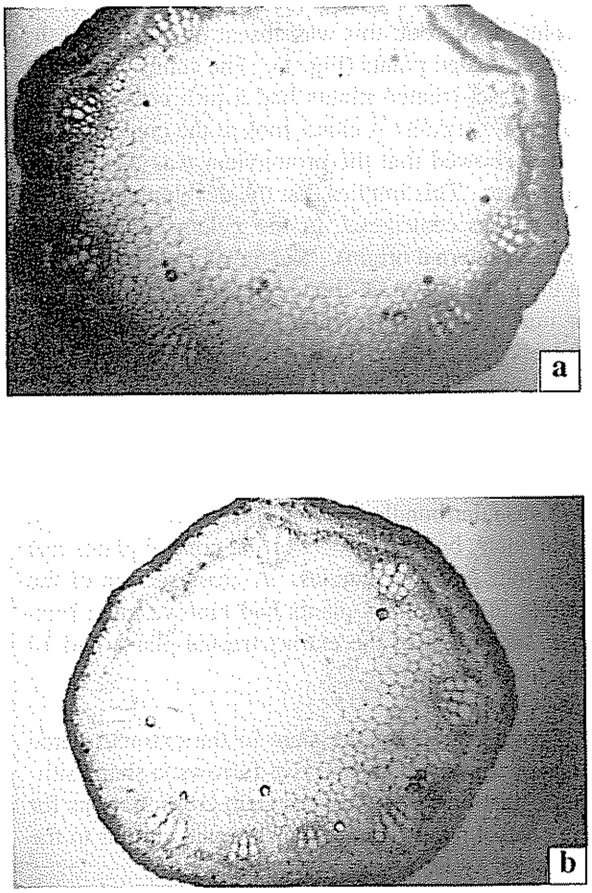

Fig. 2. Cross sections of petiole in treated (a) and control (b) cassava plants.

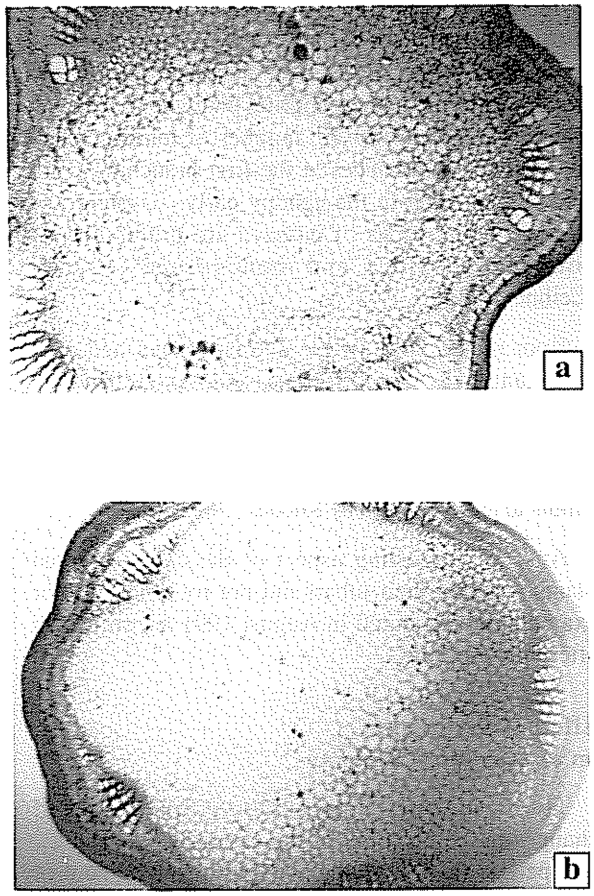

Fig. 3. Cross sections of stem in treated (a) and in control (b) cassava plants.

Calcium oxalate of the sphaero-crystal type was observed in the parenchymatous cells of the leaf and stem of both treated and control plants. The crystals were star-shaped in appearance, exactly like those reported by Dutta (1981:178, Fig. 18c).

There were stomata on the leaves and stems. Each was bordered by two subsidiary cells of equal size. The stomata belong to the paracytic type (Cutler 1978). The lower surface of the leaf had more stomata than the upper surface; on the latter, the stomata were mainly present along the main veins.

Extracts from the seeds of *B. monandra* increased tuber yield, morphological characters and induced changes in the anatomy of the cassava plants. These changes appear to be stable.

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RESEÑA DE LIBROS

INSTITUT NACIONAL DE LA RECHERCHE AGRONOMIQUE. 1989. Mise en valeur de l'écosystème forestier guyanais: Opération ECEREX. J.M. Sarrailh (coordinateur).

L'opération ECEREX, mise en place par différents organismes de recherches, est née de la volonté de développer la Guyane française par l'exploitation de la forêt dense humide naturelle à des fins papetières.

Une approche pluridisciplinaire a permis: l'évaluation des risques écologiques que présente cette exploitation passant par une déforestation totale –érosion, modifications des régimes hydrologiques, baisse de productivité, dégradations biologiques; et la proposition de modèles d'aménagement et la définition des techniques nécessaires à l'implantation d'écosystèmes simplifiés, après l'exploitation papetière.

L'opération ECEREX a obtenu l'un des 12 labels MAB (Comité français) de l'UNESCO au cours de la première décennie de fonctionnement de cet organisme.

Cet ouvrage s'adresse autant aux chercheurs qu'aux ingénieurs agronomes et forestiers chargés d'aménagements en zone forestière tropicale humide.

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