Correlation between hydrocyanic acid levels in leaf and root of cassava (**Manihot esculenta** Crantz)*[1/]

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C O M P E N D I O

Se estudió la correlación entre los niveles de ácido cianhídrico (HCN) en las hojas y cáscaras de las raíces de 26 cultivares de yuca, encontrándose que el coeficiente de correlación (r) era 0,59. Parece pues posible usar las hojas para la selección de bajos contenidos de HCN en el mejoramiento genético de la yuca. — El autor

Introduction

THE presence of the cyanogenic glucosides in cassava has long been known. In recent years, concern has arisen as to the probable toxic effects on humans using cassava as a staple food due to the hydrocyanic acid (HCN) released from the glucosides. Medical evidence has shown that tropical ataxic neuropathy in West Africa may be a manifestation of chronic cassava poisoning (2). Since cassava provides a major carbohydrate source in the diets of more than 200 million people in the tropics, the fundamental solution of the problem is to produce cassava cultivars very low in, or free from the glucosides. Breeding for low cyanoglucoside content is an objective for cassava improvement in a number of agricultural research centers.

To breed a biochemical character in plants usually requires an efficient and rapid screening technique, since a large population of plant materials may be involved in the screening process. Various assay methods for detecting the HCN released from the glucosides in plant tissues have beeen discussed by Zitnak (3). For rapid screening, we used the sodium picrate test as modified by Gilchrist *et al* (1). Once a desirable low HCN line is found and confirmed, we may use other analytical methods for more crucial determinations.

In cassava the roots are the principal part of the plant used for food; the leaves are occasionally consumed in some areas. For some early cultivars, the roots take at least six to eight months to mature after planting; and for other cultivars, they may take more than a year. Thus, to screen the cyanoglucoside content in the roots, there is usually a rather long period before the screening procedure can be performed. Moreover, excavating the roots for assay is time consuming and may possibly induce damage to the cassava plant. If other plant tissues, such as leaves, could be used to substitute for roots in screening, the efficiency of the screening procedure would be greatly increased. Thus, a study was carried out to determine whether the HCN levels in the leaf and in the root of a cassava cultivar are correlated. The experimental results are presented in this paper.

Materials and methods

Twenty-six cassava cultivars were selected for the study. In a preliminary experiment using leaves for determing HCN levels, 6 were arbitrarily classified as high, 6 as low, and the rest as intermediate. Three stem cuttings from each cultivar were grown in a row in the field. After 4 months, when the plants were a meter or more in height, the full grown and healthy leaves, usually 4th or 5th leaf from the top of the plants, were used as samples for the HCN test. The root samples were taken when the plants were more than 6 months old. Only the roots with a diameter larger than 4 cm were used for the testing.

The method used for determining the HCN levels in cassava plant tissues was essentially that described by

* Received for publication April 1, 1976.

1/ This work was supported by the U.S. Energy Research and Development Administration under contract Nº E (11-1)-2505 and supported in part by FAO/IAEA Research contract Nº 1647/RB. Publication Nº COO-2505-04.

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Table 1.—Optical densities of the eluted solution from the sodium picrate test of the leaves and root peels of 26 cassava cultivars.*

Cultivar N° in the collection	Optical density**		Cultivar N° in the collection	Optical density**	
	Leaf	Root		Leaf	Root
4	0.35	0.38	68	0.36	0.37
9	0.34	0.27	69	0.34	0.25
11	0.37	0.18	72	0.36	0.28
23	0.35	0.23	73	0.36	0.26
26	0.59	0.32	74	0.36	0.22
32	0.34	0.16	75	0.37	0.32
33	0.34	0.14	76	0.19	0.27
37	0.34	0.16	79	0.34	0.27
45	0.51	0.45	91	0.17	0.26
51	0.51	0.34	93	0.81	0.37
52	0.19	0.16	95	0.50	0.31
53	0.16	0.16	112	0.14	0.22
61	0.50	0.26	113	0.17	0.12

* Computed correlation coefficient (r) = 0.59.

** Mean of four determinations for leaf and four to six for root.

Gilchrist *et al.* (1). Twenty leaf disks were cut with a 0.5 cm metal tube (or cork borer) from a leaf and placed in a 1 x 10 cm test tube. Three drops of chloroform were added to the leaf disks. A filter paper strip, 1 x 7 cm in size, saturated with the alkaline picrate solution (25 g Na_2CO_3 and 5 g picric acid in 1 liter distilled H_2O), was immediately suspended in the test tube with a cork stopper. After 5 hours at room temperature (20 - 25 C) in the laboratory, the paper strip was removed from the tube and eluted in 10 ml of distilled H_2O. The optical density of the solution was measured by Bausch and Lomb Spectronic 20 colorimeter, set at a wavelength of 515 nm.

Since the highest concentration of the cyanogenic glucosides is in the peel of a cassava root, the peel was used to determine the HCN level. The same method used for the determination of HCN in the leaves was used for the root, except that only two peel disks were used in each test tube.

Each experimental datum recorded was an average of four different determinations of the optical density at various times for the leaf and four to six determinations for the peel.

Results and discussion

Table 1 presents the experimental data on the optical density readings of the eluted solutions from the sodium picrate test of the leaves and the root peels of the 26 cassava cultivars. These data represent the HCN recovered from the plant tissues but do not indicate the absolute amount of the HCN or the cyanogenic glucosides in the tissues. Providing that the HCN amount recovered by the present experimental method is proportional to the HCN quantity in the leaves or in the peels, the data can be used for calculating the correlation.

The correlation coefficient (r) calculated from the experimental data in Table 1 is 0.59, suggesting a rather good association between the HCN levels in the leaves and the roots. Thus, it is feasible to use leaves instead of roots for screening in a breeding program for HCN content. This procedure allows a more efficient screening process.

The site of cyanoglucoside synthesis in a cassava plant is not yet known. Whether the synthesis in taking place in the leaves and in the roots independently, or the synthesis is taking place in a primary site followed by translocation to the other plant organs has not been established. However, there is little doubt that the production of the glucosides is due to genetic control. The cultivars of high HCN content remain high after years of cultivation and those of low content remain low, although the HCN content can fluctuate to a great extent under various environmental conditions. If genetic control is a predominant factor for the glucoside production in cassava, it is likely that a cultivar of low HCN content in the leaves is also low in the roots.

Summary

An experiment was carried out to study the correlation between the hydrocyanic acid (HCN) levels in the leaves and the peels of the roots in 26 cassava cultivars. It was found that the correlation coefficient (r) was 0.59. It thus seems feasible to use leaves for screening HCN content in a cassava breeding program.

Literature cited

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