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SCREENING FOR ACYANOGENESIS IN CASSAVA

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SUMMARY

The sodium picrate test for hydrocyanic acid was used to evaluate 88,510 cassava plants for cyanogenic content. Acyanogenic plants were not found in spite of the large number of plants tested. A small number of plants exhibited low to medium degree of cyanogenesis while the rest of the plants showed a high degree of cyanogenesis. The screening method is simple and sensitive and lends itself to large-scale field screening.

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INTRODUCTION

Research on cyanogenesis of cassava *Manihot esculenta*, in recent years dealt mainly with the biosynthetic pathways of the cyanogenic glucosides (8) and the environmental conditions that affect their occurrence in the plant (1). Because of the important role that cassava plays in human and livestock nutrition, several studies were also conducted on the association between high cassava consumption and the incidence of goiter and tropical ataxic neuropathy (3, 4, 5, 9). These studies suggest a link between several human diseases and chronic cyanide intake from cassava. In view of these reports, a screening program was conducted at the International Institute of Tropical Agriculture (IITA) to identify cassava plants free or low in cyanogenesis.

MATERIALS AND METHODS

The cassava germplasm collection at IITA contains about 110,000 clones. These originated from seed introductions from Southeast Asia and South America and from a large number of crosses made between plants from those seeds and Nigerian clones. Screening this number of plants for acyanogenesis required a sensitive and practicable method for handling large numbers of plants in the field. The sodium picrate method was the most suitable of several methods tried.

A 2-cm diameter leaf disc is cut with a cork borer from a young, fully expanded leaf from each plant and inserted in a 9.5 x 2 cm screw-cap vial. A drop of toluene is added to the leaf disc and a filter paper strip (8 x 1 cm) freshly dipped in a sodium picrate solution is suspended in the vial under the cap without touching the leaf disc. The sodium picrate solution is prepared by mixing equal volumes of a 5% solution of sodium carbonate and a saturated solution of picric acid. The paper color gradually changes from yellow to shades of red depending on the amount of HCN released from the leaf disc. The intensity of the red color is scored twice, after one and four hours, and the plants are classified according to the red color intensity after four hours in one of three categories: high, medium and low. By keeping the size of the leaf disc and the filter paper strip uniform, early scoring allows for semi-quantitative detection of differences in the speed of the enzymatic reaction. Such differences disappear later when the enzymatic reaction reaches completion after four hours. In practice, six laborers and a laboratory technician perform 1,000 analyses in the morning, while afternoon hours are used for recording data, washing vials and preparing the sodium picrate solution and filter paper for use on the following day. Cassava plants showing low degree of cyanogenesis are subjected to further tests for several days with leaf discs collected from different leaves and at different times of the day. When consistent results are obtained, the plants are transplanted to another field and more plants are propagated vegetatively (stem cuttings) from the original ones for breeding purposes and for further quantitative analysis of cyanoglucosides in the roots.

RESULTS AND DISCUSSION

Acyanogenic plants were not found in spite of the large number of cassava plants tested. A small number of plants showed low to medium degree of cyanogenesis while the majority of the plants showed high degree of cyanogenesis (Table 1). It is interesting that the majority of the 92 plants low in cyanogenesis are offsprings of two cultivars, namely Isunikankiyan and 58308 (interspecific hybrid with *Manihot glaziovii*) or

Table 1. Degree of cyanogenesis in cassava leaf disc.

Degree of cyanogenesis	Number of plants	Percentage
High	87,747	99.1
Medium	671	0.8
Low	92	0.1
Negative	0	0
Total	88,510	100.0

offsprings of their crosses (Table 2). Locally, Isunikankiyan is considered a "sweet" cassava. It is not known, however, whether "sweetness" is related to low cyanogenic content or other factors.

Complete freedom from cyanogenesis in cassava was not found in this study or by others (6, 7). This is not surprising because vegetative propagation of cassava has been practiced for centuries and may have stabilized cyanogenesis to a degree that would make finding acyanogenic plants difficult (2).

Moh and Alan (7) used the sodium picrate filter paper and Esquivel and Maravalhas (6) used the copper-benzidine tests in screening cassava roots for cyanogenesis. In this method leaf discs rather than roots are used. The choice of leaf discs is mainly based on reports by Moh and Alan (7) and by de Bruijn (1) that cassava leaves are the site of cyanoglucoside synthesis and contain more cyanoglucosides than roots. Thus a cassava plant containing small quantities of cyanoglucosides in the leaves would be expected to contain trace quantities in the roots. Other advantages of using leaf discs are ease of usage and accessibility in contrast to roots, and the possibility of screening plants in the seedling stage before root enlargement.

Table 2. Cassava clones low in cyanogenesis.

Parentage	Number of plants
58308 x Isunikankiyan	25
Isunikankiyan – OP	12
58308 x Oyanrugba Funfun	11
58308 – OP	8
Unknown	8
58308 x Wild Cassava	6
58308 x Ojunkaiye	4
58308 x Oyanrugba Dudu	4
Oyanrugba Funfun – OP	3
Nsukka 2 – OP	2
58308 x Ogunjobi	2
Isunikankiyan x Oyanrugba Funfun	2
Isunikankiyan x Oyanrugba Pupa	1
58308 x 58198	1
58308 x Abayode	1
Ogu-ocha – OP	1
58272 – OP	1
Total	92

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