SCREENING FOR ACRYANOGENIC SOMATIC MUTATIONS IN CASSAVA (Manihot esculenta Crantz)*

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ABSTRACT

By irradiating the young stem cuttings (6-8 months old wood) of a cassava cultivar, Japonesa, (Manihot esculenta Crantz) with an acute dose of 4 kR from a $^{60}$Co source, it was found that in a number of cases, the induced mutant characters appeared in the whole $R_1$ plants or in large chimeric sectors. This result suggested that a cassava plant could develop from one or two initial cells in the shoot apex of a bud. This unusual biological response to radiation provides a great advantage for selection in mutation breeding.

By using the sodium picrate method, 2676 leaves from 1338 $R_1$ plants irradiated with 4 kR were screened for hydrocyanic acid content (HCN). As compared with the control, some leaves had higher and some had lower HCN level, indicating that the radiation broadened the variability. Whether or not those $R_1$ plants producing a lower level of HCN in the leaves are truly a genetic mutant cannot be ascertained at present. Further screening of the selected $R_1$ plants in the subsequent vegetative propagation...

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gation generations will help to distinguish whether they are genetic mutants.

1. INTRODUCTION

The roots of cassava (*Manihot esculenta* Crantz) are a staple food crop in the tropics and provide a major source of carbohydrates in the daily diets of more than 200 million people. It is known that the cassava is a cyanogenic plant. The cyanogenic glucosides, when hydrolysed, release hydrocyanic acid (HCN) which is poisonous. While a great part of the glucosides can be eliminated during food processing or preparation, concern has arisen as to the probable toxic effects on humans in high cassava consumption areas. Medical evidence has shown that tropical ataxic neuropathy in West Africa may be a manifestation of chronic cassava poisoning (11). The basic solution of this problem is to grow the cassava free from the glucosides. However, such cassava cultivars have not been found.

Most cassava cultivars are monoecious and have a marked protogynous flowering habit. Because of these botanical characteristics, a high degree of hybridity in this species may be expected. Many existing cassava cultivars at present are undoubtedly the derivatives of natural hybrids. As has been pointed out by Brock (5): "The variability generated by crossing is so great that there is little chance of selecting for improved types among seeding progeny and at the same time retaining the general characteristics of the original variety". Commercially, cassava cultivars are propagated vegetatively for production and the general characteristics of the adapted cultivars are thus retained from generation to generation. Thus, to improve one or two characters in a cassava cultivar, mutation breeding may be a better method.
of approach. In the present study, experiments were carried out to explore the feasibility of whether the cyanogenic glucoside level in a cassava cultivar can be lowered or eliminated by induced somatic mutation method.

2. MATERIALS AND METHODS

A locally adapted cassava cultivar (namely, Japonesa) low in HCN content and high in root quality, was selected as the plant material. Stem cuttings of 15 cm in length from 6- to 8-month old plants were irradiated with 4 kR from a $^{60}$Co source at a dose rate of 780 R/min. Previous study has shown that a dose of 4 kR was about the maximum that cassava buds can tolerate. After irradiation, the cuttings were immediately grown in the field. Cuttings without irradiation were also planted in the adjacent field as a control.

When the plants were 5-6 months old, the two fully grown opposite leaves of a plant, usually the 4th and the 5th leaf from the top, were used for the HCN test. The use of leaves to substitute for the roots in testing HCN content is less time consuming and more efficient. In a recent study of 26 cultivars, we found that the HCN levels in the leaf and in the root are correlated (8).

The method used for determining the HCN levels in cassava leaves was essentially the same as that described by Gilchrist et al. (6). 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 sodium picrate solution (25 g Na$_2$CO$_3$ and 5 g picric acid in 1 liter distilled H$_2$O), was immediately suspended in the test
tube with a cork stopper. After 5 hr at room temperate (20-25 C) in the laboratory, the paper strip was removed from the tube and eluted in 10 ml of distilled H₂O. The transmittance of the solution was measured by a Bausch and Lomb Spectronic-20 colorimeter, set at a wavelength of 515 mu.

3. RESULTS AND DISCUSSION

3.1 Size of the induced chimera

In higher plants, the shoot apex is generally a multicellular system. The growth of a shoot is governed by a group of the initial cells in the apex. Since mutation is a single cell event, the larger the number of the initials participating in the development of the shoot, the smaller the size of chimera that will appear. However, there is evidence from mutation experiments that the shoot can be derived from a single initial, such as in Saintpaulia (10), coffea (7) and Streptocarpus (3).

As has been pointed out previously, many cassava cultivars are highly heterozygous. By irradiating the buds of cassava cuttings, somatic mutations are expected to be induced. The result showed that in a number of cases, the induced mutant character occurred in the whole plant, and in some cases, about half of the plant was chimeric and the other half was normal (Fig. 1). This phenomenon suggests that a cassava plant could develop from one or two initials in the apex. However, in a previous experiment (9), most mutant characters appearing in the R₁ plants were in a chimeric form when the older cuttings of other cassava cultivars were used for irradiation. Whether the difference in chimera output is due to the difference in
cultivars or due to the age of buds is not known at present. An attempt was made to explain the one-initial-cell phenomenon in coffee by Balema (2): "Evidence for only one initial cell from chimera may be caused by lack of apical organization in the seed of this plant at the time of treatment. If the apical cells are not stable, eventually only cells from one cell lineage will be found". Whether or not this explanation could apply to cassava remains to be seen.

3.2 Screening for HCN content

Table I presents the screening results of 2676 leaves from 1338 R1 plants. It must be pointed out that the transmittance readings from the colorimetric method represent the relative amount of HCN recovered from the leaf disks by the sodium picrate test, but do not indicate the absolute amount of the HCN or the cyanogenic glucosides in these tissues. Providing that the HCN amount recovered by the present experimental method is proportional to the HCN quantity in the leaves, the data can be used for screening as compared with the control.

Fig. 2 demonstrates the frequency distributions of the leaves with different percentages of transmittance. A higher transmittance percent suggests a lower HCN amount, and vice versa. In order to facilitate the classification of the leaves with different amount of recovered HCN, the transmittance percentages were arbitrarily divided into ten classes: 0-10, 11-20, .....etc. As one can see from Fig. 2, 92% of the leaves tested from the control population had a transmittance from 50-70%, while only about 76% of the leaves from the irradiated population were in this range of transmittance. On the other hand, a higher percentage of leaves from the irradiated population
shifted into the extremely high or low classes of transmittance, indicating that some leaves had either lower or higher HCN content than the control. This experimental result is very similar to that of the yielding ability in barley described by Gaul (cf. Aastveit, 1).

It is known that the production of the cyanogenic glucoside in cassava is greatly affected by environmental conditions as has been discussed by de Bruijn (4). Whether or not those R₁ cassava plants producing either higher or lower amount of HCN in the leaves than the control are truly a genetic mutant cannot be ascertained at present. One of the reasons that the R₁ plants produce a higher HCN content may be due to the thickening of the leaf tissues in some of the morphological mutants. For those R₁ plants of low HCN content, temporary physiological inhibition of the glucoside production in these plants due to irradiation cannot be ruled out. Nevertheless, a further screening of the HCN content of the selected R₁ plants in the second vegetative generation will help to distinguish whether they are a genetic mutant.

So far, no cassava cultivars are known to be completely free of the cyanogenic glucosides; the differences between the high glucoside cultivars and the lows are quantitative. It is known that cassava is a tetraploid, and the production of the glucosides may be due to polygenic controls. This may account for the fact that an acyanogenic cultivar is difficult to obtain. Since the genetic information is completely lacking, on such a question one can only speculate.
REFERENCES


(2) Balkema, G.H., Chimerism and diplontic Selection, A.A. Balkema, Rotterdam (1971).


(8) Moh, C.C., Correlation between hydrocyanic acid levels in leaf and root of cassava (Manihot esculenta Crantz). Turrialba (in press).


TABLE I. TRANSMITTANCE OF THE ELUTED SOLUTION FROM THE SODIUM PICRATE TEST OF THE LEAF DISKS OF A CASSAVA CULTIVAR, JAPONESA, IRRADIATED WITH 4 kR

<table>
<thead>
<tr>
<th>Transmittance (%)</th>
<th>Control Leaves tested (No.) (%)</th>
<th>4 kR Leaves tested (No.) (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-40</td>
<td>2 0.50</td>
<td>112 4.19</td>
</tr>
<tr>
<td>41-50</td>
<td>29 7.25</td>
<td>403 15.06</td>
</tr>
<tr>
<td>51-60</td>
<td>186 46.50</td>
<td>1200 44.84</td>
</tr>
<tr>
<td>61-70</td>
<td>182 45.50</td>
<td>823 30.75</td>
</tr>
<tr>
<td>71-80</td>
<td>1 0.25</td>
<td>91 3.40</td>
</tr>
<tr>
<td>81-90</td>
<td>2 0.08</td>
<td></td>
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</tbody>
</table>
Fig. 1. Cassava mutants induced by irradiating the stem cuttings (4 kR gamma rays). A. Lower left corner, normal leaves; center, yellow wrinkle leaf mutant. Note the mutant character appeared in the whole R₁ plant. B. A curly leaf mutant. C. A plant with a large chimera of narrow leaf mutant and a small normal sector.
Fig. 2. Histogram showing the frequency distribution of the transmittance of the eluted solution from the sodium picrate test of cassava leaves (data from table I).