Growth Response of Tms 96/0304 Cultivar of Cassava *Manihot esculenta* (CRANTZ) Exposed to Simulated Acid Rain of Different pH

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Abstract
Southern Nigeria is a major cassava producing area that has been subjected to air pollution from increasing industrial activities and population explosion in the coastal towns and cities. Cassava being a stable food in southern Nigeria for millions of Nigerian can potentially be affected by acid rain. The level of pollution is not expected to change drastically in the immediate future. Investigations were carried out to study the changes in the morphology, survival, growth and yield of TMS 96/0304 cultivar of cassava *Manihot esculenta* (Crantz) to simulated acid rain. The plants were exposed to simulated acid rain of pH 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 (control) respectively. Simulated acid rain induced morphological changes including chlorosis, early leaf senescence, necrosis, leaf abscission, leaf folding and death. Plant height, leaf area, fresh weight, dry weight, relative growth rate, chlorophyll content and the harvest index was the highest at pH 7.0 (control) and significantly (p<0.05) decreased with increasing acidity. There was significant reduction in the growth and productivity. The results indicated that TMS 96/0304 cultivar of *Manihot esculenta* exhibited growth stimulation at low acidity of pH 2.0 and it is likely to be retarded in future due to rapid and uncontrolled industrialization.

Keywords: lesions, photosynthetic pigment, dehydration, *manihot esculenta*, root production

INTRODUCTION
Acid rain is a major polluting event harmful to terrestrial and aquatic ecosystems (Brimblecombe *et al.*, 2007). It is the wet deposition of pollutants such as oxides of sulphur and nitrogen contained in power plant emission, factory smoke and car exhaust, react with the moisture in the atmosphere (Kita *et al.*, 2004). In natural conditions atmospheric precipitation is slightly acidic due to the dissolution of atmospheric carbon dioxide. Rain that presents a concentration of H+ ions greater than 2.5µeq-l and pH values lower than 5.6 is considered as acid. Acid deposition may cause decline in health and growth of trees as well as other plants (Wyrwicha & Sklodowska, 2006). Several experiments have been carried out in the field and in greenhouses to investigate the effect of simulated acid rain in plants. Banwart, *et al.*, 1990 reported that acid rain inhibited the growth of soybean cultivars *Glycine max* and cause a significant decrease on the yield. Also, Caporn and Hutchinson (1987) reported that acid rain sprays caused both extensive visible injury to the cotyledons of cabbage (*Brassica oleracea*) and also a reduction in the seedling growth. Acid rain exposure of plants results in characteristic foliar injury symptoms, modified leaf anatomy (Stoyanora & Velikova, 2004), structural changes in the photosynthetic pigment apparatus and a decrease in the chlorophyll concentrations (Sant’ Anna-Santos *et al.*, 2006) and has reported reduction in plant growth and yield of field corn (Banwart, *et al.*, 1988), green pepper (Shripal *et al.*, 2000) and tomato (Dursun *et al.*, 2002).

Cassava (*Manihot esculenta* Crantz) belongs to the family Euphorbiaceae (Nweke *et al.*, 2002). Since the introduction into Nigeria it has become very popular throughout the country. It is grown in 70% of the Nigerian states, available from the swamp forest area to the Guinea savannah of *Nigeria*. Aside being a major source of food in Nigeria, other important products produced from cassava are starch, beer, vinegar and alcohol. The cassava cultivar TMS 96/0304 is grown in most of the Nigerian home gardens and even on large scale production. The tuber is used for human consumption in three processed forms, the toasted meal ‘garri’, the retted meal and the flour. ‘Garri’ is the most popular form of consumption in Africa. The objective of the present study was to examine the growth and productivity of cassava cultivar TMS 96/1672 under simulated acid rain.

METHODS AND PROCEDURES
Planting Procedure
A field experiment was conducted in the school farm of the Federal University of Technology, Akure, Ondo state, Nigeria from October 2012 to March 2013. Stem cuttings of 30cm long each of TMS 96/0304 cultivar of *M. esculenta* were planted horizontally with a spacing of 100cm and four stem cuttings were planted on each row. Each pH
treatment had four replicates and was arranged in a Completely Randomised Design (CRD). The plants were watered every other day and grown for a week before the application of the simulated acid rain treatment. The experimental field was hoe weeded as necessary. Simulated acid rain was sprayed to the planted cassava cultivars according to their pH values of 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 which was the control. The solutions were applied using a medium size pressurized sprayer on the plants. The plants grew for twenty five weeks before the experiment was terminated.

Preparation of Simulated Acid Rain

The acids used was an acidic mixture of concentrated sulphuric acid (H₂SO₄) and concentrated nitric acid (HNO₃) in 2:1 ratio. The acidic solution was then calibrated using distilled water with a Deluxe pH meter to get the desired pH (2.0, 3.0, 4.0, 5.0 and 6.0) and cross checked with pH pen. The control pH (7.0) had distilled water. Several parameters were used in assessing the growth and productivity of the plant. The height of shoots was measured using a tape rule in (cm) from the soil level to the terminal bud. The measurements were taken in an interval of 2 weeks from the day the acid rain treatment commenced to the day of harvest. Leaf area was determined by the proportional method of weighing a cut-out of traced area of the leaves on graph paper with standard paper of known weight to area ratio. The fresh and dry weights were determined after twenty four weeks of treatment following the method of Hunt (1990). Relative growth rate (RGR) was calculated following the methods of Hunt (1990) and the fresh weight of the whole plant was used to determine the relative growth rate.

\[ RGR = \frac{\log_{e} W_{2} - \log_{e} W_{1}}{T_{2} - T_{1}} \]

Where: \( W_{2} = \) final weight, \( W_{1} = \) initial weight, \( T_{2} = \) final time, \( T_{1} = \) initial time.

The harvest index was determined by the method of Ekanayake (1994).

\[ \text{Harvest Index (HI)} = \frac{\text{Tuber dry weight}}{\text{Total plant dry weight}} \]

Statistical Analysis

Data obtained were subjected to analysis using the Statistical Package for Social Sciences, Version 15.0 (SPSS, 2003). Treatment means were separated using the Duncan’s Multiple Range Test (Zar, 1984).

RESULT

Morphological changes were observed in TMS 96/0304 cultivar of Manihot esculenta polluted with Simulated Acid Rain 24 weeks after treatment

Table 1: Morphological changes observed in TMS 96/0304 cultivar of Manihot esculenta polluted with Simulated Acid Rain 24 weeks after treatment

<table>
<thead>
<tr>
<th>pH Treatments</th>
<th>Observed effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0 (Control)</td>
<td>Luxuriant growth</td>
</tr>
<tr>
<td>6.0</td>
<td>Good growth. Leaves had small brownish dots on its surface.</td>
</tr>
<tr>
<td>5.0</td>
<td>Production of new leaves on the stem and at the leaf apex. Growth was retarded. There was 100% survival. Older leaves showed signs of curling and chlorosis. Had increased brownish spots on the leaf surface.</td>
</tr>
<tr>
<td>4.0</td>
<td>The leaves were curled and chlorotic. Plant growth was stunted. Leaves were showing signs of burnt surfaces from the tip. Had 70% leaf abscission. There was 80% survival.</td>
</tr>
<tr>
<td>3.0</td>
<td>Plants had stunted growth. The new leaves became folded, chlorotic followed by necrosis and eventually some of the plants died. 50% survival. 60% leaf abscission.</td>
</tr>
<tr>
<td>2.0</td>
<td>Had stunted growth. 30% survival. All the surfaces of the leaves were necrotic and curled. Had 40% leaf abscission from the base of the shoot.</td>
</tr>
</tbody>
</table>

Table 2: Effect of Simulated Acid Rain (SAR) on the Plant height (cm), Leaf area (cm), Fresh weight (g), Dry weight (g) of TMS 96/0304 cultivar of Manihot esculenta, 24 weeks after treatment

<table>
<thead>
<tr>
<th>pH of SAR</th>
<th>Plant height (cm)</th>
<th>Leaf area (cm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0 (Control)</td>
<td>172.10 ± 4.26 a</td>
<td>323.40 ± 4.12 a</td>
<td>904.20 ± 0.18 a</td>
<td>422.30 ± 0.24 a</td>
</tr>
<tr>
<td>6.0</td>
<td>94.62 ± 4.14 b</td>
<td>272.44 ± 3.20 b</td>
<td>726.42 ± 0.12 b</td>
<td>376.12 ± 0.18 b</td>
</tr>
<tr>
<td>5.0</td>
<td>90.10 ± 3.30 b</td>
<td>250.32 ± 3.12 b</td>
<td>502.16 ± 0.10 c</td>
<td>324.20 ± 0.16 b</td>
</tr>
<tr>
<td>4.0</td>
<td>72.89 ± 3.12 b</td>
<td>234.26 ± 2.18 b</td>
<td>356.10 ± 0.08 d</td>
<td>242.34 ± 0.12 c</td>
</tr>
<tr>
<td>3.0</td>
<td>40.13 ± 2.34 c</td>
<td>170.16 ± 2.04c</td>
<td>280.11 ± 0.07e</td>
<td>160.16 ± 0.08 d</td>
</tr>
<tr>
<td>2.0</td>
<td>34.81 ± 2.11 d</td>
<td>140.14 ± 1.48e</td>
<td>121.20 ± 0.03 f</td>
<td>92.48 ± 0.04 e</td>
</tr>
</tbody>
</table>

The chlorophyll content of the leaves was determined by the following method. Three grams (3g) of the youngest leaves of each seedling was ground with few grains of washed sand (sand washed with concentrated \( H_{2}SO_{4} \) and then thoroughly washed with distilled water). Chlorophyll was extracted with 20 ml of 80 % acetone and centrifuged at 2500 rpm for 15 minutes. The clear supernatant was collected and the absorbance read at 645nm and 663nm on Spectronic 20 Spectrophotometer with 80 % acetone as reference blank.
Manihot esculenta parameters. The plant had the highest plant height, compared to pH 3.0 and pH 2.0.

Lesion, suppression of leaf production, leaf curling, acid rain include chlorosis, necrosis, stunted growth, affecting mainly the epidermal cells causing erosion of the cuticle and altering the leaf permeability (Evans, 1984). Symptoms of plants polluted with simulated acid rain include chlorosis, necrosis, stunted growth, lesion, suppression of leaf production, leaf curling, withering of leaves, leaf abscission and even death of plants. Silva et al, 2006 found that plants exposed to low pH rain (pH 3.0) are generally retarded with leaf chlorosis, necrotic spot coupled with dehydration of the plants. Simulated acid rain exposure caused chlorosis, necrotic lesions and leaf tip injuries at different pH levels (Table 1). Necrosis progressed from nodal region to the adjacent inter-nodal region leading to large scale leaf abscission. Marked chlorotic and marginal necrotic symptoms were observed at pH 4.0 and 5.0. However, this was less pronounced in comparison to pH 3.0 and pH 2.0. Similar symptoms were also observed by Huany et al., 2005 on wheat at pH 4.3 and 2.3.

TMS 96/0304 cultivar of Manihot esculenta showed marked decrease in growth parameters compared to the control. Simulated acid rain at pH 2.0 caused characteristic burned irregular lesions on the plant leaves. It is well reported by many workers that plants sensitive to acid rain can present changes in their morphology, anatomy, physiology and biochemistry (Neufeld et al., 1985). All the plant growth parameters studied the plant height, leaf area, fresh weight and dry weight significantly higher (p< 0.05) at the control (pH 7.0) compared to the other acidity treatments.

<table>
<thead>
<tr>
<th>pH of SAR (mg/g)</th>
<th>Relative growth rate (control)</th>
<th>Chlorophyll content (mg/g)</th>
<th>Harvest index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (pH 7.0)</td>
<td>3.6 ± 0.24 a</td>
<td>2.8 ± 0.21 a</td>
<td>0.5</td>
</tr>
<tr>
<td>6.0</td>
<td>2.8 ± 0.21 a</td>
<td>2.2 ± 0.17 a</td>
<td>0.4</td>
</tr>
<tr>
<td>5.0</td>
<td>1.9 ± 0.19 b</td>
<td>1.4 ± 0.13 b</td>
<td>0.3</td>
</tr>
<tr>
<td>4.0</td>
<td>1.7 ± 0.16 b</td>
<td>1.1 ± 0.10 b</td>
<td>0.2</td>
</tr>
<tr>
<td>3.0</td>
<td>1.0 ± 0.14 b</td>
<td>0.7 ± 0.07 c</td>
<td>0.1</td>
</tr>
<tr>
<td>2.0</td>
<td>0.6 ± 0.06 c</td>
<td>0.3 ± 0.04 c</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The results on the plant height, leaf area, fresh and dry weights are presented in Table 2. There was a significant decrease in acidity concentrations of the parameters. The plant had the highest plant height, leaf area, fresh weight and dry weight significantly higher (p< 0.05) at the control (pH 7.0) compared to the other acidity treatments. Each value is a mean of ± standard error of three replicates. Means within the same column followed by the same letter are not significantly different at (P>0.05) from each other using New Duncan Multiple Range Test.

The effect of simulated acid rain on the relative growth rate (RGR), the chlorophyll content and the harvest index is presented in Table 3. The cultivar had the relative growth rate, the chlorophyll content and the harvest index significantly higher (p< 0.05) at pH 7.0 compared to the other acidity treatments. There was a significant reduction in the relative growth rate, the chlorophyll content and the harvest index with decreasing pH level.

**DISCUSSION**

Simulated acid rain treatment deposited on leaves affect mainly the epidermal cells causing erosion of the cuticle and altering the leaf permeability (Evans, 1984). Photosynthetic pigments were also inhibited with respect to acidity levels. Chlorophyll content was significantly reduced by simulated acid rain treatment relative to the control at pH 2.0 and pH 3.0 (Table 3). The greater foliar injury noticed in plants exposed to pH 2.0 is associated with the decreased chlorophyll content and the damage to the photosynthetic apparatus. This is similar to the earlier results of Liu et al., 2010; Evans 1984. Reduction was due to the removal of Mg+ from the tetrapyrol ring of the chlorophyll molecules by H+ (Foster, 1990) or due to increase of transpiration by acid rain (Evans et al., 1997). Recently similar results were also observed on many crops like mustard, radish, potato, bean plant Chehregani, 2007; Agrawal et al., 2005; Kausar et al., 2005; Khan & Devpura 2005; Varshney et al., 2005).

Relative growth rate and harvest index was highest at pH 2.0 and pH 3.0 compared to the control plants (Table 3) and this have been reported by a number of authors (Steinfeld et al., 1998; Ekanayake 1994; Cock et al., 1977). According to Iglesias et al., (1994), harvest index of 0.5-0.6 is the optimum level because at higher values of harvest index, root production decreases due to reduced leaf area, light interception and photosynthesis.

**CONCLUSION**

It is evident from this study that simulated acid rain treatment at pH 2.0 and 3.0 markedly suppressed growth and yield of TMS 96/0304 cultivar of Manihot esculenta due to reduction of photosynthesis as a result of chlorosis, necrosis and leaf abscission. This result shows that TMS 96/1672 cultivar is likely to be retarded in future in view of rapid and uncontrolled industrialization as well as global warming. More varieties of cassava should be tested for their tolerance/sensitivities to acidic precipitation. This paper is relevance to Track Three because the incidence of acid rains have been reported in countries like Canada, Brazil, United States of America leading to loss of food. Acid rains destroy
crops and also have an adverse effect on the soil, human health and on our environment.

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