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Studies on the effect of simulated acid rain of different pH on the growth response of TMS 419 cultivar of *Manihot esculenta* (Crantz)

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The growth response of TMS 419 cultivar of cassava *Manihot esculenta* Crantz exposed to simulated acid rain of different pH was studied. The plant was 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, senescence, necrosis, leaf abscission, leaf folding and death. Plant height, leaf area, fresh weight, dry weight, relative growth rate, the chlorophyll content of the leaf and the harvest index was highest at pH 7.0 (control) and significantly (p<0.05) decreased with increasing acidity. The result shows that the TMS 419 cultivar of *M. esculenta* exhibited growth suppression at low pH level.

Key words: Simulated acid rain, *Manihot esculenta*, senescence, harvest index, leaf abscission.

INTRODUCTION

Acid rain is a major polluting agent harmful to terrestrial and aquatic ecosystems (Brimblecombe et al., 2007). It is the wet deposition that occurs when 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 (Liu et al., 2010).

Rain that presents a concentration of H^+ ions greater than 2.5 μeq^{-1} and pH values lower than 5.6 is considered acid (Reshma and Manju, 2011). When the air becomes more polluted with nitrogen oxide and sulphur oxide, the acidity can increase to a pH value of 3. Occasional pH readings in rain and fog water of well below 2.4 have been reported in industrialized areas. Acid deposition may cause decline in health and growth of trees as well as other plants (Wyrwicha and Sklodowska, 2006). Several experiments have been carried out in the field and in greenhouses to investigate the effect of acid rain in plants (Silva et al., 2005). Exposure of plants to acid rain results in characteristic foliar injury symptoms, modified leaf anatomy (Stoyanova and Velikova, 2004), structural changes in the photosynthetic pigment apparatus and a decrease in the chlorophyll concentrations (Sant' Anna-Santos et al., 2006).

Cassava (*Manihot esculenta* Crantz) is a woody shrub that belongs to the family Euphorbiaceae (Nweke et al.,

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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 area of Nigeria (Remison, 2005). The cassava cultivar TMS 419 is about 3-4 m tall and usually has no branching. The rain forest belt in Southern Nigeria is potentially susceptible to problems related to acid rain because of the increase in the consumption of petroleum oil products such as diesel, gasoline and coal used to produce energy for different economic sectors of the economy.

There is a need to examine the potential effects of acidic precipitation on cassava since it is a staple food in southern Nigeria for millions of Nigerians. There are over 2,000 cultivars of cassava in Nigeria (Ekanayake, 1994). TMS 419 cultivar was used for this research because it is a common cultivar found in the southern part of Nigeria. In view of the importance of this plant in human diet and the adverse effect of acid rain, the present study was carried out to assess the effect of simulated acid rain on this plant cultivar.

MATERIALS AND METHODS

Source of plant material

Disease free stem cuttings from matured plant of TMS 419 cultivar of *M. esculenta* were collected from the International Institute of Tropical Agriculture (IITA) Ibadan in Oyo State.

Planting procedure

A field experiment was carried out in the school farm of the Federal University of Technology, Akure, Ondo state, Nigeria. Stem cuttings of 30 cm long each of TMS 419 cultivar of *M. esculenta* were planted horizontally with a spacing of 100 cm and four stem cuttings were planted on each row. Each pH treatment of 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 had four replicates and was arranged in a completely randomized design (CRD). The plants were watered every other day and allowed to grow for a week before the application of the simulated acid rain treatment (Reshma and Manju, 2011). Simulated acid rain was sprayed to the planted cassava cultivars every three days according to their pH values. The solutions were applied using a medium size pressurized sprayer on the plants. The plants grew for 25 weeks before the experiment was terminated.

Preparation of simulated acid rain

The acids used were a mixture of concentrated sulphuric acid (H_2SO_4) and concentrated nitric acid (HNO_3) in a ratio 2:1. This is because the most important gas which leads to acidification is sulphur dioxide. The acidic solution was then calibrated using distilled water with a Deluxe pH meter to get the desired pH and cross checked with pH pen. Distilled water was used as the control of pH 7.0.

Several parameters were used in assessing the growth and productivity of the plant. The height of shoots was measured (cm) from the soil level to the terminal bud. The measurements were taken two weeks from the day the acid rain treatment commenced to the day of harvest at twenty four weeks. 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. The tuber dry weight was determined by cutting the tuber into smaller pieces and drying before weighting. 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.

$$RGR = (W_2 - W_1)/(T_2-T_1)$$

Where, W_2 = final weight, W_1 = initial weight, T_2 = final time and T_1 = initial time.

The chlorophyll content of the leaves was determined and the harvest index was determined by the method of Ekanayake (1994):

Harvest Index (HI) = Tuber dry weight/total plant dry weight

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using the Statistical Package for Social Sciences, Version 15.0 (SPSS, 2003). Treatment means were separated using the Duncan multiple range test (Zar, 1984).

RESULTS

Morphological changes were observed in TMS 419 cultivar of *M. esculenta* throughout the period of the experiment. Table 1 shows the effects of simulated acid rain on the morphology of TMS 419 cultivar of *M. esculenta* at 24 weeks after simulated acid rain treatment. Leaves turned brownish, withered with 60% leaf abscission at pH 4.0. The falling and eventual collapse of leaves stretched over a period of 16-20 weeks. At 4.0 pH treatment, leaf abscission started with the leaves at the base of the shoot falling with long petiole. Leaves were chlorotic and necrotic. At 2.0 pH treatment, the plants die from the base of the shoot and leaves had 40% leaf abscission.

The results of the plant height, leaf area, fresh and dry weights are presented in Table 2. There was a significant ($p<0.05$) decrease in the plant height, leaf area, fresh and dry weights of the cultivar with increasing acidity. The plant height, leaf area, fresh weight and dry weight were significantly higher ($p< 0.05$) at the control (pH 7.0) as compared to the other acidity treatments.

The effect of simulated acid rain on the relative growth rate (RGR), the chlorophyll content and the harvest index of TMS 419 of *M. esculenta* is presented in Table 3. The cultivar had relative growth rate, chlorophyll content and harvest index at pH 7.0 as 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

Symptoms of plants polluted with simulated acid rain

Table 1. Morphological changes observed in TMS 419 cultivar of *M. esculenta* polluted with simulated acid rain 24 weeks after treatments.

pH treatment	Observed effects
7.0 (Control)	Luxuriant growth
6.0	Plant had good growth and the leaves had necrotic dots on its surface.
5.0	Production of new leaves on the stem and at the leaf apex. Growth was retarded. There was 90% survival. Older leaves showed signs of curling and chlorosis.
4.0	The leaves were curled and chlorotic. Plant growth was stunted. Leaves were showing signs of burnt surfaces from the tip. Had 60% leaf abscission. There was 70% survival of the plant
3.0	Plants had stunted growth. The new leaves became folded, chlorotic followed by necrosis and eventually some of the plants died. 40% survival. 60% leaf abscission.
2.0	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.

Table 2. Effect of simulated acid rain (SAR) on the plant height (cm), leaf area (cm), fresh weight (g), dry weight (g) of TMS 419 cultivar of *M. esculenta*, 24 weeks after treatment.

pH of SAR	Plant height (cm)	Leaf area (cm)	Fresh weight (g)	Dry weight (g)
7.0 (Control)	274.20 ± 4.16^{a}	288.24 ± 3.25^{a}	782.11 ± 0.16^{a}	326.24 ± 0.21^{a}
6.0	96.12 ± 4.02^{b}	270.11 ± 3.14^{b}	638.30 ± 0.14^{b}	276.14 ± 0.20^{b}
5.0	90.13 ± 3.20^{b}	224.34 ± 3.13^{b}	420.16 ± 0.09^{c}	216.22 ± 0.16^{b}
4.0	84.30 ± 3.12^{b}	218.24 ± 3.10^{b}	354.20 ± 0.09^{d}	168.40 ± 0.14^{c}
3.0	62.34 ± 2.21^{c}	184.10 ± 1.24^{c}	228 .10 ± 0.05^{e}	102.36 ± 0.10^{d}
2.0	50.02 ± 1.10^{d}	166.32 ± 1.16^{c}	114.27 ± 0.02^{f}	60.14 ± 0.04^{e}

Means followed by the same letter vertically are not significantly different at 5% level of significant by New Duncan's multiple range test for the parameters tested.

Table 3. Effect of simulated acid rain (SAR) on the relative growth rate ($gg^{-1}d^{-1}$), chlorophyll content (mg/g) and harvest index of TMS 419 cultivar of *M. esculenta*, 24 weeks after treatment.

pH of SAR	Relative growth rate	Chlorophyll content	Harvest index
7.0 (Control)	4.2 ± 0.22^{a}	4.4 ± 0.20^{a}	0.6
6.0	3.4 ± 0.20^{a}	4.1 ± 0.18^{a}	0.5
5.0	2.6 ± 0.17^{b}	3.2 ± 0.14^{b}	0.4
4.0	1.8 ± 0.14^{b}	2.4 ± 0.10^{b}	0.3
3.0	1.4 ± 0.11^{b}	1.6 ± 0.07^{c}	0.2
2.0	0.6 ± 0.05^{c}	0.8 ± 0.03^{c}	0.1

Means followed by the same letter vertically are not significantly different at 5% level of significant by New Duncan's multiple range test for the parameters tested.

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 slow-growing with leaf chlorosis and necrotic spot coupled with dehydration of the plants. In this study, 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 with pH 3.0 and 2.0. Similar symptoms were also observed by Johnston and Shriner (1985) on wheat at pH 4.3 and 2.3.

The TMS 419 cultivar of *M. esculenta* showed a decrease in growth parameters. Simulated acid rain at pH 2.0 caused 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, including plant height, leaf area, fresh weight and dry weight were reduced significantly at all acidity levels as compared to the control. The highest reduction was observed at pH 2.0 level (Table 2). The adverse effects of simulated acid rain on plant growth parameters on several crops were also observed by Evans et al. (1997), Banwart et al. (1990), Chevone et al. (1984) at pH 2.0

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 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 Sheridan and Rosenstreter (1973) and Evans (1984). Chlorophyll reduction has been attributed to the removal of magnesium ion from the tetrapyrol ring of the chlorophyll molecules by hydrogen ion (Foster, 1990) or increased transpiration due to acid rain (Evans et al., 1997). Recently, similar reductions of chlorophyll content by simulated acid rain were observed on many crops like mustard, radish, potato (Agrawal et al., 2005; Kausar et al., 2005; Khan and Deopura, 2005; Varshney et al., 2005).

The highest relative growth rate and harvest index that was recorded at pH 2.0 and 3.0 (Table 3) is similar to other results for cassava reported by a number of authors (Seinfield et al., 1998; Ekanayake, 1994; Cock et al., 1977; Kawano, 1978). Harvest index is the fraction of total dry matter in the economically useful parts. In cassava, storage roots are the economic yield component. 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

The present results show that simulated acid rain with pH 2.0 and 3.0 has negative effect on the growth and yield of TMS 419 cultivar of *M. esculenta* due to reduction of photosynthesis as a result of chlorosis, necrosis and leaf abscission.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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