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Effects of Flood on the Growth and Some Aspects of Physiology of Five Genotypes of Cassava (*Manihot esculentus*)

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ABSTRACT: The effects of flood on five genotypes of Cassava (Manihot esculenta), TMEB419, 011371, I98058I, I30574 and 91934 was investigated for a period of twenty (21) days. The plants were subjected to flooding two months after establishment. Results revealed that there are three homogenous groups for chlorophyll A and chlorophyll B. It was observed that the effect of flooding on chlorophyll content of the various genotypes is dependent on time. The longer the duration of flooding the less quantity of chlorophyll in the plant. Genotype I30574 had the highest mean for relative water content in flooded condition (having an average of 90%). Genotypes with significant difference in Proline content between treatment and control showed insignificant difference in chlorophyll content. This suggests if flooded plants are treated with exogenous Proline, their chlorophyll content will be enhanced.

Keywords: chlorophyll content, Flood, Net assimilation, Genotype, *Manihot esculenta*.

Introduction

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Cassava (*Manihot esculenta*) is an important root crops that make up the daily calorie needs of people in the tropics and it is cultivated more widely as compared to grains (Omemu *et al*., 2005). Together with Maize, Rice and Sugar cane, the crop makes the most important source of energy in the diet of people living in tropical countries, where it serve as staple for over 800 million people (Burns *et al*., 2010; Perez *et al*., 2011). The annual crop production is estimated to be 241 tonnes of fresh root of which majority come from small scale farmers in Africa and Asia. About 70% of cassava roots produced are consumed by humans, while the remaining is used as animal feed and in production of industrial products such as starch, glucose and alcohol (El-Sharkawy, 2004), the young leaves of cassava are eaten as vegetable in many African countries (Fregene *et al*., 2000) to provide protein, calcium, iron and vitamin, supplementing a predominantly starchy diet in poor communities.

Cassava production faces several set-back including pests, diseases and environmental constraints. The major biotic constrain include: cassava mealy bug and cassava bacterial blight (Soyode and Oyetunji, 2009). Cassava mosaic disease and cassava brown streak disease (Hillocks and Jennings, 2003). Efforts to address biotic stress of cassava have made progress through breeding and selection for tolerance. For instance, molecular markers closely associated with cassava mosaic disease, resistant gene CMD2 have been used for marker assisted breeding for CMD resistance (Akano *et al.,* 2002). Breeding tolerance to cassava post- harvest physiological deterioration has been recently reported (Morante *et al*., 2010). However, only little progress has been recorded with respect to development of flood tolerant varieties. Yet, it has been reported that global warming and its attendant consequence; flooding has caused unforgettable losses to farmers in coastal regions and semi- arid

region. This have impacted on food security in these region, resulting in many households becoming food insecure. In 2012 alone, flooding in parts of our country, Nigeria (Anambra, Bayelsa, Cross Rivers, Rivers, Benue, Kogi, Kano, etc) resulted in food insecurity for families in these region compelling them to depend on federal government aid and that of good spirited Nigerians from non-affected areas.

Excessive moisture in the soil causes oxygen level in the soil to decrease, thus impeding root respiration and photosynthesis (Oyetunji *et al*., 2007). Besides the obvious killing of submerged branches and leaves, most plants are intolerant to having their roots submerged for long period of time. As a result of reduction in oxygen, the level of carbon dioxide, methane, hydrogen and nitrogen gas around the root increase. Roots suffocate and die (Lahai and Ekayannake, 2009). Toxic compounds such as ethanol and hydrogen sulphide, as well as numerous other harmful compounds, can build up in water saturated soils (Lahai *et al*., 1999). Photosynthesis is inhibited and plant growth slows or even stops.

The varietal differences among cassava genotypes in response to flooding, can be exploited to identify and develop cassava varieties well adapted to flood prone areas. Yet, in Nigeria information regarding genetic variability for flood tolerance amongst farmer preferred cassava genotypes is limited. This information is an important resource to help cassava breeders to accurately identify flood tolerant genotypes to act as gene source for breeding or to be directly used by farmers in flood prone areas. Development of improved crop varieties will without doubt result in improved food security and poverty alleviation amongst the resource poor farmers living in low land areas and it is in line with the Millennium Development Goal (MDG) that calls for the world to reduce the proportion of people who suffer from poverty and hunger (UN, 2010).

Materials and methods.

The experiment was carried out in the screen house of the Department of Botany, University of Ibadan, Oyo State, Nigeria. Polythene bags were bought from Oke-paddy market and perforated at the bottom. 25kg weight of soil was collected in each bag and transported to the Department of Botany's screen house. Fresh cassava stems were collected from IITA and cut into stem cuttings of 30cm. Two cuttings of the same genotype were planted in each bag and the bags were then arranged in a completely randomized block design. Flooding of the plants was done 2 months after planting. The plants which were growing in the bags were put into large basins filled with water, flooding the soil beyond field capacity with the excess water rising to about 5cm the stem.

Data collection: Data were collected on growth parameters such as: height of plants (cm), number of shoots. Plant height was measured using meter rule, while number of leaves and number of shoot were counted with manually. Data was collected three times a week on chlorophyll content, proline content and relative water content and their means calculated.

Chlorophyll content: The plant leave material was processed in fresh state immediately after collection. Finely chopped portion of the cassava leaf weighing 0.5g measured off on an analytical balance was collected from five samples of each genotype of both control and treated plants. The measured off material was then homogenized in a homogenizer with the addition of 10ml of 80% acetone. A pry acetone extract containing all chlorophyll pigments was obtained. The extract was then refrigerated at 2500rnp for 5minutes and the supernatant collected. The supernatant was then subjected to reading on spectrophotometer by taking its highest and lowest light absorbance at 663nm and 640nm respectively. Chlorophyll content of each sample was then calculated according to Welburn (1994):

Chlorophyll A= 11.75 (Abs640) – (Abs663)
Chlorophyll B=18.61 (Abs663) – (Abs640).

Proline content: Proline content was determined using the method of Marin *et al*., 2009; based on the reaction of proline with ninhydrin. A 1: 1:1 solution of proline, acid ninhydrin and glacial acetate was incubated at 100 °C for 1 hour. The reaction was arrested in ice bath and chromophore was extracted with 4ml toluene and its absorbance at 520nm in Biomate spectrophotometer was determined.

Relative water content: Relative water content was done by the method of Barr and wetherly, (1950). The fresh and dry weight, length and width of leaves were taken. The leaves were then soaked in water at 25^0c for 1hour, and their turgid weight was taken. They were then oven dried at 60°C for 24 hours and their dried weight was taken. Relative water content was then calculated using the formula:

$$RWC = \frac{\text{Fresh weight} - \text{Dried weight}}{\text{Turgid weight} - \text{Dried weight}} \times 100$$

Net assimilation: Net assimilation was done according to the method of Gorge, (1917). The fresh and dried weight, length and width of the leaves were taken. The area of the leaves was then calculated using formula:

$$\text{Net assimilation} = \frac{(W2 - W1)(\log A2 - \log A1)}{(t_2 - t_1)(A_2 - A_1)}$$

Analysis of experimental data: Analysis of variance (ANOVA) was used to show if there were significant differences in degree of tolerance of the 5 genotypes of cassava to flooding.

Results

Table 1 shows the properties of the soil used in this study. The soil is a sandy- loam with slightly alkaline pH of 7.2, organic carbon content (O.C) 37.79 g/kg, total nitrogen (T.N) 2.82 g/kg and Fe+ content of 24.2 mg/kg.

Table 1: Chemical properties of soil used

Parameter	**pH**	**T.N** (g/kg)	**O.C** (g/kg)	**Av. P** (mg/kg)	**Acidity**	**(mol/kg)**				**(mg/kg)**			
						Ca	**Mg**	**K**	**Na**	**Mn**	**Fe**	**Cu**	**Zn**
Measure	7.2	2.81	37.79	3.3	0.2	15.59	8.92	0.34	10.4	89.3	24.2	6.06	5.1

Figure1 shows the percentage composition of various soil particles present in the soil. Sand has the highest proportion 85%, silt 12% and 8% clay. It indicates the soil is a loamy soil.

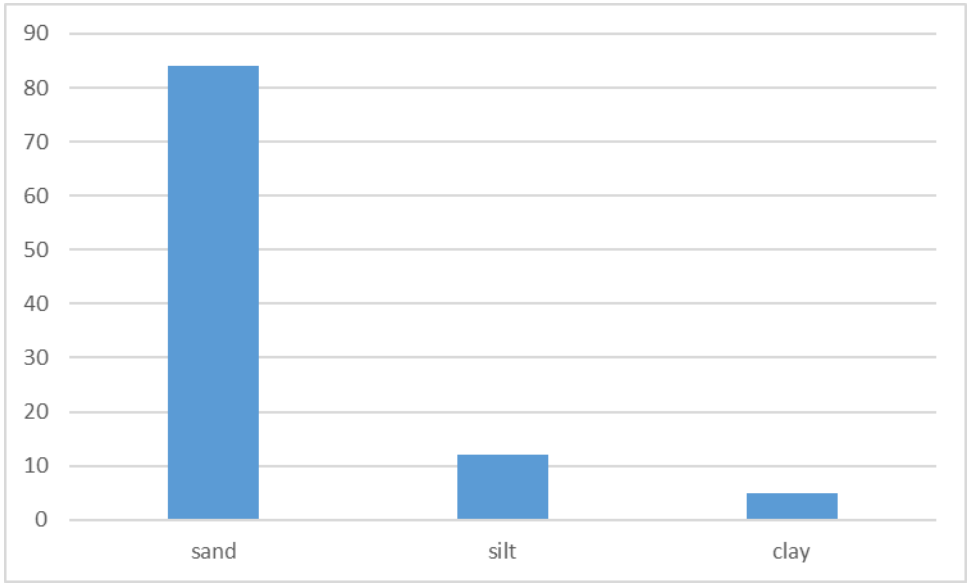

Figure 1: Percentage soil composition.

Table 2 shows the mean values of chlorophylls A and B and their total content for the five varieties. It reveals that there were three homogenous groups of means for the five varieties for chlorophyll A, such that varieties labelled same alphabet were not significantly different at 95% confidence level and they exhibited similar traits for chlorophyll A content. Genotype 011374 had the highest mean value for chlorophyll A content in weeks 1, 2 and 3 which are 18.42 µg/ml, 17.24 µg/ml and 13.73 µg/ml respectively. Three homogenous groups were also observed for chlorophyll-B. Genotype TMEB419 had the highest chlorophyll-B content in week 1 (26.17 µg/ml), which dropped slightly in week 2 (24.59 µg/ml) and increased significantly in week 3 (28.04 µg/ml). This was followed by genotypes 011371 and I980581.

The table also revealed that the effects of flooding on chlorophyll-A is dependent on time; the longer the flooding period, the less the chlorophyll present in the plant leaves whereas flooding had little or no effect on chlorophyll-B.

Table 2: Mean Chlorophyll A and B content in the five genotypes for three consecutive weeks

	Chlorophyll a			Chlorophyll b			Total		
Variety	**Week 1**	**Week 2**	**Week 3**	**Week 1**	**Week 2**	**Week 3**	**Week 1**	**Week 2**	**Week 3**
Tmeb419	14.28^{b}	13.44^{b}	10.99^{c}	26.17^{a}	24.59^{b}	28.04^{a}	40.45^{b}	38.03^{a}	39.03^{a}
011371	18.42^{a}	17.24^{a}	13.73^{a}	26.35^{a}	25.43^{a}	26.26^{a}	44.77^{a}	42.66^{a}	39.99^{a}
I980581	17.09^{a}	13.86^{b}	11.56^{b}	23.57^{b}	25.96^{a}	25.47^{b}	40.66^{b}	39.82^{a}	37.03^{b}
91934	17.08^{a}	14.79^{a}	13,32^{a}	23.11^{b}	22.13^{c}	2 6.02^{a}	40.19^{a}	36.92^{b}	26.02^{b}
I30574	16.86^{b}	14.29^{a}	12.82^{a}	22.54^{c}	23.77^{c}	25.11^{b}	39.4^{b}	38.06^{b}	37.93^{b}

Figure 2 shows net assimilation of the five varieties of cassava. Genotypes TMEB419 and 011371 had higher net assimilation in control plants (38 cm^2/g and 36 cm^2/g respectively), while their treated plants had 37 cm^2/g and 26 cm^2/g respectively whereas in Genotypes I980581 and I30572, the treated plants (27 cm^2/g and 34 cm^2/g respectively) were higher than the control plants(22 cm^2/g. and 28 cm^2/g).

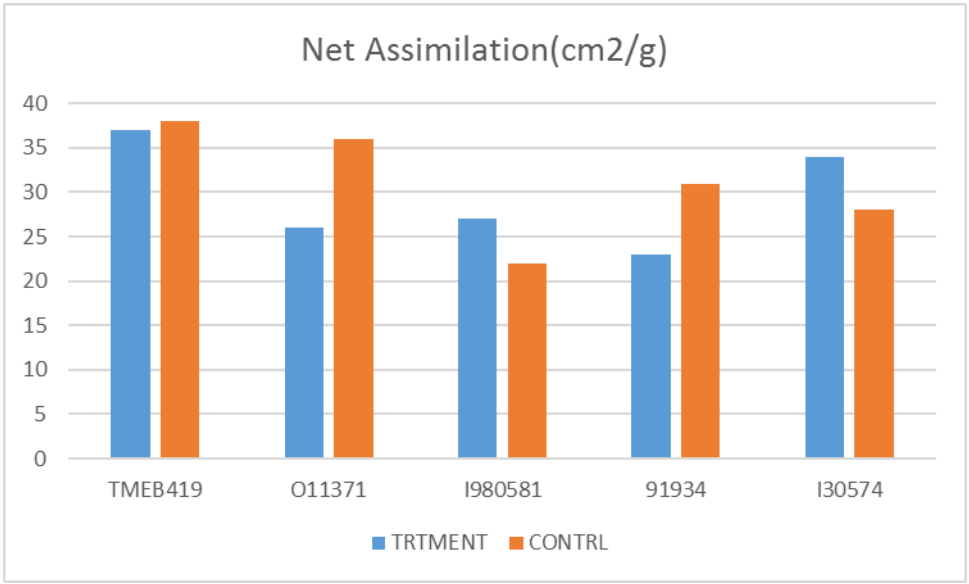

Figure 2: Net Assimilation in cm^2/g in control plants and treated plants for the five genotypes

Table 3 shows the mean relative water content of the five genotypes. The table reveals that there are two homogenous sets of means for the five varieties for relative water content in which means with same alphabets are not significantly different. Genotype TMEB419 has the highest mean in both Weeks 1and 2 (88.1% and 80.5% respectively) while Genotype I30574 had the highest relative water content in week 3 (93%). The table also revealed that relative water content is dependent on time. While some genotypes decreased with time, others increased with time.

Table 3: Mean relative water content in the five genotypes in three consecutive weeks

Genotype	Week 1	Week 2	Week 3
TMEB419	88.1[a]	80.5[a]	74[b]
o11371	86.5[a]	66[a]	75.5[b]
I980581	57.5[b]	64[b]	87.5[a]
91934	55.2[b]	78.5[a]	86.5[a]
I30574	79.4[a]	79[a]	93[a]

Figure 3 shows proline content of the treated plants compered to their control plants. Genotype 91934 had the highest proline content (18000 µg/ml) followed by TMEB419 (15000 µg/ml) in treated plants while in Genotype I980581, proline content was slightly higher in the control plants ((6700 µg/ml) and 6500 µg/ml in treated plants.

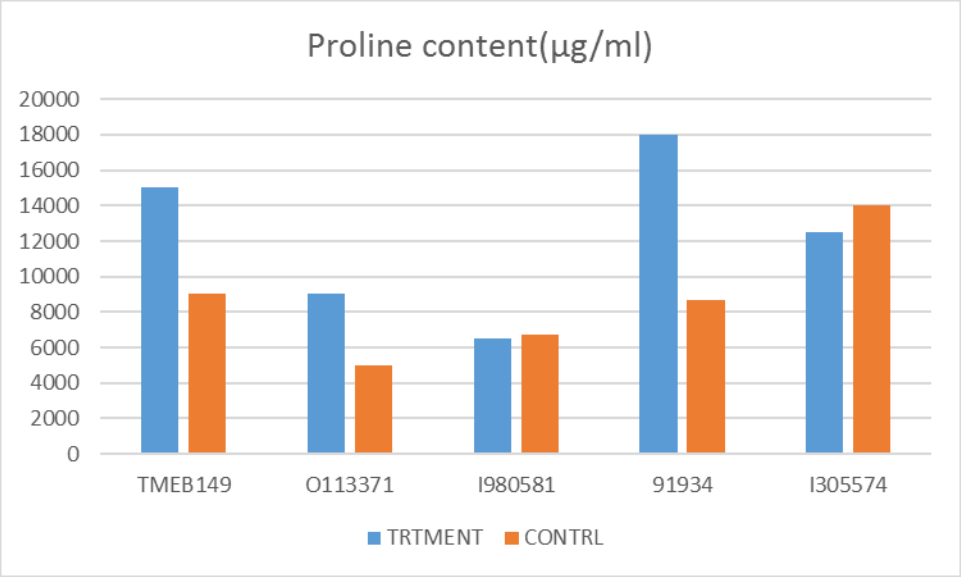

Figure 5: Proline content of the treated plant compared to their control plants

Discussion

The plants generally grew well, genotype 011371 had a good trait for plant height, measuring an average of 62.71cm at 10 weeks after planting, TMEB419 exhibited the worse trait for plant height measuring 33.2 cm at 10 weeks after planting. At the beginning all genotypes were relatively equal in height and started varying at about 6 weeks after planting.

The number of shoots remained constant throughout the study period as no new shoots were produced. Genotype 91934 had a good trait for number of shoot, producing an average of 2 shoots per stand whereas, 011371 had the lowest number of shoot, producing only 1 shoot per stand or stem cutting. Genotype 91934 with the best trait for number of shoot also had the best trait for number of leaves, producing an average of 29 leaves at 10 weeks after planting while Genotype 011371 with lowest number of shoot, and also had the lowest number of leaves, producing an average of 19 leaves at 10 weeks after planting.

Genotypes TMEB419, 011371 and 91934 had significant difference at 95% confidence level in their chlorophyll content between flooded plants and control plants. The control plants had higher chlorophyll content than flooded plants. Reduction in chlorophyll content arises when reactive oxygen species alter chlorophyll production. Reactive oxygen species are produced under low oxygen condition in flooded plants and cause alteration in biochemical reactions of photosynthesis. These biochemical alterations include restricted activity of Ribulose Bisphosphate Carboxylase (RUBPC), phosphohycollate and glycollate oxidase (Yordanova and

Popova, 2001) destruction of chloroplast membrane inhibiting photosynthetic electron transport and efficiency of photosystem II. These reactive oxygen species are extremely reactive and have been observed to cause damage in most cellular molecules and metabolites such as proteins, lipids, pigments and DNA (Ashrof, 2003).
Genotype **I**980581 and **I**30574 exhibited positive trait for relative water content under flooded condition compared to their control plants. Flooded plants had higher relative water content than their control plants. In flood tolerant plants, plants water relation during flooding depends on the season of occurrence and species response (Crowford, 2003; Lenssen *et al.,* 2004), for example in *Paspalum detortatum*, flooding had no significant effect on leaf water potential, stomatal conductance and transpiration rate. Interestingly, flooded plants recorded higher leaf water content than control plants on days with high water evaporation demand.
Genotype showed 011371 significant decrease in relative water content with time as compared to their control plants and the other genotypes. This decrease in relative water content is usually due to reduction in stomata conductance (Folzer *et al*., 2006). Plants exposed to flooding stress exhibit increased stomata resistance as well as limited water uptake, leading to internal water deficiet (Parent *et al*., 2008), for instance, flooding had negative effect on leaf water potential, stomatal conductance and transpiration rate in L. *tenuis*. This negative effect increased over the days and caused reduction of between 40 – 60% in leaf water potential, stomatal conductance and transpiration rate (Striker *et al*., 2005) compared to control plants.
Reduction in relative water content in plants could also be due to reduction in root hydraulic conductivity. In species that are very sensitive to flooding, few hours after flooding occurs, water uptake by root was reduced. Jackson and Drew, (1984) observed a fast decrease in leaf water potential within few hours in *Solanium lycopersicum, Piscum saturum* and *Nicotiana tabacum.* Reduction in root hydraulic conductivity is assumed to be associated with the acidification of the cell cytoplasm and the gating of aquaoporins (Tournarie-Roux *et al*., 2003). The excess of protons produced as a result of acidification brings about change in the conformation of water channels and subsequently result in their closure (Verdoucq *et al.,* 2008). Reduction in water uptake in most species become apparent in wilting of leaves (Bradford and Hsias, 1982: Else *et al*., 1996).
In Genotypes I30574 and I980581, the flooded plants had more chlorophyll than the control plants although this was not significant at 95% confidence level. The treated plants had slightly higher chlorophyll content than their control plants. This may be due to low production or antioxidants. Reactive oxygen species are also produce in plants under normal condition but their concentration is usually very low. However, when plants are faced with environmental stress such as flooding, the concentration of reactive oxygen species increases. Increased level of reactive oxygen species damages several cellular metabolic reactions and processes (Ashrol, 2003).
Genotypes with significant increase in proline content in flooded plants had insignificant difference in chlorophyll content between flooded plants and control plants while genotypes with good trait for chlorophyll content in flooded condition, showed good level of net assimilation. Flooding causes disruption of different chlorophyll florescence. Since chlorophyll florescence is an excellent physiological characteristic that determine energy transfer due to excitation, absorption of light and photochemical reactions occurring in photosystem II (Saleem *et al*., 2011), it therefore affects net assimilation, since photosynthesis is hampered. For instance, Han *et al*., (2006) observed a substantial decrease in maximum quantum efficiency in Cork Oak (*Qercus variabitis*) and China Wingnut (*Pterocarya steropetra*) when they were subjected to flooding. Also a decrease in quantum yield of Photosystem II plant chemistry and net assimilation was observed in field beans when subjected to varying days of water logging stress (Pociecha *et a*l., 2008). This decrease in Photosystem II quantum efficiency and net assimilation are indications that the photosynthetic apparatus (chloroplast) are sensitive to water stress and also the inability of the plants regenerate Rubisco under stress (Smethrust *et al*., 2005). Rubisco is the enzyme that catalyzes the initial reaction during CO_2 in the process of photosynthesis.

Conclusion

Genotype I30574 has good trait for chlorophyll content in flooded condition in which the flooded plants had slightly higher chlorophyll content than the control plants while Genotype 91934 has a good trait for relative water content in flooded condition with the flooded plants having slightly higher relative water content and they were both significant at 95% confidence level.

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