Effect of Siam weed mulch on ATpase activities of *Abelmoshus esculentus* in a crude oil contaminated soil.

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Abstract: The effect of Siam weed mulch on chloroplast and mitochondrial ATpase Activity of Abelmoschus esculentus grown in an oil contaminated Soil was investigated. Abelmoschus. esculentus Seeds were planted in cellophane bags with soil polluted with 200ml of crude oil representing 3% pollution level. Siam weed mulch weighing 50g was applied to the cellophane bag polluted with crude oil. Data were collected on two weeks interval and evaluated on ATpase Activities (CAA and MAA) for six times. Results showed that Siam weed mulch significantly enhanced ATpase activities of the chloroplast and mitochondria than in Abelmoschus esculentus planted in non treated soils.

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Introduction

ATpase activity in plant is part of the ATP Synthesisig machinery based on the translocation of energy resulting from photosynthesis for electron transport. Studies have shown that interaction between dicyclohexcarbonondioxide and hydrophobic portion of ATpase complex leads to increase in the chloroplast ATpase activity (Gentle 1998). From literature (Akonye 1992), chloroplast is assumed to have vast direct functions including chlorophyll synthesis, energy (ATP) formation, photosynthetic Co_2 fixation and glucocagenesis among several others. It has also been known that intact chloroplast express an inhibited photosynthetic rate when exposed to different environmental Stress. (Gibson and Sulfital, 1986). The degree and magnitude of environmental contamination and its effect on ATpase activities depends on the rate and nature of pollutant.

Severe polluted soils may inhibit components of chloroplast such as 1, 6 biphosphate activity by inhibition of light activation. (Onwugbuta and Offor 2010, Ebulamson 2002). In addition to photosynthetic chloroplast metabolism, chloroplast respiration may be affected by environmental contaminants (Kaiser and Heber 1981). There are also suggestions that chloroplast ATpase activities are capable of Osmotic adjustment in response to various levels of environmental contaminants, this could, be possible through regulation of materials capable of restoring soil potentials for plant's growth.

From available evidence (Onwuguta and Offor 2010) it seems that inhibition of photosynthesis by oil pollution or contamination is at biochemical level resulting from reversible inhibition of chloroplast enzymes and its impact by membrane damage which requires repair mechanism for reversibility brought about by chemical components in the soil.

Insights on the use of Siam weed mulch on primary metabolism are scarce. In this study, evidence is presented that Siam weed mulch has significant effect on ATpase activities of *Abelmoschus esculentus*. The choice on the use of Siam weed mulch was based on its availability, easily affordable, and environmentally friendly in soil restoration as evidenced in our previous works. (Offor and Akonye 2006, Offor, Akonye and Onwugbuta 2009).

Materials And Method

Seeds of *A. esculentus* was purchased from the Agricultural development Agency commercial unit while the crude oil was supplied from the Nigeria Agip oil company Ebocha base-Port-Harcourt. Cellophane bags of equal diameter perforated at the bottom were filled with sand weighing. 600g. 200ml of crude oil was applied to some of the cellophane bags and mixed thoroughly with the sand with the help of a trowel. 50g of Siam weed mulch leaves were weighed and applied to the soil with crude oil application. Seeds of *Abelmosclus esculentus* were then sown on the cellophane bags at the rate of five (5) seeds per bag and the experiment is represented as follows:

- 3% (200ml) pollution
- 3% (200ml) pollution with Siam weed mulch
- Control-without pollution and Siam weed mulch.

The experiment was carried out for three months

i. Chloroplast extraction

Chloroplast extraction was accomplished by finding 1-08 of leaf samples with pre-chelled mortar and pestle. The finding medium comprised of 0.4m sucrose, 0.05m tris butter and 0.81m Nacl and 1% PVP (ph.8.0). The homogenate was squeezed through a double layer of cheese cloth subjected to differential centrifugation for 90 seconds at 600xg to throw down debris and broken cells, the supernatant was centrifuged for 10 minutes at 100xg in a ms2 centrifuge. Cold conditions at 4^0c were maintained by immersing the centrifuge tubes in a centrifuge bucket containing a mixture of ice-chips and salt. Chloroplast were washed by adding 5ml fresh grinding medium and centrifuged again. Finally, the chloroplast was re-suspended in 5-10ml of the grinding medium depending on the concentration.

ii. Chloroplast ATpase Activity

The components of the standard reaction for ATP hydrolysis were 120um Tris (PH. 8.0), T.6um ATP 24um mgcl and 4um PMS (phenazine methosulphate) in 1ml chloroplast suspension. Distilled water was added to give a final volume of 8ml. The reaction was carried out with appropriate controls lacking ATP and/or chloroplast extract. Chloroplast Activity was determined by estimating the amount of inorganic phosphate in the supernatant after centrifugation at 1.000xg for 10minates. The reaction was carried out in a water bath illuminated on two sides with 100wbulbs. The temperature of the reaction was maintained at 30^0c. The reaction proceeded for 26minutes and was terminated after wards by turning off the light and adding trichloroacetic acid to a final concentration of 3% (w/v). This was centrifuged at 1000g for 5 minutes and aliquot of the supernatant were assayed for ATP hydrolysis using calorimetric method of chen, Troibara and Earner (1996). The analysis was replicated six times.

iii. Mitochondrial Extraction

Mitochondrial extraction was achieved by grinding about 1.0g of leaf sample in the ice. Cold 0.55m Sucrose (PH.7.7), 5mM EDTA and 5mM DDT (Dithiothreitol). The homogenate was filtered through layers of ice-cold cheese cloth into an ice cold 100ml Erlenmeyer flask immersed in ice chips. The filtrate was centrifuged at 100xg at 4^0c in an MSE centrifuged for 5 minutes. The supernatant was centrifuged at 6000 x g for 20minutes. (Nashashi and Hiraike, 1982).

Mitochondrial ATpase Activity

The reaction comprised of the following in micromoles per ml (μmoles/ml): mgS04, $7H2^0$-0.3 kd-0.5, sucrose-167:, Trias buffer-2.0 (PH 7.3). 1ml of mitochondrial extract prepared as described above and distilled water to give a final volume of 9.0ml. The reaction was carried out in water bath at 25^0c for 10minutes with stirring at frequent intervals. At the end of the reaction, the mitochondrial extract was immediately precipitated out by adding 10% trichloroacetic acid (TCA) and centrifuge at 600xg for 20 minutes. The ATpase activity of the mitochondrial extract was measured as the amount of inorganic phosphate present in the supernatant. This was done following the method of Fiske and subbarrow (1925).

Results

Chloroplast ATpase activity in *Abelmoschus esculentus* was generally low during the first two weeks of the study and rise from the 3rd week at both control and polluted treatments. Thereafter there was a significant increase in treatments with Siam weed mulch comparable to control. Application of Siam weed mulch to polluted soil significant enhanced chloroplast ATpase activity. Least chloroplast ATpase activity was observed in polluted soil without Siam weed mulch.

Mitochorodrial ATpase activity was equally, enhanced in polluted sol treated with Siam weed mulch. The significant increase was less when compared with mitochorodrial ATpase Activity of *Abelmoschus esculentus* planted in control experiment.

ATpase activity in the mitochondria dropped in the first week of the experiment in treatment with Siam weed mulch and rose again, but was significantly consistent from the 5th week.

Discussions

ATpase activity is part of the ATP synthesizing machinery based on the translocation of energy for election transport. The significant promotory effects shown in treatment with Siam weed mulch could be related to interactions with hydrophobic membrane portion of the ATpase complex Armstrong (1978) and Banks *at al* 2003) made similar observations. Inhibition of chloroplast ATpase activity by crude oil could be as a result of their electron-transport-conpled ATP Synthesis in chloroplast by blocking electron-coupled ATP Synthesis from electron transport or by directly inhibiting phosphorylation reactions. Queirolo *etal* (1981) made similar observations on the inhibition of ATP Synthesis and coupled electron transport by 2, 4-dihydroxy-7-methoxy1-1, 4- (2H) benzoxan – 3 (4 H)- one (DIMBOA). The observation by Quierolo *et al* (1981) shows that part of the inhibition was due to an interaction between the contaminants and the membrane bound coupling factor/component of the enzyme. Crude oil appears to have similar effect.

Mitochondria ATpase activity was equally affected when treated with Siam weed mulch. The

decrease in MAA at the initial stage of the experiment may be as a result of conformation of protoplasmic organelles, like chloroplast and mitochondria. The observed lower levels of ATpase activities in the treatments could result in the inhibition of ATpase Synthesis in both chloroplast and mitochondria and subsequent retard some metabolic activities by a reduction in the available ATP which in turn leads to reduced growth in plants.

In this study, treatment with Siam weed mulch showed significant enhancement of ATpase activity (chloroplast and mitochondria)in Abelmoschus esculentus.

Table 1: Effects of siam weed application on chloroplast/mitonchodorial ATpase activities of *Abelmoschus esculentus*

Time	3% pollution + Siam weeds		3% pollution without amendment		Control (No pollution, No Siam Weeds)	
	CAA	MAA	CAA	MAA	CAA	MAA
1	0.62 ± 0.11^{ab}	0.31 ± 0.11^{ab}	0.31 ± 11^{d}	0.16 ± 1.11^{c}	0.67 ± 0.01^{a}	0.32 ± 0.02^{a}
2	0.85 ± 0.11^{b}	0.41 ± 0.11^{a}	0.29 ± 1.01^{c}	0.33 ± 1.17^{c}	1.24 ± 1.02^{a}	0.62 ± 1.01^{a}
3	1.08 ± 0.18^{bc}	0.25 ± 1.08^{e}	1.07 ± 0.11	0.21 ± 0.16^{e}	2.16 ± 0.91^{a}	1.08 ± 1.0^{a}
4	1.54 ± 0.08^{a}	0.30 ± 1.03^{c}	1.06 ± 0.02^{ab}	0.53 ± 0.12^{a}	1.09 ± 0.92^{ab}	0.54 ± 1.1^{a}
5	1.61 ± 1.11^{a}	0.40 ± 1.12^{b}	1.00 ± 0.01^{c}	0.34 ± 0.11^{cd}	1.07 ± 0.91^{b}	0.52 ± 1.01^{a}
6	1.69 ± 1.11^{b}	0.42 ± 1.10^{b}	1.29 ± 1.10^{c}	0.41 ± 0.11^{cb}	2.01 ± 1.01^{a}	1.01 ± 1.09^{a}

Note: CAA - Chloroplast ATpase Activity, MAA – Mitochodria ATpase Activity within Column ± SEM with different superscript are significantly different at P< 0.0

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