

## Changes in the contents of carotenoid, chlorophyll and antioxidant enzymes in the leaf tissues of Pepper (*Capsicum annuum* L.) following exogenous application of bioregulators

Olaiya, C.O. and Poloamina, L.A.

Department of Biochemistry, University of Ibadan, Ibadan, Nigeria.

[coolaiya@yahoo.com](mailto:coolaiya@yahoo.com)

**Abstract:** The impact of exogenously applied bioregulators, Indole acetic acid (IAA) and Naphthalene acetic acid (NAA) on antioxidant enzyme activity and some phytochemicals was investigated in three pepper species namely *Capsicum annuum* var. *grossum*, *Capsicum annuum* var. *accuminatum* and *Capsicum chinense* (big sun). Seeds of these pepper species were respectively treated with 40, 60, 80, 100 and 120 mg/L concentrations of the bioregulators and a control was set up for each of them. The treatments were replicated three times in a completely randomized pattern and the seeds were then planted in standard polythene bags in a screen house. The plants were watered on alternate days. At maturity, their leaves were collected and used for analyses. Results showed low activity for catalase and peroxidase while the content of chlorophyll a, chlorophyll b and carotenoid were significantly increased ( $p < 0.05$ ) in the *grossum* and *accuminatum* species at 60mg/L IAA; 40 and 80mg/L NAA. The highest level of photosynthetic pigments was obtained at 40mg/L NAA in the *Capsicum chinense* species. The high concentration of 120mg/L NAA generally caused a decrease in the level of phytochemicals in the three pepper species relative to control. Though IAA and NAA have been reported to induce many physiological and biochemical processes in plants, this study revealed the negative effects of these compounds on the biochemical contents of pepper, especially at high concentrations.

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### 1. Introduction

Pepper (*Capsicum annuum* L.) is a member of the solanaceous fruity vegetables. It is an important economic food crop which is rich in bioactive nutrients and dietary antioxidants (Navarro *et al.*, 2006). The intake of these compounds in food is an important health-protecting factor. They have been recognized as being beneficial for prevention of widespread human diseases, including cancer and cardiovascular diseases, when taken daily in adequate amounts (Bramley, 2000). The role of capsicum consumption has been reported to influence carbohydrate metabolism in humans (Yoshioka *et al.*, 1995). Chili pepper leaves are used in a variety of dishes. They taste bitter but impart only a mild heat and pungency in comparison to the fruits and are hence, perfect substitutes for people who can't stand spice. Also, aqueous extracts of *Capsicum* sp. have been found to display significant antimicrobial effects (Cichewicz and Thorpe, 1996).

Bioregulators are endogenous or exogenous chemicals that affect the expression of biological responses in plant tissues (Olaiya and Adigun, 2010). They have been reported to affect the photosynthetic activity of leaves and plant metabolism (Sahu and Sabat, 2011), which might account for higher accumulation of health-promoting metabolites in reproductive organs of plants (Abou El-Yazeid,

2011). In recent years, bioregulators has drawn the attention of researchers because of their role in induction of tolerance to various abiotic stresses (Horva'th *et al.*, 2007; Salehi *et al.*, 2011), improvement of nutritional quality of food crops (Olaiya *et al.*, 2010) and unique ability to regulate plant growth, development, fruiting and senescence (El-Rokiek *et al.*, 2012). Little is known about the effect of these bioregulators on anti-oxidant enzymes and photosynthetic pigments of the pepper plant, especially in sub-Saharan Africa. Their effect on the activities of these enzymes and associated photosynthetic pigments in the leaves of plants is therefore a renewed area of research.

The production of activated oxygen species (AOS) which can damage DNA, protein, chlorophyll and membrane function is a byproduct of oxidative metabolism in chloroplasts, mitochondria and peroxisomes. Their production is usually aggravated in response to various abiotic stress, such as cold, salt, extreme temperature, drought, metal and herbicides (Zawoznik *et al.*, 2007). Plants have a number of enzymatic and non-enzymatic detoxification mechanisms to scavenge either the AOS or their secondary metabolites (Hajiboland *et al.*, 2007). This study is aimed at investigating the effect of two bioregulators namely, IAA and NAA on

anti-oxidant enzymes and some biochemical contents of the pepper plant.

## 2. Materials and Methods

### Plant material

The seeds of three species of pepper plant (*Capsicum annuum* var. *grossum*, *Capsicum annuum* var. *accuminatum* and *Capsicum chinense* (big sun) were obtained from the National Institute for Horticultural Research (NIHORT), Ibadan, Oyo State, Nigeria. The seeds were sown after pre-treatment in polyethylene bags filled with loamy soil gathered from the University of Ibadan community.

### Pre-treatment of seeds and plant cultivation

Seeds were respectively soaked in solutions of 40, 60, 80, 100 and 120mg/L concentrations of naphthalene acetic acid (NAA) and indole acetic acid (IAA). A control was set up by using distilled water in place of the bioregulators. The seeds were soaked in film containers and kept in the dark for 24 hours, after which they were air - dried before sowing. After drying, treated seeds were sown in soil-filled polyethylene bags in three replicates and the bags were kept in a greenhouse for germination and growth. The plants were watered every other day and weeds removed from the plants environment as at when due to curb competition. Watering of the plant continued until full maturity of the plant.

### Determination of Total Carotenoids, Chlorophyll a and Chlorophyll b

The spectrophotometric method of Metzner *et al* (1965) was used. 1g of leaf samples was homogenized in 85% acetone and centrifuged for 20 minutes at 15000rpm and the supernatant was stored in a refrigerator. The supernatant which contained the pigments was diluted with 85% cold aqueous acetone to an appropriate volume for spectrophotometric measurements. The extract was measured against a blank of pure 85% cold acetone solution at three wave lengths: 452nm, 663nm, and 644nm. The concentrations of chlorophyll a, chlorophyll b, and total Carotenoids were determined using Arnon's equation (1949).

### Sample Preparation for Enzyme Assay

1g of leaf samples was grinded in 10ml solution containing 0.1 M Potassium phosphate buffer, pH 7.5, and containing 0.5mM Ethylenediaminetetraacetic acid (EDTA). The brie was centrifuged for 20 minutes at 15000 rpm and the supernatant was collected for enzyme assays.

### Determination of Catalase Activity

The method of Beers and Sizer (1952) in which the disappearance of peroxide is followed spectrophotometrically at 240 nm was used. One unit decomposes one micromole of H<sub>2</sub>O<sub>2</sub> per minute at 25°C and pH 7.0 under the specified conditions.

### Procedure:

The Lambda 25 UV/Vis spectrometer (Perkin Elmer) was adjusted to 240 nm. The assay mixture contained 1.9 ml of phosphate buffer, 1.0ml of H<sub>2</sub>O<sub>2</sub> and 0.1 ml of the extract. The decomposition of H<sub>2</sub>O<sub>2</sub> was recorded as the decline in absorbance at 240 nm for 3 minutes. Then,  $\Delta A_{240}/\text{min}$  was calculated from the initial (45 second) linear portion of the curve.

### Determination of Peroxidase Activity

This was determined by measuring the increase in absorbance at 510 nm resulting from the decomposition of hydrogen peroxide (Trinder, 1966). The Lambda 25 UV/Vis spectrometer (Perkin Elmer) was adjusted to 510 nm. The blank was a mixture of 1.4 ml of phosphate buffer and 1.4 ml of H<sub>2</sub>O<sub>2</sub> in the cuvette. The assay mixture contained 1.4 ml of phosphate buffer, 1.4 ml of H<sub>2</sub>O<sub>2</sub> and 0.2 ml of the extract. The increase in absorbance at 510nm was recorded for 4 minutes. Then,  $\Delta A_{240}/\text{min}$  was calculated from the initial (45 second) linear portion of the curve.

### Statistical analysis

This was done using the methods described by Snedecor and Cochran (1982). The mean differences were compared with the Duncan multiple range test (DMRT).  $P < 0.05$  was considered statistically significant.

## 3. Results and Discussion

### Chlorophyll a, Chlorophyll b and Carotenoid Content

In plants, photosynthetic efficiency depends on photosynthetic pigments such as chlorophylls 'a' and 'b', which play an important role in the photochemical reactions of photosynthesis (Taiz and Zieger, 2006). The data in Table 1 indicate that both IAA and NAA stimulated the concentrations of chlorophylls 'a' and 'b', in comparison to the untreated control. However, high levels of the bioregulators, especially the 120mg/L treatment caused a significant decrease in the chlorophyll concentration of the pepper plants. IAA was suggested to prevent the loss of chlorophylls throughout the aging of chloroplasts in wheat both *in vivo* and *in vitro* conditions (Misra and Biswal, 1980). Chlorophyll and protein loss in leaves are conventionally used biochemical parameters to

indicate senescence (Ghanem *et al.*, 2008). The changes in leaf chlorophyll content at high concentrations of the bioregulators may have been due to reduced biosynthesis or increased degradation of chlorophyll. Leaf senescence is marked by degradation in chlorophyll levels (Ghanem *et al.*, 2008). The 120mg/L concentration of the bioregulators is therefore expected to cause greater senescence of the leaves of pepper plants used in this study.

Carotenoids are an important class of antioxidants; their destruction through oxidation decreases the power of this defence molecular system (Chedea and Jisaka, 2013). The increasing bioregulator concentrations (except the 120mg/L concentration) increased the carotenoid content of the '*grossum*' and '*accuminatum*' pepper species (Table 2), but there was a significant decrease in the carotenoid content of the '*chinense*' species, especially at higher bioregulator concentrations. Similar observations of high concentrations of bioregulators impacting negatively on carotenoid content have been reported in wheat plants (Sahu *et al.* 2011). The 80mg/L IAA treatment seems to give the highest increase in carotenoid content relative to the control in the '*grossum*' and '*accuminatum*' pepper species while varying results were obtained in the '*chinense*' species. This suggests that the response to treatments with the bioregulators is

species dependent. The increase of chlorophyll and carotenoid contents observed in this work may enhance the photosynthetic efficiency and consequently increase plant growth (Wanas, 2006) since these bioregulators have been reported to affect the balance between photosynthesis and photorespiration in plants (Olaiya, 2010).

#### Antioxidant enzyme activity

Antioxidant enzymes have the ability to scavenge AOS. The activity of the antioxidant enzymes investigated in this study though generally low, seems to be species dependent as varying patterns of expression were observed (Table 3). The peroxidase activity for all treatments of IAA and NAA was not significantly different ( $p > 0.05$ ) from the reference control in the pepper plants, except for the 80mg/L NAA – treated '*grossum*' species which showed significant difference ( $p < 0.05$ ) (Table 3). Catalase, the major  $H_2O_2$  scavenging enzyme suffered a decline in activity in all IAA treatments in the '*grossum*' species while an increase was noticed in the '*accuminatum*' species, relative to control. The NAA treatments also gave a decline in catalase activity in the '*grossum*' species but caused elevation of activity in the '*accuminatum*' and '*chinense*' species relative to reference controls, especially at the 40mg/L concentration.

**Table 1:** Effect of bioregulator treatment on chlorophyll a and chlorophyll b content of the leaves of the three cultivars of *Capsicum* spp.

Bioregulator (mg/L)	Chlorophyll a (mg/g F.W)*			Chlorophyll b (mg/g F.W)*		
	<i>Capsicum annuum</i> var. <i>grossum</i>	<i>Capsicum annuum</i> var. <i>accuminatum</i>	<i>Capsicum chinense</i> (big sun)	<i>Capsicum annuum</i> var. <i>grossum</i>	<i>Capsicum annuum</i> var. <i>accuminatum</i>	<i>Capsicum chinense</i> (big sun)
Control	4.482f	3.431e	3.356f	2.509cd	1.790bcd	1.819cd
IAA 40	6.757c	4.107e	5.068c	3.106a	2.089bcd	2.708b
60	5.782d	5.667b	5.349b	2.491cd	2.777bc	2.736b
80	4.511f	8.255a	3.734e	1.514g	3.963a	2.116c
100	5.419e	5.339bc	4.549d	2.366de	2.438bcd	1.701d
120	4.022g	4.329cde	3.466f	2.036f	2.225bcd	1.714d
NAA 40	4.422f	3.416e	7.440a	2.253e	1.620cd	3.723a
60	7.286b	5.464b	5.298b	2.834b	3.057ab	2.492b
80	7.682a	4.592bcd	0.025g	2.606c	2.502bc	0.140e
100	6.632c	2.170f	0.017g	2.359de	1.217d	0.110e
120	4.843c	0.014g	0.010g	2.658c	0.012e	0.023e

Means with the same letters are not significantly different at  $p = 5\%$  according to the DMRT

\*Fresh weight

The observed existence of species differences in the level of enzyme activity is of concern in comparative studies and quantitative or qualitative alterations in antioxidant enzyme system are often related to level of resistance to stress (Nagesh and Devaraj, 2008). The generally low antioxidant enzyme activity in the pepper plants studied may partly be due to their growth under prevailing screenhouse conditions devoid of biotic or

abiotic stress. This is because the activities of leaf mitochondrial and chloroplastic antioxidant enzymes (primary scavenger in the detoxification of active oxygen species in plants) often become elevated in response to stress or stress - related conditions (Barakat, 2011). These enzymes convert superoxide to  $H_2O_2$  and  $O_2$ , and protect cells against superoxide induced oxidative stress (Rajaeian *et al.*, 2011).

**Table 2:** Effect of bioregulator treatment on carotenoid content of the leaves of the three cultivars of *Capsicum* spp.

Bioregulator (mg/L)	Carotenoid (mg/g F.W)*		
	<i>Capsicum annuum</i> var. <i>grossum</i>	<i>Capsicum annuum</i> var. <i>accuminatum</i>	<i>Capsicum chinense</i> (big sun)
Control	2.917c	2.128d	1.709d
IAA 40	3.325b	2.563c	2.670b
	60 2.868cd	2.772c	2.903a
	80 2.663e	4.154a	1.605d
	100 3.408b	2.690c	1.648d
120	1.862f	2.124c	1.329e
	NAA 40 2.736de	1.786e	2.399c
	60 3.348b	3.437b	1.590d
	80 3.418b	2.517c	0.210f
100 4.457a	1.514e	0.154f	
120 3.254b	0.100f	0.027f	

Means with the same letters are not significantly different at  $p = 5\%$  according to the DMRT

\*Fresh weight

**Table 3:** Effect of bioregulator treatment on antioxidant enzyme activity of the leaves of the three cultivars of *Capsicum* spp.

Bioregulator (mg/L)	Peroxidase (unit mg <sup>-1</sup> protein/min)			Catalase (unit mg <sup>-1</sup> protein/min)		
	<i>Capsicum annuum</i> var. <i>grossum</i>	<i>Capsicum annuum</i> var. <i>accuminatum</i>	<i>Capsicum chinense</i> (big sun)	<i>Capsicum annuum</i> var. <i>grossum</i>	<i>Capsicum annuum</i> var. <i>accuminatum</i>	<i>Capsicum chinense</i> (big sun)
Control	0.198ab	0.723cd	0.071d	1.584a	0.120gh	0.510c
IAA 40	0.081c	0.027g	0.138c	0.073h	1.012b	0.255d
	60 0.104c	0.105b	0.152b	0.575e	0.832c	0.106e
	80 0.053e	0.103b	0.040e	0.076h	0.319f	0.460c
	100 0.053e	0.162a	0.070d	1.226b	0.656d	0.954b
120 0.025f	0.056ef	0.038e	1.107c	0.166g	0.899b	
NAA 40	0.106c	0.023g	0.194a	0.204g	0.417e	1.397a
	60 0.189b	0.063de	0.038e	0.790d	0.268f	0.176e
	80 0.215a	0.0813c	0.032f	0.313f	2.410a	0.130e
	100 0.122c	0.051f	0.030f	0.024h	0.070h	0.077e
120 0.125c	0.031h	0.022f	0.029h	0.030i	0.032e	

Means with the same letters are not significantly different at  $p = 5\%$  according to the DMRT

#### 4. Conclusion

This study shows that exogenous application of IAA and NAA affects enzymatic activities within the pepper plant. They enhanced carotenoid and chlorophylls 'a' and 'b' contents, which suggests a protective role of the bioregulators on photosynthetic apparatus against oxidative damage, thereby ensuring

photosynthetic efficiency of the chloroplasts. The study therefore led credence to the fact that these bioregulators could serve as tools for improving the pepper plant to the benefit of agricultural producers. However, further studies into the best method of application of the bioregulators to achieve optimum effects should be encouraged and possible combined

treatments of bioregulators to improve plant productivity should also be considered.

#### Corresponding Author:

Dr. Charles O. Olaiya  
Department of Biochemistry,  
University of Ibadan,  
Ibadan 200005, Nigeria  
E-mail: [cooolaiya@yahoo.com](mailto:cooolaiya@yahoo.com)

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