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Determination of Endogenous Hormones Content in Cotton Varieties (*Gossypiumhirsutum*) as Influenced by Phosphorus and Potassium Nutrition

Adebusoye O. Onanuga[1], Ping'an Jiang[2] & Sina Adl[1]

[1] Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax, Nova Scotia, B3H 4J2, Canada

[2] College of Natural Resources and Environmental Sciences, Xinjiang Agricultural University, No 42 Nanchang Road, Urumqi 830052, China

Correspondence: Adebusoye O. Onanuga, Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax, Nova Scotia, B3H 4J2, Canada. Tel: 1-902-494-2753, 902-404-9452. E-mail: Adebusoye.Onanuga@Dal.Ca

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Abstract

Two experiments were conducted to evaluate the endogenous hormones content of cotton plants grown hydroponically, and supplied with varying levels of phosphorus (P) and potassium (K) mineral nutrients in the first cropping. In the second cropping, varying levels of exogenous indole-3-acetic acid (IAA), gibberellic acid (GA_3) and zeatin (Z) and their combinations were applied to high level of PK in the hydroponic. In the first experiment, low K and low P treated plants had higher content of gibberellic acid (GA_3) in the leaves than high PK treated plants, and low K treated plants also produced more Zeatin (Z) in the leaves than either low P or high PK treated plants. Furthermore, low K treated plants had higher Zeatin (Z) content in the root than either low P or high PK treated plants. However, the two cotton varieties expressed different hormones content in the leaves, stem and root. In the second experiment, GA_3 concentration was highest in the cotton stem treated to IAA x GA_3 x Z. Moreover, Zeatin content in the leaves, stem and root was highest in the cotton treated to IAA x GA_3 x Z and 2IAA x $2GA_3$ x 2Z for leaves, IAA x GA_3 x Z for stem and Z, IAA x $2GA_3$ x Z for root. Nevertheless, two cotton varieties possessed different hormones content in the leaves, stem and root. In general, there was no relationship between the first cropping and second cropping. The results demonstrated influence of exogenous hormones application in altering the endogenous hormones content of cotton plants.

Keywords: gibberellic acid, indoacetic acid, zeatin, Zhong cotton Xin cotton, phosphorus, potassium

1. Introduction

Plant hormones are used in crop production to promote growth, development and yield (Nickell, 1982). In this study, indole-3-acetic acid (an auxin), gibberellic acid (gibberllin) and zeatin, (cytokinin) endogenous content were measured affected by phosphorus, potassium nutrients and exogenous hormones application. Auxin forms in the meristemic tissues of stem, root apices and in young developing leaves (Wareing & Phillips, 1970), root growth and development (Torrey, 1950; Blakely et al., 1982; Muday & Haworth, 1994; Casimiro et al., 2001; Jose et al., 2002). Gibberellic acid is an important hormone that regulates physiological activities in plant such as nutrients transport, inducing stem elongation, increase in dry matter production and weight, leaf area expansion and flowering (Wareing & Phillips, 1981; Brock, 1993; Azuma et al., 1997; Bhaskar et al., 1997; Gupta & Dutta, 2001; Takei et al., 2002; Khan & Samiullah, 2003; Shah et al., 2006). Cytokinins promote cell division and differentiation, stimulate photosynthesis (chlorophyll formation), flower production and senescence process (Hartman et al., 1981; Frankenberger & Arshad, 1995; Arshad & Frankenberger, 1998; Zahir et al., 2001). Findings of Siobhanand Peter (2003) revealed that GA_3 and auxin interacted together to stimulate cell elongation. Previous studies have shown that exogenous application of synthetic cytokinins significantly affected nutrient uptake by plants both in soil and nutrient culture by direct action on the roots (Stoyanov & Drev, 1978; Maksimov et al., 1979). Increased in NPK concentration of straw and grain by application of cytokinin caused a tremendous increase in yield of rice (Zahir et al., 2001).

Phosphorus plays an important role in plant growth, development and reproduction (Jose et al., 2002). Phosphorus can be immobilized in soil and unavailable to roots through physical and chemical interaction (Holford, 1997), and root size (Dinkelaker et al., 1995, Bates & Lynch, 1996; Borch et al., 1999). It has been

reported by Schachtman et al. (1997); Lopez-Bucio et al. (2000) that organic acid excretion, alteration of pH and great potential of plant roots to explore different soil layers are the important factors that can alleviate P-deficiency in the soil or rooting medium.

Potassiumcan also be immobilized in soil and an important nutrient for cotton cultivation (Zhiyong et al., 2009). The investigation by Brouder & Cassman (1990, 1994) revealed that K-deficiency in cotton during late season growth was significantly related to poor shallow root system and that K efficient cultivar could increase the rate of root extension by acquiring more total root surface area. Nevertheless, a large root system could facilitate better K availability to plants.

The main objective of this research effort was to study comparative endogenous hormones content of cotton plants without exogenous hormones application and endogenous content of hormones with exogenous application of hormones in two cotton plant varieties.

2. Materials and Methods

2.1 First Experiment

The two Chinese cotton varieties used in this experiment were early maturing and highly resistant to disease and pest. Single plant of Zhongmian 36 cotton has 9-10 matured medium size boll with average weight around 5.0 g. Cotton lint is 36.6% and seed weight is 11g. This variety possessed the ability to open broadly, which makes it easy to pick. The Zhongmian 36 cotton variety is known for high yielding with estimated yield to be 802kg/ha and 846kg/ha for gin cotton (seed cotton) and ginned (no seed cotton), respectively prior to frost. Intercropping wheat with cotton, wheat production was found to be 4500-5250kg/ha and ginned cotton to be 1230-1350kg/ha. In the Northern part of Xinjiang, the ginned cotton was estimated to be 2310kg/ha, increased for 15.0% compared with locally disseminated variety. The fiber tested by Cotton Quality Testing and Supervision Center, Ministry of Agriculture, showed that 2.5% fiber length is 29.3 mm, relative strength is 23.2 gf/tex (gram-force per texture) with thickness value of 4.4 micron.

Single plant of XinLuoZao 13 maintains 8-10 fruiting branches, the ball is ovoid shape, the single ball weights around 5.5g and the cottons pure white. It has a lint weight of 7.04 g, lint of 40-41% and seed weight of 9.96g. Lint cotton yield is 1641.15 kg/ha, lint cotton yield before frost is 1459.95 kg/ha. The quality test result showed that fiber length was 30.6mm, fiber evenness was 48.2%, stretching rate was 7.1%, fiber strength was 21.2gf/tex with thickness value of 4.3 micron.

The two Chinese cotton varieties (ZhongMian 36 and XinLuoZao 13) were cultivated in quartz sand. The roots of cotton seedlings were allowed to grow up to 8 – 10cm, thereafter, the seedlings were transplanted into 6L pots of nutrients solution. Temperature was kept between 20 – 35^{o}C by spreading black blanket over the roof of the greenhouse, the surrounding sunlight was maintained above 12 hours by using electrical lighting and a constant supply of oxygen to the roots was supplied using electrical air pump. Nutrient solution should be changed and replaced at every nutrients change because nutrient deep technique (NDT) hydroponic system was used in this experiment. The separation and determination of endogenous plant hormones were carried out with high performance liquid chromatography (HPLC) at the end of the first experiment, when the plants were fully matured. The separation and determination of hormones were estimated at 148 DAT. The nutrient solutions were Hoagland and micro anion nutrients (Hoagland & Aron, 1950). The low P level was 5 .0 x 10^{-5}M P, low K level was 1 x 10^{-3}M K and high PK level was 1 x 10^{-3}M P and 6 x 10^{-3} M K (Table 1) at pH 6.5 with two varieties of cotton. The experiment was replicated three times with total number of 18 experimental pots (3x3x2).

Table 1. Hydroponics nutrients composition

Nutrients	**P**	**P**	**K**	**K**
		MolL^{-1}		
	1 x 10^{-3}	**0.05 x 10^{-3}**	**6 x 10^{-3}**	**1 x 10^{-3}**
1 molL^{-1} KNO_3	5 (30 ml)	5 (30 ml)	5 (30ml)	1 (6ml)
1 molL^{-1} Ca $(NO_3)_2$	5 (30 ml)	5 (30ml)	5 (30ml)	7 (42ml)
1molL^{-1} $MgSO_4$	2 (12 ml)	2 (12ml)	2 (12ml)	2 (12ml)
1 molL^{-1} KH_2PO_4	1 (6ml)	Nil	1 (6ml)	Nil
0.1molL^{-1} KH_2PO_4	Nil	0.5 (3ml)	Nil	Nil
1 molL^{-1}KCl	Nil	0.95 (5.7ml)	Nil	Nil
1molL^{-1} $NH_4H_2PO_4$	Nil	Nil	Nil	1 (6ml)
1 molL^{-1} $FeCl_3.6H_2O$	1 (2ml)	1 (2ml)	1 (2ml)	1 (2ml)
Trace element	1 (6ml)	1 (6ml)	1 (6ml)	1 (6ml)

2.2 Second Experiment

In the second phase of the experiment, the cultivation and other necessary measures were followed as in the first experiment. The exogenous hormones concentration in micro gram per liter were applied twice at 36 and 67 days after transplanting to Hoagland complete nutrient solution, the two applications were carried out so as to know the best time hormones can be applied. The hormones were applied by spraying on the cotton leaves at single rate of 0, 50, 40 and 50 micro gram per liter for control, indole-3-acetic acid, giberellic acid and zeatin, respectively and combined rate of 50IAA x $40GA_3$ x 50Z, 100IAA x $40GA_3$ x 50Z, 50IAA x $80GA_3$ x 50Z, 50IAA x $40GA_3$ x 100Z and 100IAA x $80GA_3$ x100Z with three replications using two cotton varieties supplied with the highest P and K nutrient levels of 1 x 10^{-3}M/ P and 6 x 10^{-3}M/K (Table 1) at pH 6.5 in the hydroponics solution, resulting to total number of 54 experimental pots (9 x 3 x 2). The extraction, purification, determination and separation of of hormones were estimated at 90 DAT

2.3 The Plant Hormones Extraction and Measurement

Purification and extraction of endogenous plant hormones were carried out according to Chen et al. (1996) with some modifications. About 0.5 – 1.0g of fresh cotton plant samples were weighted and quickly refrigerated. After refrigeration, cotton plant samples were grinded to powder and 5 ml of 80% methyl alcohol solution was added to a ratio of W: V (1:10-20). The extract was completely sealed and refrigerated at 4^oC for 12 hours, then centrifuged for 30 minutes at 2000rpm. The leached solution was removed, and 3ml (80%) cold methyl alcohol solution was added and shaken for several hours, then centrifuged for 20 minutes. The supernatant solution was dried with Nitrogen in a water bath until half solution evaporated. Petroleum ether and distil liquid (supernatant solution) at ratio of 1:1 were shaken until the distinct differences were observed. The solution was left to settle and the petrol ether was removed and the methyl alcohol solution was kept. The methyl alcohol extract was dried with nitrogen on the water bath at pH 2 and extracted three times with equal volume of glacial acetic acid and shaken on a mechanical shaker. All the methanol organic phase was combined and adjusted the water phase to pH 2.8.Two ml glacial acetic acid and ethyl acetate were added and shaken. Extraction was carried out three times with 2ml of ethyl acetate. The entire ethyl acetate phase combined and dried with nitrogen on water bath at 40^oC. Extracted three times with 2 ml buthanol, and dried with nitrogen on water bath until it reduced to 1ml. The filtrate passed through 0.45μm membrane and 0.1 μ L samples were analyzed by HPLC to separate and determine the concentration of indole-3-acetic acid, gibberellic acid and zeatin endogenous hormones concentration in cotton varieties with mobile phase mixture of acetonitrile and water (volume ratio 4:6) at flow rate of 1 $mlmin^{-1}$ with an injection volume of 0.1μL detector wavelength set at 254 nm.

2.4 Data Analysis

The data were subjected to analysis of variance (ANOVA) using general linear model (GLM) with SPSS software. Least significant difference was calculated using Duncan's multiple range test (DMRT) for separation of treatment and variety means. Pearson's correlation statistical analysis was used to compare first and second phase of the experiment.

3. Result

3.1 Hormones Content in Cotton Varieties Plant Parts Grown Hydroponically Treated to Phosphorus (P), Potassium (K) and PK Harvested at 148 Days after Transplanting (DAT)

3.1.1 Treatments Effect

Figure 1 summarizes the effects of low P, low K and high PK treatments on cotton plant partitioning hormones content. The leaves of cotton plants treated to low K had the greatest endogenous gibberellic acid content of 2.63μgg^{-1} than other treatments while high PK treated pot had the least endogenous gibberellic acid. Furthermore, low P, low K and high PK treated pots showed no significant difference in endogenous indole-3-acetic acid content of the leaves. However, low K treated pot produced more zeatin (1.01μgg^{-1}) in the leaves than either low P or high PK (Figure 1).

Low P, low K and high PK treatments had no effect on gibberellic acid, indoacetic acid and zeatin contents in the stem (Figure 1).

The application of low P, low K and high PK significantly had no effect in gibberellic acid and indole-3-acetic acid contents in the roots. However, low K treatment gave higher zeatin content (0.222 μ g g^{-1}) in the root than either gibberellic acid or indole-3-acetic acid (Figure 1).

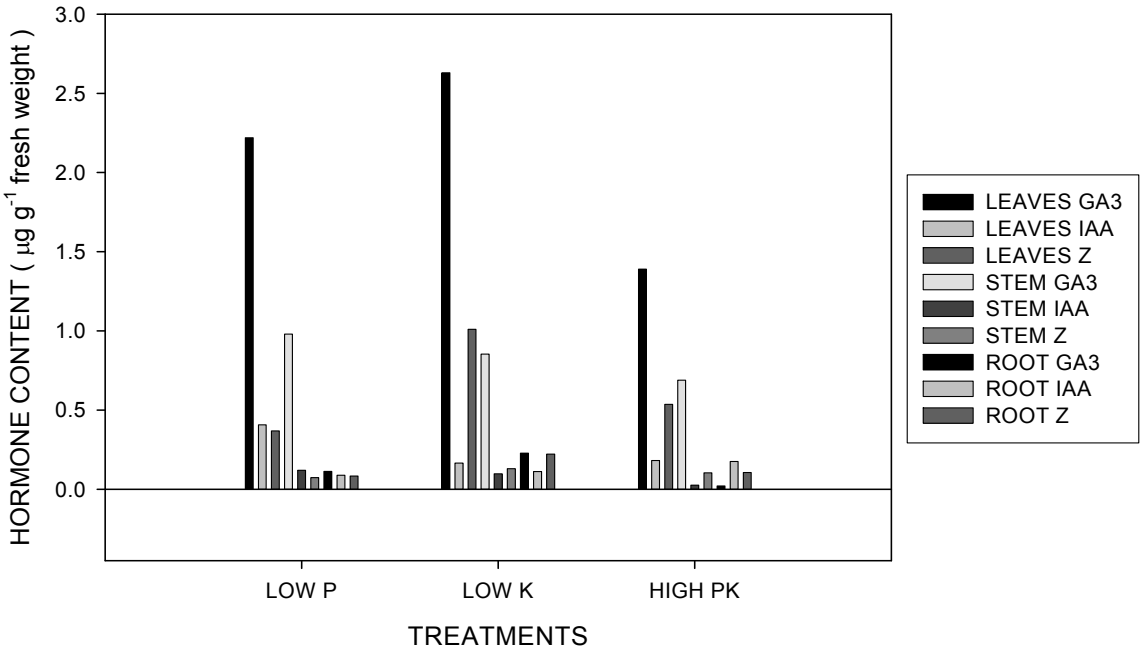

Figure 1. Hormones content of cotton plants partitioning grown hydroponically treated to phosphorus, potassium and their combinations harvested at 148 Days after transplanting

3.1.2 Variety Effect

Figure 2 shows that gibberellic acid and Indole-3-acetic acid contents in the leaves were not significant, but Xin cotton variety significantly produced more zeatin than Zhong cotton variety.

Xin Cotton variety contained higher Gibberellic acid content in the stem than Zhong cotton variety. There was no significant difference between the two varieties as regards indole-3-acetic acid content. However, Zhong cotton variety produced more zeatin in the stem than Xin cotton variety (Figure 2).

Figure 2 reveals that Xin Cotton variety produced more gibberellic acid and zeatin contents in the root than Zhong cotton variety. Nevertheless, no significant differences were found between the two varieties in relation to indole-3-acetic acid content in the root.

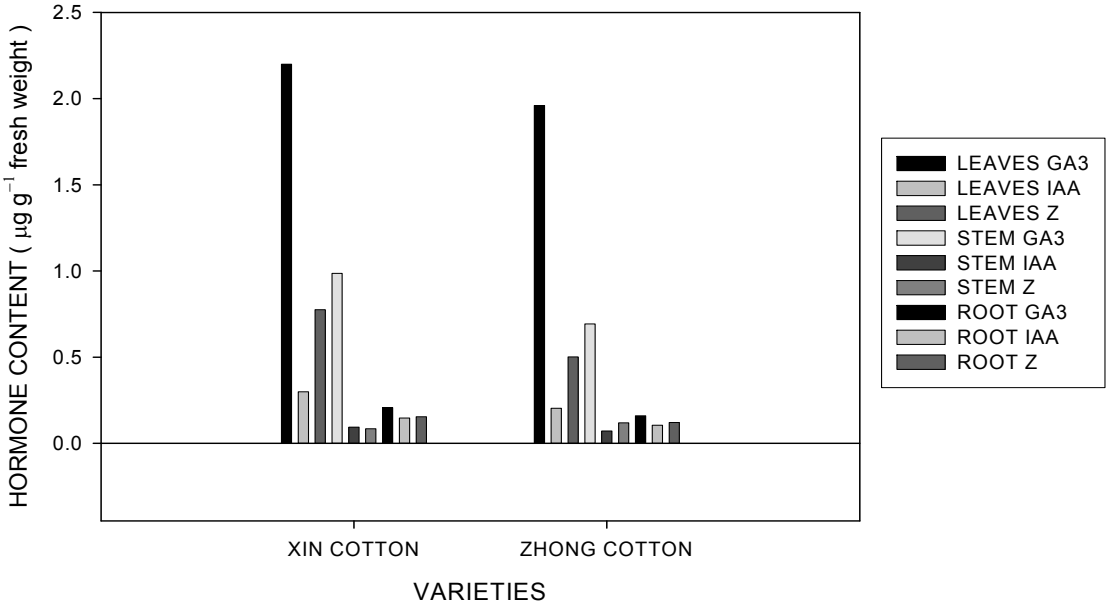

Figure 2. Hormones content of cotton plant varieties partitioning grown hydroponically treated to phosphorus, potassium and their combinations harvested at 148 Days after transplanting

3.2 Effect of Gibberellic Acid, Indole-3-acetic Acid, Zeatin and Their Combinations on Cotton Variety Plant Parts Grown Hydroponically Treated to High Level of Phosphorus and Potassium Harvested at 90 Days after Transplanting

3.2.1 Treatments Effect

Figure 3 shows the effect of Gibberellic acid (GA_3), Indole-3-acetic acid (IAA), Zeatin (Z) and their combinations on each plant partitioning grown hydroponically treated to high level of phosphorus and potassium. There was no significant difference between the plants treated to hormones and control in relation to GA_3 and IAA in leaves content (Figure 3). It is quite obvious from the figure that combined use of IAA x GA_3 x Z treated pot with the content value of 0.717 $\mu g g^{-1}$ had the highest Z content in the leaves than other treatments and control.

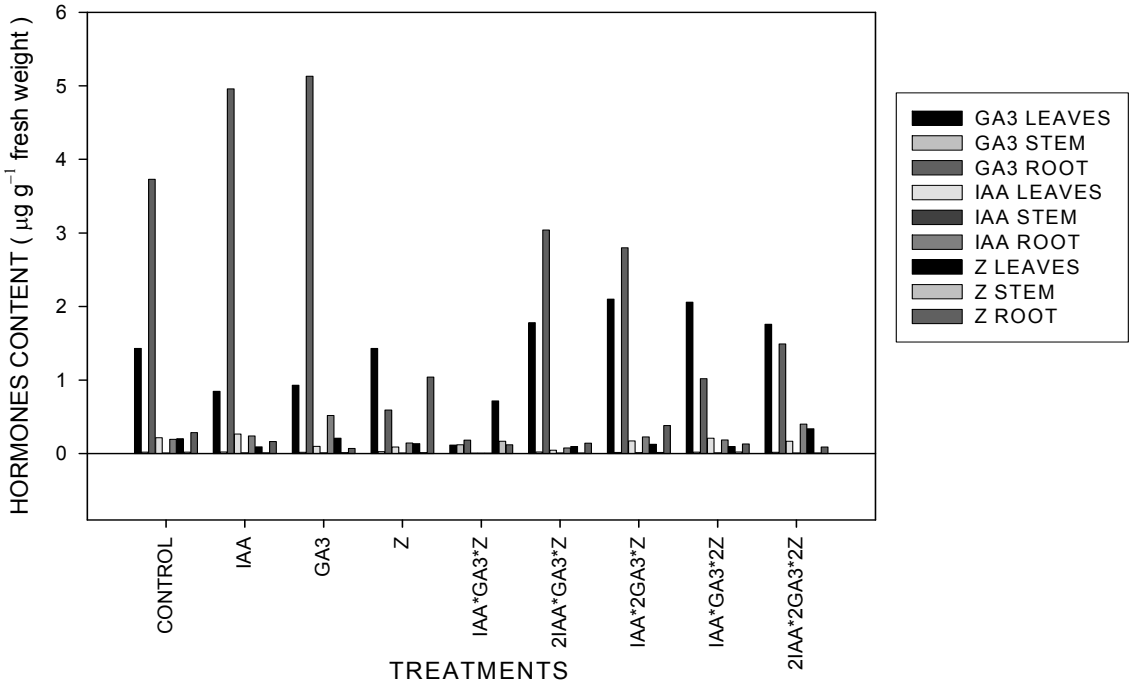

Figure 3 Effect of Gibberellic acid (GA_3), Indoacetic acid (IAA) and Zeatin (Z) and their combinations on cotton plants partitioning grown hydroponically treated to high level of PK harvested at ninety days after transplanting

Combined use of IAA with GA_3 and Z enhanced more gibberellic acid content in the stem than other treatments.There was no significant difference between hormones treated plants and controls in relation to endogenous IAA content in the stem. Furthermore, the treatments applied significantly increased Z content in the stem, the plants treated with IAA x GA_3 x Z hormone significantly produced greater endogenous Z (0.168 $\mu g g^{-1}$) in the stem than the other treatments including control (Figure 3).

It is clearly shown in the Figure 3 that there was no significant difference between plants treated to different hormones and untreated plants in relation to gibberellic acid (GA_3) and Indole-3-acetic acid (IAA) content in the roots. However, plants treated with Zeatin increased Z level in the root (1.044 μ $g g^{-1}$) compared to those plants treated with IAA, GA_3, IAA x GA_3 x Z, 2IAA x GA_3 x Z, IAA x GA_3 x 2Z, 2IAA x $2GA_3$ x 2Z and controls with values of 0.164μ g g^{-1}, 0.071μ g g^{-1}, 0.122μ g g^{-1}, 0.143μ g g^{-1}, 0.134μ g g^{-1}, 0.091 $\mu g g^{-1}$, respectively.

3.2.2 Variety Effect

Xin and Zhong cotton varieties were significantly affected by the hormones content in the leaves, stem and root. Zhong cotton variety had higher Gibberellic acid, Indole-3-acetic acid and Zeatin content in the leaves than the Xin variety (Figure 4).

However, there was no significant difference between Xin and Zhong cotton varieties in GA_3 content in the stem. The Zhong cotton variety had higher IAA and Z hormones contents in the stem than the Xin cotton variety (Figure 4).

The Xin cotton variety had higher GA_3 and IAA content in the root than the Zhong cotton variety. There was no significant difference between Xin and Zhong cotton varieties in Z root content (Figure 4).

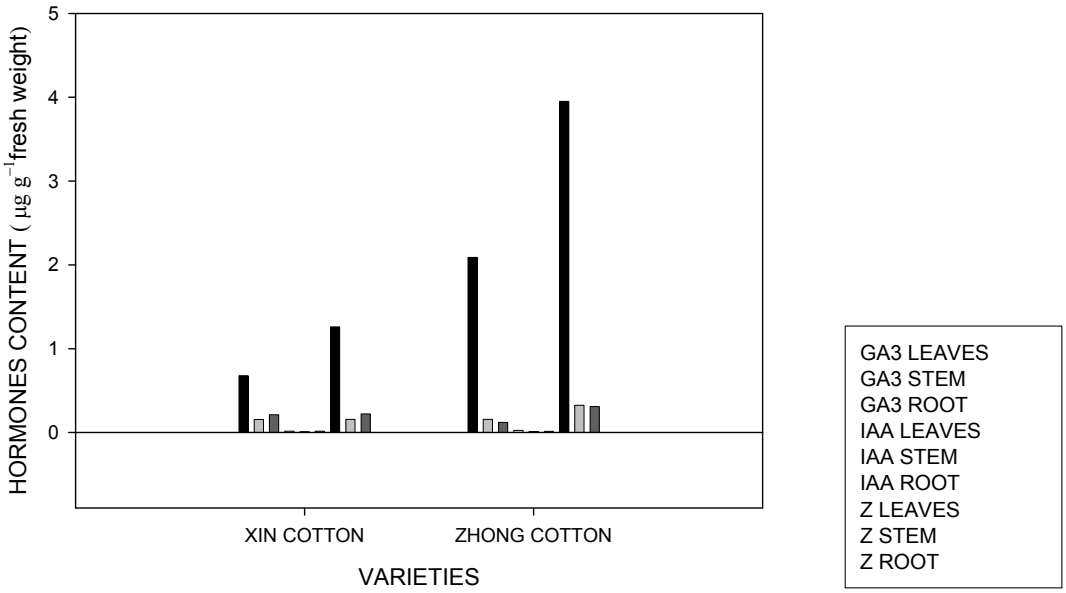

Figure 4. Effect of Gibberellic acid (GA_3), Indoacetic acid (IAA) and Zeatin (Z) and their combinations on cotton plant varieties partitioning grown hydroponically treated to high level of PK harvested at ninety days after transplanting

3.3 Correlation Co-efficient Relating Low P, Low K and High PK Treated Plants at 148 DAT to Hormones Treated Plants at High Level of PK at 90 DAT.

The correlation co-efficient relating treatments applied to cotton plants are given in Table 2.There was no relationship between treatments applied in the first cropping and treatments applied in the second cropping in relation to endogenous hormones contents in the leaves, stem and root.

Table 2. Correlation co- efficient relating low P, low K and high PK plant treatments at 148 days after transplanting to hormones plant treatments at high level of PK at 90 days after transplanting

Growth and yield parameters	Correlation Co-efficient (r) relating low P, low K and high PK to hormones applied		
CONTENTS	Leaves	Stem	Root
GA	- 0.887ns	- 0.438ns	- 0.330ns
IAA	0.994ns	0.967ns	- 0.493ns
Z	0.993ns	0.543ns	- 0.446ns

ns:not significant at $p<0.005$.

4. Discussion

4.1 Endogenous Hormones Content of Cotton Plants as Influenced by Phosphorus and Potassium Mineral Nutrients

Endogenous plant hormones tremendously promote growth and development of plant (Marscher, 1995, Palmer et al., 1996, Martin et al., 2000; Yan et al., 2004). Low K and low P treatments significantly influenced endogenous gibberellic acid content in the leaves and Zeatin content in the root while the treatments produced no effect in the stem at 148 days after transplanting. This suggests that plants with adequate endogenous phytohormones content could be used to offset the cost incurred on mineral fertilizers. Moreover, plants with right amount of endogenous phytohormones obtained could be used as substitute for high dosage of mineral fertilizers that have negative impact on environment. Furthermore, high level of GA_3 in the leaves in this study agrees with the report of Dong and Artceca, (1981) that GA_3 concentration in the leaves influenced photosynthesis in tomatoes and endogenous zeatin content in the rice plants increased the amount of zeatin in the root (Zahir et al., 2001).

Xin and Zhong cotton varieties contained different endogenous plant hormones content. The gibberellic acid and indole-3-acetic acid contents in the leaves of both varieties were the same while Xin variety gave high content of Zeatin in the leaves. Moreover, high level of gibberellic acid was observed in Xin cotton stem whereas high Zeatin level was observed in Zhong cotton stem. However, in the root, the Xin cotton variety gave high levels of both gibberellic acid and Zeatin. These results revealed the genetic variations in these two cotton varieties

(Gialvalis & Seagull, 2001).

4.2 Cotton Hormones Content as Influenced by Exogenous Hormones Application Grown Hydroponically in High Level of Phosphorus and Potassium

Plant hormones, gibberellic acid, indole-3-acetic acid, Zeatin and their combinations supported plant growth, development and yield at low concentration (Birgit et al., 2005). The Zeatin content in the leaves was high in plants treated with IAA x GA_3 x Z. Gibberellic acid and zeatin contents in the stem were high in the plants treated with IAA x GA_3 x Z while zeatin content in the root was high in the plants treated withZ only. Based on these results, Van Standen (1976c); Van Standen (1977); Van Standen and Brown, (1977) and Vysot-Skaya et al. (2007) reported that leaves are the site of cytokinins (Zeatin) synthesis. Conversely,cytokinin (Zeatin) produced in the root tip promote lateral stem growth (Godwin & Morris, 1979; Zahir et al., 2001). Furthermore, Gibberellic acid induced stem elongation in hydroponically grown little Marvel pea (Anderson et al., 1988). Early workers showed that root tip is a major site for cytokinins (Zeatin) synthesis. (Short & Torey, 1972; Beever & Woolhouse, 1974; Takei et al., 2002).

The differences were quite obvious in the two cotton varieties. Zhong cotton leaves had more hormones content (IAA, GA_3 and Z) than Xin cotton leaves variety. Both varieties had the same GA_3 content in the stem but Zhong variety had more content of IAA and Z, in the stem than Xin. The Zhong cotton varieties possessed the same Zeatin content in the root while GA_3 and IAA in the cotton root were more in the Xin variety than that of Zhong variety. All these variety differences can be attributed to genetic variation between the two varieties (Gialvalis, and Seagull, 2001).

4.3 Relationship between Treatments Applied during the First and Second Cropping

Comparing the two experiments (Table 2), the first without exogenous hormones application and the second with hormones application showed that the results were not statistically significant. The exogenous hormones applied during the second experiment altered growth and development of cotton plants. This result was similar toTao et al. (1993) on pea seedling growth, Zahir et al. (2001) on growth and yield of rice,Gialvalis, and Seagull, (2001) on plant hormones altered fiber initiation in unfertilized cultured ovules of *Gossypiumhirsutum*,Chunrong et al. (2008) on endogenous and exogenous hormones of water stress in maize seedling.

5. Conclussion

The results of this study reveal the great potential of endogenous plant hormones in reducing the amount of mineral nutrients needed for plant growth and development. Low P and low K nutrients applied to hydroponically grown cotton were supplemented for by endogenous hormones content of GA_3, Z in the leaves and Z in the root which enhanced cotton plants growth and development. However, exogenous hormone application of IAA x GA_3 x Z stimulated endogenous GA_3 content in the stem, also stimulated endogenous Z content in the leaves and stem. Nevertheless, Z exogenous application stimulated Z content in the root. This study was conducted with the ultimate goal to compare endogenous hormones content in the cotton plants with no exogenous application of hormones and with application of hormones for the benefit of hydroponics cotton production.

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