A HYDROPONIC APPROACH TO EVALUATE RESPONSES TO NUTRIENTS AND PHYTOHORMONES IN COTTON PLANTS (*Gossypium hirsutum L.*) GROWTH AND DEVELOPMENT

by

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DEDICATION

For
my dad, mom Onanuga for their words of encouragement, my lovely wife, (Kenny) and my children, Faith, Favour and Flourish,

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ABSTRACT

Cotton plant growth and development, as well as monitoring nutrient use efficiency were evaluated using hydroponic approach. Two set of experiments were conducted to determine the influence of phosphorus (P), potassium (K) and PK and exogenous application of Indole-3-acetic acid (IAA), gibberellic acid (GA_3), zeatin (Z) and their combinations on growth and development of cotton plants (*Gossypium hirsutum*) grown hydroponically. In the nutrient solution experiment, cotton vegetative growth was positively influenced by low P (half strength Hoagland standard solution), low K (one-sixth strength Hoagland standard solution) and high PK treatments (Hoagland standard solution). Phytohormone experiment negatively supported vegetative growth except root length at 43 days after transplanting (DAT). The nutrients levels applied significantly favoured NPK uptake by cotton plants while exogenous phytohormones application did not affect NPK uptake by cotton plants, except N uptake by stem. Low P and low K treatments estimated to have high nutrient use efficiency (NUE). For chlorophyll formation, low K and high PK significantly increased formation of chlorophyll a, b and total ab while the application of GA_3, IAA, Z and IAA x GA_3 x 2Z treatments significantly increased chlorophyll a, b and total ab at 80 DAT only. Low K and low P treatments stimulated endogenous phytohormone contents in the cotton plants. In the phytohormone experiment, cotton plants treated to IAA x GA_3 x Z increased endogenous phytohormone contents in the cotton plants. Low P, low K, high PK treatments and phytohormones treatments significantly increased root area, root volume and root activity. Low P, low K and high PK treatments applied significantly influenced residual level of P and K in the hydroponics while phytohormone treatments did not affect residual level of P and K except at 43 DAT. Evapotranspiration rate was high at early and reproductive stages of plant growth. This report shows the response of mineral nutrients and phytohormones to support growth and development of cotton plants grown hydroponically.

LIST OF ABBREVIATIONS AND SYMBOLS USED

AAA	Active absorption area
AFB	Auxin F-box protein
AHA	Australian Hydroponics Association
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AUXI	Auxin influx carrier
AVRDC	Asian Vegetable Research and Development Centre
axr1	Auxin response 1
axr4	Auxin response 4
BA	Benzylaminopurine
$Ca(NO_3)_2$	Calcium nitrate
CPD	2-carboxyphenyl-3-phenylpropane-1, 3-dione
DMRT	Duncan's multiple range test
dil	Dilute
ET	Evapotranspiration
FAO	Food agriculture organization of United Nations
$FeCl_3.6H_2O$	Iron (III) chloride hexahydrate
GA_3	Gibberelic acid
g f/tex	gram-force per texture
HPLC	High performance liquid chromatography
$H_2\ PO4^{2-}$	dihydrogen phosphate ion
HPO_4^{2-}	monohydrogen phosphate ion

H^+/K^+	Hydrogen ion/ potassium ion
IAA	Indole-3-acetic acid
IBA	Indole butyric acid
Ipt	isopently transfarease
IAAH	Protonated Indole -3-acetic acid
K_2HPO_4	Potassium mono hydrogen phosphate
KH_2PO_4	Potassium di hydrogen phosphate
KNO_3	Potassium nitrate
$\mu E\ m^{-2}\ s^{-1}$	Micro Einstein meter per second
$mg\ pot^{-1}$	Milligram per pot
M	Molarity
N	Normal
Na^+/K^+	Sodium ion/ potassium ion
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NOA	1-napthoxyacetic acid
NPA	1-N-naphthylphthalamic acid (NPA
NUE	Nutrient use efficiency
$(NH_4)_2HPO_4$	Mono ammonium phosphate
$NH_4H_2PO_4$	Di ammonium phosphate
PAAAR	Percentage active absorption area ratio
PGR-IV	Plant growth regulator four
PGP	P-glycoprotein

RIRDC,	Rural Industries Research and Development Corporation
rpm	Revolution per minute
RA	Root activity
RV	Root volume
SNAP	Simple Nutrient Addition Program
SSA	Specific surface area
TAA	Total absorption area
TCA	Tri carboxylic acid
TIR1	Transport inhibitor response 1
TIBA	2, 3, 5- triiodobenzoic acid
TTC	Triphenyl tetrazolium chloride
Z	Zeatin

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CHAPTER 1

INTRODUCTION

Hydroponic systems have been in use to evaluate growth and development of vegetables, fruits and flowers for decades (RIRDC 2001). Hydroponic systems use nutrient solutions to grow crops under soilless condition. Recently, there has been an increased interest in hydroponics to evaluate growth and development of crops such as wheat and rice in Asia. Wheat crops absorbed more nutrients such as iron and zinc grown in hydroponic systems than plants grown in the field due to direct contact of root hairs with the nutrient solution (Brian et al. 2009). Hydroponic systems planted with rice genotype IR651 and cotton plants reduced osmotic and toxic effects of salinity (Nemati et al. 2011; Natalia Castillo 2011). The hydroponic medium also increased growth and yield of rice plants (Nemati et al. 2011). Hydroponic systems also make it easier to monitor nutrient uptake, root morphology, physiological development status and yield (Lynch 1995).

Other inputs such as synthetic plant hormones and mineral nutrients also boost crop yield when using hydroponics (Wareing and Phillips 1981). Auxin, gibberellin and cytokinin are three classes of plant hormones that influenced plant growth and development at low concentrations (Zahir et al. 2001). Gibberellic acid functions to improve physiological process in plant growth, flowering and nutrient uptake in hydroponically grown vegetable and fruit crops (Wareing and Phillips 1981). Gibberellic acid works by promoting stem elongation (Godwin and Morris 1979; Anderson et al. 1998; Shan et al. 2006). Indole-3-acetic acid (IAA) is a derivative of auxin and is believed to form in the apical meristem of shoot and root apices. IAA is best known for

promoting root development, especially lateral root formation (Casson and Lindsey 2003; De Smet et al. 2006; Zhiyong et al. 2009). It has been shown that exogenous application of IAA increased endogenous IAA for root formation (Sitbon et al. 1992; Evan et al. 1994; Himanen et al. 2002). Zeatin is a derivative of cytokinin and is involved in cell division, cell enlargement and tissue differentiation. Zeatin stimulates flower formation and senescence retardation (Hartman et al. 1981; West and Harada 1993; Peres and Kerbany 1999). Zahir et al. (2001) reported that exogenous application of zeatin at the root zone increased growth and yield of rice plants.

A standard solution used in most hydroponic cultivations is called the Hoagland nutrient solution and it contains all essential macronutrients and micronutrients required by plants for growth, development and yield (Hoagland and Arnon, 1950). Hoagland nutrient solutions are typically modified to fit specific crop need (Spomer et al. 1997). For instance phosphorus and potassium are two macronutrients required by plants for growth, development and yield. These two nutrients are taken into consideration in this thesis because they are needed in large quantities for cotton growth and development. Moreover, they can appear in non-available forms in soils. Phosphorus has a tremendous influence on the branching patterns of plants, total root length, root hair elongation and lateral root formation (Dinkelaker et al. 1995; Bates and Lynch 1996; Borch et al. 1999; Jose et al. 2002). Maizlisch et al. (1980) observed a strong relationship between root growth and P-uptake. Extensive root growth for nutrient acquisition, as well as bumper yield is achieved when the crop is treated with phosphorus (Linkohr et al. 2002; Yan et al. 2004). It has been reported that a high level of potassium nutrition also significantly increased formation of chlorophyll pigment in the plants (Lamrani et al. 1996).

Oosterhuis and Bednarz (1997) reported that chlorophyll a and total chlorophyll concentrations were reduced in cotton plants as a result of potassium deficiency. Adequate supply of potassium increases chlorophyll content of plants, improves the plant's green colour and makes the plants healthy. (Stromberg 1960; Fletcher et al. 1982; Maples et al. 1988; Oosterhuis and Bednarz 1997; Duli et al. 2001).

This study explored growing cotton hydroponically using standard Hoagland solution as reference nutrient fertilizer and modified standard Hoagland solution by reducing the concentration of macronutrients (phosphorus and potassium) in the standard Hoagland solution by half for phosphorus and one-sixth for potassium. Additionally, responses of cotton plants to applied phytohormones planted in standard Hoagland nutrient solution was also investigated. This is to ascertain the concentration of nutrients and phytohormones needed to grow cotton hydroponically, as well as determining the optimal timing of phytohormones application.

The objectives of this research were:

1. To evaluate the influence of P, K on growth, nutrient uptake, nutrient use efficiency, chlorophyll formation, endogenous hormones content, root area, root volume, residual and nutrient solution level of P and K nutrients, and evapotranspiration of standard and modified P and K Hoagland solutions in a hydroponically grown cotton species.

2. To determine exogenous application of indole-3-acetic acid, gibberellic acid, zeatin and their combinations applied at 36 and 67 days after transplanting on growth, nutrients uptake, chlorophyll formation, endogenous hormones content, root area, root volume,

residual and nutrient solution level of P and K nutrients, and evapotranspiration of standard Hoagland solution in a hydroponically grown cotton species.

Hypothesis: Plant nutrients and phytohormones enhance growth, nutrient uptake and physiological responses in cotton plants.

CHAPTER 2

LITERATURE REVIEW

2.1 BRIEF HISTORY AND CONVENTIONAL CULTIVATION OF COTTON PLANTS

The cotton plant (*Gossypium hirsutum L.*) is an indeterminate woody perennial fibre crop. It originated in Africa, Australia, Arabia and Mesoamerica (FAO 2001). Cotton is a dicotyledonous plant also known as dicot plants. It belongs to flowering plants whose seed typically has two embryonic leaves or cotyledons with long tap roots for effective nutrients uptake. Cotton is propagated conventionally for fibre and seed production. World production of cotton estimated to be about 21 million tonnes of lint and 59.7 million tonnes of seeds from about 33.98 million ha (FAO, 2001). In 2012, world production was estimated to be 26.6 million tonnes of lint and 65 million tonnes of seeds from about 34 million ha. (OECD and FAO, 2013)

Cotton plant growth is sensitive to temperature. Cool night and low daytime temperatures result in the production of few fruiting branches, but support vegetative growth. However, the effect of day length on germination, flowering and boll formation are influenced by temperature. The optimum temperature of 20^{o}C to 30^{o}C, 18^{o}C to 30^{o}C, 27^{o}C to 35^{o}C are required for vegetative growth, flowering, and boll development, respectively. Temperatures above 38^{o}C are detrimental to growth, development and yield of cotton (FAO, 2001). The cotton plant is also sensitive to frost and a minimum of 200 frost free days is required for growth and yield.

Conventionally, cotton can be cultivated in a wide range of soil types, from medium to heavy textured. Cotton performs best in deep soils with good water holding

capacity. Acidic or compact soils limit root penetration. The pH range of 5.5 to 6.5 is considered optimum for the cultivation of cotton in the field. The fertilizer requirement is based on the inherent soil fertility status of the soils. Soil physical and chemical analysis should be carried out so as to determine how much nutrient should be applied. In a continuous cropping system, a common general application level of NPK is based on 100 to 180 kg N/ha, 20 to 60 kg P/ha and 50 to 80 kg K/ha for N, P and K, respectively. Two-thirds of nutrient uptake occurs during the first 60 days after sowing. N and K should be applied in a split application, the first application after sowing and the second application before flowering; all P should be applied before sowing. Band application is preferred over broadcasting of fertilizer. The plant spacing on the field normally varies between 50 cm/100 cm x 30 cm/50cm.

Water requirements for growth and yield of cotton plants are between 700 to 1300 mm precipitations per year. The highest demand for water is during the reproductive stage. Moreover, evapotranspiration is high during the mid-season stage, i.e. 50 to 60 days after sowing (FAO, 2001).

There are scanty literatures on the use of phytohormones for cotton crop in the field. Khan et al. (2002a) reported that application of gibberellic acid at 10^{-5} M with nitrogen at 80 kg/ha could increase growth and development of mustard plants in the field. Phytohormones such as mepiquat chloride have commonly been used to control plant height in the field (FAO, 2001). The active ingredient in mepiquat chloride is N, N-dimethylpiperidinum chloride. Furthermore, pentia can also be used as a growth regulator. The active ingredient in pentia is mepiquat pentaborate. These growth regulators control plant height. Mepiquant functions to hasten maturity, reduce plant

height, decrease boll rot, reduce insect infestation and increase yield. Mepiquat can be also applied at early bloom with an application rate of 2, 023 to 4,047 m^2 at 1.5 g/l

Nevertheless, adhering to these conventional practices, it has been estimated that cotton grown under irrigation produce 4 to 5 tonnes/ha seed cotton in which 35% is lint (FAO, 2001)

2.2 HYDROPONICS

2.2.1 HYDROPONIC SYSTEM

The main component of a hydroponic system is the nutrient solution and media. Nutrient solutions are typically based on Hoagland nutrient solution and modified versions of Hoagland's (Table 1.1).

Table 2.1 Hydroponic nutrient solution composition

Compound	Molecular weight	Concentration of stock solution	Concentration of stock per litre of solution	Stock solution final solution
Modified Hoagland solution				
Macronutrients				
KNO_3	101.10	1,000	101.10	6.0
$Ca(NO_3)_2.4H_2O$	236.16	1,000	236.16	4.0
$NH_4H_2PO_4$	115.08	1,000	115.08	2.0
$MgSO_4.7H_2O$	246.48	1,000	246.49	1.0
Trace				
KCI	74.55	25	1.864	
H_3BO_3	61.83	12.5	0.773	
$MnSO_4.H_2O$	169.01	1.0	0.169	
$ZnSO_4.7H_2O$	287.54	1.0	0.288	2.0
$CuSO_4.5H_2O$	249.68	0.25	0.062	
H_2MoO_4 (85% MoO_3)	161.97	0.25	0.040	
NaFeDTPA (10% Fe)	468.20	64	30.0	0.3-1.0
Full strength Hoagland solution				
$Ca(NO_3)_2$ stock	236.16	1,000	As-above	7
KNO_3	101.10	1,000	As-above	5
KH_2PO_4	136	1,000	As-above	2
$MgSO_4$	246.48	1,000	As-above	2
Trace elements	As-above	1,000	As-above	1
FeEDTA	As-above	1,000	As-above	1

Source: Epstein (1972), Hoagland Arnon (1950).

The required nutrients and their concentrations in the hydroponics must be adequately supplied. Parameters such as water quality, salinity level, and sodium and chlorides levels should be constantly monitored in hydroponic systems. As well the nutrient balance must be monitored. Electrical conductivity can be used to monitor

nutrient balance (dissolved salts), but it cannot give an accurate measurement of individual nutrients in the hydroponic nutrient solution. The pH of the nutrient solution must be taken into consideration when preparing a nutrient solution and during the plant growth period. Islam et al. (1980) and Bugbee (2003) reported a pH range 5.5 – 6.5 respectively, as suitable for the availability of essential nutrients for the plant uptake. The availability of Mg, Ca, K and P are slightly decreased at higher pH while the availability of Mn, Cu, Zn and especially Fe are significantly reduced (Bugbee 2003).

Different media to act as anchors for the roots have been used in hydroponic systems including perlite, pumice, gravel, peat, sand, rock wool, clay pebbles and sawdust (RRDIC 2001). They have little to no effects on plant nutrition. The nutrient solution is the only source of nutrients in hydroponic systems.

Advantages of growing media are high crop yield in a limited area and control over fertilization and irrigation. It is quite simple and easy to recycle waste water. The disadvantage of growing media is low nutrient holding capacity resulting in a need to replace the solution due to low buffering capacity of the solution.

2.2.2 TYPES OF HYDROPONIC SYSTEMS

There are two types of hydroponic systems: a) open or outdoor and b) closed or greenhouse hydroponic systems. In the closed system, the nutrient solution can be recycled and the nutrient concentration can be recirculated and adjusted at least once a week. The closed system is quite challenging and laborious because the nutrient balance must be monitored carefully. The closed hydroponic systems include:

i) Deep Water culture hydroponic system which is a common practice whereby the plants are suspended in an oxygen-enriched nutrient solution;

ii) The Wick hydroponic system uses a wick which runs from the base of the plant container or pot down to a reservoir and draw nutrients upward;

iii) Nutrient film techniques: This system uses continuous flow of nutrient solution over the roots, resulting to thin film of nutrients over the root. This allows aeration and nutrients access; and

iv) Ebb and flow: In this system, growing medium is flooded with nutrient solution and then it is allowed to drain. The duration and frequency of the flood depends on type of growing medium, size of containers and water requirement of the plants. These hydroponic systems mentioned above use electricity for aeration. Asian Vegetable Research and Development Centre (AVRDC) has developed a low cost hydroponics system called the AVRDC hydroponics method (AVRDC, 1992). This hydroponics system was designed to use solar energy instead of electrical energy to aerate the root of the plants by exposing upper plant roots to take oxygen from the immediate environment. The Simple Nutrient Addition Program (SNAP) has also been developed (Santos and Ocampo 2005) for household growers and small scale vegetable producers in the Philippines.

The open system; introduces a new nutrient solution during each irrigation cycle. The nutrient solution is usually supplied to the plants using a drip irrigation system. The nutrient balance in the roots must be maintained by adequate run-off. There are differences between open (outdoor) and closed (greenhouse) hydroponic systems, closed system consume less water and fertilizers than the open system (Stanghellini 1993;

Fernandez et al. 2003). The differences can be explained by influence of evaporative factors which are greater in the open system than close system. The hydroponic system selected in this study was based on a closed system (greenhouse) that uses less fertilizers and water for hydroponic crop production (Stanghellini 1993; Fernandez et al. 2003).

2.2.3 CROP NUTRIENTS USE IN HYDROPONIC SYSTEMS

Most hydroponics solutions are based on the work of Hoagland and Arnon (1950) and have been adapted for numerous crops (Whipker and Hammer, 1998). Spomer et al. (1997) recommended a nutrient solution equal to about one-half strength of the original Hoagland's nutrient solution for vegetable crops. Randle (2000) observed that an increase in nitrogen (N) content in hydroponically grown onion increased total N content and potassium (K) content and decreased boron (B), calcium (Ca) and magnesium (Mg) content, but showed no direct effect on copper (Cu), iron (Fe), phosphorus (P) and zinc (Zn). Asher and Ozanne (1967) found that an increase in K in the nutrient solution increased the K content and yield of both the shoots and roots of several pasture crop species, but a decrease was observed for root: shoot ratio and dry matter percentage. The percentage of K in the leaves and total uptake of K by tomatoes were controlled significantly by the N concentration in the nutrient solutions (Adams et al. 1973).

2.3 PHOSPHORUS AND POTASSIUM FORMS, FUNCTIONS AND UPTAKE IN THE HYDROPONICS

2.3.1 PHOSPHORUS

Phosphorus is an important major nutrient component of plants. It is involved in sugar phosphate intermediate respiration and photosynthesis and phospholipids that make up cell membrane. It is also an important nutrient in energy storage such as adenosine triphosphate (ATP) (Mengel and Kirby 1987). The ATP is the source of energy that all living organisms use to power necessary chemical reactions in plant and animal cells. Phosphorus exists in solution with either potassium or nitrogen in the form of ammonium [$(NH_4)_2HPO_4$ or $NH_4H_2PO_4$] or potassium [K_2HPO_4 or KH_2PO_4] phosphates. Phosphorus in plants including cotton plants exists in solution as monohydrogen phosphate (HPO_4^{2-}) and dihydrogen phosphate (H_2 $PO4^{2-}$) (Ullrich-Eberius et al. 1981; Tu et al. 1990). The abundance of H_2PO4^{2-} and HPO_4^{2-} ions are pH dependent. Monohydrogen phosphate pH is abundant near neutral (pH approx. 7.0) and dihydrogen phosphate abundant in an acidic pH range. Cotton has received less attention as regards P nutrition unlike K nutrition. However, the optimal level of P in the plants varies between 0.25 to 0.6 % (Hochmuth et al. 2009). Phosphorus is also mobile in the plants. The optimum P uptake by plants leads to expanded root surface area through increase root growth and root hair development (Gilroy and Jones 2000). Phosphorus is an essential nutrient for root formation, flowering, fruiting and ripening. A complete hydroponics nutrient solution planted with different vegetable crops revealed that increases in stem width, height, and leaf length were observed when adequate phosphorus was added into nutrient solution (Gayle et al. 2001). Phosphorus deficiency is noticed in the older leaves leading to

stunted growth in young plants, dark green colouration of leaves and reddish colouration resulting to display of anthocyanin pigment. Severe deficiency can cause necrotic spot in leaves. Excess of P in the root making Zn unavailable for plant uptake (Hochmuth et al. 2009).

2.3.2 POTASSIUM

Potassium exists within the cotton plants as a cation (K^+). Potassium is supplied in the hydroponics as potassium nitrate (KNO_3) or potassium sulphate (K_2SO_4). If there is no other source of chloride in the solution, potassium is also supplied as potassium chloride (KCl). It plays an important role in plant growth, development and osmotic regulation potential in the plant cell (Schachtman and Schroeder 1994; V´ery and Sentenac 2003; Yi-Fang Chen et al. 2008). Potassium also activates many enzymes for photosynthesis and respiration. The estimated range of 1.5 to 4.0% has been shown to produce healthy plants including cotton plants (Hochmuth et al. 2009). Potassium is mobile in plants. Adequate application of potassium to cotton plant is known to influence fruit formation in plants and plays a significant role in cotton boll enlargement, fibre elongation and deposition of cellulose into fibre cell (Ritchie et al. 2004). Potassium controls opening and closing of stomata through guard cells by regulating the concentration of potassium in the guard cells. Potassium also controls plant transpiration rate. This has been investigated in crops such as cotton and barley, hot wind increased transpiration rate which took long time to react to close the stomata, but adequate K supply alleviated the problem (Gething 1990; Zia-Ul-Hassan and Arshad 2010). An adequate level of potassium in cotton plants produced photosynthate at a faster rate for

longer period of time than K deficient plants (Zia-Ul-Hassan and Arshad 2010). Potassium deficiency is observed in the lower leaves with mottled or marginal chlorosis, severe deficiency leads to necrosis. Potassium deficiency results in plants susceptibility to root-rot fungi (Hochmuth et al. 2009).

2.3.3 PHOSPHORUS AND POTASSIUM TRANSPORT AND UPTAKE

The movement of ions across cell membrane is by electrical neutrality, for example bulk flow. Bulk flow is electrical neutrality which neutralizes negatively charged plasma membrane and positively charged K ion. This electrical neutrality permits easy passage of potassium ions into the plant cell. Potassium can also be transported by ion channel and transporters. In K^+ channel, ion can be transported to plants by passive uptake, because K^+ influx across the cell membrane does not require energy produced by adenosine triphosphate (ATP) but depends on the electrochemical potential of cell membrane. Potassium transporters act as carrier and co-transporters which involves active uptake that require energy produced by ATP e.g. Na^+/K^+ or H^+/K^+ transport (Yi-Fang Chen et al. 2008). Plant roots absorb phosphate through proton gradient influx (H^+ - HPO_4^{2-} symporter) generated by plasma membrane incorporated into a variety of organic compound such as sugar phosphates, phospholipids and nucleotides (Ullrich-Eberius et al. 1981; Sakano 1990; Yi-Fang Chen et al. 2008).

Phosphorus and K ions in the solution enter plant roots through the cell wall apoplast moving to the cell membrane to another membrane. Ions can also move by symplast, move directly to plant cell through plasmodermata that connect cells together.

The nutrients move from the cortex to endodermis. In the endodermis the ion movement is blocked by a suberized layer called the casparian strip, the ion moves into pericycle finally into the xylem tracheary, where the nutrients are distributed to other parts of the plant. The casparian strip also influenced high ion concentration in the plant cell (Marschner 1995; Epstein and Bloom 2005). Sugar produced during the photosynthesis can be transported by diffusion from the leaves in the palisade layer of the spongy mesophyll through the phloem sieve to the plant parts (Haehnel et al. 1989).

2.4 BENEFITS AND FUNCTIONS OF PHYTOHORMONES TO CROPS

The term hormone was developed by animal physiologists to denote naturally occurring organic substances, produced at a specific site, effective at very low concentrations, whose action may be involved at sites far removed from their origin (Janick 1979; Benková and Hejátko 2009*)*. The term growth regulator has been used to include all naturally occurring and synthetically produced substances that affect plant growth and development (Janick 1979; Benková and Hejátko 2009). Phytohormones influence the availability of nutrients and can speed up nutrient uptake by plants resulting in more efficient use of chemical fertilizers. Thus, with the addition of phytohormones, less nutrients need to be used. With an adequate supply of nutrients and phytohormones, we can achieve much better growth of plants. Phytohormone treatment of seeds and plants therefore, is more important than hybrid seed development (Janick 1979; Benková and Hejátko 2009). The full genetic potential of seeds is achieved and yields are greatly improved through the use of phytohormones. Micronutrients and phytohormones are

naturally occurring elements and compounds. Thus the use of which are environmentally safe and highly desirable.

In general, auxin is an important phytohormone that promotes root development, through initiation and emergence of the lateral root, root apical meristem, gravitropism and root elongation (Ljung et al. 2005). Auxin biosynthesis occurs in the shoot and roots of the plant (Ljung et al. 2005). Bhalerao et al. (2002) reported that during early seedling stage of plant development, Arabidopsis root system synthesized IAA. The IAA is one of the phytohormones known for root growth and development (Muday 2001; Casimiro et al. 2003; Friml 2003; Ljung et al. 2005). For example, an increase in the formation of lateral roots in search of nutrients and water can stimulate increase in leaf development. The IAA synthesis can be inhibited by application of naphthylpthalamic acid (NPA) (Ljung et al. 2005). The directional transport of auxin is by polar transport influx of IAA through passive diffusion of the protonated form (IAAH) from any direction across the phospholipids bilayers (Raven 1975; Goldsmith 1977; Mitchison 1981; Blakeslee et al. 2005; Kramer 2006; Lewis et al. 2007). Robert et al. (2009) reported that auxin influx also takes place by secondary active transport of the dissociated form (IAA^-) via a $2H^+$-IAA (symporter). The auxin uptake depends on apoplastic pH (Raven 1975). The passive uptake by plant cell increases as the extracellular pH is lowered from neutral to a more acidic value. The secondary active uptake of auxin allows more auxin accumulation than simple diffusion because it is being driven into the cell by proton motive force (H^+-ATPase) at the cell membrane (Kramer 2006; Blakeslee 2006; Jönsson et al. 2006; Wu et al. 2007; Petrášek 2009). The auxin influx carrier, AUXI, influx auxin into the vascular parenchyma polar transport stream (Bennett et al. 1996; Yang et al. 2006). In addition to

AUXI, an ATP also helps influx auxin into the cell (Kramer and Bennett 2006). Auxin efflux is the movement of auxin out of the cell. Efflux of auxin out of the cell is through PIN protein and P-glycoprotein (PGP). Blakeslee et al. (2007) reported that auxin efflux carrier coded PINs and PGP can function both independently and synergistically to catalyze auxin efflux. Inside the cell, 1-N-naphthylphthalamic acid (NPA), 2-carboxypheny l-3-phenylpropane-1, 3-dione (CPD) and 2, 3, 5- triiodobenzoic acid (TIBA) are auxin efflux inhibitors while 1-napthoxyacetic acid (NOA) is the auxin influx inhibitor (Blilou 2005; Blakeslee 2007; Dhonukshe 2007; Bailly 2008). The optimal growth of peas and black cumins plants is obtained by applying auxin at 10^{-6} to 10^{-5} μ g/L (Anderson 1988; Shah 2006). In Arabidopsis, it is slightly lower.

Gibberelic acid (GA_3) stimulates stem elongation (Yang et al. 1996), seed germination (Bethke et al. 1997), shoot growth (Fujioka et al. 1990), transition to flowering (Levy and Dean 1998), anther development, pollen tube growth, floral development (Bethke et al. 1997; Bl´azquez 1998), fruit set and growth and seed development (García-Martínez 1991; García-Martínez et al. 1997). The gibberellic acid (GA_3) functions in seed development (Swain et al. 1993, 1995; Hedden and Kamiya 1997) and pod growth (Garcia-Martinez et al. 1991, Rodrigo et al. 1997; Hedden and Kamiya 1997). Verma (2003) reported that gibberellic acid increased carnation (*Dianthus caryophyllus L*) stem height and flower formation when gibberllic acid was applied at 50 mg/L and 100 mg/L, respectively.

Cytokinins mobilize nutrients from the root to leaves. Exogenous cytokinin supplied is directly proportional to nitrate level and transported to the leaves from the root via xylem. As plants increase in height and biomass, there is tremendous change in

cytokinin levels (Morris et al. 1993; Benkova et al. 1999; Dewitte et al. 1999; Emery et al. 2000; Yang et al. 2001; Jacqmard et al. 2002). This change was attributed to mineral nutrition such as nitrogenous nutrients (Goring and Mardonov 1976; Salama and Wareing 1979; Samuelson and Larsson 1993; Takei et al. 2002). Nitrate increase cytokinin concentration in the xylem sap. It can also be affected by a decrease in water as a result of drought (Yang et al. 2001). Cytokinin modifies apical dominance and promotes root growth. Senescence is delayed by cytokinins by triggering the expression of ipt gene within the leaf cell (Miyawaki et al. 2004). Cytokinin level promotes shoot growth as well as chlorophyll formation. Zeatin stimulates cell division in the presence of auxin. Rice plant height and yield were increased when rice plants were treated with cytokinins at 10^{-5} M for plant height and 10^{-4} M for yield (Zahir et al. 2001). Examples of cytokinins are Kinetin, Zeatin (Trans -6- (4- hydroxy- 3- methylbut- 2- enylamino) purine), Benzyladenine, NN Diphenylurea, Thidazuron (for herbicide killer). Researchers have not used cotton crop as a model to investigate the basic research discussed above. However, this study aims to shed more light on the response of cotton crop to P and K nutrients and phytohormones grown hydroponically.

2.5 AUXIN, GIBBERELLIN, CYTOKININS, PHOSPHORUS AND POTASSIUM INTERACTIONS

Plant growth regulators (auxin, gibberellin and cytokinins) and nutrients (P and K) promote growth and development in plants. The exogenous application of auxin together with P increase lateral root development and root hair formation (Vance et al. 2003). Data collected by Gilbert et al. (2000) revealed that cluster root response to P –

deficiency in white lupin is solely controlled by auxin transport. However, auxin insensitive mutants of Arabidopsis (aux1, axr1 and axr4) that have been found to reduce lateral root formation, increase production of lateral roots on low P media (Williamson et al. 2001). Phosphorus and N deficiency influenced decrease in cytokinins content in the plants tissue (Vance et al. 2003). Furthermore, exogenous application of cytokinins could be used to counteract lateral root growth stimulated by N and P deficiency in Arabidopsis (Kuiper et al. 1988) but stimulate tiller growth and yield of rice (Zahir et al. 2001). The interaction of potassium with phytohormones (IAA, GA_3 and Z) in hydroponic cotton crops has received less attention.

2.6 EFFECT OF PHOSPHORUS AND POTASSIUM ON COTTON PLANTS

2.6.1 GROWTH, NUTRIENT UPTAKE AND NUTRIENT USE EFFICIENCY

Ivana, et al. (1986) found that uptake and translocation of mineral nutrients is being controlled by plant hormones. Phytohormones such as gibberellic acid and indole-3-acetic acid induced P-uptake by crops including cotton crop. Jose et al. (2002) made an assertion that auxin sensitivity played an important role in root modification and P-availability. Nutrients uptake such as NPK depend on cotton root growth (Barber and Mackay 1986; Yan et al. 2004) and phytohormones content (Wang et al. 2009). Furthermore, Wang et al. (2009) reported close correlation between root growth of strawberry and N application which could suggest that phytohormones can influence N uptake. Marischner (1995) and Takei et al. (2001) reported the significant role of cytokinins (Zeatin) in N uptake by crops such as cotton.

Efficient use of mineral fertilizers is paramount from economic and

environmental points of view. Nutrient use efficiency can be improved by cutting down rising farm production cost through selecting a cultivar with high harvest index (ratio of grain yield to total plant biomass (Bufogle et al. 1997). Second, by adopting optimum nutrient application rate through soil testing or standardized nutrient solution or predicting yield expectation of the crop. Third, by improving application time for nutrient uptake (Bandel et al. 1990; Scharf and Alley; 1993) and fertilizer sources (Eghball and Sander 2001). Growers can improve their production systems by using these practices.

Excessive nutrients in the soils or adding excess nutrients to hydroponics can cause environmental hazard. Nutrients losses to the environment can also have a negative effect on human health. In order to safeguard against this, researchers have discovered some tools to tackle these problems. Agronomic nutrient use efficiency is the fundamental basis for economic and environmental efficiency (Robert, 2008). Nutrient use efficiency can be expressed in many ways; Ladha et al. (2005) defined eighteen different ways to calculate nutrient use efficiency. Mosier et al. (2004) simplified nutrient use efficiency into four agronomic indices: partial factor productivity (crop yield in kg per nutrient applied in kg); agronomic efficiency (increase crop yield in kg per nutrient applied); apparent recovery efficiency (nutrient taken up in kg per nutrient applied) and physiological efficiency (increase in yield in kg per nutrient taken up in kg). Robert et al. (2008) postulated crop removal efficiency (removal of nutrient in harvested crops per percent of nutrient applied). The P-efficiency is lower than N efficiency because P is least unavailable and least mobile in the soil. Robert et al. (2008) estimated a recovery range of less than 10% to 30%. Conversely, K use efficiency is higher than N and P because it is mobile in soils and it cannot be volatilized. First year recovery of applied K was

estimated to be 20% to 60%. Therefore, to obtain optimum nutrient use efficiency, appropriate application rate, correct time and placement of nutrients should be taken into consideration. Jovicich et al. (2007) reported that nutrient use efficiency was greater in the greenhouse under hydroponics than field production of cucumber plant. Nitrogen and K use efficiency in the greenhouse hydroponic production systems are estimated to be 1,260 kg and 1,620 kg, respectively, against 1,757 kg for N and 2,103 kg for K in the field or conventional production (Jovicich et al. 2007).

2.6.2 CHLOROPHYLL PRODUCTION

Plant nutrients especially N, P and K are primary or essential nutrients that plants required for their physiological development. The physiological changes in plant status resulted from the measured changes in leaf pigmentation e.g. chlorophyll a, chlorophyll b (Fridgen and Varco 2004). Studies have indicated that less than optimum N availability required for plants lead to reduced total chlorophyll concentration in cotton leaves (Thomas and Gausman 1977; Longstreth and Nobel 1980; Everitt et al. 1985). It has been demonstrated by Oosterhuis and Bednarz (1997) that chlorophyll a and total chlorophyll concentration were reduced in cotton due to K deficiency. Lamrani et al. (1996) reported that high levels of K nutrition promoted formation of chlorophyll a and b in cucumber plant (*Cucumis sativus* cv Brumex). It has been reported that adequate supply of K nutrient increase chlorophyll content of plants (Stromberg 1960; Fletcher et al. 1982; Maples et al. 1988; Duli et al. 2001). From the previous studies, P needed for chlorophyll formation received less attention. It is reported in the literature that K and N are essential for chlorophyll synthesis in plants.

2.6.3 ROOT GROWTH

Since the 1980s hydroponic units have been commercialized for vegetables and flowers production and more than 60,000 ha of vegetables were grown hydroponically in greenhouses worldwide (Jone et al. 1997; Sattar et al. 2010). The improved agricultural practice using hydroponic nutrients solution to produce crops is common (Irshad et al. 2004; Sattar et al. 2010). Hydroponic nutrient solution provides an ideal medium to evaluate root morphology and physiology. Lynch (1995) reported that root morphology changes with their development status which directly related to nutrients uptake from soil or nutrient solution and therefore affect plant growth, biomass and yield. The branching pattern of plants, total root length, root hair elongation, and lateral root formation explores greater volume of soil or hydroponic system if treated with phosphorus (Dinkelaker et al. 1995; Bates and Lynch 1996; Borch et al. 1999, Jose et al. 2002). Marschner (1995) reported that root size is an important factor for nutrient acquisition such as P and K that are immobile in the soil. Root system of cotton plant's architecture brings effective nutrient acquisition and bumper yield (Linkohr et al. 2002; Yan et al. 2004). Barber and Mackay (1986) found a positive correlation between yield and root size in maize hybrid B73 x Mo17. A strong relationship between the maize root growth and P uptake was reported by Maizlisch et al. (1980); Barber and Mackay (1986).

2.6.4 RESIDUAL LEVEL OF PHOSPHORUS AND POTASSIUM NUTRIENTS AND EVAPOTRANSPIRATION OF NUTRIENT SOLUTION

Environmental pollution occurs as a result of over use of chemical fertilizer. Chemical fertilizer must be used in appropriate quantities to mitigate environmental

impact and reduce production cost. The absorption of water and mineral nutrients depend on the early growth of cotton roots (Oosterhuis and Zhao 1994), but there are some occasions whereby high levels of P and K are left behind in excess after cropping in hydroponic systems. However under some circumstances, residual nutrients in soils or hydroponics may provide a nutrient resource for cropping in subsequent cropping seasons. Previous studies have indicated that residual P-nutrient had a greater effect on foliar, forest floor and soil nutrient content than K-residual level (Crous et al. 2008). Furthermore, Benbi and Biswas (1999) reported that residual levels of P in the soil depend on rate and the total amount of P added and its removal by the crops. Obigbesan and Akinrinde (2000) confirmed the beneficial effect of residual P (rock phosphate source) on height and biomass of millet planted in strongly acidic soils. Akinrinde et al. (2003) stated that residual P (single super phosphate fertilizer source) had a favourable effect of releasing unused P in the last cropping season to the crop during the subsequent new planting season.

Plant growth, development and yield depend on adequate water and nutrients (nutrient solution) supply to the crop, especially during the seedling and reproductive stages. Kozlowski (1972) and Stevenson (1982) reported that water and plant nutrients are the most important variables for producing profitable yield.

Evapotranspiration is defined as the transport of water into atmosphere from surfaces which include soil (soil evaporation) or from nutrient solution (nutrient evaporation) and from vegetation or leaves canopy (transpiration) (Slabbers et al. 1979). Evapotranspiration estimate is an important tool to reveal the influence of water supply on crop growth and yield (Slabbers et al. 1979). Burba et al. (2006) reported that

evapotranspiration at any location in the globe is controlled by energy availability, physical attributes to the vegetation (vegetative cover, plant height, leaf area index and leaf shape), stomata resistance and soil characteristics (water holding capacity). It has been discovered that minimum evapotranspiration rates normally occur during the coldest month of the year (winter) and maximum evapotranspiration (ET) occurs during summer season (Burba et al. 2006).

In the hydroponic systems, evapotranspiration is measured by determining water applied and drainage. The differences between water applied and water drainage is the actual evapotranspiration. The differences between open (outdoor) and closed system (greenhouse) in relation to evaporation have been discussed in section 2.2.2

2.7 RESPONSE OF PHYTOHORMONES ON COTTON PLANTS

2.7.1 GROWTH, NUTRIENT UPTAKE AND NUTRIENT USE EFFICIENCY

Phytohormones are organic compounds that influence plant growth and development at extremely low concentration (Zahir et al. 2001). Phytohormones are applied either naturally or synthetic to influence growth, development and yield of plants. Gibberellic acid is very important hormone known to be actively involved in physiological process in plants such as growth, flowering and nutrients transport (Wareing and Phillips 1981; Takei et al. 2001; Khan and Samiullah 2003). Gibberellic acid stimulates stem and internode elongation (Godwin and Morris 1979; Anderson et al. 1988; Shan et al. 2006). Indole-3-acetic acid which is one of the derivatives of auxin is best known for root development (Casson and Lindsey 2003; De Smet et al. 2006; Zhiyong et al. 2009). Sitbon et al. (1992); Evan et al. (1994); Himanen et al. (2002) advocated the importance of exogenous application of IAA and increase of endogenous

auxin for the root formation. Auxins appear to be formed in the meristemic tissue of stem and root apices (Wareing and Phillips 1970). Zeatin is the one of the cytokinins derivative, cytokinins actively promote cell division. It also influences cell enlargement, tissue differentiation, flower formation and senescence retardation (Hartman et al. 1981; West and Harada 1993; Peres and Kerbany 1999). It has been reported that exogenous supply of cytokinins at the root zone improved growth and yield of treated crops (Zahir et al. 2001).

2.7.2 CHLOROPHYLL PRODUCTION AND ENDOGENOUS HORMONES CONTENTS

Phytohormones have been known to regulate the physiological process in plants. Greening in plant indicates the healthy condition of a plant. Among the phytohormones, cytokinins (zeatin) regulate various growth and development process in plants. Fletcher and McCullagh (1971) reported that cytokinins played an important role in the chlorophyll formation. It is well documented that cucumber plant pretreated in the dark with cytokinins promote chlorophyll formation during subsequent continuous illumination. Furthermore, Mitsuru and Hideo (1978) made an assertion that the pre-treated cotyledon of cucumber with Benzylaminopurine (BA), Gibberellic acid (GA_3), and Indole-3-acetic acid (IAA) stimulated chlorophyll formation. Several workers have reported that cytokinin effect on chlorophyll formation in light depends on the length of time of dark preincubation with cytokinins (Fletcher and McCullagh, 1971; Ford et al., 1977; Uheda and Kuraishi, 1977; Dei and Tsuji 1978). However, cytokinins (zeatin) is one of the plant hormones known to promote greening (chlorophyll synthesis) in plants

using light as a final step catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH) (Koski et al. 1951; Griffiths 1978; Apel et al. 1980; Yoshiki et al. 2003).

Endogenous phytohormones promote growth, development and yield (Nickell, 1982). Auxin forms in the meristemic tissues of stem, root apices and in young developing leaves (Wareing and Phillips 1970), root growth and development (Torrey 1950; Blakely et al. 1982; Muday 2001; Casimiro et al. 2001; Jose et al. 2002). Gibberellic acid is an important hormone that regulates physiological activities in plant such as nutrient transport, inducing stem elongation, increase in dry matter production and weight, leaf area expansion and flowering (Wareing and Phillips 1981; Brock 1993; Azuma et al. 1997; Bhaskar et al. 1997; Gupta and Dutta 2001; Takei et al. 2002; Khan and 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 and Arshad 1995; Arshad and Frankenberger 1998; Zahir et al. 2001). Findings of Siobhan and 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 and 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 crops e.g. cotton, wheat, barley rice (Zahir et al. 2001).

2.7.3 ROOT GROWTH AND ROOT ACTIVITY

Auxins have been known to alter primary root growth and promote root hair and

lateral root formation (Torrey 1976; Jose et al. 2002). The application of synthetic indole-3-acetic acid (IAA) increased lateral root formation whereas auxin transport inhibitors did not (Torrey 1950, Blakely et al. 1982; Muday and Haworth 1994, Casimiro et al. 2001; Jose et al. 2002, Wang et al. 2009). Furthermore, application of auxin either by synthetic exogenous and/or natural biosynthesis, increase root growth, root hair and lateral root formation (Ivanchenko et al. 2008, Benkova and Hejatko, 2009). Indo -3-acetic acid (IAA) enters the plant cell through protonated IAA or anion IAA^- in relation to pH of the cell compartment. The protonated and anion IAA^- diffused into the cell through the phospholipids cell membrane (Kramer and Bennett 2006). In relation to molecular point of view, auxin signal occurs as a result of interaction of the transport inhibitor response 1 (TIR1) protein of auxin F-box protein (AFBs). The TIR1 and AFBs provide a good avenue for E3 ubquitin-ligase complex of SKP1-cul1-TIR1. This interaction gives way to AUX/IAA ubiquitination, which is later degraded by 26S proteasome (Chapman and Estelle 2009). AUXI has been recognized as auxin influx facilitator. This auxin influx protein reveals the expression in the elongation of root and lateral root growth. The proteolytic degradation of AUXI/IAA brings about genetic expression whereby root growth is stimulated when auxin is applied (Swarup et al. 2005). Therefore, in the absence of AUX 1, the rate of IAA diffusion would be too slow for extensive root growth to occur, resulting to reduced root growth. In contract, PIN auxin efflux protein facilitates the establishment of direct transport of auxin out of the plant cell.

Application of high P with auxin to Arabidopsis root stimulates lateral root formation (Jose et al. 2002). On the other hand; cytokinins reduced the elongation of

roots and the formation of lateral root (Lopez-Bucio et al. 2003; Lohar et al. 2004). Furthermore, Goodwin and Moris (1979) reported that cytokinins produced at the root tip of pea inhibit the lateral root formation, but support lateral stem growth. Zahir et al. (2001) found that exogenous application of cytokinin at the root zone supported luxuriant growth and yield of rice. There are few literatures on auxin and potassium interaction, notable work was carried out by Shin et al. (2007). These researchers reported that significant auxin transport was reduced under potassium nutrient deficiency when auxin transport Myb 77-1 was compared with wild type (Myb 77-ox line). The reduced auxin transport under deprived K nutrient resulted to significantly lower lateral root density in Myb 77-1 and Myb 77-2 than wild type whereas Myb 77 -1 and 2 and wild type lateral root were the same under nitrogen and phosphorus nutrients. Furthermore, the same result was achieved when receptor mutant (TIR1) was measured for control and deprived K nutrient.

Root activity measured by triphenyl tetrazolium chloride (TTC) is related to the aerobic respiration of root. Root aeration brings about effective nutrients absorption at the root for growth and yield of crops. Moreso, TTC activities in the roots is associated with dehydrogenase activity of tricarboxylic acid (TCA) cycle that regulate sugar (Carbohydrate) and mineral absorption in the plants. Atkins (1992) and Clark et al. (1992) revealed that PGR-IV a plant growth regulator which consists of gibberellic acid (GA_3) and synthetic indole butyric acid (IBA) increased root mass and root activity of cotton plants. Cotton root activity measured by 2, 3, 5-triphenyl tetrazolium chloride reduction is also temperature dependent. It has been suggested that optimum temperature range of 33 to 36^{o}C (Arndt 1945; Pearson 1970) could support growth and yield of

cotton. The differences in optimum temperature are related to changes in stored seed reserves for cotton root growth (McMicheal and Burke 1994).

2.7.4 INFLUENCE OF PHYTOHORMONES ON RESIDUAL LEVEL OF PHOSPHORUS AND POTASSIUM NUTRIENTS AND EVAPOTRANSPIRATION OF NUTRIENT SOLUTION IN HYDROPONIC SYSTEM

Plant hormones can also be used to stimulate effective water use. Duli Zhao and Derrick Oosterhuis, (1997) concluded that PGR IV (combination of Gibberellic acid and auxin) has the ability to partially remove detrimental effect of water stress on biomass accumulation and photosynthesis in order to improve the growth and nutrients absorption of cotton. Reports on interaction between hormones and mineral nutrients on evapotranspiration are limited. Kudoyarova et al. (2007) reported that cytokinin (zeatin type) loading into xylem decreased as the soil dries (evapotranspiration).

CHAPTER 3

MATERIALS AND METHODS

3.1 CHARACTERISTICS OF COTTON PLANTS USED IN THE EXPERIMENTS

Zhong mian 36 cotton and Xin Luo Zao 13 cotton cultivars were selected for this experiment because they are indeterminate plants; they continue to grow after first harvest. They are also early maturing and high yielding cultivars.

A single plant of Zhong mian 36 cotton cultivar yields 9-10 matured medium size bolls with average weight around 5.0 g, cotton lint 36.6% and seed weight is 11 g. This cultivar possesses the ability to open broadly, which makes it easy to pick. The Zhong mian 36 cotton cultivar is known for high yielding with an estimated yield to be 802 kg/ha for gin cotton (seed cotton) and 846 kg/ha for ginned (no seed cotton) prior to frost. In Northern part of Xinjiang, China the ginned cotton yield was estimated to be 2310 kg/ha, an increase of 15.0% compared with a locally disseminated cultivar. The fibre tested by Cotton Quality Testing and Supervision Centre, Ministry of Agriculture, showed that 2.5% fibre length is 29.3 mm, relative strength is 23.2 g f/tex (gram-force per texture) with a thickness value of 4.4 micron (Unpublished data from Xinjiang Provincial Government).

A single plant of Xin Luo Zao 13 cultivar maintains 8-10 fruiting branches, the ball is ovoid shape, the single ball weighs around 5.5 g and the cotton is pure white. It has a lint weight of 7.04 g, lint of 40-41% and seed weight of 9.96 g. Lint is the mass of soft fibre surrounding the seed of unginned or gin cotton. Lint cotton yield is 1641.15 kg/ha, lint cotton yield before frost is 1459.95 kg/ha. The quality test result showed that fibre

length was 30.6 mm, fibre evenness was 48.2%, stretching rate was 7.1%, and fibre strength was 21.2 g f/tex with thickness value of 4.3 micron (Unpublished data from Xinjiang Provincial Government). The attributes of these two cultivars discussed above merit their uses for this study.

3.2 NUTRIENT SOLUTION EXPERIMENT AND PHYTOHORMONES EXPERIMENT

The experiments were conducted in the greenhouse and laboratory, the nutrient solution experiment from July 24, 2006 to December 29, 2006 and the phytohormone experiment from December 15, 2006 to August 21, 2007 at Xinjiang Agricultural University, Urumqi, China. Xinjiang lies in North West China (latitude $34^0\ 25^1$-$49^0\ 10^1$ N and longitude of $73^0\ 25^1$ – $96^0\ 23^1$ E). The two cultivars of cotton (*Gossypium hirsutum*) Zhong Mian 36 and Xin Luo Zao 13 used for the study are widely grown in China. The experiments were conducted under intensively controlled greenhouse environmental conditions simulating field conditions for cotton production. The nutrient solution experiment was performed by cultivating the two cotton seedlings in a 6 litres nutrient solution plastic bucket containing Hoagland standard nutrient solution (high PK) and modified Hoagland standard nutrient solution of low P (half strength Hoagland standard P solution) and low K (one-sixth strength Hoagland standard K solution). The stock solution of macronutrients, trace elements and FeEDTA were made. One litre of Hoagland nutrient solution from the stock was made by adding 7 ml $Ca(NO_3)_2$, 5 ml KNO_3, 2 ml KH_2PO_4, 2 ml $MgSO_4$, 1 ml trace elements (H_3BO_3 2.8 g, $MnCl_2.4H_2O$ 1.8 g, $ZnSO_4.7H_2O$ 0.2 g, $CuSO_4.5H_2O$ 0.1 g, $NaMoO_4$ 0.025 g) and 1ml FeEDTA (10.4 g EDTA.2Na, 7.8 g $FeSO_4.7H_2O$, 56.1 g KOH added to 1 L KOH) to 1 litre distil water.

However, the second experiment was done with application of phytohormones (IAA, GA_3, Z and their combinations) to two cotton crops cultivated in Hoagland standard nutrient solution (high PK). The phytohormones were applied by spraying on cotton plant leaves at 36 and 67 days after transplanting. The concentration of phytohormones used in this present study was in line with Shah et al. (2006), Anderson et al. (1988) with slight modification. Phytohormones are needed in small quantity which influenced plant growth and development. The variables measured were subjected to analysis of variance in a completely randomized design using Proc Univariate of SPSS version 15 for the all variables excluding fresh biomass weight using Proc Mixed of SAS v 9.3. Means were compared by Duncan's multiple range test (DMRT) for the variables except fresh biomass weight that was compared using Tukey's HSD. The completely randomized design is focused on comparison of t population means. In terms of experimental design, we assumed that there are $n_1 + n_2 + n_3 + \ldots + n_t$ homogenous experimental unit (the smallest unit of experimental materials to which a treatment is applied). The treatments were randomly allocated to the experimental units in such a way that n_1 units received treatment 1, n_2 received treatment 2 and n_3 received treatment 3. The hypothesis was tested using analysis of variance (ANOVA) table. The ANOVA is based on calculating total sum of square (TSS). The TSS is the difference between treatment sum of square (SST) and the sum of square error (SSE), TSS = SST- SSE. The TSS was partitioned into two separate sources of variability: one due to variability among treatments and second was variability in observation of experimental unit that received treatment. The means square treatment (MST) was calculated by dividing SST by degree of freedom of treatment while means square error (MSE) was obtained by dividing SSE by degree of

freedom of error. When null hypothesis is true, both MST and MSE are unbiased estimate of variance, the variance of the experimental error is E (MST) = variance and E (MSE) = variance. This implies that null hypothesis that says all treatments are equal should be rejected if F calculated exceeds F tabulated, but if F calculated is lower than F tabulated all the treatments should be accepted as equal i.e. homogenous. This statistical approach was used because completely randomized design is extremely easy to construct, the design is easy to analyze even though the samples sizes might not be the same for each treatment and design can be used for any number of treatments.

The transplanting of cotton plants to nutrients solution was done after seven days of growth. Nutrient solutions were changed and replaced at every nutrient change because nutrient deep technique (NDT) closed hydroponic system was used in this experiment. Nutrient solutions were also changed so as to measure various plants parameters.

3.3 DESIGN OF NUTRIENT SOLUTION EXPERIMENT AND PHYTOHORMONES EXPERIMENT

3.3.1 NUTRIENT SOLUTION EXPERIMENT

The three treatments used in the nutrient solution experiment were low P level (5.0×10^{-5} M), low K level (1×10^{-3} M) and Hoagland standard nutrient solution high PK level (1×10^{-3} and 6×10^{-3} M) at pH 6.5. Each of the three treatments (low P, low K and high PK) were replicated thirty times given a total number of 90 plants and set up on a completely randomized design. Parameters measured were plant height, root length, number of leaves, nutrient solution level and residual level of P and K in the nutrient solution, NPK nutrients uptake by cotton plant parts, nutrient use efficiency and endogenous phytohormone content. At 83, 91, 104, 120 and 148 DAT six representative

plants from each treatment replicate were removed to measure root area, root volume, root activity and chlorophyll formation. The tissue NPK nutrient uptake, nutrient use efficiency and endogenous phytohormone content were determined from ten plants removed from each treatment replicate at 148 DAT

3.3.2 PHYTOHORMONE EXPERIMENT

In the phytohormone experiment, the exogenous phytohormones concentration were applied at 36 and 67 days after transplanting to standard complete and amended Hoagland nutrient solution (Table 3.1) by spraying on the cotton leaves at single application of 0, 50, 40 and 50 μ gL^{-1} for untreated pot, indole-3-acetic acid, gibberellic 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 100 IAA x $80GA_3$ x 100Z. Each treatment replicated twelve times supplied with the Hoagland standard solution containing high P and K nutrients levels of 1 x 10^{-3} and 6 x 10^{-3} M, respectively (Table 3.1) at pH 6.5 resulting in a total number of 108 plants. Parameters measured were plant height, root length, number of leaves, nutrient solution level and residual level of P and K in the nutrient solution. Subsampling plants were taken from the nine treatments. For each treatment, six plants were removed at 80 and 90 DAT to measure other parameters such as root area, root volume, root activity and chlorophyll formation. For NPK nutrients uptake and endogenous phytohormone content four plants were removed from each treatment replicate at 90 DAT for the analysis.

3.4 ENVIRONMENTAL CONDITION OF COTTON IN THE GREENHOUSE

The two cotton seed cultivars were planted in the quartz sand and water was sprinkled on the cotton seedlings every day. The roots of cotton seedlings were allowed to grow up to 8 – 10 cm, before transplanting into nutrient solution pot of 6 L at seven days of growth. The seedlings were supported with cotton wool so as to hold the seedlings to stand firm.

These specifications were followed in the greenhouse when the plants were transplanted into the nutrient solution: The daytime and night time temperature was kept at 35^{o}C and 20^{o}C respectively, this was achieved by spreading a black blanket over the roof of greenhouse, the surrounding daylight was maintained at 12 to 14 hours daylight by using cool-white and incandescent electrical light bulb intensity at the plant canopy of 600 μE m^{-2} s^{-1}, for the photosynthesis process, there was constant supply of oxygen to the plant roots in the hydroponic system using electrical air pump so as to increase metabolic activity at root rhizosphere. The nutrient solution was Hoagland and micro anion nutrients (Table 3.1).

Table 3.1 Hydroponic nutrient solution composition

Nutrients	High P 1 x 10^{-3} M	High K 6 x 10^{-3}M	Low P 5.0 x 10^{-5}M	Low K 1 x 10^{-3}M
1 molL^{-1} KNO_3	5 (30 ml)	5 (30ml)	5 (30 ml)	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 (12 ml)	2 (12 ml)	2 (12 ml)
1 molL^{-1} KH_2PO_4	1 (6 ml)	1 (6 ml)	Nil	Nil
0.1molL^{-1} KH_2PO_4	Nil	0.5 (3 ml)	Nil	Nill
1 molL^{-1} KCl	Nil	0.95 (5.7 ml)	Nil	Nill
1molL^{-1} $NH_4H_2PO_4$	Nil	Nil	Nill	1 (6 ml)
1 molL^{-1} $FeCl_3.6H_2O$	1 (2 ml)	1 (2 ml)	1 (2 ml)	1 (2 ml)
Trace element	1 (6 ml)	1 (6 ml)	1 (6 ml)	1 (6 ml)

Low P = 5.0 x 10^{-5} M (Half strength Hoagland solution)

Low K = 1 x 10^{-3} M (One-sixth strength Hoagland solution)

High PK = 1 x 10^{-3} / 6 x 10^{-3} M (Hoagland standard solution)

pH = 6.5

P-Phosphorus,

K-Potassium

3.5 VARIABLES MEASURED IN THE NUTRIENT SOLUTION EXPERIMENT AND PHYTOHORMONE EXPERIMENT

3.5.1 NUTRIENT SOLUTION EXPERIMENT

GROWTH AND NUTRIENT UPTAKE

Plant height and root length were measured with graduated ruler while leaves number was measured by counting. The NPK uptake by cotton plants partitioning were measured at the end of the nutrient solution experiment.

NUTRIENT USE EFFICIENCY IN THE NUTRIENT SOLUTION EXPERIMENTS

Phosphorus and potassium use efficiency of apparent recovery efficiency using numerical analysis estimated by Mosier et al. (2004) was used to calculate nutrient use efficiency from nutrient uptake:

$$\text{Apparent recovery efficiency} = \frac{\text{Nutrient taken up by the plants (kg)}}{\text{Nutrient applied (kg)}}$$

CHLOROPHYLL PRODUCTION

At 83, 91, 104, 120 and 148 days after transplanting, one cotton plant from each of the three treatments (low P level was 5.0×10^{-5}M P, low K level was 1×10^{-3} M K and Hoagland standard solution high PK level was 1×10^{-3} M P and 6×10^{-3}M K) to measure chlorophyll a, b and ab. Each treatment was replicated six times. The details of the laboratory techniques are described in section 3.6.2

ENDOGENOUS HORMONE CONTENT

The separation and determination of endogenous phytohormones were carried out with high performance liquid chromatography (HPLC) at the end of the nutrient solution 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 and 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 Hoagland standard solution high PK level was 1 x 10^{-3} M P and 6 x 10^{-3}M K (Table 3.1) at pH 6.5, each treatment was replicated ten times with total number of 30 plants.

ROOT AREA, ROOT VOLUME AND ROOT ACTIVITY

At 83, 91, 104, 120 and 148 days after transplanting, random selection of one cotton plant from each of the three treatments (low P, low K and Hoagland standard solution high PK treatments) were used to measure root area, root volume, and root activity. There were six replicated treatments. The total number of plants used to carry out root area, root volume and root activity throughout the experimental period was 90.

NUTRIENT SOLUTION LEVEL AND RESIDUAL LEVEL OF P AND K

At 21, 46, 57, 72, 83 and 91 days after transplanting, the nutrient solution level in the hydroponic containers or pots was measured with graduated ruler. Also, phosphorus and potassium contents left in the solution in each pot were measured using standard laboratory techniques developed by Olsen and Sommer (1982) for phosphorus and Richards (1954) for potassium. The description of experimental unit is in section 3.3.1.

At 104, 120 and 148 days after transplanting, 18 subsampling pots in each period of assessment were taken to measure other parameters

EVAPOTRANSPIRATION OF NUTRIENT SOLUTION IN THE HYDROPONICS

Nutrient solution level was measured using a graduated ruler. Evapotranspiration of nutrient solution in the hydroponics was estimated using method described by Slabbers et al. (1979). The data obtained from nutrient solution level was used to calculate evapotranspiration of nutrient solution:

Evapotranspiration = Water applied (L) – Water drained (L)

3.5.2 PHYTOHORMONE EXPERIMENT

GROWTH AND NUTRIENT UPTAKE

The graduated ruler was used to measure plant height and root length. The number of leaves was measured by counting. Random selection of nine cotton plants from each treatment replicated four times resulting in 36 plants were used to measure NPK uptake by cotton plant parts partitioning at the end of the phytohormone experiment.

CHLOROPHYLL FORMATION

At 80 and 90 days after transplanting, nine cotton plants from each treatment replicated six times were used to measure chlorophyll a, b and ab.

ENDOGENOUS PHYTOHORMONE CONTENT

The nine phytohormones treated plants were randomly selected for endogenous phytohormone content supplied with the Hoagland standard solution high P and K nutrient levels of 1 x 10^{-3} M/ P and 6 x 10^{-3}M/ K, respectively (Table 3.1) at pH 6.5 in the hydroponics solution, resulting to total number of 36 plants. The extraction, purification, determination and separation of phytohormones were estimated at 90 DAT. More details on extraction, purification, determination and separation of phytohormones is in section 3.5.3.

ROOT AREA, ROOT VOLUME AND ROOT ACTIVITY

The root area, root volume and root activity measurements were carried out at 80 and 90 days after transplanting (DAT), eight phytohormone treated plants and control were randomly selected for these parameters. Each treatment replicated six times resulting in 54 plants at each period of assessment (80 and 90 DAT). The total number of plants used for the whole experiment was 108 cotton plants.

NUTRIENT SOLUTION LEVEL AND RESIDUAL LEVEL OF PHOSPHORUS AND POTASSIUM

At day 43 and 74 after transplanting, nutrient solution level in the hydroponic pots were measured with graduated ruler, phosphorus and potassium contents left behind in the hydroponic pots were measured using method described in first nutrient solution experiment (Section 3.5.1). The eight phytohormone treatments and control were randomly selected; each treatment replicated twelve times resulting to 108 experimental hydroponic pots. At day 80 and 90 after transplanting, nine phytohormones treated pots

including control were randomly selected, each treatment replicated six times, resulting to a total number of 54 pots in each period of parameters measurement. The hydroponic nutrient solution was supplied with the Hoagland standard high PK nutrients level of 10^{-3} M and 6 x 10^{-3} M (Table 3.1) at pH 6.5.

EVAPOTRANSPIRATION OF NUTRIENT SOLUTION

Nutrient solution level in the hydroponic system was measured using graduated ruler. Evapotranspiration of nutrient solution in the hydroponics was estimated using method described by Slabbers et al. (1979). The data from nutrient solution level was used to calculate evapotranspiration of nutrient solution.

Evapotranspiration = Water applied (L) – Water drained (L)

3.6 LABORATORY ANALYTICAL METHOD USED TO MEASURE GROWTH AND DEVELOPMENT OF COTTON

3.6.1 PLANT ANALYSIS FOR NITROGEN, PHOSPHORUS AND POTASSIUM

NITROGEN

The 0.25 g dried plant parts were digested with 20 ml sulfuric-salicylic acid mixture as an alternative to digestion procedure for replacing Kjeldahl-N determination in soils and plants. The 2.5 g sodium thiosulphate was added while swirling gently. The digest was allowed to stand overnight. The 4 g catalyst mixture (K_2SO_4-Se, 100:1 w/w ratio) was added and placed on block digester, heated to 400^0C until the mixture became clear. Distilled water of 250 ml was added to the digested material. Nitrogen content was measured in the digest by the distillation method (Buresh et al. 1982).

PHOSPHORUS AND POTASSIUM

Dry ashing method was used in this experiment to digest plant leaves, stem and root. The procedure used to carry out this analysis was in line with that of Chapman and Pratt (1961). The 0.5 g portion of grounded leaves, stems and roots were weighed in 50 ml porcelain crucibles. The plant materials were ashed at 550^{o}C for 5 hours. Cooled plant materials were dissolved in 5 ml of 2N hydrochloric acid. The volume was made up to 50 ml with distilled water. The mixtures were thoroughly mixed together and filtered with No. 42 filter paper and were analyzed the aliquots for P by spectrophotometer (Ammonium Vanadate-Ammonium Molybdate yellow color method) at 410 nm, brand name JINGKE, Model number: JK-FS-930A, for K by flame photometry (Chinese brand name KEQI, Model number: FP640) at 410 nm.

3.6.2 CHLOROPHYLL MEASUREMENT

The 0.5 g of leaf pigment was extracted from cotton leaves. The 8 ml of 80 percent acetone was added, and incubated for 12 – 14 hours. The 1 ml solution was taken out of the latter, 9 ml of 80 percent acetone was added, and then centrifuge at 2000 rpm. The 10 ml of 80 percent acetone was added to the supernatant. The chlorophyll a and b at wavelength of 663 nm for chlorophyll a and 645 nm for chlorophyll b were measured with spectrophotometer, Brand name JINGKE, Model number: JK-FS-930A,

3.6.3 PLANT ENDOGENOUS 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 – 1.0 g of fresh

cotton plant samples were weighted and quickly refrigerated. After refrigeration, cotton plant samples were ground to powder and 5 ml of 80% methyl alcohol solution was added at a ratio of W:V (1:10-20). The extract was completely sealed and refrigerated at $4^{o}C$ for 12 hours, then centrifuged for 30 minutes at 2000 rpm. The leached solution was removed, and 3 ml (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 a 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 in 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 2 ml of ethyl acetate. The entire ethyl acetate phase combined and dried with nitrogen on water bath at $40^{o}C$. Extracted three times with 2 ml buthanol, and dried with nitrogen on water bath until it reduced to 1 ml. The filtrate passed through 0.45 μm membrane and 0.1 μL 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 ml min^{-1} with an injection volume of 0.1 μL detector wavelength set at 254 nm.

3.6.4 ROOT AREA MEASUMENT

The root area parameters were measured according to method of analysis described by Zhao (1998). Methyl blue of 0.0064 g was added to distilled water of 1000 ml. The sample roots of each plant were immersed into the first beaker containing methyl blue for 90 seconds. Then the roots were moved to second beaker of the same methyl blue concentration and roots were moved to third beaker of the same concentration. The process was repeated three times. One ml sample was removed with a pipette into the test tube from each of the beakers. Nine ml distil water was added to all the test tubes. Thereafter, shaken on a mechanical shaker for 1 minute. The absorbent was measured with spectrophotometer (brand name JINGKE, Model number: JK-FS-930A) at a wavelength of 660 nm. Standard curve was measured by pipetting 1, 2, 3, 4, 5, 6 micro milliliters of methyl blue and read with spectrophotometer at wavelength of 660 nm. The following steps were used to estimate various parameters:

Total absorption area = milligram of methyl blue from 3^{rd} beaker

$$\text{Percentage active absorption area} = \frac{3^{rd}\ \text{beaker} \times 1.1m^2 \times 100}{1^{st}\ \text{and}\ 2^{nd}\ \text{beaker} \times 1.1m^2}$$

$$\text{Specific surface area} = \frac{1^{st}\ \text{and}\ 2^{nd}\ \text{beaker} \times 1.1m^2}{\text{Root volume}}$$

Active absorption area = 3^{rd} beaker x $1.1m^2$

3.6.5 ROOT VOLUME

A 1000 ml volumetric cylinder was used. Root samples were immersed into 900

ml of distilled water. Root volume was calculated by water displacement in the volumetric cylinder (Zhao 1998).

3.6.6 ROOT ACTIVITY

Root activity was measured by standard laboratory method described by Zhao (1998). Measured 0.2 g fresh root was mixed with 5 ml of 4% TTC and 5 ml Na_2HPO_4, the mixture incubated at 37^oC for 1.5 hours. After 10 minutes, 2 ml H_2SO_4 was added, and then filtered with filter paper, the colour changed from white to red colour. Six ml ethyl acetate was added and the root was ground with mortar and pestle then filtered with Whatman filter paper. Four ml of ethyl acetate were added to the filtrate and shook on a mechanical shaker for 1 minute. It was then measured with a spectrophotometer at wavelength of 485 nm. Spectrophotometer Brand name JINGKE, Model number: JK-FS-930A. A standard solution was prepared by measuring 0.25 ml 4% of TTC into 10 ml test tube. Two mg of sodium hyposulphite was added and 10 ml ethyl acetate. 9.75 ml was taken out, ethyl acetate was used to make up 10 ml and pipette 0.25 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 3.0 ml.

$$\text{TTC reduction strength} = \frac{\text{TTC reduction}}{\text{Fresh Root weight x hrs of incubation x 10(dil)}}$$

3.6.7 METHOD USED TO MEASURE RESIDUAL LEVEL OF P AND K

Five ml nutrient solution was added to 5 ml portion of 2N HCl and mix with a plastic rod. After 15 - 20 minutes, 50 ml of distilled water was added. The solution was thoroughly mixed and allowed to stand for about 30 minutes. The supernatant was filtered through Whatman No. 42 filter paper. Phosphorus was analyzed using method

described by Olsen and Sommer (1982) while potassium was determined using standard method described by Richards, (1954). Phosphorus in the nutrient solution was determined by spectrophotometer (Brand name JINGKE, Model number: JK-FS-930A) at 410 nm, and K by flame photometry (Chinese brand name KEQI, Model number: FP640) at 410 nm.

3.7 DATA ANALYSIS

The two cotton cultivars (Zhong mian 36 and Xin Luo Zao 13) were subjected into the same environmental condition as well as the same operational condition; mean, variance and analysis of variance (ANOVA) on each cultivar were performed to establish statistical differences between them. There were no significant differences between the two cultivars. The two cultivars were homogenous and there was no interaction between them, when analysis of variance was performed on each of the two cultivars. As a result of this, the two cultivars variable data were pooled together. The F test was used to compare the two variance. If F test reject the hypothesis of equality (i.e. variances of the two cultivars are equal), then the data can be pooled together. It can be safely assumed that both cultivars come from normal population with the same variance. The full details on normality of statistical analysis are discussed in section 3.2.

The ability of nutrient solution and phytohormones experiments to influence cotton plants growth and development were conducted in a completely randomized design whose statistical model can be written in the form (Montgomery 1991)

$Y_{ij} = \mu_\iota + e_{ij}$ with $\mu_\iota = \mu + T_\iota$

Where the terms of the model are defined as follows:

Y_{ij} = Observation on jth experimental unit receiving treatment i (plant height, root length, number of leaves, chlorophyll etc);

μ_i: ith treatment mean

μ: Overall treatment means;

$_{T1}$: An effect due to treatment i;

e_{ij} = A random error associated with the response from the jth experimental unit receiving treatment i.

The above model can be explained by having t treatment means in t + 1parameters: μ and $_{T1}$ .....$T\tau$. In other to calculate the least square estimate, it is necessary to put constraints on these sets of parameters. A commonly used constraint is to set $_{T\tau} = 0$. The interpretations of these parameters are as follows:

$\mu = \mu_i$, $_{T1} = \mu_1 - \mu_\tau$,....., $_{T\tau - 1} = \mu_{\tau-1} - \mu_\tau$, $\mu_\tau = 0$

To test the hypothesis

H_0: $\mu_1 = \mu_2 = \ldots. = \mu_\tau$ versus H_a: Not all μ_i's are equal,

which is equivalent to test

H_0: $_{T1} = _{T2} = \ldots\ldots. = _{T\tau}$ versus H_a : Not all $_{Ti}$'s are 0

An analysis of variance (ANOVA) table was used. The ANOVA full details are described in section 3.2.

The data collected were analyzed using univariate procedure of SPSS software version 15. Overall effects of the treatments over the growth period were evaluated at periodic interval using the General Linear Model (GLM) procedure of SPSS. Mean comparisons were performed using Duncan's multiple range test (DMRT) at a 0.05 level of probability of significant treatment identified by the GLM. Statistical analysis using

SAS mixed procedure was used for fresh weight biomass and means separation was done with Tukey-Kramer.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 NUTRIENT SOLUTION EXPERIMENT

4.1.1 EFFECT OF LOW PHOSPHORUS (P), LOW POTASSIUM (K) AND HIGH PK TREATMENTS ON COTTON GROWTH AND FRESH BIOMASS PRODUCTION IN THE HYDROPONIC NUTRIENT SOLUTION

4.1.1.1 PLANT HEIGHT

The High PK treatment significantly increased plant height compared with either the low P treatment or low K treatment from 46 to 148 days after transplanting (DAT), but there was no significant difference detected between the low K treatment and high PK treatment at 46 DAT (Table 4.1). High PK treatment showed rapid plant height change in height while low P treated plants had the slowest change in height. Low K showed intermediate height change.

Table 4.1 Main effect of nutrients solution on plant height of cotton grown hydroponically treated to phosphorus and potassium

	Plant height (cm)								
	Days after transplanting								
Treatments	21	46	57	72	83	91	104	120	148
Low P	3.50a	8.16b	9.03c	10.02c	11.54c	13.28c	17.09c	19.41c	26.61c
Low K	3.76a	14.21a	19.45b	23.92b	31.06b	32.40b	33.90b	39.24b	46.92b
High PK	3.91a	15.38a	22.40a	30.41a	39.35a	43.33a	47.30a	51.77a	60.65a
SE	0.40	0.640	0.780	0. 960	1.58	1.68	2.17	2.97	4.32

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.1.2 ROOT LENGTH

The effectiveness of the low P, low K and high PK treatments to support root growth was significantly increased throughout experimental period except at 21 DAT. High PK treatment significantly produced longest root growth while low P treatment gave the shortest root length. Root length had similar growth trend with plant height (Table 4.2).

Table 4.2 Root length of cotton plants grown hydroponically treated to phosphorus and potassium combinations

Treatment Combinations	Root Length (cm) Day after transplanting								
	21	46	57	72	83	91	104	120	148
Low P	17.40a	30.90c	33.30c	38.04c	39.60c	44.90b	49.70b	51.50c	52.80b
Low K	16.20a	36.10b	39.90b	44.40b	46.00b	46.30b	47.10b	46.00b	46.60b
High PK	17.20a	41.90a	46.40a	51.70a	56.80a	57.90a	59.00a	59.10a	58.00a
SE	0.652	1.28	1.50	1.79	1.64	1.61	1.68	2.21	2.88

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.1.3 NUMBER OF LEAVES

Low P and high PK treatments significantly favoured cotton leaves production than low K treatment during the 21 DAT. At 46 DAT, low P treatment significantly produced least leaves number whereas low K and high PK treatments produced greater number of leaves than low P treatment (Table 4.3). At 57 DAT, high PK and low K treatments significantly produced more leaves than low P treatment. However, the effectiveness of the treatments were observed at 104 DAT, low P and high PK treatments jointly favoured leaf production while low K treatment produced least number of leaves. At 120 DAT, low P treatment significantly gave highest number of leaves than other treatments while low K and high PK treatments produced least. The highest leaf production was observed at 46 DAT, thereafter decreased towards the end of the experiment. Massive leaves senescence was observed after 46 DAT, corresponding to the start of the reproductive stage.

Table 4.3 Leaf numbers of cotton plants grown hydroponically treated with phosphorus and potassium combinations

Treatments	Number of Leaves							
	Days after transplanting							
	21	46	57	72	83	91	104	120
Low P	1.97a	2.44b	1.56b	1.71a	2.06a	1.60a	1.70a	1.67a
Low K	1.77b	5.27a	1.68ab	1.48a	2.23a	1.27a	1.00b	1.00b
High PK	1.97a	5.20a	2.00a	1.86a	2.60a	1.13a	1.86a	1.00b
SE	0.84	0.19	0.195	0.16	0.057	0.078	0.596	1.00

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.1.4 FRESH BIOMASS PRODUCTION

The treatments did not affect stem and root fresh biomass weight, but the treatments were significantly produced increase in fresh leaves biomass, high PK treatment produced more fresh leaves biomass than either low P treatment or low K treatment. There was 35.2% increase in biomass while low P and low K both had 32.4% (Table 4.4).

Table 4.4 Effect of low P, low K and High PK on fresh biomass weight of hydroponically grown cotton plants harvested at 148 days after transplanting

	Fresh Biomass weight		
Treatments	Leaves fresh weight (g)	Stem fresh weight (g)	Root fresh weight (g)
Low P	0.3176b	0.3346a	0.3181a
Low K	0.3180b	0.3359a	0.3289a
High PK	0.3455a	0.3155a	0.3215a
SE	0.006	0.008	0.0062

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Tukey Kramer. Low P –Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.2 NITROGEN, PHOSPHORUS AND POTASSIUM UPTAKE BY COTTON PLANTS PARTITIONS

LEAVES: The low K treatment significantly enhanced N uptake by leaves while low P treatment gave least N uptake by cotton leaves. The low K and high PK treatments significantly produced the highest P-uptake by cotton leaves while low P treatment had the shortest. Regardless of K level, P uptake is still strong. In case of K-uptake by cotton leaves, high PK treatment significantly enhanced higher K uptake by cotton leaves with increase of 39.5 % than low P and low K treatments by 34.5 % and 26 %, respectively. It appears that efficient K uptake depends on high level of PK (Table 4.5).

STEM: Low P treatment produced significantly gave highest K-uptake by cotton plants followed by high PK treatment and low K treatment had the least. Low P had increase of 43.3 % while low K and high PK had 20.2 % and 36.5 %, respectively (Table 4.5).

ROOT: The low K treatment significantly produced the highest N-uptake while low P treatment produced the least, but not significantly different from high PK treatment. Conversely, low K and high PK treatments significantly produced higher P-uptake than low P treatment. It is noteworthy that low P treatment significantly favoured K-uptake by cotton root (41.2 %) compared with high PK and low K treatments (35.7 % and 23.1 %, respectively) (Table 4.5).

Table 4.5 The uptake (mg g^{-1}) of nitrogen (N), phosphorus (P) and potassium (K) by cotton plant parts grown in hydroponically treated to phosphorus and potassium harvested at 148 days after transplanting

Treatment Combinations	P, K and PK – Uptake (mg g^{-1})								
	LEAVES			STEM			ROOT		
	N	P	K	N	P	K	N	P	K
Low P	25.10c	4.57b	32.63b	14.26a	10.19a	40.66a	15.59b	5.30b	40.53a
Low K	36.21a	10.18a	24.53c	13.89a	8.93a	18.97c	25.85a	16.82a	22.70c
High PK	30.85b	9.79a	37.38a	7.73a	8.82a	34.30b	16.38b	20.34a	35.08b
SE	1.97	0.639	1.39	3.96	1.99	2.09	2.79	1.85	1.94

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.3 PHOSPHORUS AND POTASSIUM NUTRIENTS USE EFFICIENCY TREATED TO LOW P, LOW K AND HIGH PK.

LEAVES: Both low P and high P treatments significantly gave highest P use efficiency (NUE) by the leaves compared to low K and high K treatments. Low P treatment significantly had the highest K use efficiency in the leaves followed by high P treatment, followed by low K treatment while high K treatment gave the least K use efficiency by the leaves (Fig. 4.1).

STEM: Low P treatment significantly gave the highest P use efficiency by the stem than low K, high P and high K treatments. A similar trend was observed for K use efficiency

by the stem (Fig. 4.1).

ROOT: Low P treatment significantly favoured highest P use efficiency by cotton root than low K, low P and high K treatments. A similar trend was also observed for K use efficiency by the root (Fig. 4.1).

Potassium in low P treatment is needed for the growth and development of cotton plant. Low P treatment significantly supported K use efficiency by the leaves, stem and root.

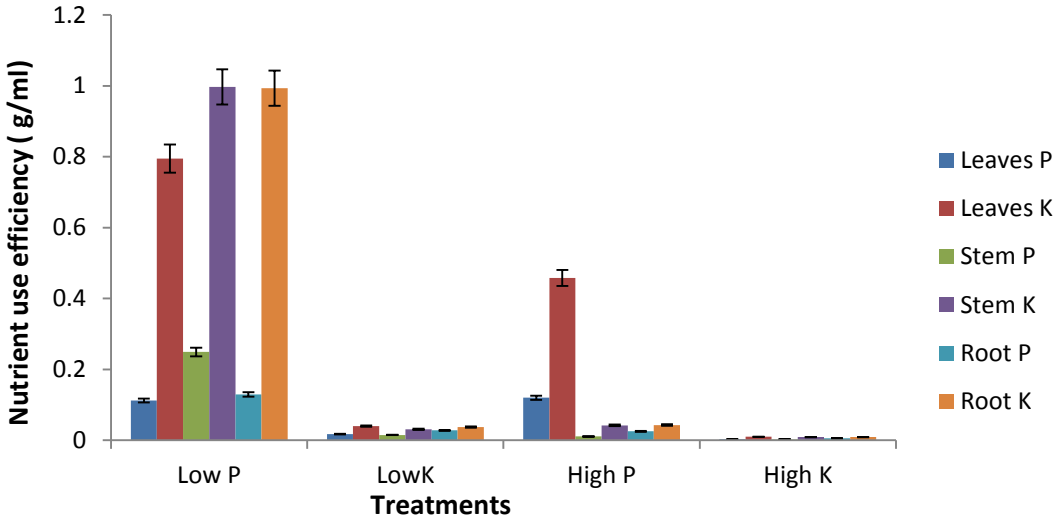

Figure 4.1 Phosphorus and potassium use efficiency in cotton plants treated with varying levels of phosphorus and potassium hydroponic solution. Low P –Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution). P – Phosphorus, K- Potassium

4.1.4 EFFECT OF PHOSPHORUS (P), POTASSIUM (K) AND PK TREATMENTS ON COTTON PLANTS CHLOROPHYLL A, B AND AB PRODUCTION IN HYDROPONIC NUTRIENT SOLUTION

The high PK treatment significantly produced the highest chlorophyll content,

which was not significantly different from low K treatment (Table 4.6). The low P treatment gave the least chlorophyll a content at 83 days after transplanting (DAT). However, at 91 DAT, low K and high PK treatments significantly produced more chlorophyll a than low P treatment. As from 104 to 148 DAT, there was no significant difference in the ability of the treatments applied to promote chlorophyll formation.

Table 4.6 The chlorophyll a content of cotton plants grown hydroponically treated to phosphorus, potassium and their combinations

	Chlorophyll a (μ gml^{-1})				
	DAT				
Treatments	83	91	104	120	148
Low P	0.456b	0.504b	1.76a	1.24a	1.02a
Low K	1.23a	1.76a	0.727a	1.76a	0.989a
High PK	1.92a	1.90a	2.39a	1.07a	0.673a
SE	0.453	0.351	0.860	0.458	0.335

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

At 83 DAT, low K and high PK treatments significantly produced more chlorophyll b than low P treatment. Similar performance was observed at 91 DAT. It is noteworthy that treatments applied had no effect on chlorophyll formation from 104 to 148 DAT (Table 4.7). The same result was obtained for chlorophyll ab (Table 4.8).

Table 4.7 The chlorophyll b content of cotton plants grown hydroponically treated to phosphorus, potassium and their combinations

	Chlorophyll b (μ gml^{-1})				
	DAT				
Treatments	83	91	104	120	148
Low P	0.084b	0.227b	0.979a	0.631a	0.185a
Low K	0.635a	1.22a	0.415a	0.876a	0.216a
High PK	0.636a	1.29a	0.402a	0.537a	0.015a
SE	0.195	0.239	0.436	0.206	0.123

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

Table 4.8 The chlorophyll ab content of cotton plants grown hydroponically treated to phosphorus, potassium and their combinations

	Chlorophyll ab (μ gml^{-1})				
	DAT				
Treatments	83	91	104	120	148
Low P	0.540b	0.731b	2.74a	1.87a	1.21a
Low K	1.87a	2.97a	1.14a	2.64a	1.21a
High PK	2.56a	3.20a	2.79a	1.61a	0.688a
SE	0.615	0.495	0.882	0.658	0.429

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.5 HORMONES CONTENT IN COTTON PLANT PARTS GROWN HYDROPONICALLY TREATED TO PHOSPHORUS (P), POTASSIUM (K) AND PK HARVESTED AT 148 DAT

Low P, low K and high PK treatments were not significant in endogenous gibberellic acid and indole-3-acetic acid content of the leaves. However, low K treatment significantly produced more zeatin in the leaves than either low P treatment or high PK treatment (Table. 4.9). Low P, low K and high PK treatments had no effect on gibberellic acid, indole-3-acetic acid and zeatin contents in the stem (Table.4.9). The application of low P, low K and high PK treatments significantly had no effect in gibberellic acid and indole-3-acetic acid contents in the roots. However, low K treatment significantly gave higher zeatin content in the root than either gibberellic acid or indole-3-acetic acid.

Table 4.9 Hormones content of cotton plants partitioning grown hydroponically treated to phosphorus, potassium and their combinations harvested at 148 Days after transplanting.

	Hormones (micro g g^{-1} fresh weight)								
Treatments	LEAVES			STEM			ROOT		
	GA_3	IAA	Z	GA_3	IAA	Z	GA_3	IAA	Z
Low P	2.22a	0.407a	0.368b	0.980a	0.124a	0.073a	0.113a	0.089a	0.084b
Low K	2.63a	0.166a	1.01a	0.853a	0.098a	0.130a	0.228a	0.112a	0.222a
High PK	1.39a	0.181a	0.537ab	0.689a	0.026a	0.104a	0.209a	0.176a	0.106b
SE	0.573	0.206	0.257	0.211	0.058	0.036	0.058	0.099	0.042

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.6 THE EFFECTS OF LOW P, LOW K AND HIGH PK TREATMENTS ON COTTON ROOT AREA PARAMETERS, ROOT VOLUME AND ROOT ACTIVITY

4.1.6.1 TOTAL ROOT ABSORPTION AREA

The low P, low K and high PK treatments did not significantly affect total root absorption area except at 104 DAT that low K and high PK treatments significantly performed better than low P treatment. The highest total root absorption was observed at 91 DAT, which could be the period when plant mostly absorbed the nutrient in the hydroponic for growth and development of cotton plants (Table 4.10).

Table 4.10 Effect of low P, low K and high PK nutrients solution on the mean root total absorption area (m^2) of cotton plants grown hydroponically over time

	Mean Total Absorption Area (m^2)				
	Days after transplanting				
Treatments	83	91	104	120	148
Low P	30.67a	133.87a	6.83b	4.65a	4.59a
Low K	21.13a	132.49a	20.15a	2.65a	2.52a
High PK	32.83a	133.48a	25.27a	2.66a	8.61a
SE	9.44	0.818	5.01	2.84	7.74

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.6.2 ACTIVE ABSORPTION AREA

At 83 DAT, low P and high PK treatments significantly supported active absorption area while low K treatment had the shortest active absorption area. The significant treatment was not observed in the nutrients applied at 91 DAT, but at 104 DAT, low K and high PK treatments significantly influenced more active absorption area than low P treatment. Conversely, at 120 DAT, low P treatment significantly increased active absorption area while low K treatment gave the least. However, at 148 DAT, low P and low K treatments significantly gave the greater active absorption area than high PK treatment. The active absorption area peak was also noticed at 91 DAT (Table 4.11).

Table 4.11 Effect of low P, low K and high PK nutrients solution on mean active absorption area (m^2) of cotton plants grown hydroponically over time

	Mean Active Absorption Area (m^2)				
	Days after transplanting				
Treatments	83	91	104	120	148
Low P	8.81a	67.34a	0.531b	5.99a	6.06a
Low K	4.28b	66.64a	10.24a	0.892c	5.56a
High PK	15.76a	66.48a	10.66a	3.67b	3.14b
SE	5.28	0.604	4.38	1.88	2.47

SE - Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.6.3 PERCENTAGE ACTIVE ABSORPTION AREA

At 83 DAT, high PK treatment significantly produced the highest percentage active absorption area, followed by low P treatment, but both treatments were not significant. The low K treatment had the least. From 91 to 148 DAT, treatments applied did not affect percentage active absorption area (Table 4.12).

Table 4.12 Effect of low P, low K and high PK nutrients solution on mean percentage active absorption area (%) of cotton plants grown hydroponically over time

	Percentage Active Absorption Area (%)				
	Days after transplanting				
Treatments	83	91	104	120	148
Low P	0.380a	0.503a	0.370a	0.626a	1.31a
Low K	0.156b	0.503a	0.579a	1.28a	1.27a
High PK	0.506a	0.498a	0.415a	0.620a	0.822a
SE	0.240	0.005	0.385	1.41	10.08

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.6.4 SPECIFIC SURFACE AREA

The low P treatment significantly gave the highest specific surface area at 83 and 91 DAT than other treatments. At 104 DAT, low K treatment significantly influenced greater specific surface area than either low P treatment or high PK treatment. At 120

DAT, low P significantly produced greater specific surface area than low K and high PK treatments. No significant differences were observed among the treatments applied at 148 DAT. Large surface area was noticed at 91 DAT (Table 4.13).

Table 4.13 Effect of low P, low K and high PK nutrients solution on mean specific surface area (m^2ml^{-1}) of cotton plants grown hydroponically over time

	Mean Specific Surface Area (m^2ml^{-1})				
	Days after transplanting				
Treatments	83	91	104	120	148
Low P	4.28a	19.52a	0.206b	0.660a	0.272a
Low K	1.23b	7.21b	3.68a	0.111b	0.126a
High PK	1.35b	5.01b	0.904b	0.089b	0.196a
SE	1.196	1.06	1.09	0.209	0.319

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.6.5 ROOT VOLUME

High PK treatment consistently had the significant largest root volume while low P treatment gave the least throughout the experimental period except at 104 DAT, no significant difference was detected (Table 4.14). It was also observed at 148 DAT that no significant differences in root volume were observed for low K and high PK treatments in one hand and low P and low K treatments on the other hand. Low P significantly produced largest volume at 104 DAT while smallest volume at 91 DAT. Low K treatment significantly gave the largest volume at 120 DAT, whereas smallest volume was noticed

at 104 DAT. High PK treatment significantly produced largest volume at 120 DAT while smallest volume was observed at 148 DAT. This reveals that high strength of nutrients in low K and high PK sustained the root growth overtime while low P nutrient was quickly used up (Table 4.14).

Table 4.14 Effect of low P, low K and high PK nutrients solution on mean root volume (ml^3) of cotton plants grown hydroponically over time

	Mean Root Volume (ml^3)				
	Days after transplanting				
Treatments	83	91	104	120	148
Low P	7.25c	7.00c	18.00a	7.50c	15.00b
Low K	17.25b	18.50b	16.00a	30.00b	20.00ab
High PK	25.25a	27.00a	28.00a	42.50a	25.00a
SE	0.871	1.28	6.33	4.66	2.58

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.6.6 ROOT ACTIVITY

The significant treatment was not observed in the ability of low P, low K and high PK treatments to support cotton root activity at 83 and 91 DAT. Beyond this period, there was a significant change; high PK treatment significantly supported more cotton root activity than the other treatments at 104 DAT. However, at 120 DAT, low P and low K treatments significantly increased root activity. There was no significant difference

detected at 148 DAT (Table 4.15).

Table 4.15 Effect of low P, low K and high PK nutrients solution on mean root activity (TTC reduction strength) of cotton plants grown hydroponically over time

	Mean Root Activity (TTC reduction strength)				
	Days after transplanting				
Treatments	83	91	104	120	148
Low P	0.0003a	0.0019a	0.0012b	0.0009a	0.0012a
Low K	0.0011a	0.0021a	0.0014b	0.0016a	0.0008a
High PK	0.0007a	0.0019a	0.0044a	0.0005b	0.0010a
SE	0.00045	0.00059	0.00077	0.00033	0.00016

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.7 NUTRIENT SOLUTION LEVEL AND RESIDUAL LEVEL OF P AND K CONTENTS IN A HYDROPONIC SYSTEM USED FOR CULTIVATING COTTON PLANTS TREATED WITH LOW P, LOW K AND HIGH PK

The nutrient solution level in the hydroponics pot low P treatment significantly had the highest nutrient solution level in all the treatments applied at 72, 91, 104 and 120 DAT while high PK treatment had the lowest level. The plants grown in the low K and high PK treatments were significantly the same at 21, 46, 57, 83 and 148 DAT (Table 4.16).

Table 4.16 Nutrient solution level of cotton plants grown hydroponically treated to phosphorus and potassium

	Nutrient solution level (cm)								
	Days after transplanting								
Treatments	21	46	57	72	83	91	104	120	148
Low P	20.04a	19.00a	19.73a	19.34a	20.34a	19.40a	18.63a	18.18a	18.38a
Low K	19.79b	16.95b	17.37b	17.25b	16.44b	16.93b	16.43b	15.45b	16.83b
High PK	19.84b	17.28b	16.93b	15.29c	15.06b	16.00c	14.60c	12.56c	15.81b
SE	0.087	0.244	0.282	0.652	0.773	0.339	0.520	1.10	0.69

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

There was no significant difference detected in residual P content in the nutrient solutions treated to low P, low K and high PK treatments at 21 DAT. However, the low K treatment significantly had the highest residual P content in all the treatments except at 46 DAT that low P and high PK treatments significantly had the same residual P level. In contrast, the low P treatment significantly gave the lowest residual P content. The data shows that P in low P treatment quickly finished while P in low K treatment accumulated in the hydroponic pot (Table 4.17). This signifies that the low P treatment had sufficient amount of P that the cotton plant could uptake. However, the residual P content in low K treatment suggests that inadequate K limited the P uptake by the cotton plants

Table 4.17 Residual phosphorus nutrient level in the hydroponics at the end of every nutrient change planted with cotton plants

	Phosphorus (ml pot^{-1})								
	Days after transplanting								
Treatments	21	46	57	72	83	91	104	120	148
Low P	0.746a	0.863b	1.49c	0.396c	0.449c	0.765c	0.563c	0.705c	0.458c
Low K	0.801a	14.67a	14.53a	16.40a	11.72a	12.15a	12.97a	11.21a	15.48a
High PK	0.775a	0.548b	4.93b	5.44b	4.65b	9.76b	5.56b	3.84b	3.58b
SE	0.083	0.697	0.541	0.482	0.375	0.697	0.946	0.704	0.509

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P –Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

The low P treatment significantly produced the higher residual K content than either high PK treatment or the low K treatment in the hydroponic nutrient solution at 21 DAT. In contrast, high PK treatment significantly had the highest concentrations of residual K in all the treatments applied. The low K treatment significantly gave the lowest concentrations of residual K from 46 to 148 DAT in all the treatments. From the data, high left over of K was observed in high PK while plant utilized K in low K treatment (Table 4.18).

Table 4.18 Residual level of Potassium nutrient in the hydroponics at the end of every nutrient change grown with cotton plants

	Potassium (ml pot^{-1})								
	Days after transplanting								
Treatments	21	46	57	72	83	91	104	120	148
Low P	291.52a	274.19b	214.85b	256.88b	260.95b	270.18b	270.52b	277.49b	270.30b
Low K	37.37c	29.49c	38.27c	32.46c	35.39c	39.68c	40.11c	45.61c	47.49c
High PK	261.77b	283.91a	249.82a	304.38a	313.88a	323.99a	338.81a	339.22a	302.50a
SE	10.06	2.57	2.86	7.86	5.93	6.13	5.56	9.27	10.57

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.1.8 EVAPOTRANSPIRATION OF NUTRIENT SOLUTION GROWN HYDROPONICALLY WITH COTTON PLANTS

High nutrient solution ET was recorded at day 21 to 46 after transplanting for the two treatments (low P, low K), low K treatment significantly gave highest nutrient solution ET than low P and High PK treatments, except nutrient solution treated to high PK treatment at 120 to 148 DAT while low ET was recorded at 72 to 83 days after transplanting (DAT) for high PK treatment, 120 to 148 DAT for low P treatment and 57 to 72 DAT for low K treatment (Fig.4.2).

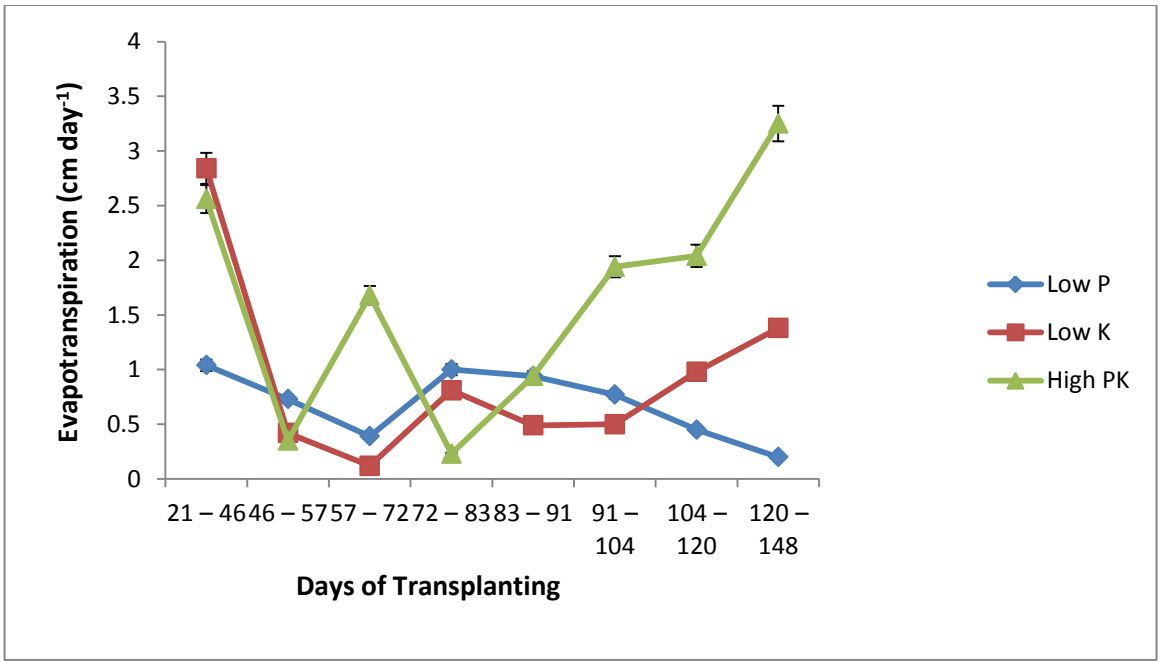

Figure 4.2 Evapotranspiration of nutrient solution treated to low P, low K and high PK grown in hydroponics nutrient solution. P- Phosphorus and K- Potassium. Low P – Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

High nutrient solution ET per day was significantly observed for high PK treatment at 91 to 104 DAT, for low P treatment, high ET per day occurred at 83 to 91 DAT and for low K treatment, high ET per day was recorded at 21 to 46 DAT. The low ET per day at 120 to 148 DAT for low P treatment, 57 to 72 DAT for low K treatment and 72 to 83 DAT for high PK treatment (Fig. 4.3).

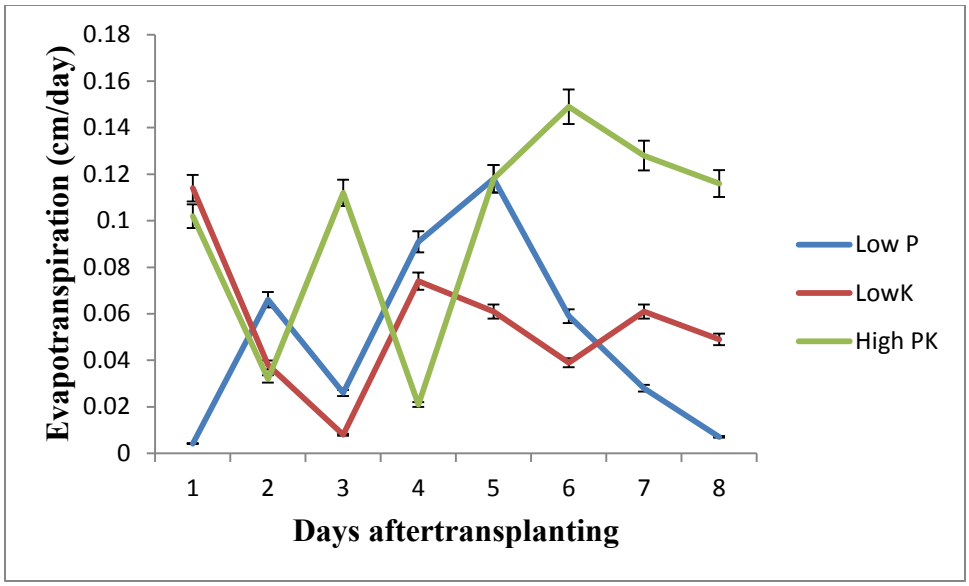

Figure 4.3 Evapotranspiration per day of nutrient solution treated to low P, low K and high PK grown in hydroponics nutrient solution. P - Phosphorus and K - Potassium. 1 Day after transplanting (DAT) = 21-46 DAT, 2 DAT = 46-57 DAT, 3 DAT = 57-72 DAT, 4 DAT = 72-83 DAT, 5 DAT = 83-91 DAT, 6 DAT = 91-104 DAT, 7 DAT = 104 – 120 DAT, 8 DAT = 104-120 DAT. SE-Standard Error. Low P –Phosphorus (half standard Hoagland solution), Low K-Potassium (one-sixth standard Hoagland solution). High PK (standard Hoagland solution)

4.2 DISCUSSION

4.2.1 EFFECT OF PHOSPHORUS (P) AND POTASSIUM (K) ON COTTON GROWTH AND FRESH BIOMASS PRODUCTION

The application of adequate mineral nutrients promotes plant vegetative growth and biomass production. The data from plant height, root length, number of leaves and leaves fresh weight showed that cotton plants treated to high phosphorus (P) and potassium (K) supported cotton growth due to availability and accessibility of nutrients in the high PK treatment in the hydroponics. Availability of nutrients to growing plants in the nutrient solution leads to increase in cell division, elongation and tissue differentiation. This agrees with findings of Moaed et al. (2010) that K nutrient content of hydroponically grown tomato influenced growth of tomato crop and Marschner (1995)

reported that greatest plant growth occurs with high concentrations of K in the soil or plants. High K content that enhanced tomato growth in the hydroponic grown tomato crop also supported growth of cotton plants. Furthermore, José et al. (2002) reported that less than 50 μM P supported major changes in Arabidopsis root formation. This suggested nutrient level of 50 μM P contributed to increase in root length, root surface area, as well as height. Ciro et al. (1999) found that P level up to 83 mg kg^{-1} supported root length and surface of cotton root. Hallmark and Barber (1984) observed that increase in soil P or solution P influenced increased in shoot and root of soybean plants. This current study noticed that 10^{-3} M P influenced plant height and root length of cotton plants grown hydroponically. Number of leaves decreased towards the end of experiment, this suggests that most of the photosynthate diverted to flower and boll formation. Standard Hoagland nutrient solution (high PK) used in this study promoted luxuriant growth of cotton plants. However, it is important to note that cotton plants with excessive nutrients and water as in high PK treatment can grow tall and develop heavy vegetative growth. This kind of growth could promote boll rot, flower and fruit abscission and prevent easy picking of good quality cotton during harvest (Marschner 1995). The nutrients in the plant tissue especially nutrients in the leaf tissue is a good indicator that could enhance cotton yield (Marschner 1995).

4.2.2 NITROGEN, PHOSPHORUS AND POTASSIUM UPTAKE BY COTTON PLANT GROWN HYDROPONICALLY

Nutrient uptake positively influenced plant growth and yield. The results obtained in NPK uptake experiment supported the assertion made by Linkohr et al. (2002);

Casimiro et al. (2003); José et al. (2002); Yan et al. (2004); Rengel and Damon (2008); Zhiyong et al. (2009) that root growth and nutrients availability are important factors for NPK nutrient uptake by plants. The considerable amount of N concentration in the low K treatment hydroponic pots and high amount of K concentration in the high PK treatment hydroponic pots (Table 3.1) enhanced N and K nutrients uptake by the cotton plants because of availability of these essential nutrients in the hydroponics Hoagland solution. Furthermore, Adams et al. (1973) confirmed that the content of K in the leaves and total uptake of K by tomatoes were controlled significantly by the N concentration in the nutrient solutions. This signifies the ability of root system to absorb different concentration of nutrients depends on nutrients availability in the hydroponics and /or soil for nutrients uptake and growth. Furthermore, potassium is needed for cotton growth and development; it influenced development of cotton plant from vegetative growth to the yield. The considerable quantity of potassium in the tissue of the cotton plant would influence cotton fibre filling, elongation, maturation and harvest in later reproductive stage (Yan et al 2004). Phosphorus enhanced root growth of cotton plant for easy nutrient uptake (Zhiyong et al. 2009)

4.2.3 PHOSPHORUS AND POTASSIUM NUTRIENT USE EFFICIENCY

Low P nutrient application at 5.0×10^{-5} M P supported high nutrient use efficiency in the hydroponically grown cotton plants. This result suggests that high P application to hydroponic nutrient solution at 10^{-3} M P or more could lead to waste; a similar result was obtained by Robert (2008). However, NUE estimated for P in low P application ranged between 11.2% to 24.9%, this range corresponded with the range of 10% to 30%

observed by Robert (2008). The higher range was recorded for K use efficiency (79.5% to 99.7%) under the low P application, but Robert (2008) recorded 20% to 60%. This suggests that K nutrition greatly enhanced cotton growth and yield (Jiang et al. 2008; Zia-UI-Hassan and Arshad 2008; Yingxia et al. 2011; Zia-uI-Hassan et al. 2011; Jiang et al. 2011; Zia-uI-Hassan and Muhammad 2011).

4.2.4 INFLUENCE OF PHOSPHORUS AND POTASSIUM ON CHLOROPHYLL FORMATION

The greenish pigment of plants indicates a healthy physiological condition of the plants. The chlorophyll a measured all photosynthesis in the plants and protozoan including cyanobacteria while chlorophyll b measured plant accessory pigment e.g carotenoid, xanthophyll (Fridgen and Varco 2004). The low K and high PK treatments increased chlorophyll a, b and ab production at 83 and 91 DAT. This corresponds to the report stated earlier that high levels of K nutrition promoted formation of chlorophyll a and b in cucumber (*Cucumis sativus cv Brunex*) leaves (Lamrani et al. 1996). Duli et al. (2001) also stated that K deficient in cotton is associated with low chlorophyll content. Beyond 91 days after transplanting, the nutrients applied did not have a significant effect. This could be attributed to plants carbohydrate in the leaves manufactured during photosynthesis is directed to cotton plant's reproductive stage. This process is also known as source-to-sink, whereby carbohydrates are transported from the leaves via phloem, supply area called source to area of growth or reproduction called sink.

4.2.5 EFFECT OF PHOSPHORUS AND POTASSIUM ON ENDOGENOUS HORMONE CONTENT

Endogenous plant hormones promote growth and development of plant (Marscher 1995; Palmer et al. 1996; Martin et al. 2000; Yan et al. 2004). Low K treatment significantly influenced zeatin content in the leaves and 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 the 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 zeatin in the leaves in this study agrees with the report of Dong and Artceca (1981) that Z concentration in the leaves influenced photosynthesis in tomatoes and endogenous zeatin content in rice plants increased the amount of zeatin in the root (Zahir et al. 2001).

4.2.6 RESPONSE OF PHOSPHORUS AND POTASSIUM ON ROOT AREA AND ROOT ACTIVITY

The P and K nutrients significantly affected cotton root area. Low P, low K and High PK treatments significantly enhanced root area formation at the beginning of the experiment while lower values were obtained towards the end of the experiment, it could be due to the fact that there was no increase in root length after 80 – 90 days after transplanting. Decrease in growth towards the end of experiment could also be due to rigorous competition for nutrients between the two plants in the same pots or containers. It could also be due to root loss especially root hairs as the plants matured (Nayakekorala

and Taylor 1990). Moreover, lower root area values were obtained in this experiment because of differences in the nutrient concentrations. Furthermore, the lower values obtained towards the end of experiment could be related to source-to-sink phenomenon where plant nutrients are transported from the roots through xylem to other parts of the plants for growth. Cotton percentage active absorption area (PAAAR) was high with plants treated to high PK at 83 DAT. Silberbush and Barber (1983) indicated that greater P and K uptake by maize may also result from the greater root growth as root growth is closely related to P and K uptake. However, High PK treatment produced the greatest cotton root volume (RV) throughout the experimental period except at 104 DAT. The high PK treatment significantly supported root volume which indicates that root hairs and lateral root hairs assist in acquisition of nutrients such as P and K by exploring a greater soil volume and by increasing the absorptive surface of the root (Hallmark and Barber 1984; Jose et al. 2002). Specific surface area (SSA) was significantly affected by nutrients solution. Low P treatment gave highest specific surface area at 83 and 91 days after transplanting. At 104 DAT, low K treatment gave highest specific surface area. At 120 DAT, low P treatment positively influenced specific surface area. Low P and low K treatments gave high specific surface area and active absorption area; this could be due to roots having direct contact with nutrients solution in hydroponic. Moreover, nutrients could be toxic to plants at high concentration greater than low P and low K.

Cotton root activity measured by triphenyltetrazolium chloride (TTC) had significant effect on treatments applied under the varying temperature of 20 to 35^{o}C at nutrient solution pH 6.5. There was no significant difference among the treatments applied from 83 to 91 days after transplanting. However, high PK, low K and low P

treatments significantly affected cotton root activities beyond 91 DAT. This could be due to variation in room temperature of dehydrogenase root activity as evaluated by the TTC method during the growth stage (McMicheal and Burke 1994). The planting environment of this current study varies between 20 to 35^{o}C

4.2.7 NUTRIENT SOLUTION LEVEL AND RESIDUAL LEVELS OF PHOSPHORUS AND POTASSIUM OF HYDROPONICALLY GROWN COTTON PLANTS

The results of this study illustrate the effectiveness of cotton plants to utilize the nutrient solutions (such as P and K nutrients) in the hydroponics system during growth and development stages of cotton plants. The residual levels of P and K at every sampling period showed that the low K treatment gave highest residual P concentrations in all the treatments while low P treatment gave the least. On the other hand, residual K content in the Hoagland standard solution high PK treatment had the highest residual K in all the treatments while low K treatment had the least. This indicates that an increase accumulation of nutrient concentrations in the hydroponics or soils far above recommended nutrient concentration can lead to fertilizer wastage (Hallmark and Barber 1984). Furthermore, plants in low K treatment effectively utilized K nutrient in the hydroponics for growth and development. This result was in line with Asher and Ozanne (1967) who noticed that K content in the nutrient solution increased the K content and yield of both the shoots and roots of several pasture crop species. Zhiyong et al. (2009) confirmed that high K nutrient is needed for cotton growth and yield. Conversely, P decrease in low P treatment in the nutrients solution implies that high content of P is required by cotton plants at this period for growth, development and yield (Hallmark and

Barber 1984; José et al. 2002). However, frequent addition of nutrients to the soil or in hydroponic systems can result in high residual level of nutrients in solution. This may provide ample available nutrients or its level may be toxic to the crops.

4.2.8 EVAPOTRANSPIRATION OF NUTRIENT SOLUTION TREATED TO PHOSPHORUS AND POTASSIUM.

The high nutrient solution evapotranspiration rate was noticed at the early stage of growth (21-46 DAT) in the hydroponic pots treated to low P and low K treatments excluding high PK treatment at 120-148 DAT. This indicates that potassium nutrition is needed for growth of cotton plants (Ebdon et al. 1998). Furthermore, potassium concentration in the guard cell of the leaves influenced opening and closing of stomata which regulates entry of water and carbon dioxide in and out of the leaves for the process of photosynthesis (Burba et al. 2006). High ET per day was also noticed at 21-46 DAT for low K treatment and 83-91 DAT for low P treatment during the reproductive stage which indicates that phosphorus is also needed at this stage for flowers and fruits production (Linkohr et al. 2002; Yan et al. 2004).

4.3 PHYTOHORMONE EXPERIMENT

4.3.1 EFFECT OF EXOGENOUS HORMONES TREATMENTS ON COTTON GROWTH AND FRESH BIOMASS PRODUCTION AT THE HIGH LEVEL OF PHOSPHORUS AND POTASSIUM NUTRIENTS GROWN HYDROPONICALLY

4.3.1.1 PLANT HEIGHT

The significant difference was not observed in the effectiveness of treatments to support plant height throughout the experimental period except at 80 DAT, Z treatment

significantly increased plant height while 2IAA x GA_3 x Z and IAA x $2GA_3$ x Z treatments produced the least height, although Z treatment was not significantly different from control. At 80 DAT, single application of phytohormones performed better than combined application. This reveals that there is no synergistic interaction between the concentrations of phytohormones applied to support plant height (Table 4.19).

Table 4.19 Effect of hormones on cotton plants height grown in high level of phosphorus and potassium hydroponic nutrients solution

Treatment levels	Plant Height (cm)			
	DAT			
	43	74	80	90
Control	13.75a	26.73a	28.67ab	37.00a
IAA	14.95a	30.78a	31.08ab	38.73a
GA_3	14.98a	30.95a	30.80ab	42.37a
Z	15.24a	32.45a	34.70a	41.10a
IAA x GA_3 x Z	18.77a	28.32a	28.00ab	37.83a
2IAA x GA_3 x Z	14.60a	26.51a	24.15b	37.77a
IAA x $2GA_3$ x Z	14.88a	26.01a	25.03b	36.13a
IAA x GA_3 x 2Z	14.10a	29.39a	27.57ab	43.82a
2IAA x $2GA_3$ x 2Z	13.91a	28.26a	28.08ab	37.63a
SE	1.69	2.97	3.86	3.74

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. IAA-Indole-3-acetic acid, GA_3-Gibberellic acid and Z- Zeatin. High PK (standard Hoagland solution)

4.3.1.2 ROOT LENGTH

The combined use of IAA x GA_3 x Z treatment significantly produced the longest root length when compared to other treatments while GA_3 treatment produced the least root length, but GA_3 treatment was not significantly different from other treatments including control at 43 DAT. The IAA x GA_3 x 2Z treatment significantly gave longest root, but not significant different from other treatments including control while 2IAA x GA_3 x Z treatment had the least root length at 74 DAT (Table 4.20).

Table 4.20 Effect of hormones on cotton root length grown in high level of phosphorus and potassium hydroponic nutrients solution

Treatment levels	Root Length (cm)			
	Days After Transplanting			
	43	74	80	90
Control	30.48b	45.51a	48.85a	52.13a
IAA	29.42b	44.16a	43.70a	51.82a
GA_3	28.97b	46.03a	46.68a	50.27a
Z	30.85b	46.48a	47.72a	49.47a
IAA x GA_3 x Z	35.80a	44.56a	43.88a	48.63a
2IAA x GA_3 x Z	30.27b	37.05b	39.13a	52.02a
IAA x 2GA_3 x Z	29.59b	42.02ab	40.33a	49.38a
IAA x GA_3 x 2Z	29.56b	46.55a	44.73a	51.70a
2IAA x 2GA_3 x 2Z	29.83b	44.39a	42.77a	49.35a
SE	2.02	2.94	4.56	3.94

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. IAA-Indole-3-acetic acid, GA_3-Gibberellic acid and Z- Zeatin. High PK (standard Hoagland solution)

4.3.1.3 NUMBER OF LEAVES

The treated plants and untreated plants significantly appeared the same. The significant treatment was not detected in the number of leaves produced in the phytohormone amended hydroponic solution (Table 4.21).

Table 4.21 Leaf numbers of cotton plants treated to different hormones concentration grown hydroponically in high level of phosphorus and potassium nutrients

Treatment levels	Number of Leaves	
	Days After Transplanting	
	43	74
Control	5.08a	8.25a
IAA	4.58a	9.17a
GA_3	4.67a	8.42a
Z	4.71a	9.75a
IAA x GA_3 x Z	5.46a	8.50a
2IAA x GA_3 x Z	4.83a	8.00a
IAA x 2GA_3 x Z	4.88a	8.83a
IAA x GA_3 x 2Z	4.29a	7.92a
2IAA x 2GA_3 x 2Z	4.79a	9.33a
SE	0.329	0.656

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. IAA-Indole-3-acetic acid, GA_3-Gibberellic acid and Z- Zeatin. High PK (standard Hoagland solution)

4.3.1.4 FRESH BIOMASS PRODUCTION

The significant treatment was not observed between untreated plants and treated plants. Therefore, biomass fresh weight was not affected by spraying phytohormones to the growing medium for cotton plants (Table 4.22).

Table 4.22 Effect of phytohormones on fresh biomass weight of hydroponically grown cotton plants harvested at 90 days after transplanting

	Fresh Biomass weight		
Treatments	Leaves fresh weight (g)	Stem fresh weight (g)	Root fresh weight (g)
Control	0.5294a	0.4378a	0.4519a
IAA	0.4831a	0.4595a	0.4537a
GA_3	0.4754a	0.4664a	0.4917 a
Z	0.5306a	0.5238a	0.4937a
IAA x GA_3 x Z	0.4642a	0.4610a	0.4351a
2IAA x GA_3 x Z	0.4758a	0.4314a	0.4289a
IAA $2GA_3$ x Z	0.5119a	0.5094a	0.4938a
IAA x GA_3 x 2Z	0.4832a	0.4495a	0.4824a
2IAA x $2GA_3$ x 2Z	0.5147a	0.4515a	0.5028a
SE	0.0174	0.024	0.033

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Turkey-Kramer test. IAA-Indole-3-acetic acid, GA_3-Gibberellic acid and Z- Zeatin. High PK (standard Hoagland solution)

4.3.1.5 NITROGEN (N), PHOSPHORUS (P) AND POTASSIUM (K) UPTAKE BY COTTON PLANTS PARTITIONING TREATED TO DIFFERENT HORMONES GROWN HYDROPONICALLY HARVESTED AT 90 DAT

LEAVES: The IAA treatment significantly enhanced more N uptake by the leaves than other treatments while Z treatment gave the least, but IAA treatment was not significantly different from other treatments including control. The combination of IAA x $2GA_3$ x Z treatment significantly influenced P uptake by the leaves while Z treatment gave the least, but IAA x $2GA_3$ x Z treatment was not significantly different from other treatments including control. There was no significant difference detected in the effectiveness of phytohormones applied to influence K uptake by the leaves (Table 4.23).

STEM: The 2IAA x GA_3 x Z treatment significantly gave the highest N uptake by the stem than other treatments while control gave the least, although control was not significant different from other treatments. The double level of IAA in synergistic interaction with GA_3 and Z significantly produced N uptake by the stem. Both Z and IAA x GA_3 x Z treatments significantly supported P uptake by the leaves whereas IAA x GA_3 x 2Z treatment gave the least, but Z and IAA x GA_3 x Z treatments were not significantly different from other treatments including control. The table 4.23 also shows that Z treatment significantly produced highest P uptake in the stem than the other treatments whereas Z treatment gave the lowest P uptake in the leaves. The 2IAA x GA_3 x Z treatment significantly enhanced more K uptake by the stem than the other treatments

while IAA, GA_3, IAA x GA_3 x 2Z and 2IAA x $2GA_3$ x 2Z treatments gave the least, but 2IAA x GA_3 x Z treatment was not significantly different from other treatments including control.

ROOT: The plants treated with IAA x GA_3 x Z treatment significantly influenced greatest N uptake by the root than other treatments while IAA treatment gave the least N uptake by the root, but IAA x GA_3 x Z treatment was not significantly different from other treatments and control. The IAA x $2GA_3$ x Z treatment significantly gave the highest P uptake by the root than other treatments applied, but IAA x $2GA_3$ x Z treatment was not significantly different from control while IAA, GA_3 and Z treatment gave the least P uptake by the root. There was a synergistic interaction of the three treatments to influence P uptake by the root. There was no significant difference observed in the ability of the treatments to support K uptake by the root (Table 4.23).

Table 4.23 The uptake (mg g^{-1}) of NPK by cotton plants partitioning treated to different hormones concentration grown hydroponically in high PK level harvested at 90 days after transplanting.

Treatment Combinations	NPK Uptake (mg g^{-1})								
	LEAVES			STEM			ROOT		
	N	P	K	N	P	K	N	P	K
Control	36.32ab	8.67ab	47.46a	14.77b	3.61ab	54.83ab	32.62ab	8.38ab	62.88a
IAA	42.71a	7.98ab	43.82a	17.36ab	3.70ab	51.04b	24.97b	7.24b	47.43a
GA_3	37.69ab	8.49ab	44.56a	20.07ab	3.49ab	49.41b	29.93ab	7.71b	56.22a
Z	31.20b	7.38b	48.49a	26.81ab	4.32a	53.07ab	28.69ab	8.03b	57.00a
IAA*GA_3*Z	38.11ab	9.43ab	52.12a	24.50ab	4.31a	55.70ab	39.59a	11.48ab	63.60a
2IAA*GA_3*Z	36.10ab	8.24ab	46.63a	38.23a	4.02ab	66.73a	38.77ab	9.90ab	61.53a
IAA*2GA_3*Z	35.44ab	11.40a	50.58a	21.85ab	4.07ab	59.44ab	29.73ab	12.29a	63.60a
IAA*GA_3*2Z	36.42ab	8.58ab	48.69a	23.93ab	2.96b	48.84b	37.33ab	8.05ab	56.04a
2IAA*2GA_3*2Z	39.35ab	7.80ab	44.44a	17.12ab	3.27ab	50.45b	37.33ab	8.05ab	56.04a
SE	5.43	1.58	4.08	9.49	0.591	6.25	7.04	2.04	9.08

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. IAA-Indole-3-acetic acid, GA_3-Gibberellic acid and Z- Zeatin. High PK (standard Hoagland solution)

4.3.1.6 EFFECT OF HORMONES TREATMENTS ON COTTON PLANTS CHLOROPHYLL A, B AND AB PRODUCTION IN HYDROPONICS NUTRIENT SOLUTION

The single applied IAA and Z treatments significantly enhanced chlorophyll a production, but there was no significant difference detected between plants treated with

IAA, Z treatments and those plants treated with GA_3, IAA x GA_3 x 2Z treatments while combined use of 2IAA with GA_3 and Z including untreated plants gave the least chlorophyll a production at 80 DAT. Surprisingly, at 90 DAT, the untreated pots and treated pots were not significantly different (Table 4.24).

The combined application of IAA x GA_3 x 2Z treatment significantly gave the highest chlorophyll b production, although combined hormone application of IAA x GA_3 x 2Z treatment was the same as with a single applied IAA, GA_3 and Z phytohormone treatments. However, combined use of 2IAA x GA_3 x Z treatment including untreated plants gave the least chlorophyll b formation at 80 DAT. Thereafter, at 90 DAT, untreated plants and treated plants gave the same results (Table 4.24).

The single hormone application of IAA, Z and combined hormone application of IAA x GA_3 x 2Z significantly gave the highest chlorophyll ab production but these treatments (IAA, Z, and IAA x GA_3 x 2Z) were not significant to single phytohormone application of GA_3 at 80 DAT. However, at 90 DAT, control experiment had more chlorophyll ab than other treated pots, but there was no significant difference between control and either IAA treatment or 2IAA x GA_3 x Z treatment (Table 4.24)

Table 4.24 Effect of hormones concentration on chlorophyll content of cotton plants grown hydroponically in high level of PK

	Chlorophyll (μ gml^{-1})					
	80			Days after transplanting (DAT)		90
Treatments	a	b	ab	a	b	ab
Control	0.798cd	0.327de	1.13bc	1.34a	0.716a	2.05a
IAA	2.53a	1.23ab	3.76a	0.783ab	0.489ab	1.27ab
GA_3	1.62abc	0.933abc	2.55ab	0.261b	0.196b	0.457b
Z	2.48a	1.27ab	3.75a	0.473b	0.307b	0.783b
IAA x GA_3 x Z	0.670cd	0.407cde	1.08bc	0.409b	0.193b	0.561b
2IAA x GA_3 x Z	0.209d	0.149e	0.359c	0.910ab	0.377b	1.29ab
IAA x 2GA_3 x Z	1.05cd	0.491cde	1.54bc	0.342b	0.206b	0.549b
IAA x GA_3 x 2Z	2.33ab	1.46a	3.79a	0.514b	0.329b	0.843b
2IAA x 2GA_3 x 2Z	1.34bc	0.732bcd	2.08b	0.328b	0.259b	0.587b
SE	0.476	0.256	0.708	0.298	0.160	0.446

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. IAA-Indole-3-acetic acid, GA_3-Gibberellic acid and Z- Zeatin. High PK (standard Hoagland solution)

4.3.1.7 EFFECT OF EXOGENOUS APPLICATION OF GA_3, IAA, Z AND THEIR COMBINATIONS ON COTTON PLANT PARTS ENDOGENOUS HORMONE CONTENTS GROWN HYDROPONICALLY TREATED TO HIGH LEVEL OF PHOSPHORUS AND POTASSIUM HARVESTED AT 90 DAT

The effect of Gibberellic acid (GA_3), Indole-3-acetic acid (IAA), Zeatin (Z) treatments and their combinations on each cotton plant partitioning grown hydroponically

treated to Hoagland standard solution high phosphorus and potassium.

LEAVES: A significant difference was not detected between the plants treated to phytohormones and control in relation to endogenous content of GA_3 and Z in the leaves. The significant highest endogenous IAA content in the leaves was produced by IAA treatment while 2IAA x GA_3 x Z treatment gave the lowest. This shows that double application level of IAA with GA_3 and Z combination reduced the endogenous content of IAA in the leaves (Table 4.25).

STEM: A significant difference was not observed between phytohormones treated plants and controls in relation to endogenous IAA content in the stem. However, the treatments applied significantly increased GA_3 and Z contents in the stem, the plants treated with IAA x GA_3 x Z hormone significantly produced more endogenous GA_3 and Z in the stem than the other treatments including control (Table 4.25).

ROOT: A significant difference was not detected between plants treated to different phytohormones and untreated plants in relation to gibberellic acid (GA_3) and Indole-3-acetic acid (IAA). However, Z treatment significantly gave the highest zeatin (Z) content in the roots than the other treatments, although there was no significant difference between Z treatment and IAA x 2GA_3 x Z treatment including control while other treatments gave the least Z content (Table 4.25).

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Table 4.25 Effect of exogenous application of GA_3, IAA, Z and their combinations on cotton plant parts endogenous hormones contents grown hydroponically treated to high level of phosphorus and potassium harvested at 90 DAT

Treatment Combinations	micro g g^{-1} fresh weight								
	Gibberellic acid (GA_3)			Indo-3-acetic acid (IAA)			Zeatin (Z)		
	L	S	R	L	S	R	L	S	R
Control	1.43a	0.022b	3.73a	0.216ab	0.011a	0.195a	0.204ab	0.022b	0.287ab
IAA	0.848a	0.025b	4.96a	0.267a	0.012a	0. 241a	0.093b	0.013b	0.164b
GA_3	0.930a	0.020b	5.13a	0.101ab	0.012a	0.519a	0.211b	0.014b	0.071b
Z	1.43a	0.028b	0.592a	0.091ab	0.011a	0.145a	0.137b	0.015b	1.04a
IAA*GA_3*Z	0.117a	0.121a	0.184a	0.0092b	0.0086a	0.0094a	0.717a	0.168a	0.122b
2IAA*GA_3*Z	1.78a	0.024b	3.04a	0.048b	0.011a	0.079a	0.098b	0.010b	0.143b
IAA*2GA_3*Z	2.10a	0.017b	2.80a	0.174ab	0.014a	0.228a	0.128b	0.016b	0.382ab
IAA*GA_3*2Z	2.06a	0.023b	1.02a	0.211ab	0.012a	0.187a	0.099b	0.024b	0.134b
2IAA*2GA_3*2Z	1.76a	0.018b	1.49a	0.171ab	0.011a	0.402a	0.339ab	0.011b	0.091b
SE	1.20	0.024	2.94	0.09	0.0031	0.223	0.208	0.032	0.343

SE-Standard Error. IAA-Indole -3-acetic acid,GA_3-Gibberellic acid, Z-Zeatin Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. High PK- Standard Hoagland solution. L-Leaves, S- Stem, R- root

4.3.1.8 THE INFLUENCE OF HORMONES ON COTTON ROOTS AREA, ROOT VOLUME AND ROOT ACTIVITY GROWN HYDROPONICALLY IN HIGH LEVEL OF PHOSPHORUS AND POTASSIUM.

4.3.1.8.1 TOTAL ROOT ABSORPTION AREA (TAA)

The plants treated to GA_3 treatment significantly gave the highest total absorption root area than the other treatments while IAA treatment gave the least, although the

highest and lowest treatments were not significantly different from control at 80 DAT (Table 4.26). Moreover, IAA, IAA x GA_3 x 2Z treatments and control significantly influenced more total absorption area than other treatments while GA_3 treatment gave the least at 90 DAT. (Table 4.27).

4.3.1.8.2 ACTIVE ROOT ABSORPTION AREA (AAA)

All the treatments applied significantly increased root active absorption area except 2IAA x GA_3 x Z treatment and control experiment at 80 DAT (Table 4.26).

The combined application of IAA x GA_3 x 2Z treatment significantly supported largest cotton root active absorption area than other treatments while the combined use of IAA x GA_3 x Z treatment gave the least cotton root active absorption area at 90 DAT (Table 4.27).

4.3.1.8.3 PERCENTAGE ACTIVE ABSORPTION AREA (AAAR)

The IAA x GA_3 x Z treatment significantly gave the highest percentage active absorption area, but not significantly different from other treatments including control while 2IAA x GA_3 x Z and IAA x GA_3 x 2Z treatments gave the least at 80 DAT (Table 4.26).

The plants treated with GA_3 phytohormone treatment performed better than plants treated to other single and combined applied phytohormones treatments. It was observed that plants treated with IAA x GA_3 x Z treatment had a least percentage active absorption area ratio (Table 4.27).

4.3.1.8.4 SPECIFIC SURFACE AREA

The GA_3 treatment significantly gave the largest specific surface area than the other phytohormones treated plants, but GA_3 and 2IAA x GA_3 x Z, IAA x $2GA_3$ x Z treatments and control were not significantly different while IAA, Z and 2IAA x $2GA_3$ x 2Z treatments gave the smallest specific surface area at 80 DAT (Table 4.26). However, at 90 DAT, the significant largest specific surface area was observed in plants treated with 2IAA x GA_3 x Z treatment while those treated with IAA x GA_3 x 2Z treatment gave the least, but no significant difference detected from other phytohormones treatments except GA_3 treatment (Table 4.27).

4.3.1.8.5 ROOT VOLUME

The IAA, Z and 2IAA x $2GA_3$ x 2Z treatments significantly had more root volume than those treated with IAA x GA_3 x Z, 2IAA x GA_3 x Z, IAA x $2GA_3$ x Z treatments and control at 80 days after transplanting (Table 4.26).

Nevertheless, IAA x GA_3 x 2Z treatment and control significantly favoured root volume whereas plant treated with 2IAA x GA_3 x Z treatment gave the least (Table 4.27).

Table 4.26 Effect of hormones concentration on mean roots area of cotton plants grown hydroponically in high level of PK at 80 days after transplanting

ROOT AREA/ ROOT VOLUME					
	Days After Transplanting				
Treatments	TAA(m^2)	AAA(m^2)	PAAAR(m^2)	RV(ml)	SSA(m^2ml^{-1})
Control	29.60ab	1.55b	0.098ab	2.00b	20.85ab
IAA	18.83b	12.03a	0.615ab	7.00a	3.34c
GA_3	43.86a	6.94a	0.189ab	5.50ab	30.53a
Z	30.68ab	8.96a	0.359ab	8.00a	3.84c
IAA x GA_3 x Z	21.94b	12.62a	0.733a	1.50b	14.11b
2IAA x GA_3 x Z	30.78ab	4.61b	0.135b	1.50b	22.23ab
IAA x 2GA_3 x Z	33.59ab	9.16a	0.500ab	1.50b	26.40ab
IAA x GA_3 x 2Z	19.52b	12.08a	0.005b	5.50ab	14.76b
2IAA x 2GA_3 x 2Z	31.29ab	7.03a	0.654ab	6.50a	7.02c
SE	8.28	5.20	0.358	1.90	8.79

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. IAA- Indo-3-acetic acid, GA_3 -Gibberellic acid, Z- Zeatin, TAA-Total absorption area, AAA-Active absorption area, PAAAR – Percentage active absorption area, RV-Root volume, SSA-Specific surface area. High PK Standard Hoagland solution

Table 4.27 Effect of hormones on mean roots area of cotton plants grown hydroponically in high level of PK at 90 days after transplanting

	ROOT AREA/ ROOT VOLUME				
	Days After Transplanting				
Treatments	TAA(m^2)	AAA(m^2)	PAAAR(m^2)	RV(ml)	SSA(m^2/ml)
Control	43.14a	13.20b	0.298bcd	15.50ab	3.27bc
IAA	45.25a	6.12b	0.130cd	10.00b	4.53bc
GA_3	13.20d	13.37b	1.10a	2.00de	9.89b
Z	21.60c	11.80b	0.158cd	6.00cde	4.66bc
IAA x GA_3 x Z	34.28abc	2.84c	0.092d	6.50cde	6.78bc
2IAA x GA_3 x Z	26.77bc	13.01b	0.510bc	1.50e	21.39a
IAA x 2GA_3 x Z	31.81bc	13.49b	0.442bc	8.00cd	4.51bc
IAA x GA_3 x 2Z	42.10a	23.64a	0.691b	20.00a	2.19c
2IAA x 2GA_3 x 2Z	36.93ab	10.20b	0.282bcd	7.50cde	5.57bc
SE	5.95	4.17	0.20	2.84	3.03

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. IAA- Indo-3-acetic acid, GA_3 -Gibberellic acid, Z- Zeatin, TAA-Total absorption area, AAA-Active absorption area, PAAAR – Percentage active absorption area, RV-Root volume, SSA-Specific surface area. High PK – Standard Hoagland solution

4.3.1.8.6 ROOT ACTIVITY

Single applied GA_3 phytohormone treatment and combined applied IAA x GA_3 x 2Z phytohormone treatment significantly favoured highest cotton root activity than other phytohormones treated plants while combined use of 2IAA x GA_3 x Z, IAA x 2GA_3 x Z treatments and control gave lowest root activity, but not significantly different with plants

treated to single applied Z treatment, combined applied IAA x GA_3 x Z and 2IAA x $2GA_3$ x 2Z treatments at 80 days after transplanting. It is noteworthy at 90 days after transplanting that combined application of IAA x GA_3 x 2Z treatment significantly performed better than the other treatments and control (Table 4.28).

Table 4.28 Influence of hormones concentration on mean cotton root activity (TTC reduction strength) grown hydroponically in high level of PK

	Days after transplanting (TTC reduction strength)	
Treatments	80	90
Control	0.0011c	0.0011b
IAA	0.0032ab	0.0028b
GA_3	0.0040a	0.0013b
Z	0.0024abc	0.0012b
IAA x GA_3 x Z	0.0014bc	0.0012b
2IAA x GA_3 x Z	0.0006c	0.0013b
IAA x $2GA_3$ x Z	0.0010c	0.0015b
IAA x GA_3 x 2Z	0.0040a	0.0058a
2IAA x $2GA_3$ x 2Z	0.0022abc	0.0013b
SE	0.001	0.003

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. IAA- Indo-3-acetic acid, GA_3 -Gibberellic acid, Z- Zeatin, TAA-Total absorption area, AAA-Active absorption area, PAAAR -Percentage active absorption area, RV-Root volume, SSA-Specific surface area. High PK – Standard Hoagland solution

4.3.2 EFFECT OF HORMONES TREATMENTS ON NUTRIENT SOLUTION LEVEL AND RESIDUAL LEVEL OF P AND K IN THE HYDROPONICALLY GROWN COTTON

A significant difference was not detected between treated hydroponic pots and untreated hydroponic pots at 74 and 90 DAT, because those plant treated with 2IAA x GA_3 x Z treatment and other treatments including control were significantly similar at 74DAT. Furthermore, plant treated with IAA x GA_3 x Z treatment, control and other treatments excluding the IAA x GA_3 x 2Z treatment significantly appear the same at 90 DAT. It is noteworthy that at 43 DAT, IAA x GA_3 x Z treatment significantly had the lowest nutrient solution level while the other treatments including control had the highest level. However, at 80 DAT, IAA x GA_3 x Z, 2IAA x GA_3 x Z and IAA x $2GA_3$ x Z treatments significantly had the highest nutrient solution level whereas other treatments had the lowest level (Table 4.29).

Table 4.29 Nutrient solution level as influenced by hormones grown hydroponically in high PK nutrients level.

	Nutrient solution level (cm)			
	Days After Transplanting (DAT)			
Treatment levels	43	74	80	90
Control	18.78a	15.90ab	18.42b	12.50ab
IAA	18.43a	15.94ab	18.17b	14.32ab
GA_3	18.57a	15.49ab	18.25b	13.42ab
Z	18.50a	14.63b	18.18b	13.17ab
IAA x GA_3 x Z	17.55b	15.41ab	19.42a	16.00a
2IAA x GA_3 x Z	18.24a	16.96a	19.90a	15.15ab
IAA x 2GA_3 x Z	18.48a	15.99ab	19.03ab	14.47ab
IAA x GA_3 x 2Z	18.14a	14.53b	18.23b	11.47b
2IAA x 2GA_3 x 2Z	18.38a	14.93b	18.42b	14.33ab
SE	0.296	0.830	0.432	1.71

SE- Standard Error. IAA-Indole -3-acetic acid, GA_3-Gibberellic acid, Z-Zeatin. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. P-Phosphorus and K-Potassium. High PK – Standard Hoagland solution

The untreated pots significantly produced highest residual P than other treatments while IAA x GA_3 x Z treatment gave the least at 43 DAT. The data showed that IAA x GA_3 x Z treatment influenced effective phosphorus utilization by cotton plants. Similarly, those that are treated with GA_3, Z, GA_3 treatments and control significantly produced highest residual P than the other treatments at 74, 80 and 90 DAT, respectively. The plants

treated with IAA x GA_3 x Z treatment at 43 DAT, 2IAA x $2GA_3$ x 2Z treatment at 74 DAT, 2IAA x GA_3 x Z treatment at 80 and 90 DAT significantly had the lowest residual P (Table 4.30). This signifies that these treatments enhanced utilization of phosphorus by cotton plants.

Table 4.30 Residual phosphorus nutrient solution analysis treated to different hormones concentration grown hydroponically in high level of PK nutrients planted with cotton plants

	Phosphorus (ml pot^{-1})			
	Days After Transplanting (DAT)			
Treatment levels	43	74	80	90
Control	1.35a	0.988ab	2.70ab	1.52ab
IAA	1.22ab	1.01ab	2.84ab	1.89ab
GA_3	1.22ab	1.17a	3.12ab	2.10a
Z	1.21ab	0.675ab	3.53a	1.86ab
IAA x GA_3 x Z	1.11b	0.498ab	2.62ab	1.81ab
2IAA x GA_3 x Z	1.18ab	0.779ab	2.35b	1.29b
IAA x $2GA_3$ x Z	1.27ab	0.729ab	3.11ab	1.94ab
IAA x GA_3 x 2Z	1.31a	0.453ab	3.27ab	1.55ab
2IAA x $2GA_3$ x 2Z	1.31a	0.383b	2.68ab	1.52ab
SE	0.087	0.328	0.399	0.328

SE-Standard Error. IAA-Indole -3-acetic acid,GA_3-Gibberellic acid, Z-Zeatin. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. P-Phosphorus and K-Potassium. High PK – Standard Hoagland solution

At 43 DAT, the pot treated to IAA x GA_3 x Z treatment significantly had the highest residual K content while the other treatments including control had the lowest residual K content in the hydroponic nutrient solution. Contrary to the result obtained for P, the IAA x GA_3 x Z treatment build up K content in the hydroponic system. At 74 DAT, IAA x GA_3 x Z treatment, other treatments and control except 2IAA x GA_3 x Z and IAA x $2GA_3$ x Z treatments significantly increased residual K. At 80 DAT, GA_3 treatment significantly had the highest residual K, but no significant difference observed from other treatments including control while IAA treatment had the least, but significant difference was not detected between IAA and other treatments including control. The effectiveness of phytohormones treatments on K residual level was not significant at 90 DAT (Table 4.31).

Table 4.31 Residual potassium nutrient solution analysis treated to different hormones concentration grown hydroponically in high level of PK nutrients planted with cotton plants

	Potassium (ml pot^{-1})			
	Days After Transplanting (DAT)			
Treatment levels	43	74	80	90
Control	136.86b	153.02ab	145.15ab	163.36a
IAA	144.53b	154.33ab	133.44b	190.67a
GA_3	139.53b	154.65ab	155.55a	164.66a
Z	137.19b	152.37ab	145.15ab	187.42a
IAA x GA_3 x Z	160.22a	165.41a	136.04ab	159.45a
2IAA x GA_3 x Z	146.20b	149.76b	137.99ab	165.31a
IAA x 2GA_3 x Z	148.21b	150.74b	143.85ab	172.46a
IAA x GA_3 x 2Z	142.87b	157.26ab	143.85ab	180.91a
2IAA x 2GA_3 x 2Z	140.86b	159.54ab	149.70ab	172.46a
SE	5.04	6.14	8.59	27.89

SE-Standard Error. Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test. IAA-Indole-3-acetic acid, GA_3-Gibberellic acid and Z- Zeatin. High PK (standard Hoagland solution)

4.3.3 INFLUENCE OF PHYTOHORMONES ON EVAPOTRANSPIRATION OF NUTRIENT SOLUTION IN THE HYDROPONICS PLANTED COTTON

Evapotranspiration of nutrient solution was high in all the treatments at 80-90 DAT. Hydroponic pot treated to IAA x GA_3 x 2Z treatment significantly gave the highest evapotranspiration whereas the hydroponic pot treated to IAA x GA_3 x Z treatment had

the least evapotranspiration of nutrient in the hydroponic nutrient solution (Fig. 4.3).

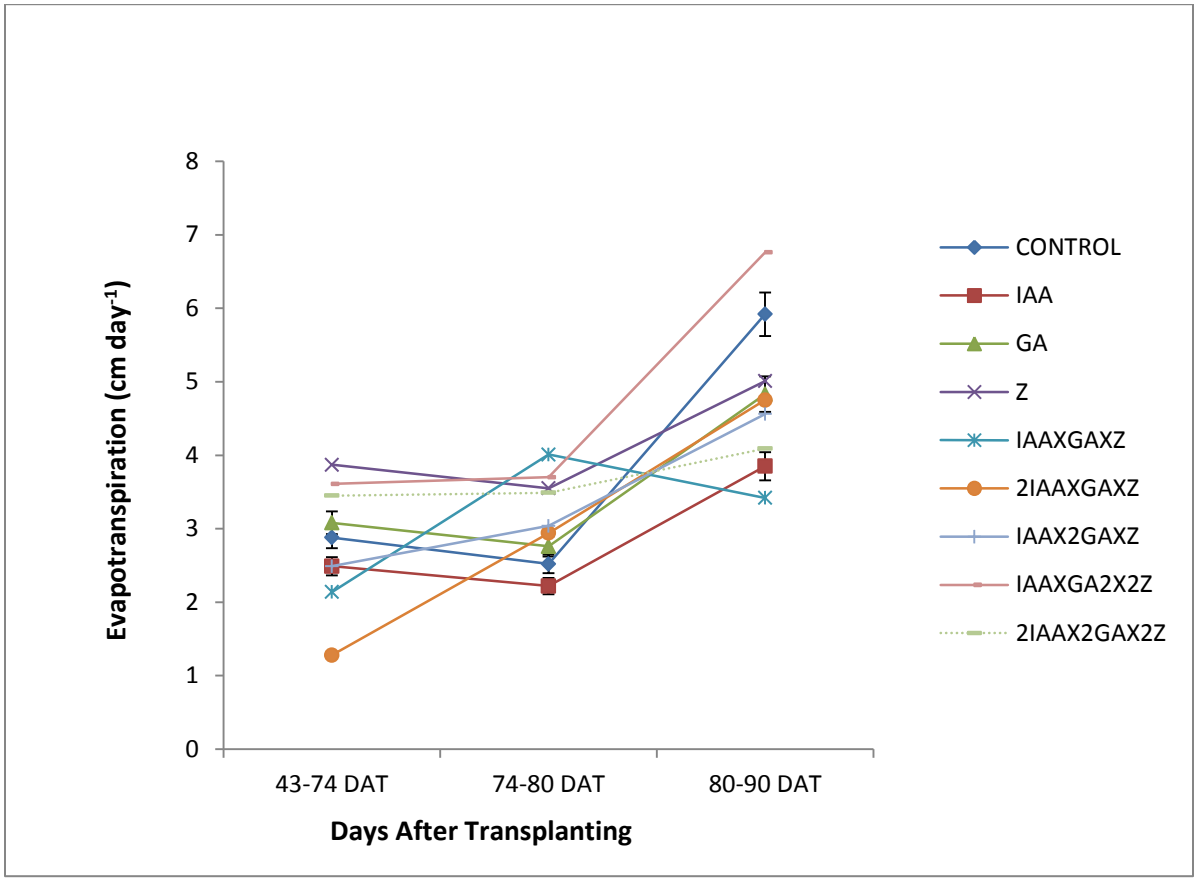

Figure 4.4 Influence of hormones on nutrient solution evapotranspiration of cotton plants grown hydroponically. IAA-Indole-3-acetic acid, GA_3 - Gibberellic acid, Z- Zeatin

Contrary result was obtained for nutrient solution ET per day, highest ET per day was noticed at 74 to 80 DAT (Fig 4.4). The hydroponic pot treated to IAA x GA_3 x Z significantly gave highest ET per day whereas IAA treatment gave the least.

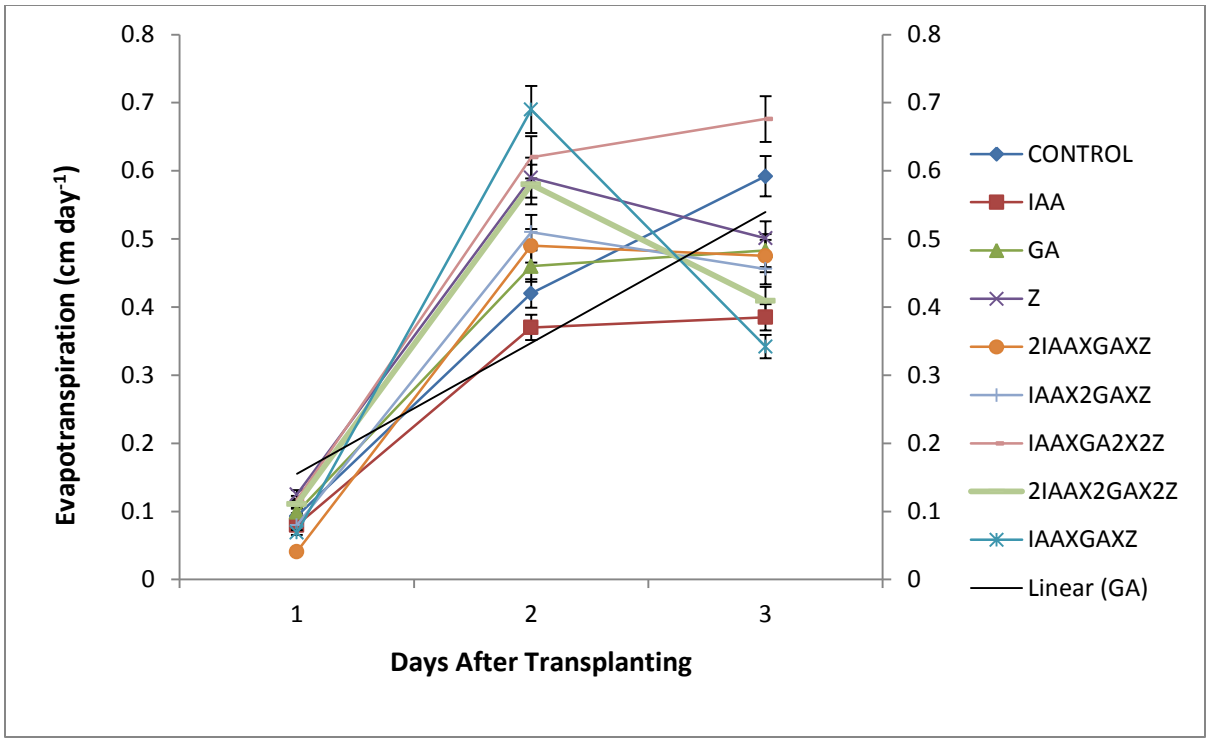

Figure 4.5 Influence of hormones on nutrient solution evapotranspiration per day of cotton plants grown hydroponically. IAA-Indole-3-acetic acid, GA_3 - Gibberellic acid, Z-Zeatin. 1 DAT = 43-74, 2 DAT = 74-80, 3 DAT = 80-90

4.4 DISCUSSION

4.4.1 INFLUENCE OF EXOGENOUS HORMONES APPLICATION ON COTTON PLANT GROWTH AND FRESH BIOMASS PRODUCTION GROWN HYDROPONICALLY

Excessive hormones application jeopardized plant growth and biomass production. Hormones treated plants and untreated plants were not significantly different in cotton plant height, root length (except 43 DAT), number of leaves and fresh biomass weight. Shah et al. (2006) reported that highest growth of Black Cumin (*Nigella sativa* L.) was obtained when sprayed with hormones such as GA_3 at 40 days after sowing (DAS). However, application of hormones at 36 and 67 DAT had negative effect on plant

height, root length, number of leaves and cotton fresh weight. The negative effect was observed due to the fact that additional concentrations of phytohormones were applied at 67 DAT which resulted to physiological disorder that turned green pigment of the leaves to yellow.

4.4.2 EFFECT OF EXOGENOUS HORMONES APPLICATION ON NITROGEN, PHOSPHORUS AND POTASSIUM UPTAKE BY COTTON PLANTS GROWN HYDROPONICALLY

Exogenous hormones applications are the most important factor influencing positive growth of plants (Marschner, 1995; Palmer et al. 1996; Martin et al. 2000; Fan et al. 2002). Plant hormones applied did not affect NPK uptake by cotton plant parts except N uptake by cotton stem. This could be due to the time of hormones application and concentration of hormones applied (Shah et al. 2006). Concentration of hormones applied at 36 DAT and additional hormone concentrations applied at 67 DAT may hinder the effectiveness of nutrients uptake by cotton plants as a result of excessive hormones applications. Shah et al. (2006) reported 40 days after sowing for the best time to apply GA_3 at concentration of 10^{-5} M for growth and yield of Black Cumin (*Nigella sativa L*). Anderson et al. (1988) observed that GA_3 hormone supported stem elongation of Little Marvel peas at 4 days or longer treatment period when excess of 29 μM could be applied to produce healthy plant growth. However, adequate level, as well as time of application of phytohormones promotes increase in mineral nutrients uptake. All the phytohormones applied influenced nitrogen uptake by cotton stem. The result obtained in this current study corresponded with Wang et al. (2009). They reported that phytohormones (IAA, GA_3 and Z) applied supported N uptake by strawberry which resulted to close correlation

between extensive root growth and N uptake. This implies that application of phytohormones stimulate root growth that aid N uptake by plants. Takei et al. (2001) also reported increase N uptake by rice plants when phytohormones such as cytokinin were applied.

4.4.3 EFFECT OF PHYTOHORMONES ON CHLOROPHYLL FORMATION

Single hormone application of IAA, Z, GA_3 and combined hormone application of IAA x GA_3 x 2Z significantly increased chlorophyll a, b and ab at 80 DAT. However, Mitsuru and Hideo (1978) reported that pre-treatment of cotyledons with BA, GA_3, or IAA stimulates chlorophyll formation by subsequent illumination. Fletcher and McCullagh. (1971); Roger and Hideo (1982); Yoshiki et al. (2003) reported that cytokinins (Zeatin) have an important role in the formation of chlorophyll. Beyond 80 DAT, the treatments failed to promote chlorophyll formation; this could be due to another hormone concentrations added at 67 DAT.

4.4.4 EFFECT OF EXOGENOUS PHYTOHORMONES APPLICATION ON ENDOGENOUS HORMONES CONTENT

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 gibbeerellic acid and zeatin contents in the stem increased in the plants treated with IAA x GA_3 x Z treatment. Based on these results, Van Standen (1976c), Van Standen (1977), Van Standen and Brown (1977) and Vysot-Skaya et al. (2007) reported that stem is the site of gibberellic acid and cytokinins (Zeatin) synthesis. Conversely, cytokinin (Zeatin) produced in the root tip promote lateral stem growth (Godwin and

Morris 1979; Zahir et al. 2001). Furthermore, Gibberellic acid has been known to induced stem elongation in hydroponically grown little Marvel pea (Anderson et al. 1988).

4.4.5 EFFECT OF PHYTOHORMONES TO ROOT AREA AND ROOT ACTIVITY

It has been recorded that an extensive root system is responsible for K (Zhiyong et al. 2009) and P (Jose et al. 2002) acquisition. Exogenous phytohormones applied to high level of P and K nutrients solution did not affect cotton total absorption area (TAA), percentage active absorption area (PAAAR) and and specific surface area (SSA). Although exogenous phytohormones applied significantly had high active absorption area (AAA), and root volume (RV) when individually applied IAA, Z, GA_3 and combined applied 2IAA x GA_3 x Z, IAA x GA_3 x Z, IAA x $2GA_3$ x Z, IAA x GA_3 x 2Z, and 2IAA x $2GA_3$ x 2Z treatments at 80 DAT. Furthermore, active absorption area, percentage active absorption area and specific surface area were increased when GA_3, IAA x GA_3 x 2Z and 2IAA x GA_3 x Z applied at 90 DAT. Zahir et al. (2001) reported that exogenous supply of cytokinin (Zeatin) or its precursor in the root zone improved growth and yield of rice. Furthermore, Pillet (1983) found that root growth is controlled by indole-3-acetic acid and abscisic acid both of these phytohormones being complimentary to each other. The result of Baluska et al. (1993) indicated that gibberellins are morphogenetically active substances in the shoot and root of crops such as maize. These results showed that phytohormones (IAA, GA_3, and Z) and their combinations could be used to stimulate root growth.

Root activity measured with triphenyl tetrazolium chloride (TTC) is related with *nicotinamide adenine dinucleotide* (NAD) dehydrogenase during aerobic respiration of

roots (Shimada 1969). Exogenous phytohormones applied significantly affected root activity. Single applied GA_3 and combined treatment applications of IAA x GA_3 x 2Z at 80 DAT and IAA x GA_3 x 2Z at 90 DAT stimulated high level of root activity. Present results agree with the finding of Atkins (1992) and Clark et al. (1992) who reported that plant growth regulator (PGR-IV) that consisted of gibberellic acid and synthetic indolebutyric acid (auxin derivative) increased root mass and root activity. The result of this present study also includes zeatin (cytokinin) as a phytohormone that could be used to stimulate cotton root activity.

4.4.6 NUTRIENT SOLUTION LEVEL AND RESIDUAL LEVEL OF PHOSPHORUS AND POTASSIUM OF HYDROPONICALLY GROWN COTTON PLANTS TREATED TO DIFFERENT HORMONES

Hormone treated pots were significantly affected by the nutrient solution level in the hydroponics system. It became obvious at 43 DAT that phytohormone treated pots with IAA x GA_3 x Z had lowest level of the nutrient solution. This implies that cotton plants were utilizing nutrients more efficiently through the influence of the added hormones (José et al. 2002). However, results obtained at 80 days after transplanting showed an opposite trend in all hydroponic pots treated with hormones and untreated pot except for the IAA x GA_3 x Z, 2IAA x GA_3 x Z and IAA x $2GA_3$ x Z treatments. This could be due to higher level of phytohormone concentrations added at day 67 after transplanting which altered cotton growth and development.

Exogenous application of phytohormones did not affect residual P mineral nutrient in the hydroponics nutrient solution except at 43 DAT, it might also be due to higher level of hormones applied at 67 DAT, as a result of this, treated pots and untreated pots

significantly appeared the same. Moreover, IAA x GA_3 x Z influenced effective use of P in the solution at 43 DAT, which implies that using this phytohormone combination would influence P utilization by plants.

On the contrary, the pot treated with IAA x GA_3 x Z had the highest residual K-content in the nutrients solution at 43 days after transplanting This implies that this treatment influenced K-content to remain in the solution at 43 DAT whereas other treatments including untreated plants caused effective K utilization by the cotton plants. This result could also be attributed to the higher concentration of K in the hydroponics nutrient solution (Table 3.1). Furthermore, phytohormone treatments did not influence residual K from 74 to 90 days after transplanting could be due to higher concentration of hormone added at 67 DAT. Shah et al. (2006) reported that highest growth, NPK accumulation and seed yield of Black Cumin (*Nigella sativa* L) was obtained when GA_3 is sprayed at 40 days after sowing (DAS).

4.4.7 EVAPOTRANSPIRATION OF NUTRIENT SOLUTION TREATED TO PHYTOHORMONES.

Evapotranspiration of nutrient solution from the surface of hydroponic was observed at 80-90 DAT. Hydroponic pot treated to combination of indole-3-acetic acid (50 μ gL^{-1}), gibberellic acid (40 μ gL^{-1}) and high level of zeatin (100 μ gL^{-1}) influenced high evapotranspiration of nutrient solution. Kudoyarova et al. (2007) reported that cytokinin (zeatin type) greatly enhanced evapotranspiration. Highest evapotranspiration per day occurred at 74 to 80 DAT during the reproductive stage. The combined phytohormones applied at 50 μ gL^{-1} for IAA, 40 μ gL^{-1} for GA_3 and 50 μ gL^{-1} for zeatin influenced evapotranspiration. It has been mentioned earlier that zeatin enhanced

evapotranspiration (Kudoyarova et al. 2007), this study suggests combination of zeatin with IAA and GA_3.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

This study suggests that Hoagland standard solution (high PK level at 1 x 10^{-3} M P and 6 x 10^{-3} M K, respectively) supported an increase in plant height and root length. Reduced concentration (modified version) of Hoagland standard solution, low P (half strength standard Hoagland solution) and low K (one-sixth strength standard Hoagland solution) could be used to facilitate the effective nutrients uptake for the growth and development of cotton plants. High PK could only support K uptake by leaves. Moreover, there is a need to pay close attention to concentration and time of hormones application for the better performance of crops. Phytohormones applied did not favour cotton growth especially plant height and root length except at 43 DAT, where IAA x GA_3 x Z treatment positively influenced root length. Low application of P and K at 5 x 10^{-5} M P and 1 x 10^{-3} M K, respectively positively influenced nutrient use efficiency. These application rates could be applied to curb economic and environmental wastage of nutrients. Application of high concentration of hormones at inappropriate times (67 DAT) negatively affected plant growth and NPK uptake except N uptake by stem. All the phytohormones applied could be used to increase N uptake by stem only.

The results of this investigation indicate a significant role of potassium (K) at low concentration (1 x 10^{-3} M P) or Hoagland standard solution high PK (1 x 10^{-3} M P, and 6 x 10^{-3}M P, respectively) and plant hormones at concentration of 50, 40 and 50 μ gL^{-1} for IAA, GA_3, Z, respectively, and Z at 100 μ gL^{-1} play in chlorophyll formation in plants. The cotton plant greenish pigment (chlorophyll a, b, and ab) was noticed up to 91 DAT for the nutrient solution experiment without hormones application and 80 DAT for the

phytohormone experiment with hormones application. 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 K nutrient applied to hydroponically grown cotton were supplemented for by endogenous phytohormone content of Z in the leaves and roots which enhanced cotton plants growth and development. However, exogenous phytohormone application of IAA x GA_3 x Z stimulated endogenous GA_3 content in the stem, also stimulated endogenous Z content in the stem. The stem of a plant has been known for GA_3 and Z biosynthesis. The experiment on plant hormone content suggests low level of potassium mineral nutrient with combined hormone application (IAA x GA_3 x Z) for the benefit of hydroponic cotton growth and development. Hence, the result of this present study suggests an early application of hormones at 36 DAT would be better.

Phosphorus and potassium nutrients influenced cotton root growth and development. Low P and low K applications greatly influenced increase in root area (total absorption area, active absorption area, percentage active absorption area, specific surface area) and root activity while high PK influence root volume. On the other hand, IAA x GA_3 x Z treatment increased active absorption area while Z increased root volume at 80 DAT. At 90 DAT, IAA x GA_3 x 2Z, GA_3 and 2IAA x GA_3 x Z favoured active absorption area, percentage active absorption area and specific surface area, respectively. The GA_3 and IAA x GA_3 x 2Z treatments increased root activity at 80 and 90 DAT. All these phytohormones are well known to increase root growth.

The results of this investigation demonstrate the use of nutrients and phytohormones to support growth and development of cotton. Nutrient concentrations

applied in this study indicated that low K and High PK treatments had high residual P and K nutrients content in the hydroponics nutrient solution, respectively. This contributes to P and K wastage that could lead to environmental pollution. The application of phytohormones did not change P and K residual level in the nutrients solution except at early growth stages for P and K nutrients (43 DAT). The IAA x GA_3 x Z treatment plays an antagonistic role whereby IAA x GA_3 x Z supported P utilization by the crops and K accumulation in the hydroponic system. Evapotranspiration of nutrient solution was noticed at early and reproductive stages which indicate that great demand of nutrient is needed at these periods. Report in this thesis advocated for economical use of phytohormones, excessive phytohormones application can jeopardize nutrients acquisition by cotton plants. Judicious use of nutrients fertilizer and plant hormones should be given priority consideration in hydroponics systems with regards to cotton plants. The nutrient solution experiment recommended low P and K application for NPK nutrients uptake by cotton plants, endogenous phytohormone contents, chlorophyll formation, root area and P and K nutrients use efficiency unlike cotton planted in the field that requires heavy fertilizer application for growth and nutrient uptake. The results achieved in this study could enhanced overall production of cotton. Future research is needed to investigate the full cycle of cotton crop grown hydroponically for commercial production. In phytohormone experiment, the phytohormone responses indicate that its application would not be beneficial for commercial application. The future study for phytohormones must consider lower range and time of application of phytohormones. The approach of using hydroponic system as a tool for understanding physiological responses in cotton production has been demonstrated, however, the full cycle of growth

and yield of cotton crop must be further investigated.

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APPENDIX 2 OTHER PUBLICATIONS IN PREPARATION

- **Adebusoye O. Onanuga**, Samuel K. Asiedu, Norman Goodyear and Gordon W. Price. Role of phosphorus, potassium and phytohormones on growth of plants in hydroponic systems. (Review manuscript in preparation)
- **Adebusoye O. Onanuga**, Samuel K. Asiedu, Norman Goodyear and Gordon W. Price. Estimation of phosphorus and potassium use efficiency of hydroponically grown cotton plants (*Gossypium hirsutum*). (Manuscript in preparation)
- **Adebusoye O. Onanuga**, Samuel K. Asiedu, Norman Goodyear and Gordon W. Price. Evaluation of phosphorus and potassium nutrient solution evapotranspiration of hydroponically grown cotton *(Gossypium hirsutum*). (Manuscript in preparation)
- **Adebusoye O. Onanuga**, Samuel K. Asiedu, Norman Goodyear and Gordon W. Price. Influence of Phosphorus, Potassium and Exogenous application of Indoacetic acid, Gibberellic acid, Zeatin on growth and nutrients uptake of Cotton (Gossypium hirsutum) plant species grown in hydroponics nutrient solution. Journal of Plant nutrition (Manuscript in preparation).