

# Effect of Phytohormones, Phosphorus and Potassium on Cotton Varieties (*Gossypium hirsutum*) Root Growth and Root Activity Grown in Hydroponic Nutrient Solution

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## Abstract

Modern agriculture using hydroponics allows in-depth study of root morphology and physiology. Two experiments were conducted to evaluate effectiveness of mineral nutrients, phosphorus (P) and potassium (K) and phytohormones, indole-3-acetic acid (IAA), gibberellic acid, ( $GA_3$ ) and zeatin (Z) on cotton plant varieties root area, root volume and root activity. During the first cropping, Low P, low K and high PK treated plants significantly influenced total root absorption area, active absorption area, percentage active absorption area ratio, specific surface area and root volume. Furthermore, treatments applied did not favour root activity at the early growth stage but effects were known as from 104 to 148 days after transplanting. During the second experiment, however, cotton plants treated with hormones significantly affected active absorption area, percentage active absorption area ratio, root volume, specific surface area and root activity at 80 and 90 days after transplanting. In general, there was no relationship between the first cropping without hormones application and second cropping with hormones application except percentage active absorption area ratio (-0.999\*) which was negatively correlated. This report shows effect of mineral nutrients and stimulatory tendency of hormones in root growth regardless of different cropping time between first and second cropping.

**Keywords:** Hydroponics, Phytohormones, Root area, Root volume, Root activity, Cotton variety

## 1. Introduction

Since 1980s hydroponics units have been commercialized for vegetables and flowers production and more than 60,000 ha of vegetables are grown hydroponically in greenhouse worldwide (Jone *et al.* 1997). Nowadays, improved agricultural practice using hydroponics 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 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 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. Crop yield is very important to farmers, 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). Root system architecture brings effective nutrient acquisition and bumper yield (Linkohr *et al.*, 2002; Yan *et al.*, 2004).

Auxins have been known to alter primary root growth and promoting 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 where as 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<sup>-</sup> in relation to pH of the cell compartment. The protonated and anion IAA<sup>-</sup> 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 ubiquitin-ligase complex of SKP1-cull1-TIR1. This interaction gives way to AUX/IAA ubiquitination, which is later degraded by 26S proteasome (Chapman and Estelle, 2009). AUX1 has been recognised as auxin influx facilitator. This auxin influx protein reveals the expression in the elongation of root and lateral root growth. The proteolytic degradation of AUX1/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 root gravitational 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 scanty 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 tri carboxylic 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<sub>3</sub>) 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°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).

The present study was conducted to evaluate the effect of phosphorus and potassium nutrition, exogenous plant hormones application on root area, root volume and root activity of cotton plants and to compare with and without hormones application on cotton root growth.

## 2. Materials and Methods

Two cropping systems were adopted in this experiment. The first cropping system was cropped without hormone application while the second cropping system was cropped with application of hormones. Apart from using this system to grow vegetables and flowers, this cropping system is also common practice for growing other crops such as wheat and rice (Brian *et al.*, 2009; Nemati *et al.*, 2011). Zhong mian 36 and Xin luo zao 13 are Chinese cotton varieties. These two varieties were selected for this experiment because they are indeterminate plant; they continue to grow after first harvest. They are also early maturing and high yielding varieties.

### 2.1 First Cropping.

Nutrients solution experiment: The two cotton species were cultivated in quartz sand. The cotton seedlings were transplanted into nutrients solution pot of 6L at seven days of growth.

The greenhouse environmental specifications were followed: The day time temperature was recorded and kept at 20 – 35°C, with 12 hours of sunlight, there was constant supply of oxygen to the roots. The nutrients solutions were Hoagland and micro anion nutrients. The low P level was  $5.0 \times 10^{-5}$  M, low K level was  $1 \times 10^{-3}$  M and high PK level was  $1 \times 10^{-3}$  and  $6 \times 10^{-3}$  M (Table 1) at pH 6.5 with two varieties of cotton. At 83, 91, 104, 120 and 148 days after transplanting, random selection of one cotton plant from each of low P, low K and high PK treatments of the two cotton varieties was carried out so as to measure root area, root volume, and root activity. The experiment was replicated three times with total number of 18 pots ( $3 \times 3 \times 2$ ). The total number of plant used to carry out root area, root volume and root activity throughout the experimental period was 90.

#### 2.1.1 Second Cropping

In the second phase of the experiment, the cultivation and other necessary measures were followed as in the first experiment. The exogenous hormones concentration in micro gram per litter ( $\mu \text{g L}^{-1}$ ) were applied twice at 36 and 67 days after transplanting to Hoagland complete nutrients fluid by spraying on the cotton leaves at single rate of 0, 50, 40 and  $50 \mu \text{g L}^{-1}$  for indole-3- acetic acid (IAA), gibberellic acid ( $\text{GA}_3$ ) and zeatin (Z), respectively and combined rate of 50IAA\*40GA\*50Z, 100IAA\*40GA\*50Z, 50IAA\*80GA\*50Z, 50IAA\*40GA\*100Z and 100IAA\*80GA\*100 with three replication using two cotton varieties supply with the highest P and K nutrient levels of  $1 \times 10^{-3}$  and  $6 \times 10^{-3}$  M ( Table 1 ) at pH 6.5 in the hydroponic solution, resulting to total number of ( $9 \times 3 \times 2$ ) 54 experimental pots. The concentration of hormones used in this present study was inline with Shah *et al.*, (2006), Anderson *et al.*, (1988) with slight modification. The root area, root volume and root activity measurement were carried out at 80 and 90 days after transplanting (DAT).The total number of plants that was used for the whole experiment was 108 cotton plants.

### 2.2 Root Area Measurement

#### 2.2.1 Root Area

The root area parameters were measured according to method of analysis described by Zhao Shijie (1998). Methyl blue of 0.0064 g was added to distil water of 1000 ml. The sample roots were immersed into the beaker containing methyl blue for 90 seconds. The process was repeated three times in three beakers and pipette 1 ml into test tube. Nine ml distil water was added to all the test tube. Thereafter, shake on a mechanical shaker for 1 minute. The absorbent was measured with spectrophotometer at 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.

Total absorption area = milligram of methyl blue from 3<sup>rd</sup> beaker

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

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

Active absorption area = 3<sup>rd</sup> beaker  $\times 1.1\text{m}^2$

### 2.3 Root Volume measurement.

#### 2.3.1 Root Volume

1000 ml volumetric cylinder was used. Root samples were immersed into 900 ml of distil water. Root volume was calculated by water displacement in the volumetric cylinder.

#### 2.3.2 Root activity

Measured 0.2 g fresh root was mixed with 5 ml of 4% TTC and 5 ml  $\text{Na}_2\text{HPO}_4$ . Incubate the mixture at 37°C for 1.5 hours. After 10 minutes, 2 ml  $\text{H}_2\text{SO}_4$  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 grinded with mortar and pestle then filtered with whatman filter paper. Four ml of ethyl acetate was added to the filtrate and shake on mechanical shaker for 1 minute. It was then measured with spectrophotometer at wavelength of 485 nm.

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.

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

### 2.3.3 Data Analysis

The data was analyzed using general linear model (GLM) univariate with SPSS software version 15. Least significance difference using Duncan's multiple range test (DMRT) for separation of treatment means irrespective of variety. This was done so as to evaluate the general performance of cotton plants. Variety means was also evaluated using DMRT; this was carried out in order to ascertain differences between the two varieties. Correlation statistical analysis was used to compare root parameters in the first and second phase of the experiment. The results of this experiment were presented in tables and figures for treatment means and variety means, respectively.

## 3. Results

### 3.1 First Experiment

3.1.1 The effects of low P, low K and high PK treatments on cotton root area parameters, root volume and root activity

#### *Total absorption area*

The effect of low P, low K and high PK treatments on cotton total absorption area are shown in Table 2. The low P, low K and high PK treatments did not significantly affect total root absorption area except at 104 days after transplanting (DAT), that low K and high PK treatments significantly performed better than low P treatment.

#### *Varieties*

Figure 1 shows that Xin and Zhong cotton varieties climbed the peak total absorption area at 91 days after transplanting (DAT) and thereafter there was a sharp decrease. However, Xin Cotton variety had the greater total root absorption area than Zhong cotton at 83 and 104 DAT. It was noticed that Xin varieties had a negative total root absorption area at 120 and 148, although there were no significant differences in total absorption area between the Xin and Zhong cotton variety at 91, 120 and 148 DAT.

#### *Active Absorption Area*

The effect of low P, low K and high PK treatments on cotton root active absorption area are shown in Table 3. At 83 DAT, low P and high PK treatments significantly supported active absorption area while low K treatment had the shortest area with negative growth. There was no significant difference 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 with negative growth. Conversely, at 120 DAT, low K and high PK treatments negatively influenced active absorption area while low P treatment gave the least negative growth but there were no significant differences between low P and high PK treatments. However, at 148 DAT, high PK treatment had the greatest active absorption area with value of 3.14 m<sup>2</sup> compared to low K treatment with negative value of 5.56 m<sup>2</sup> and low P treatment with negative value of 6.06 m<sup>2</sup>.

#### *Varieties*

Figure 2 reveals that at 91 days after planting both varieties had the highest active absorption area while lowest was noticed at 120 days after planting. Zhong Cotton variety performed better than Xin cotton variety at 83 DAT. At 91 DAT the result was contrary, Zhong cotton variety was inferior to Xin cotton variety. It is noteworthy that both varieties had negative root active absorption area at 120 and 148 DAT, but the varieties differences were insignificant as from 104 to 148 DAT (Fig. 2).

#### *Percentage active absorption area ratio*

The effects of low P, low K and high PK treatments on cotton Percentage root active absorption area ratio are summarized in Table 4. At 83 DAT, high PK treatment had the highest percentage active absorption area ratio of 0.506 m<sup>2</sup>, followed by low P treatment with value of 0.380 m<sup>2</sup> but both treatments were insignificant. The low K treatment had the least negative value of 0.156 m<sup>2</sup>. From 91 to 148 DAT, the treatments applied significantly appeared the same.

#### *Varieties*

It is clearly seen in Figure 3 that two varieties had different growth habit, Zhong cotton variety slightly increased

positively in growth throughout the experimental period while Xin variety negatively decreased at 83, 120 and 148 DAT. Zhong Cotton varieties was superior to Xin cotton variety at 83 and 104 DAT but inferior to Xin cotton variety at 91 DAT. There were no significant differences between the two varieties at 120 and 148 DAT (Fig. 3).

#### *Specific Surface Area*

Table 5 shows the effect of low P, low K and high PK treatments on cotton specific surface area. The low P treatment gave the highest specific surface area with value of  $4.28 \text{ m}^2 \text{ ml}^{-1}$  and  $19.25 \text{ m}^2 \text{ ml}^{-1}$  at 83 and 91 DAT, respectively than other treatments. At 104 DAT low K treatment significantly influenced greater specific surface area with value of  $3.68 \text{ m}^2 \text{ ml}^{-1}$ , than either low P or high PK treatments. At 120 DAT, low K and high PK treatments jointly produced greater specific surface area than low P. No significant differences were observed among the treatments applied at 148 DAT.

#### *Varieties*

Figure 4 reveals that highest specific surface area was noticed at 91 days after transplanting while lowest was obtained at 120 days after transplanting for both varieties. However, Xin and Zhong cotton varieties had negative specific surface area at 120 DAT. Furthermore, at 148 DAT, it was observed that Xin had negative specific surface area. Nevertheless, Xin Cotton variety performed better than Zhong cotton variety at 83 and 91 DAT while at 104 DAT, Zhong cotton variety supported more specific surface area than Xin cotton variety. The Xin cotton and Zhong cotton varieties showed no significant differences at 120 and 148 DAT (Fig. 4).

#### *Root volume*

The effect low P, low K and high PK treatments on cotton root volume are given in Table 6. The high PK treatment consistently had the greatest root volume while low P treatment gave the least throughout the experimental period except at 104 DAT that result showed insignificant. 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.

#### *Varieties*

The two varieties possessed different root volume, at 104 days after transplanting; Xin cotton variety had highest root volume while lowest volume was recorded for Zhong cotton variety (Fig. 5). Xin Cotton and Zhong cotton varieties showed no significant differences at 83 and 120 DAT but there were significant changes between Xin cotton and Zhong cotton varieties at 91, 104 and 148 DAT. Zhong Cotton variety proved superior to Xin cotton variety at 91 and 148 DAT but reversed was the case at 104 DAT, Xin cotton variety performed better than Zhong cotton variety (Fig. 5).

#### *Root Activity*

Table 7 summarizes the effect of low P, low K and high PK on cotton root activity. There was no significant difference 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 K treatment significantly affected root activity but there were no differences between low P and low K treatments in one hand and low P and high PK treatments in other hand. It is quite obvious from the data that low P treatment significantly had the larger root activity than low K treatment, but high PK treatment showed no significant difference to low P treatment at 148 DAT.

#### *Varieties*

Figure 6 shows that the two varieties possessed different root activity; Xin variety had highest root activity at 91 DAT while it was 104 DAT for Zhong cotton variety. Zhong Cotton variety performed better than Xin cotton variety at 83 and 148 DAT but the situation changed as from 91 to 120 DAT, Xin cotton variety had greater root activity than Zhong cotton variety.

### *3.2 Second Cropping*

3.2.1 The influence of hormones on cotton root area, root volume and root activity grown hydroponically in high level of phosphorus and potassium.

#### *Total Absorption Area (TAA)*

The influence of plant hormones on cotton root total absorption area grown hydroponically in high level of phosphorus and potassium are given in Table 8 & 9. Plant hormones applied did not favour total absorption

area. The Treated pots with hormones and untreated pots showed similar result at 80 and 90 days after transplanting (DAT) (Table 8 & 9).

#### *Active Absorption Area (AAA)*

Hormones applied significantly affected cotton root active absorption area (Table 8 & 9). All the treatments applied significantly influenced root active absorption area except 2IAA x GA<sub>3</sub> x Z treatment and control experiment at 80 DAT (Table 8).

Table 9 summarizes the influence of hormones on cotton root active absorption area. The combined application of IAA x GA<sub>3</sub> x 2Z significantly supported largest cotton root active absorption area than other treatments while the combined use of IAA x GA<sub>3</sub> x Z gave the least cotton root active absorption area at 90 DAT.

#### *Percentage Active Absorption Area Ratio (PAAAR)*

Hormones applied significantly had effect on cotton root percentage active absorption area ratio (Table 8 & 9). The combined use of IAA hormone with GA<sub>3</sub> and Z significantly had more percentage active absorption area ratio than 2IAA x GA<sub>3</sub> x Z, IAA x GA<sub>3</sub> x 2Z and control but combined use of IAA x GA<sub>3</sub> x Z hormone was insignificant to single applied IAA, GA<sub>3</sub>, Z, combined applied IAA x 2GA<sub>3</sub> x Z and 2 IAA X 2 GA<sub>3</sub> X 2 Z at 80 days after transplanting (Table 8).

The influence of hormones on cotton root percentage active absorption area ratio at 90 days after transplanting is presented in Table 9. The plants treated with GA<sub>3</sub> hormone significantly had a tremendous land slam performance over plants treated to single and combined applied hormones. It was observed that plants treated with IAA x GA<sub>3</sub> x Z had a negative least growth.

#### *Specific Surface Area*

The hormones applied significantly had profound effect on cotton root specific surface area (Table 8 & 9). At 80 DAT, the treatments had no effect on cotton root specific surface area. The treated plants with hormones and untreated plant significant appeared the same (Table 8).

However, at 90 DAT, the largest specific surface area was observed in plants treated with 2IAA x GA<sub>3</sub> x Z hormones with value of 21.39 m<sup>2</sup> ml<sup>-1</sup> while those treated with IAA x GA<sub>3</sub> x 2Z gave the least but not significantly different from other hormones treatments except GA<sub>3</sub> hormones. (Table 9).

#### *Root Volume*

Hormones applied significantly had effect on cotton root volume (Table 8 & 9) The plant treated to IAA, Z and 2IAA x 2GA<sub>3</sub> x 2Z significantly had more root volume than those treated with IAA x GA<sub>3</sub> x Z, 2IAA x GA<sub>3</sub> x Z, IAA x 2GA<sub>3</sub> x Z and control at 80 days after transplanting (Table 8).

Nevertheless, the hormones applied did not support cotton root volume at 90 days after transplanting (Table 9). It is clearly seen that untreated pot had better performance than other treatments.

#### *Varieties*

The two cotton varieties expressed different root growth. It was noticed that Xin cotton variety increased in total absorption area while Zhong cotton variety decreased. Furthermore, both varieties constantly increased in active absorption area and root volume. It is noteworthy that Xin cotton decreased in percentage active absorption area ratio while Zhong cotton increased. Nevertheless, both varieties decreased in specific surface area from 80 to 90 DAT.

The hormones treated to cotton varieties significantly influence root area parameters at 80 days after transplanting. Zhong cotton variety did better than Xin cotton variety in total absorption area and specific surface area (Fig. 7 & 10). Furthermore, Xin cotton variety outclassed Zhong cotton variety in active absorption area and percentage active absorption areas ratio (Fig. 8 & 9). There was no significant difference between two varieties as regards root volume (Fig. 11).

The effect of hormones applied on Xin cotton and Zhong cotton root area at 90 days after transplanting are given in Figure 7 to 11. The Xin cotton variety significantly proved superior to Zhong cotton variety in all the root area parameters measured except percentage active absorption area ratio which showed no significant difference between two varieties planted in the hydroponics nutrients solution treated to high level of phosphorus and potassium..

#### *Root Activity*

Table 10 shows effect of hormones on cotton root activity grown hydroponically treated to high level of

phosphorus and potassium. It is obvious from the data that single applied GA<sub>3</sub> hormone and combined applied IAA x GA<sub>3</sub> x 2Z hormone significantly favoured highest cotton root activity than other hormones treated plants while combined use of 2IAA x GA<sub>3</sub> x Z, IAA x 2GA<sub>3</sub> x Z and control gave lowest root activity but not significantly different with plants treated to single applied Z, combined applied IAA x GA<sub>3</sub> x Z and 2IAA x 2GA<sub>3</sub> x 2Z at 80 days after transplanting. It is noteworthy at 90 days after transplanting that combined application of IAA x GA<sub>3</sub> x 2Z performed better than the other treatments.

#### Varieties

Figure 12 reveals the effect of hormones on Xin cotton and Zhong cotton root activity grown hydroponically treated to high level of phosphorus and potassium. It is obvious from the Figure 12 that Xin cotton increased in root activity while opposite result was obtained for Zhong cotton variety. However, Xin cotton variety significantly had a greater performance than Zhong cotton variety at 80 and 90 days after transplanting.

#### 3.3 Correlation co-efficient relating low P, low K and high PK plant treatments at 148 DAT to hormone plant treatments (Indoacetic acid, Gibberellic acid, Zeatin and their combinations) at high level of PK at 90 DAT

The correlation co-efficient relating treatments applied to cotton plants are given in Table 11. There was no relationship between treatments applied in first cropping and treatments applied in second cropping in relation to crop performance except root active absorption area ratio which was negatively related (-0.999\*).

#### 4. Discussion

The phosphorus and potassium nutrients significantly affected cotton root area. Low K and High PK treatments performed more than low P treatment at 104 DAT for total absorption area (TAA), at 104 and 148 DAT for active absorption area (AAA) while at 120 DAT negative growth was observed, it could be due to the fact that there was no significant increase in root length after 80 – 90 days of emergence (Nayakekorala and Taylor, 1990). Cotton percentage active absorption area ratio (PAAAR) was high with plants treated to high PK and Low P at 83 DAT. Silberbush and Barber (1983) indicated that greater phosphorus (P) and potassium (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 treated plants produced the greatest cotton root volume (RV) throughout the experimental period except at 104 and 148 DAT. The high PK treated plants 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 gave highest specific surface area. At 120 DAT, low K and high PK produced larger specific surface area than Low P. The treatments appeared similar at 148 DAT. Low P and K gave high specific surface area this could be as a result of direct root contact with nutrients solution in hydroponic nutrients solution.

The differences between Xin and Zhong cotton on root area parameters (TAA, AAA, PAAAR, RV and SSA) throughout the experimental period revealed the differences between nutrient efficiency cotton genotypes on uptake, transportation, accumulation, distribution and utilization (Mi, *et al.*, 1998; Jiang *et al.*, 2008).

Cotton root activity measured by triphenyltetrazolium chloride (TTC) had significant effect on treatments applied. There was no significant difference among the treatments applied from 83 to 91 days after transplanting. However, high PK, low K and low P significantly affected cotton root activities beyond 91 DAT. This could be due to variation in room temperature of dehydrogenase activity root 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 °C. On the other hand, differences between the varieties (Zhong and Xin cultivars) reveal the genetic makeup of the plants (Jiang *et al.*, 2008).

It has been recorded that a large root system is responsible for K (Zhiyong *et al.*, 2009) and P (Jose *et al.*, 2002) acquisition. Exogenous hormones applied to high level of P and K nutrients solution did not affect cotton total absorption area (TAA), although exogenous hormones applied was significantly had high active absorption area (AAA), percentage active absorption area ratio (PAAAR), root volume (RV) and specific surface area (SSA) when single applied IAA, Z, GA<sub>3</sub> and combined applied 2IAA x GA<sub>3</sub> x Z, IAA x GA<sub>3</sub> x Z, 2IAA x 2GA<sub>3</sub> x Z at 80 DAT and GA<sub>3</sub>IAA x GA<sub>3</sub> x Z, IAA x GA<sub>3</sub> x 2Z and 2IAA x GA<sub>3</sub> x Z 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 maize. These results showed

that phytohormones (IAA, GA<sub>3</sub>, and Z) and their combinations could be used to stimulate root growth. The differences between Zhong and Xin cotton varieties likely resulted from their genetic constitution (Jiang *et al.*, 2008).

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<sub>3</sub> and combined treatment applications of IAA x GA<sub>3</sub> x Z at 80 DAT and IAA x GA<sub>3</sub> x Z at 90 DAT stimulated high level of root activity. Present results agree with the finding of Atkins (1992), Clark *et al.*, (1992), who reported that plant growth regulator (PGR-IV) that consists 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. Nevertheless, Xin cotton variety possessed higher root activity than Zhong cotton variety this could be due as a result of differences in their genetic makeup.

The exogenous hormones applied during the second experiment proved to be effective for growth and development of cotton root. All the parameters measured in the first cropping without hormones application and second cropping with hormone application were insignificant except for percentage active absorption area which was negatively correlated (-0.999\*). This result was similar to Tao *et al.*, (1993) on pea seedling growth, Chunrong *et al.*, (2008) on endogenous and exogenous hormones of water stress in maize seedling, Zahir *et al.*, (2001) on growth and yield of rice, Kerbauy and Colli (1997) on root of *C. fimbriatum*.

## 5. Conclusion

The results obtained showed that Phosphorus (P) and Potassium (K) and their combination in first cropping and hormones applied at highest PK level in the second cropping significantly influenced the performance of two cotton varieties planted. The present study reveals high root growth when low P, low K and high PK nutrients applied to cotton plants species. On the other hand, Gibberellic acid, indoacetic acid and zeatin at concentration of 80  $\mu\text{g L}^{-1}$  for gibberellic acid, 100  $\mu\text{g L}^{-1}$  for indoacetic acid and 100  $\mu\text{g L}^{-1}$  for zeatin significant contributed to high cotton root area parameters and root volume while 40, 50 and 50  $\mu\text{g L}^{-1}$  for gibberellic acid, indoacetic acid and zeatin, respectively needed for root activity. There was no relationship between first cropping and second cropping for all the root parameters measured except active root absorption area ratio. Therefore, hydroponics farmers should pay close attention to the nutrients and hormones usage to attain ultimate desire for improving growth and yield of the crops.

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Table 1. Hydroponics nutrients composition

Nutrients	MolL <sup>-1</sup>			
	P 1 x 10 <sup>-3</sup>	P 0.05 x 10 <sup>-3</sup>	K 6 x 10 <sup>-3</sup>	K 1 x 10 <sup>-3</sup>
1 molL <sup>-1</sup> KNO <sub>3</sub>	5 (30 ml)	5 (30 ml)	5 (30ml)	1 (6ml)
1 molL <sup>-1</sup> Ca (NO <sub>3</sub> ) <sub>2</sub>	5 (30 ml)	5 (30ml)	5 (30ml)	7 (42ml)
1molL <sup>-1</sup> MgSO <sub>4</sub>	2 (12 ml)	2 (12ml)	2 (12ml)	2 (12ml)
1 molL <sup>-1</sup> KH <sub>2</sub> PO <sub>4</sub>	1 (6ml)	Nil	1 (6ml)	Nil
0.1molL <sup>-1</sup> KH <sub>2</sub> PO <sub>4</sub>	Nil	0.5 (3ml)	Nil	Nil
1 molL <sup>-1</sup> KCl	Nil	0.95 (5.7ml)	Nil	Nil
1molL <sup>-1</sup> NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub>	Nil	Nil	Nil	1 (6ml)
1 molL <sup>-1</sup> FeCl <sub>3</sub> .6H <sub>2</sub> O	1 (2ml)	1 (2ml)	1 (2ml)	1 (2ml)
Trace element	1 (6ml)	1 (6ml)	1 (6ml)	1 (6ml)

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

Treatments	Days after transplanting				
	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. P-phosphorus, K-potassium.

Table 3. Effect of low P, low K and high PK nutrients solution on mean active absorption area (m<sup>2</sup>) of cotton plants grown hydroponically over time

Treatments	Days after transplanting				
	83	91	104	120	148
Low P	8.81a	67.34a	-0.531b	-5.99b	-6.06b
Low K	-4.28b	66.64a	10.24a	-0.892a	-5.56b
High PK	15.76a	66.48a	10.66a	-3.67ab	3.14a
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. P-phosphorus, K-potassium.

Table 4. Effect of low P, low K and high PK nutrients solution on mean active absorption area ratio (m<sup>2</sup>) of cotton plants grown hydroponically over time

Treatments	Days after transplanting				
	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	-12.69a
High PK	0.506a	0.498a	0.415a	0.026a	0.282a
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. P-phosphorus, K-potassium.

Table 5. Effect of low P, low K and high PK nutrients solution on mean specific surface area ( $\text{m}^2\text{ml}^{-1}$ ) of cotton plants grown hydroponically over time

Treatments	Days after transplanting				
	83	91	104	120	148
Low P	4.28a	19.52a	0.206b	-0.660b	-0.272a
Low K	1.23b	7.21b	3.68a	0.111a	-0.126a
High PK	1.35b	5.01b	0.904b	-0.089a	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. P-phosphorus, K-potassium.

Table 6. Effect of low P, low K and high PK nutrients solution on mean root volume ( $\text{ml}^3$ ) of cotton plants grown hydroponically over time

Treatments	Days after transplanting				
	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. P-phosphorus, K-potassium.

Table 7. 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

Treatments	Days after transplanting				
	83	91	104	120	148
Low P	0.0003a	0.0019a	0.0012b	0.0009ab	0.0012a
Low K	0.0011a	0.0021a	0.0014b	0.0016a	0.0008b
High PK	0.0007a	0.0019a	0.0044a	0.0005b	0.0010ab
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. P-phosphorus, K-potassium.

Table 8. Effect of hormones concentration on mean root area of cotton plants grown hydroponically in high level of PK at 80 days after transplanting

Treatments	ROOT AREA				
	TAA( $\text{m}^2$ )	AAA( $\text{m}^2$ )	PAAAR( $\text{m}^2$ )	RV( ml )	SSA( $\text{m}^2\text{ml}^{-1}$ )
Control	29.60ab	1.55b	0.098b	2.00b	20.85ab
IAA	18.83b	12.03a	0.615ab	7.00a	3.34c
GA <sub>3</sub>	43.86a	6.94a	0.189ab	5.50ab	30.53a
Z	30.68ab	8.96a	0.359ab	8.00a	3.84c
IAA x GA <sub>3</sub> x Z	21.94b	12.62a	0.733a	1.50b	14.11abc
2IAA x GA <sub>3</sub> x Z	30.78ab	- 4.61b	- 0.135b	1.50b	22.23ab
IAA x 2GA <sub>3</sub> x Z	33.59ab	9.16a	0.500ab	1.50b	26.40ab
IAA x GA <sub>3</sub> x 2Z	19.52b	12.08a	0.005b	5.50ab	14.76abc
2IAA x 2GA <sub>3</sub> 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<sub>3</sub> -Gibberellic acid, Z- Zeatin, TAA-Total absorption area, AAA-Active absorption area, PAAAR -Percentage active absorption area ratio, RV-Root volume, SSA-Specific surface area.

Table 9. Effect of hormones concentration on mean root area of cotton plants grown hydroponically in high level of PK at 90 days after transplanting

Treatments	ROOT AREA				
	TAA( m <sup>2</sup> )	AAA( m <sup>2</sup> )	PAAAR( m <sup>2</sup> )	RV( ml )	SSA( m <sup>2</sup> /ml)
Control	43.14a	13.20b	0.298bcd	15.50ab	3.27bc
IAA	45.25a	6.12b	0.130cd	10.00b	4.53bc
GA <sub>3</sub>	13.20d	13.37b	1.10a	2.00de	9.89b
Z	21.60d	11.80b	0.158cd	6.00cde	4.66bc
IAA x GA <sub>3</sub> x Z	34.28abc	- 2.84c	-0.092d	6.50cde	6.78bc
2IAA x GA <sub>3</sub> x Z	26.77bc	13.01b	0.510bc	1.50e	21.39a
IAA x 2GA <sub>3</sub> x Z	31.81bc	13.49b	0.442bc	8.00cd	4.51bc
IAA x GA <sub>3</sub> x 2Z	42.10a	23.64a	0.691b	20.00a	2.19c
2IAA x 2GA <sub>3</sub> 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<sub>3</sub> -Gibberellic acid, Z- Zeatin, TAA-Total absorption area, AAA-Active absorption area, PAAAR -Percentage active absorption area ratio, RV-Root volume, SSA-Specific surface area.

Table 10. Influence of hormones concentration on mean cotton root activity (TTC reduction strength) grown hydroponically in high level of PK

Treatments	Days after transplanting	
	80	90
Control	0.0011c	0.0011b
IAA	0.0032ab	0.0028b
GA <sub>3</sub>	0.0040a	0.0013b
Z	0.0024abc	0.0012b
IAA x GA <sub>3</sub> x Z	0.0014bc	0.0012b
2IAA x GA <sub>3</sub> x Z	0.0006c	0.0013b
IAA x 2GA <sub>3</sub> x Z	0.0010c	0.0015b
IAA x GA <sub>3</sub> x 2Z	0.0040a	0.0058a
2IAA x 2GA <sub>3</sub> 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<sub>3</sub> -Gibberellic acid, Z- Zeatin, TAA-Total absorption area, AAA-Active absorption area, PAAAR -Percentage active absorption area ratio, RV-Root volume, SSA-Specific surface area.

Table 11. Correlation co- efficient relating low P, low K and high PK cotton plant treatments at 148 days after transplanting to hormones plant treatments at high level of PK at 90 days after transplanting

Growth and yield parameters	Correlation Co-efficient (r) relating low P, low K and high PK to hormones applied
TOTAL ABSORPTION AREA (TAA)	- 0.403ns
ACTIVE ABSORPTION AREA (ABA)	0.357ns
PERCENTAGE ACTIVE ABSORPTION AREA RATIO (PAAAR)	- 0.999*
ROOT VOLUME (RV)	- 0.500ns
SPECIFIC SURFACE AREA (SSA)	- 0.191ns
ROOT ACTIVITY	0.837ns

Ns :not significant.

\*Significant at p<.005

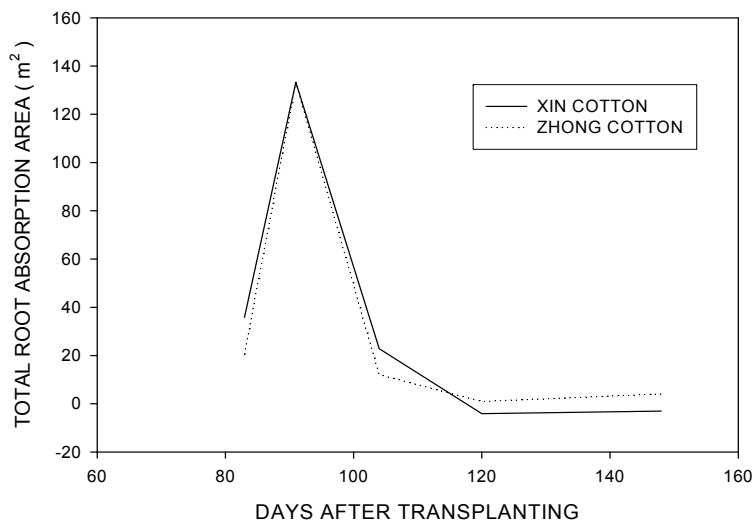


Figure 1. Root total absorption area of cotton plant varieties grown hydroponically treated to phosphorus and potassium

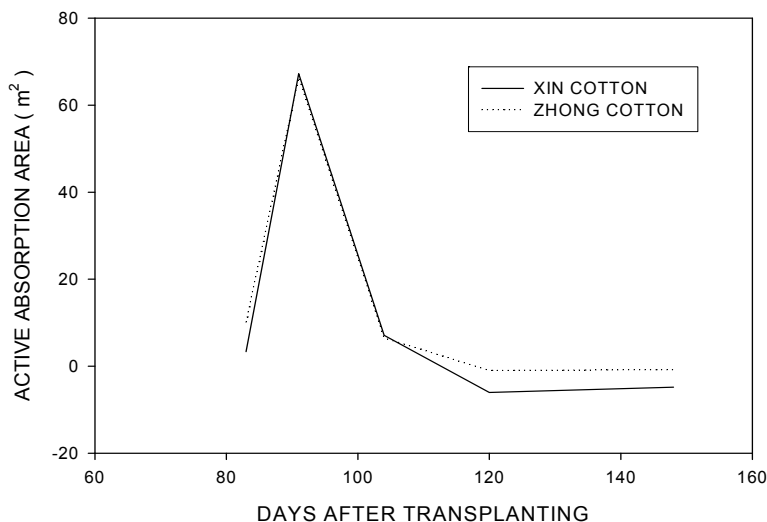


Figure 2. Root active absorption area of cotton plant varieties grown hydroponically treated to phosphorus and potassium

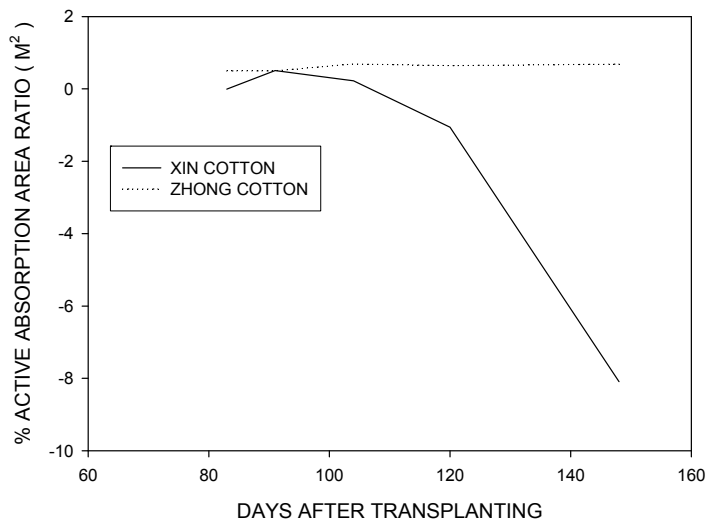


Figure 3. Root percentage active absorption area ratio of cotton plant varieties grown hydroponically treated to phosphorus and potassium

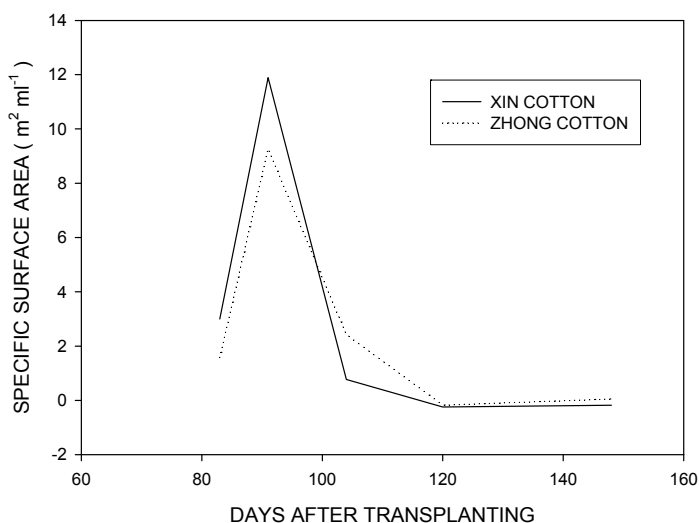


Figure 4. Root specific surface area of cotton plant varieties grown hydroponically treated to phosphorus and potassium

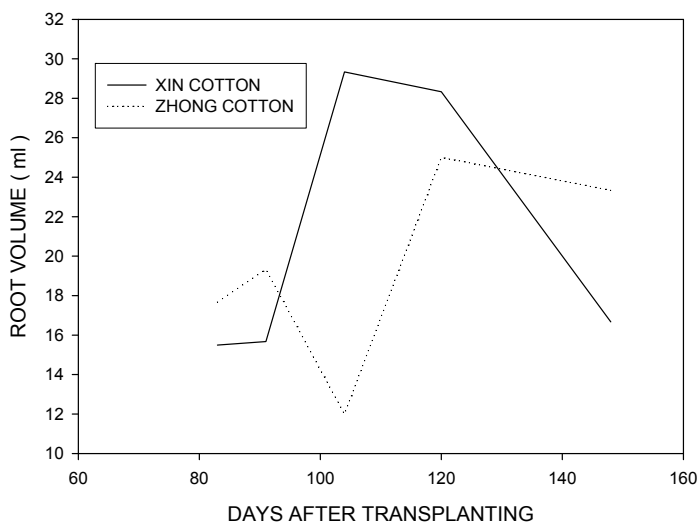


Figure 5. Root volume of cotton plant varieties grown hydroponically treated to phosphorus and potassium

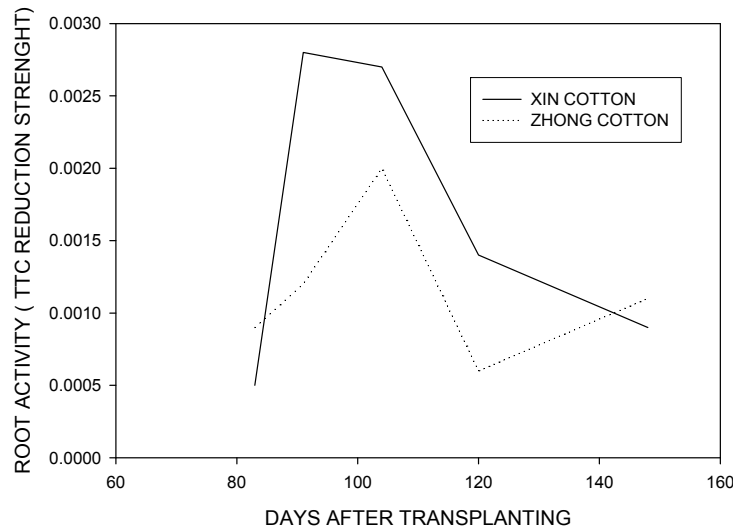


Figure 6. Root activity of cotton plant varieties grown hydroponically treated to phosphorus and potassium

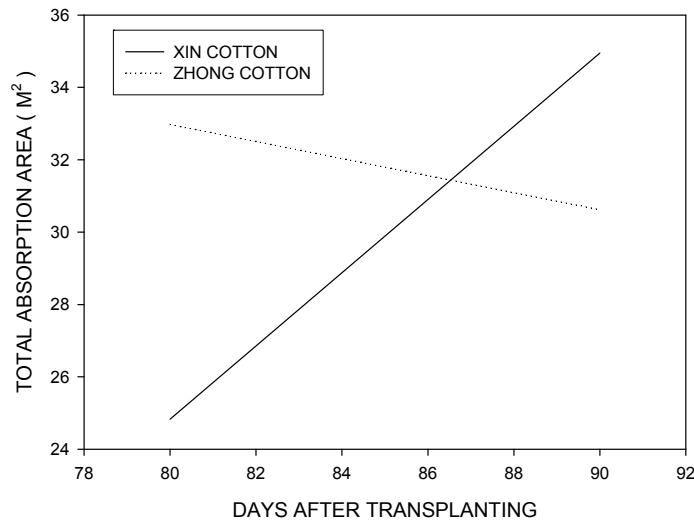


Figure 7. Effect of hormones concentration on root total absorption area of cotton varieties grown hydroponically in high level of PK

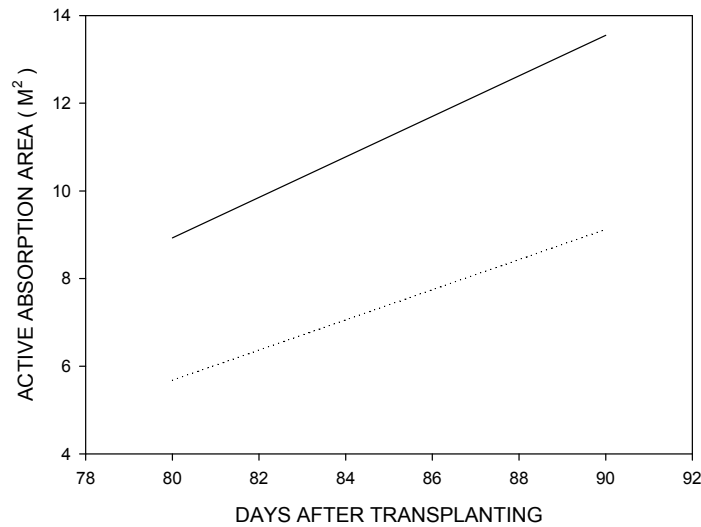


Figure 8. Effect of hormones concentration on root active absorption area of cotton varieties grown hydroponically in high level of PK



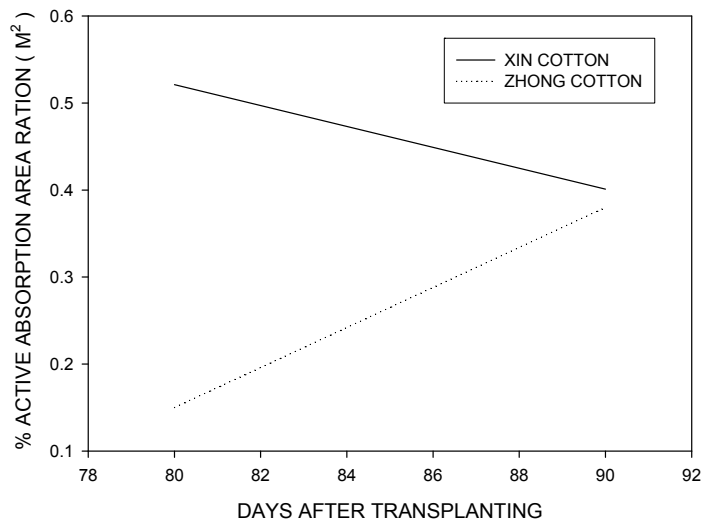


Figure 9. Effect of hormones concentration on percentage root active absorption area of cotton varieties grown hydroponically in high level of PK

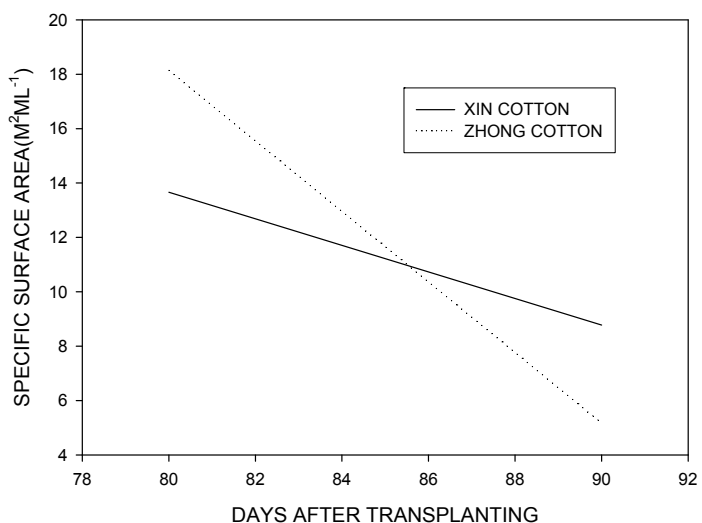


Figure 10. Effect of hormones concentration on root specific surface area of cotton varieties grown hydroponically in high level of PK

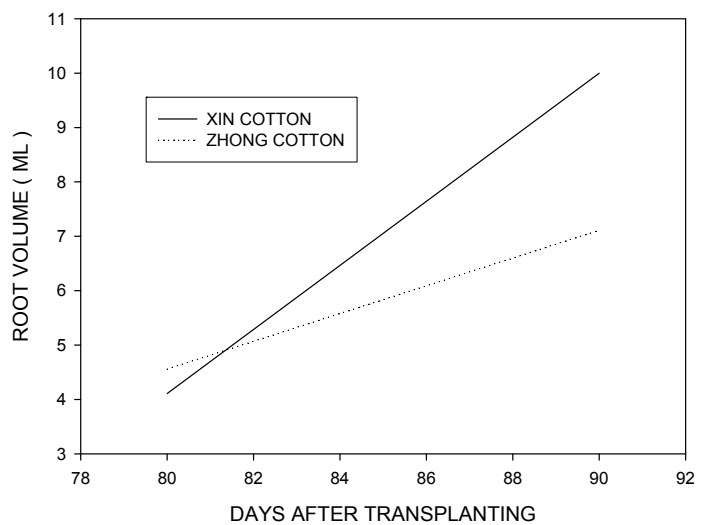


Figure 11. Effect of hormones concentration on root volume of cotton varieties grown hydroponically in high level of PK

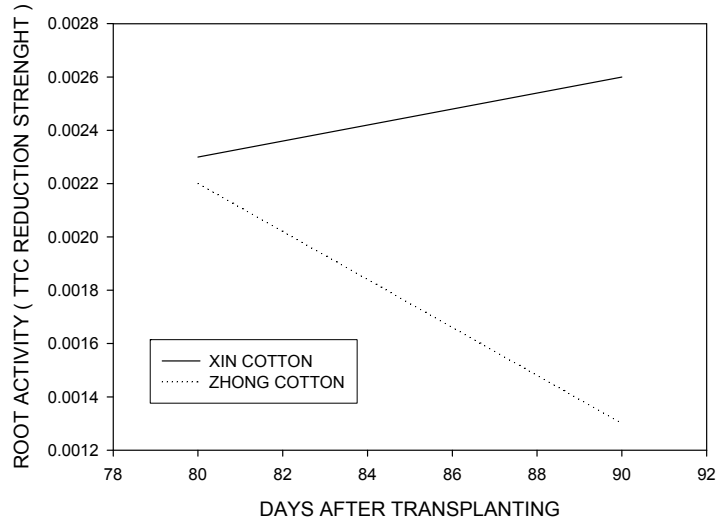


Figure 12. Influence of hormones concentration on cotton root activity varieties grown hydroponically in high level of PK