Phosphorus, Potassium and Phytohormones Promote Chlorophyll Production Differently in Two Cotton (*Gossypium hirsutum*) Varieties Grown in Hydroponic Nutrient Solution

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Received: July 1, 2011 Accepted: July 12, 2011 Online Published: December 21, 2011
doi:10.5539/jas.v4n2p157 URL: http://dx.doi.org/10.5539/jas.v4n2p157

Abstract

The effectiveness of phosphorus (P), potassium (K), indole-3-acetic acid (IAA), gibberellic acid (GA_3), zeatin (Z) and their combinations on chlorophyll a, b and total chlorophyll production was investigated in the hydroponic nutrient solution. The cotton plants were cropped without hormones application in different levels of P and K in the first cropping and with hormones application in the second cropping in high level of PK. In the first cropping, low K and high PK significantly influenced formation of chlorophyll a, b and ab from 83 to 91 days after planting (DAT) while the application of GA_3, IAA, Z and IAA x GA_3 x 2Z treatments significantly increased chlorophyll a, b and ab only at 80 DAT. However, two cotton varieties planted in two cropping seasons expressed different abilities in time to promote chlorophyll formation.

Keywords: Indole-3-acetic acid, Gibberellic acid, Zeatin, Chlorophyll, Hydroponic, Phosphorus, Potassium, Cotton

1. Introduction

Plant hormones have been known to regulate the physiological process in plant. Greening in plant indicates a well being 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 (*Cucumis sativus cv Brumex*) 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).

Plant nutrients especially Nitrogen, Phosphorus and Potassium (NPK) are primary nutrients elements that plants require in large quantity for the physiological development. The physiological changes in plant status may be 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 potassium nutrient increase chlorophyll content of plants (Stromberg 1960; Fletcher *et al.,* 1982; Maples *et al.,* 1988; Duli *et al.,* 2001). From the previous studies, phosphorus needed for chlorophyll formation received less attention. It is reported in the literature that potassium and cytokinins stimulate chlorophyll synthesis in plants. The main objective of this research was to investigate the influence of exogenous application of phytohormones (GA_3, IAA, Z and their combinations) in chlorophyll production.

2. Materials and Methods

2.1 First cropping

Nutrients solution experiment: The two cotton varieties (Zhong Mian 36 and Xin Luo Zao 13) were cultivated in the quartz sand. The roots of cotton seedlings were allowed to grow up to 8 – 10cm, before transplanting into nutrient solution pot of 6L.

These specifications were followed: The day time temperature was kept at a range of 20 – 35°C, the surrounding sunlight was maintained above 12 hours, there was a constant supply of oxygen and the room temperature was recorded. The nutrient solutions were Hoagland and micro anion nutrients, the low P level was 0.05 x 10^{-3}molL^{-1}, the low K level was 1 x 10^{-3} molL^{-1} and the high PK level were 1 x 10^{-3} and 6 x 10^{-3}molL^{-1} at pH 6.5 (Table 1). At every nutrient change, one cotton plant from each of three treatments of the two cotton varieties of the experiment replicated three times (3 x 2 x 3 = 18) to measure chlorophyll a, b and ab.

2.2 Second cropping

In the second cropping of the experiment, the cultivation and other necessary measures were as in the first cropping. The exogenous hormones concentration were applied at 36 and 67 days after transplanting to Hoagland complete nutrients fluid by spraying on cotton leave at single rate of 0, 50, 40 and 50 μ gL^{-1} for untreated pots, indole-3-acetic acid, gibberellic acid and zeatin, respectively and combined rate of 50IAA*40GA*50Z, 100IAA*40GA*50Z, 50IAA*80GA*50Z, 50IAA*40GA*100Z and 100 IAA*80GA*100 supply with the highest PK nutrient levels of 1 x 10^{-3} and 6 x 10^{-3}molL^{-1} at pH 6.5 in the hydroponics solution (Table 1). At every nutrient change, nine cotton were selected from the each of the two varieties, replicated three times (9*3*2 = 54) so as to measure chlorophyll a, b and ab.

2.3 Chlorophyll measurement

0.5g of leaf pigment was extracted from cotton leaves. 8ml of 80 percent Acetone was added, and incubated for 12 – 14 hours. 1 ml solution was taken out of the latter, 9 ml of 80 percent acetone was added, and then centrifuge. 10 ml of 80 percent acetone was added to the supernatant. Thereafter, measured with spectrophotometer at wavelength of 663nm and 645nm for chlorophyll a and b, respectively.

2.4 Data Analysis

The data was analyzed using general linear model (GLM) with SPSS software version 15. Least significance differences using Duncan's multiple range test (DMRT) for separation of treatment and variety means. Correlation statistical analysis was used to compare chlorophyll a, b and ab formation in the first and second cropping of the experiment.

3. Results

3.1 First Cropping Experiment

Effect of phosphorus (P), potassium (K) and PK treatments on cotton chlorophyll a, b and ab production grown in hydroponics nutrients solution.

The effects of low P, low K and high PK treatments on chlorophyll a are shown in Table 2. The high PK treatment significantly produced the highest chlorophyll a with the value of 1.92 μ g ml^{-1} which was insignificant to low K treatment with value of 1.23 μ g ml^{-1}. The low P treatment gave the lowest chlorophyll a production

(0.456 g ml^{-1}) which was insignificant to low K treatment at 83 days after transplanting (DAT). However, at 91 DAT, low K and high PK treatments significantly produced more chlorophyll a (1.76 and 1.90 μ g ml^{-1}, respectively) than low P treatment with value of 0.504 g ml^{-1}. As from 104 to 148 DAT, there was no significant difference in the ability of the treatments applied to promote chlorophyll formation.

The effects of low P, low K and high PK treatments on chlorophyll b and ab production are summarized in Table 3 and 4. At 83 DAT, it is obvious that low K and high PK treatments enhanced more chlorophyll b production 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. The same result was obtained for chlorophyll ab.

3.2 Varieties

Figure 1a shows that Xin Cotton variety significantly influenced more chlorophyll a production than Zhong cotton variety at 83 DAT. The two cotton varieties produced no change in chlorophyll a production from 91 to 120 DAT. However, at 148 DAT, Zhong cotton variety proved superior to Xin cotton variety in chlorophyll a production.

Figure 1b reveals that Xin Cotton variety produced more chlorophyll b than Zhong cotton variety at 83 and 104 DAT. As from 91 to 120 DAT, the two varieties were not significantly different from each other in chlorophyll b production. Zhong cotton variety performed better than Xin cotton variety at 148 DAT. The same result was obtained for the two varieties in relation to chlorophyll a and chlorophyll ab production (Fig 1c).

3.3 Second Experiment

3.3.1 Effect of hormones treatment on cotton plants chlorophyll a, b and ab production grown in hydroponics nutrient solution

Table 5 summarizes the effect of hormones on chlorophyll a production grown hydroponically in high level of phosphorus and potassium nutrients. The single applied IAA and Z treated plants significantly enhanced chlorophyll a production, but there was no significant difference between plants treated with IAA, Z and those plants treated with GA_3, IAA x GA_3 x 2Z 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 pot significantly gave more chlorophyll a production than other treatments although untreated pot was insignificant to IAA and 2IAA x GA_3 x Z treated plants.

Effect of hormones on chlorophyll b production grown in hydroponic nutrient solution of high level phosphorus and potassium nutrients are given in Table 5. The combined application of IAA x GA_3 x 2Z caused significant chlorophyll b production than other treatments although combined hormones application of IAA x GA_3 x 2Z was the same as with a single applied IAA, GA_3 and Z hormone application. However, combined use of 2IAA x GA_3 x Z including untreated plants gave the least chlorophyll b formation at 80 DAT. Thereafter, at 90 DAT, amazingly, untreated plants had significantly more chlorophyll b content than other treatments but the effect was the same as with IAA treated plants.

The effect of hormones on chlorophyll ab production grown in hydroponics nutrients solution of high level phosphorus and potassium are presented in Table 5. The single hormone application of IAA, Z and combined hormone application of IAA x GA_3 x 2Z gave the highest chlorophyll ab production than the other treatments but these treatments (IAA, Z, and IAA x GA_3 x 2Z) were insignificant to single hormone 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 or 2IAA x GA_3 x Z treated pots.

3.4 Varieties

Figures 2a, 2b and 2c show that high content of chlorophyll formation was observed at the beginning of the experiment and decreased downward toward the end of the experiment. The Zhong cotton variety produced significantly more chlorophyll a, b and ab than Xin cotton variety at 80 DAT. Beyond 80 DAT, Zhong cotton variety still proved superior to Xin cotton variety in chlorophyll b production but it apparently appeared the same for the two varieties in chlorophyll a and ab production (Fig.2a, 2b and 2c).

3.5 Correlation co-efficient relating low P, low K and high PK plant treatments at 148 DAT to hormones (IAA, GA_3, Z and their combinations) plant treatments at high level of PK at 90 DAT

The correlation co-efficient relating first and second crop performances are given in Table 6. There was no relationship between the first cropping experiment and the second cropping experiment. Chlorophyll a and ab formation in the first cropping were not related to chlorophyll a and ab formation in the second cropping while chlorophyll b was negatively insignificant (r= - 0.005).

4. Discussion

The low K and high PK treated plants enhanced chlorophyll a, b and ab production at 83 and 91 DAT. This confirms 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 confirmed 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. However, variety differences were noted at 83 DAT and 148 DAT for chlorophyll a and ab, Xin cotton variety performed better than Zhong cotton variety at 83 DAT, reversed was the case at 148 DAT, Zhong cotton variety outclassed Xin cotton variety. Nevertheless, differences between the two varieties for chlorophyll b production was also observed at 83 and 104 DAT, where Xin cotton outclassed Zhong cotton variety. The opposite observation was seen at 148 DAT. This agrees with finding of Cassman *et al.,* (1989) that different varieties exhibited variations in nutrients efficiency utilization such as K.

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.

Nevertheless, there were obvious differences between varieties in chlorophyll production. Zhong cotton produced greener pigment (Chlorophyll a, b and ab) than Xin cotton; this could be caused by genotypic differences between both varieties (Cassman *et al.,* 1989; Jiang *et al.,* 2008).

There was no relationship between chlorophyll a, b and ab in the first cropping without hormones application harvested at 148 DAT and the second cropping with hormones application harvested at 90 DAT. This could be as a result of levels of hormone concentration applied twice at 36 and 67 DAT. The higher levels of the second hormones application at 67 DAT, thereafter, caused the leaves green pigment to change to yellow pigment.

5. Conclusion

The results of this investigation indicate a significant role of potassium (K) at low (1×10^{-3}), high (6×10^{-3} $molL^{-1}$) levels, phosphorus (P) at high (1×10^{-3} $molL^{-1}$) level 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 1st experiments without hormones application and 80 DAT for the 2nd experiment with hormones application. The second cropping should have looked healthier than first cropping, if the second hormones application were not done at 67 DAT. Hence, the result of this present study suggests an early application of hormones at 36 DAT would be better. There is a need for hydroponics farmers to pay close attention to time and concentration of hormones so as to obtain optimum harvest.

Acknowledgement

Many thanks to Faculty of graduate study Dalhousie University, Halifax, Canada for research Scholarship. The author appreciated the collaboration study carried out with College of Natural Resources and Environmental Sciences research team of Xinjiang Agricultural University, Urumqi, China. The gratitude goes to Prof.Wang Chunli, Shen Dong and Chenboliang for the interpretation and technical support during the project.

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Table 1. Hydroponic nutrient composition

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

P-Phosphorus
K-Potassium

Table 2. 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	1.23ab	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.

Table 3. The chlorophyll B content of cotton plants grown hydroponically treated to phosphorus and 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.

Table 4. The chlorophyll AB content of cotton plants grown hydroponically treated to phosphorus and 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.

Table 5. Effect of hormones concentration on chlorophyll content of cotton plants grown hydroponically in high level of PK

	CHLOROPYLL (μ gml^{-1}) Days after transplanting (DAT)					
	80			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.

Table 6. Correlation co- efficient relating low P, low K and high PK plant treatments at 148 days after transplanting to hormones plant treatments at high level of PK at 90 days after transplanting

Chlorophyll Content (μ gml^{-1})	Correlation Co-efficient (r) relating low P, low K and high PK to hormones applied
A	0.188ns
B	- 0.005ns
AB	0.114ns
ns-not significant	

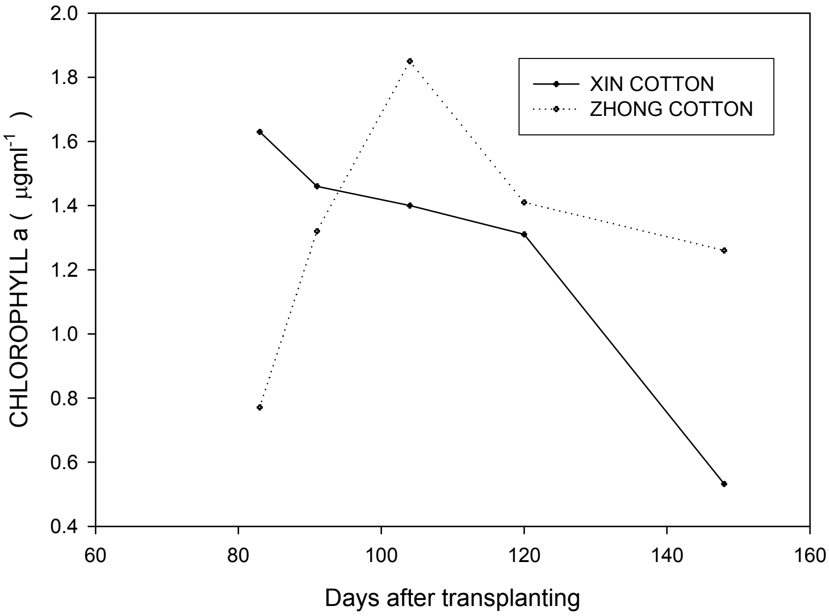

Figure 1a. The chlorophyll a content of cotton plant varieties grown hydroponically treated to phosphorus, potassium and their combination

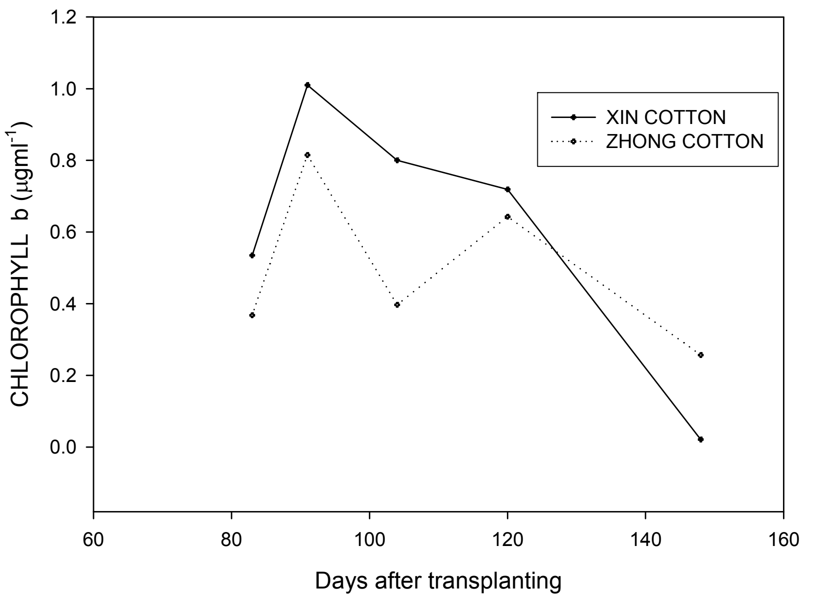

Figure 1b. The chlorophyll b content of cotton plant varieties grown hydroponically treated to phosphorus and potassium and their combination

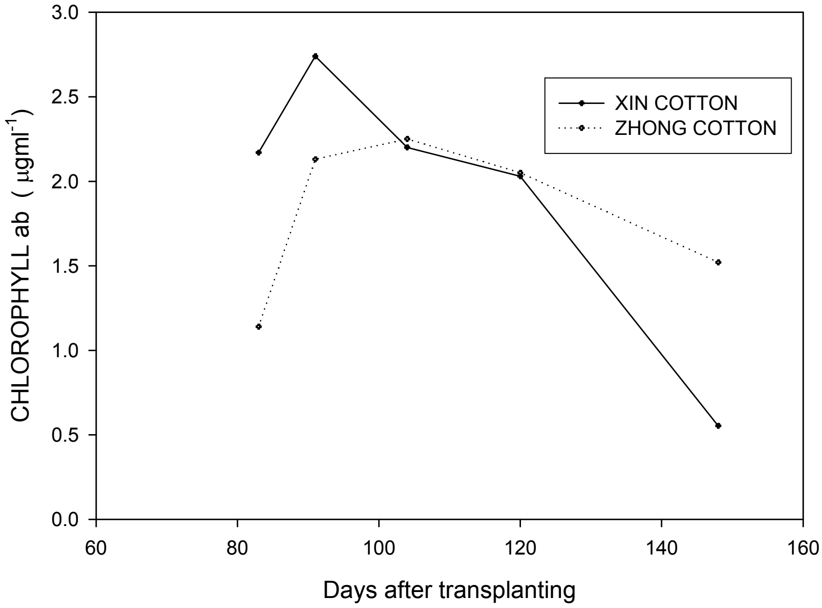

Figure 1c. The chlorophyll ab content of cotton plant varieties grown hydroponically treated to phosphorus and potassium and their combination

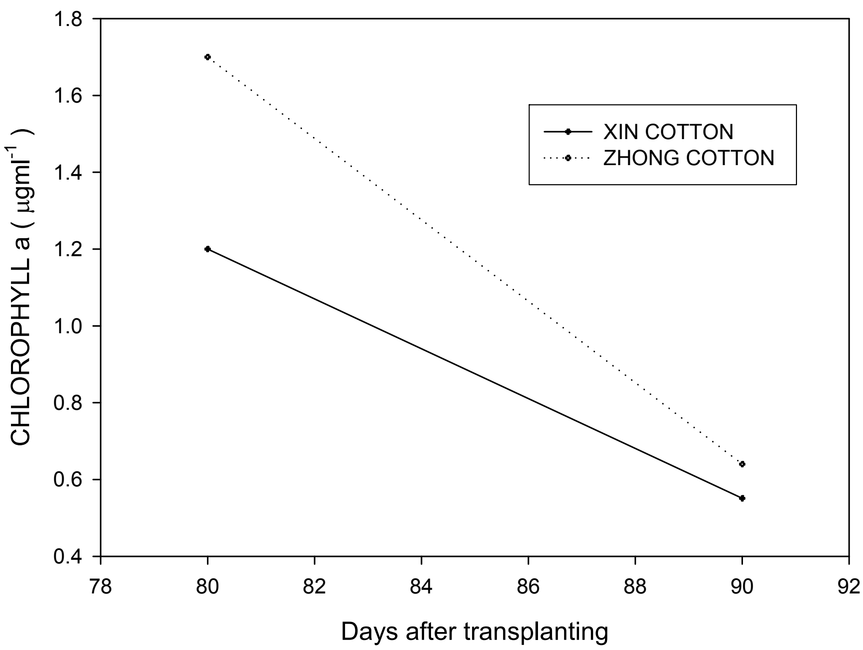

Figure 2a. Effect of hormones concentration on chlorophyll a content of cotton varieties grown hydroponically in high level of PK

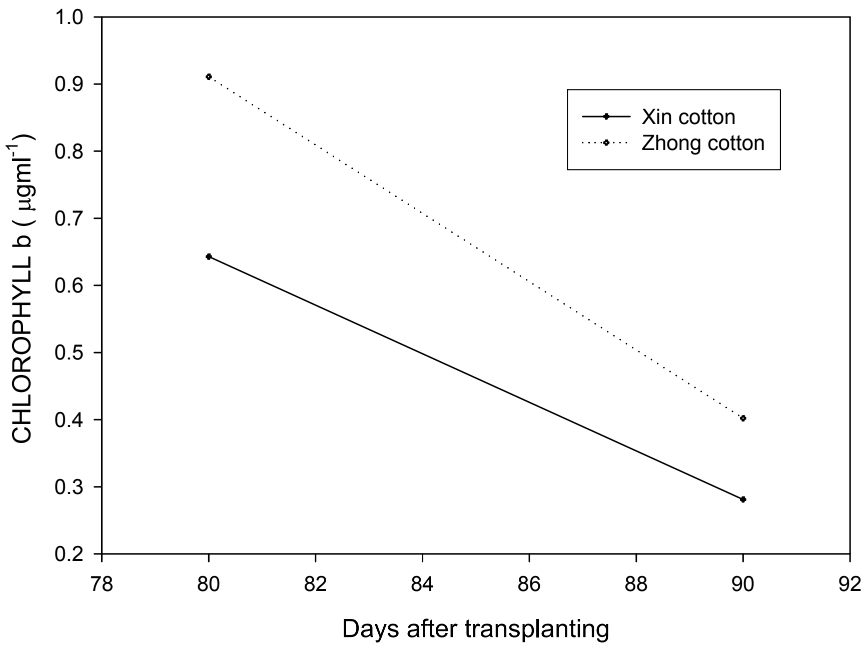

Figure 2b. Effect of hormones concentration on chlorophyll b content of cotton varieties grown hydroponically in high level of PK

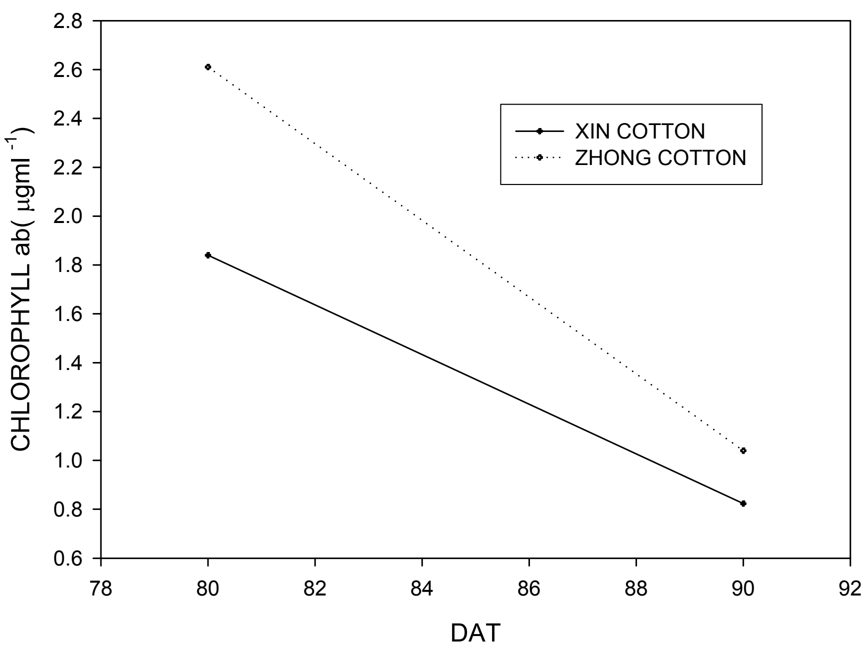

Figure 2c. Effect of hormones concentration on chlorophyll ab content of cotton varieties grown hydroponically in high level of PK