

EFFICACY OF INDOLE ACETIC ACID, GIBBERELLIC ACID OR KINETIN ON PIGMENTS PRODUCTION, PHOTOSYNTHETIC CHARACTERISTICS AND CARBOHYDRATES CONTENT IN LEAVES OF *VIGNA SINENSIS* PLANTS

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ABSTRACT

The objective of this study was conducted to investigate the effect of seed priming with different concentrations of growth bioregulators (i.e. indole acetic acid, gibberellic acid or kinetin) on pigments production, photosynthetic characteristics and carbohydrates content in leaves of *Vigna sinensis* L. Cream 7 plants throughout various stages of plant growth and development.

In the majority of cases seed priming with different concentrations of different growth regulators increased photosynthetic pigments, photosynthetic characteristics, Hill activity as well as carbohydrates content in leaves of cowpea plants.

Key words: Carbohydrates, Cowpea, Gibberellic acid, Hill activity, Indole acetic acid, Kinetin, Photosynthetic activity.

INTRODUCTION

Certain gibberellins have been found to influence chloroplast development; acting in some cases as stimulator, while, in others as inhibitor [El-Gendy *et al.*, (1995) and Abdel-Razik (1996)]. In this respect, [Farahat *et al.*, (1995)] showed that GA₃ at the highest concentrations (500 and 600 ppm) led to the formation of the highest contents of chlorophylls (a and b) and carotenoids, but the low concentrations (200, 300 and 400ppm) led to reduction in all of these

pigments. In addition exogenous application of GA₃ increased chlorophyll content and yield of cereal plants [Kadiri & Hussani (1999)].

Shawkat (2000) showed that the foliar application of GA₃ treatments (25, 50 and 75 ppm) enhanced the photosynthetic activity of *Gossypium arborium* plants grown in either clay or sandy soil. Also, the soluble, insoluble and consequently the total photosynthetic metabolites showed highly significant increase in response to the different used concentration of GA₃. In addition [Naidu & Swamy (1995)] stated that photosynthetic rate is increased in various plants treated with gibberellic acid. Moreover, [Pedhadiya et al., (1987)] found that both photosystem I (PSI) and photosystem II (PSII) were significantly increased by GA₃ application.

It is clear by many investigators that gibberellic acid application on different plant species resulted in an increase in total carbohydrates content [Abdallah et al., (1992)]. Furthermore, [Kordei & Kustschera (1998)] concluded that, induction of growth of pea plants by GA₃ was associated with an increase in glucose level in shoot of pea plants. [Aldesuquy (2000)] showed that grain priming with indole acetic acid at 25 mg kg⁻¹ stimulated the growth of flag leaf, fresh and dry weights and leaf area of wheat plants. Furthermore, he concluded that, the application of IAA increased chlorophyll contents and carotenoids, reducing sugars, Hill reaction activity and photosynthetic characteristics in flag leaf of wheat plants.

Mansour et al., (1987) showed that kinetin had a stimulatory effect on chlorophyll (a,b) and carotenoids synthesis during the early stages of growth. However at the later stages of plant development, the pigments contents were decreased significantly in wheat plant. In this respects, [El-Afifi (1990)] stated that the changes in photosynthetic pigments due to kinetin-included treatments could be related to its role in chloroplast division. Also, [Nieto & Frankenberger (1990)] reported that kinetin not only induced a higher accumulation of chlorophylls and protochlorophyllide but also changed the amount of *in vivo* chlorophyll forms which accelerate photosynthesis in plants. Moreover, [Reena et al., (1999)] found that soaking soyabean seeds in 25-150 ppm kinetin increased leaf chlorophyll and N contents, seed yield and seed protein and oil content of treated plants.

MATERIALS AND METHODS

Plant growth and culture conditions:

A homogenous lot of *Vigna sinensis* seeds were selected. The seeds were surface sterilized by soaking in 0.01% HgCl₂ solution for three minutes, then washed thoroughly with distilled water and then divided into ten sets that were separately soaked for three hours in the following solutions:

- | | |
|-------------------------------|--------------------------------|
| 1-Distilled water. | 2- Indole acetic acid; 100ppm. |
| 3-Indole acetic acid; 150ppm. | 4- Indole acetic acid; 200ppm. |
| 5- Gibberellic acid; 25ppm. | 6- Gibberellic acid; 50ppm. |
| 7- Gibberellic acid; 75ppm. | 8- Kinetin; 25ppm. |
| 9- Kinetin; 50ppm. | 10- Kinetin; 75ppm. |

A preliminary test was done to choose the suitable concentrations and soaking period. The results showed that the most suitable and effective period of soaking is three hours in the mentioned concentrations of each growth regulator. Soaking the seeds in the different solutions was carried out at room temperature.

The soaked seeds of *Vigna sinensis* were sown on the 15th May 2000 in earthenware pots (30 cm in diameter) containing equal amount of homogenous soil (sand and clay 1:2). The soil contained a phosphorus fertilizer which was applied in the form of triple superphosphate. The irrigation was carried out according to the usual practice by adding equal amounts of water. After two weeks, thinning was performed where five uniform seedlings were left in each pot for experimentation. The plants were exposed to normal day length and illumination.

Hill reaction, photosynthetic pigments and carbohydrates content were measured at four stages of experimental period. These stages represented 30, 45, 60 and 75 days from sowing and referred to two successive vegetative, flowering and fruiting stages respectively. Photosynthetic activities and Hill reaction were measured in 3rd compound leaf (numbered from base) during two harvest from vegetative stage.

The data were statistically analyzed using the Least Significant Differences (LSD) at ($P \leq 0.05$) level of probability [Snedecor & Cochran (1976)].

Estimation of photosynthetic pigments:

The plant photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined at different stages of plant development according to the spectrophotometric method as recommended by [Metzner *et al.*, (1965)].

Determination of photosynthetic activity:**Hill reaction assay:**

As described by [Arnon (1949)], 4 g of detached leaves were ground three times for 5 seconds at full speed in a chilled blender in 50 ml of 50 mM N-Tricine (N-Trishydroxyl methylglycine, pH 7.8), 0.3M-sucrose and 2mM - MgCl₂. The resulting homogenate was filtered through four layers of nylon mesh. Chloroplast pellets were obtained by centrifugation at 2000 xg for 10 minutes. The pellets were resuspended in 30 ml of 0.1 M-NaCl and then centrifuged again at 5000 xg for 5 minutes. The resulting pellets were resuspended in 1 mM N-Tricine (pH 7.8), 10 mM-NaCl and 10 mM-MgCl₂ and then kept at 0-4°C until used for the analysis.

Photosystem II activity, as indicated by the rate of 2,6 dichlorophenolindophenol (2,6-DCPIP) photo-reduction [Trebst (1967)] was monitored at 600 nm using spectronic 21 D spectrophotometer. The sample cuvette was illuminated by tungsten lamp (6000 lux) using head filter (CuSO₄ solution). The photosystem II reaction mixture contained 200 mM-Na phosphate (pH 6.7), 2 mM-MgCl₂ and 0.5mM-DCPIP. The chlorophyll concentration range for this experiment was 10 µg /ml of the reaction mixture (4 ml).

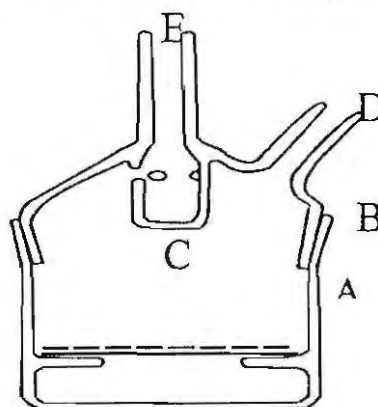
¹⁴CO₂ - light fixation:

The method used in the present study was described by [Shadad (1979)]. A definite fresh weight of leaves was introduced into the fixation apparatus (Fig.1), the lid of which has two side arms and a small container (1 ml capacity) attached at its inner lower side. 0.2 ml of sodium bicarbonate-C¹⁴ aqueous solution of known activity (100 µ Ci/ml) were pipetted through the upper vertical arm into the small inner container, the apparatus was then covered with its lid. The two side arms were covered with rubber caps, then 0.2 ml of H₂SO₄ (10%) were injected through the upper vertical arm to react with the NaH¹⁴CO₃ in the inner container. The evolving ¹⁴CO₂ passed through the pores, in the

upper part, of the inner container, to the main apparatus where it could be photosynthesized by the green leaf discs. The experiment was carried out in natural sunlight. At the end of the fixation period (10 minutes), the leaf discs were quickly transferred to boiling 85% (v/v) aqueous methanol, to be killed and extracted.

After being cooled, the insoluble fraction was separated from the soluble one by centrifugation at 2000 xg for 10 minutes. The soluble and insoluble fractions were then made up to volume (35 ml) and an aliquot (2 ml) of which was pipetted for radioactivity measurements Fig. (1):

$^{14}\text{CO}_2$ -Fixation apparatus
 A: Main container.
 B: Lid.
 C: Inner container (one ml. capacity)
 D, E: Side arms.



The counts per minute (Cpm) obtained were then calculated to disintegration per minute (Dpm) according to efficiency of the apparatus. The radioactivity measured is directly proportional to the amount of $^{14}\text{CO}_2$ fixed in the organic compounds and this was calculated as Cpm/mg fresh weight of leaves.

Estimation of carbohydrates:

Extraction of plant tissue:

Sugars were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25 °C with periodic shaking.

Total soluble sugars and sucrose were determined using modifications of the procedures of Yemm and Willis (1956) and modified by [Handle (1968)] respectively.

Estimation of total soluble sugars (T.S.S.):

Total soluble sugars were analyzed by reacting 0.1ml of the alcoholic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone

+ 100 ml of 72% H₂SO₄) in a boiling water bath for 10 min and reading the cooled samples at 625 nm in a spectronic 21 D spectrophotometer.

Estimation of sucrose:

Sucrose content was determined by first degrading reactive sugars present in 0.1 ml extract with 0.1 ml 5.4 N KOH at 97 °C for 10 min. Three ml of freshly prepared anthrone reagent were then added to the cooled reaction product, and the mixture was heated at 97 °C for 5 min, cooled, and read at 620 nm.

Estimation of glucose:

Glucose contents were estimated using the o-toluidine procedure of [Feteris (1965)] as modified by [Riazi *et al.*, (1985)].

Estimation of polysaccharides:

Polysaccharides content were estimated according to the procedure adopted by [Younis *et al.*, (1969)].

RESULTS

Changes in Photosynthetic Pigments:

The effect of different concentrations of IAA, GA₃ or kinetin on total chlorophylls (Chl a + b), Chl a/b ratio and carotenoids of cowpea leaves during the experimental stages (30, 45, 60 and 75 days old) is illustrated in figures 2, 3 and 4 respectively.

As appeared from figure 2, the total chlorophylls (Chl a + b) showed a marked increase from the first harvest of vegetative stage (30 days old) to the second harvest of vegetative stage (45 days old) followed by a sharp decrease during the flowering and fruiting stages (60 and 75 days old) in untreated and IAA-treated plants. As compared with control plants the different IAA treatments caused significant increases ($P \leq 0.05$) in the content of total chlorophylls (Chl a + b) throughout the four successive stages of experimental period.

As regards the Chl a/b ratio, in relation to the control value, the lowest concentration of IAA (200ppm) induced a significant increase ($P \leq 0.05$) and decrease respectively in this ratio during the two successive harvest of vegetative stage. On the other hand, at flowering and fruiting stages, the different concentrations of IAA induced marked decreases ($P \leq 0.05$) in the ratio of Chl a/b below the control values. The magnitude of

response was more pronounced with the increase in the concentration of IAA.

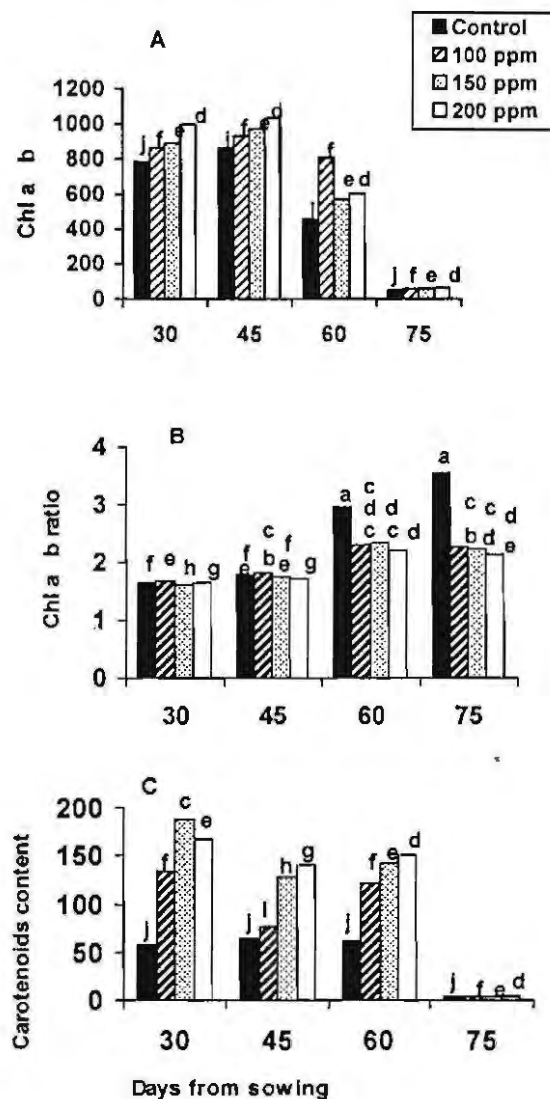


Fig. (2): Effect of seeds presoaking in different concentrations of indole acetic acid (IAA) on pigments content (mg g⁻¹ f. wt.) (A) Chl a+b, (B) Chl a/b & (C) carotenoids of cowpea leaves during growth and development. Bars in a grouping labeled with the same letter are not significant as indicated by LSD at (P < 0.05).

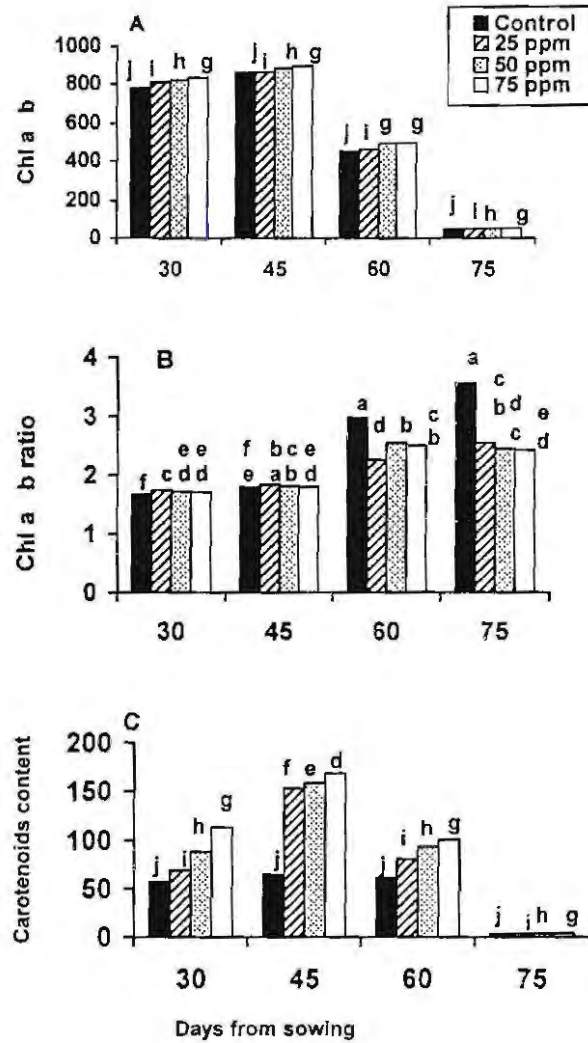


Fig. (3): Effect of seeds presoaking in different concentrations of gibberellic acid (GA₃) on pigments content (mg g⁻¹ f. wt.) (A) Chl a+b, (B) Chl a/b & (C) carotenoids of cowpea leaves during growth and development. Bars in a grouping labeled with the same letter are not significant as indicated by LSD at (P < 0.05).

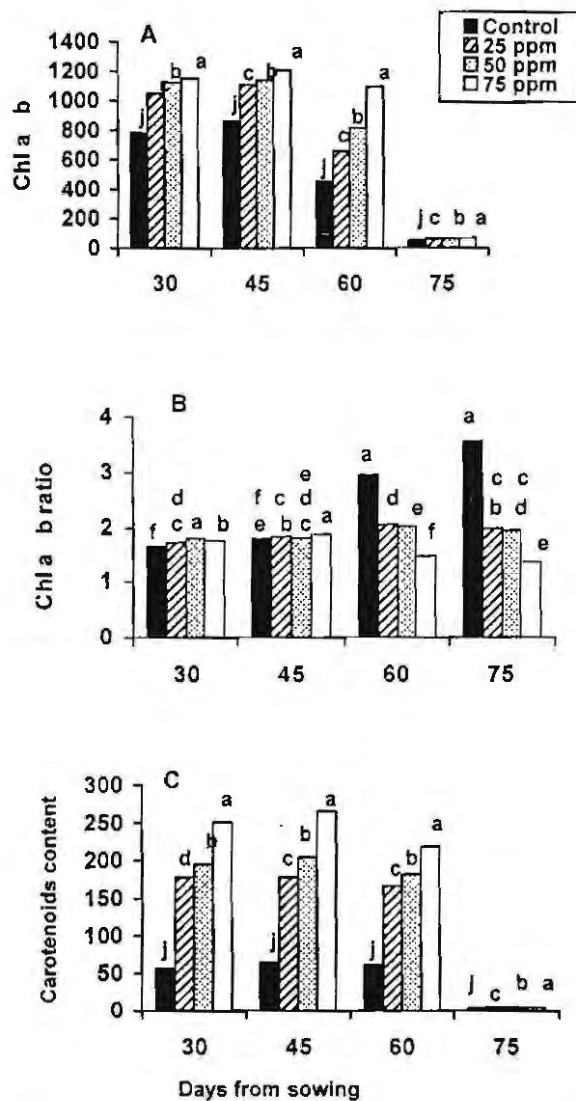


Fig. (4): Effect of seeds presoaking in different concentrations of kinetin (K) on pigments content (mg g⁻¹ f. wt.) (A) Chl a+b, (B) Chl a/b & (C) carotenoids of cowpea leaves during growth and development. Bars in a grouping labeled with the same letter are not significant as indicated by LSD at (P < 0.05).

The data represented in figure 2 showed that, seed presoaking in IAA at different concentrations increased significantly ($P \leq 0.05$) carotenoids in the cowpea leaves throughout all stages of plant growth as compared with control values. Except for the plants that treated with 150ppm of IAA which showed the maximum value of carotenoids at the first harvest of vegetative stage (30 days old), the increasing in the concentration of IAA increased the value of carotenoids of treated plants all over the experimental period.

As compared with control plants, seed priming with GA_3 at 25, 50 and 75 ppm increased ($P \leq 0.05$) total chlorophylls (Chl a +b) and carotenoids throughout all stages of plant growth. Furthermore, the Chl a/b ratio of the different GA_3 -treated plants showed a significant increase over the control value during the first and second harvest of vegetative stages (30 and 45 days old). Whereas a reversible situation was observed at flowering and fruiting stages (60 and 75 days old).

Except for the sharp decline in carotenoids content of untreated plants when compared with that treated with different concentrations of GA_3 throughout the experimental stages particularly at the first and second harvest of vegetative stage (30 and 45 days old) of experimental period; the changes in carotenoids of the variously treated cowpea plants followed a trend similar to that of total chlorophylls (Chl a+b) all over the growth period.

The values of total chlorophylls (Chl a + b) in leaves of untreated and variously kinetin-treated plants showed a slight increase from the first harvest of vegetative (30 days old) to the second harvest of vegetative stage (45 days old) followed by marked and sharp decreases at flowering and fruiting stages respectively. In relation to the control values seeds presoaking in kinetin at different concentrations induced a significant increases ($P \leq 0.05$) in total chlorophylls (Chl a + b) in cowpea plants during the different growth stages of the experimental period.

Regarding Chl a/b ratio, at the first and second harvest of vegetative stage, the different concentrations of kinetin induced significant increases ($P \leq 0.05$) in this ratio over that of control. Slight inconsistent variations, were, however among the variously treated plants. On the other hand, a marked decline ($P \leq 0.05$) in Chl a/b ratio was induced by the different kinetin treatment in relation to Chl a/b ratio of control.

Carotenoids content in leaves of the differently kinetin-treated plants showed a slight increase from the first harvest to the second harvest of vegetative stage followed by slight and marked decreases at flowering and fruiting stages respectively. On the other, hand carotenoids content in leaves of untreated plants showed slight changes from vegetative stage to flowering stage followed by sharp decline at fruiting stage.

As compared with the control, kinetin treatments (25, 50 and 75 ppm) increased ($P \leq 0.05$) significantly the level of cartenoids throughout the experimental stages; the magnitude of increase was more pronounced in the plants treated with the highest concentration of kinetin (75ppm) at second vegetative stage (45 days old).

Photosynthetic characteristics:

Hill reaction:

Photoreduction of dichlorophenol indophenol (DCPIP) by isolated illuminated cowpea chloroplasts of the different IAA, GA₃ or kinetin-treated plants during the two harvest of vegetative stage of plant growth and development is shown in figure 5.

Perusal of the data represented in figure 5 indicated that presoaking of cowpea seeds in indole acetic acid at 100,150 and 200ppm, GA₃ or kinetin at 25, 50 and 75ppm significantly increased ($P \leq 0.05$) the reduction of DCPIP over that observed in control plants. Increasing the concentration of the used growth regulators increased the rate of reduction of DCPIP except at the first harvest of vegetative stage when the following sequence (50 > 25 > 75ppm) with respect to the effect of kinetin as compared with control. Moreover, the magnitude of increase in the Hill reaction activity was more pronounced in response to the effect of different concentrations of kinetin when compared with the other used growth regulators (IAA and GA₃).

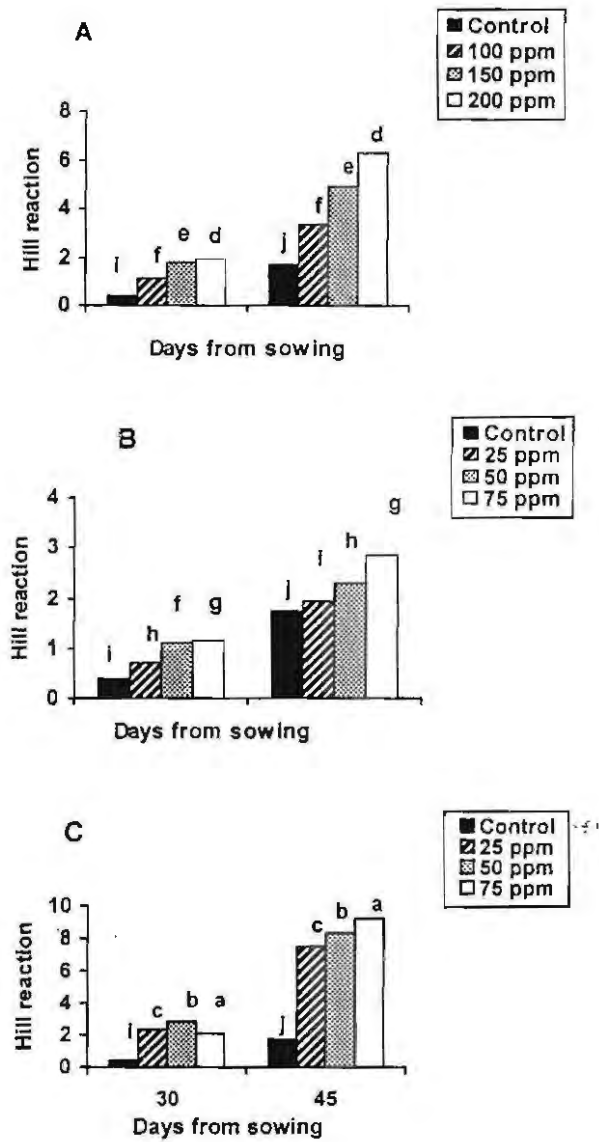


Fig. (5): Effect of seeds presoaking in different concentrations of IAA (A), GA₃ (B) or kinetin (C) on Hill reaction activity (mM DCPIP m chl/hr) of cowpea leaves. Bars in a grouping labeled with the same letter are not significant as indicated by LSD at (P < 0.05).

¹⁴CO₂ – light fixation:

The pattern of changes in ¹⁴CO₂-photo-assimilation by cowpea leaves in addition to the rate of metabolization of photosynthetic soluble intermediates to insoluble ones during the two harvest of vegetative stage in response to IAA, GA₃ or kinetin are represented in figures 6,7 and 8 respectively.

Seed presoaking in all IAA concentrations (100,150 and 200 ppm) caused significant increases ($P \leq 0.05$) in soluble photosynthates, insoluble photosynthates and consequently total photosynthates during the two harvest of vegetative stage (30 and 45 days old) over the control values. The magnitude of increase in soluble and insoluble photosynthates was more pronounced in response to the increase and decrease in the concentration of IAA respectively throughout the two harvest of vegetative stage, except for IAA treatment at 150 ppm which induced the highest amount of insoluble photosynthates at the first harvest of vegetative stage (30 days old). On the other hand the following sequence (150ppm > 100ppm < 200ppm > control) was displayed with respect to the values of total photosynthates.

Regarding soluble/insoluble ratio, in response to different concentrations of IAA. The increase in the rate of metabolization of insoluble to soluble photosynthates over that of control was observed at the first harvest of vegetative stage (30 days old). On the other hand, in general, the highest value of soluble/insoluble ratio was detected in the leaves of the control plants when compared with the values of different IAA treatments at the second harvest of vegetative stage. It is interesting to mention that, at first and second harvest of vegetative stage, increasing the concentration of IAA increased this ratio.

Concerning the effect of GA₃ on ¹⁴CO₂ photo-assimilation, at the two harvest of vegetative stage, the soluble, insoluble and consequently total photosynthetic metabolites showed a significant increase ($P \leq 0.05$) in response to different GA₃ concentrations (25, 50 and 75 ppm) except for soluble photosynthates, at the second harvest of vegetative stage, in response to 25 and 50ppm where the values of soluble photosynthates were lower than that of control. In respect to the values of soluble photosynthates in different GA₃-treated plants, increasing the concentration increases these metabolites whereas a reversible situation was observed for insoluble photosynthates at the two harvest of vegetative stage. In the meantime the highest amount of total

photosynthates was induced by 50ppm GA₃ followed by 75 ppm and 25 ppm during the first harvest of vegetative stage where as the following sequence, at the second harvest of vegetative stage, (50 ppm > 25 ppm < 75 ppm) was displayed with respect to the values of total photosynthates. At the two harvest of vegetative stage, in regard to the rate of soluble/insoluble ratio, the lower concentrations of GA₃ (25ppm and 50ppm) and the highest one (75 ppm) markedly decreased and increased the mobilization of soluble photosynthates to insoluble ones respectively in relation to the control value.

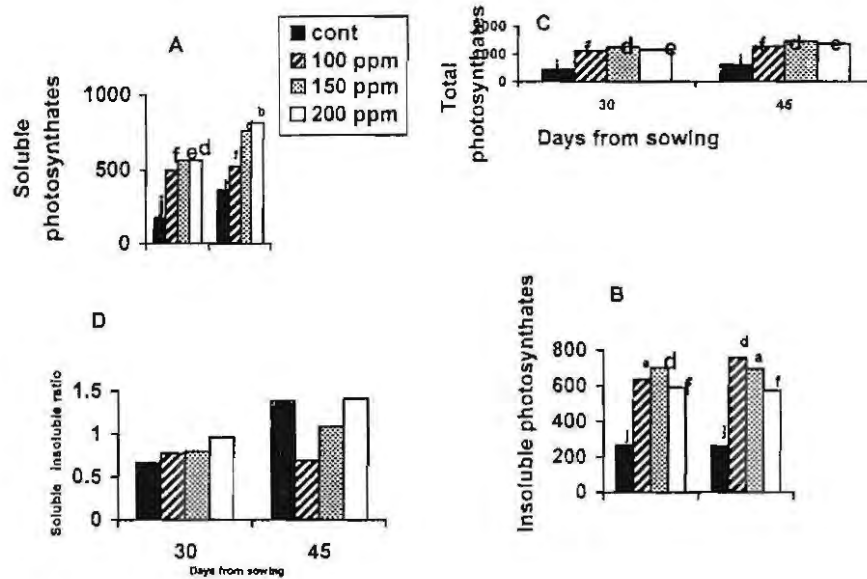


Fig. (6): Effect of different concentrations of indole acetic acid (IAA) on photosynthetic activity (Cpm g⁻¹ f wt) (A, B, C & D) of cowpealeaves. Bars in a grouping labeled with the same letter are not significant as indicated by LSD at (P < 0.05).

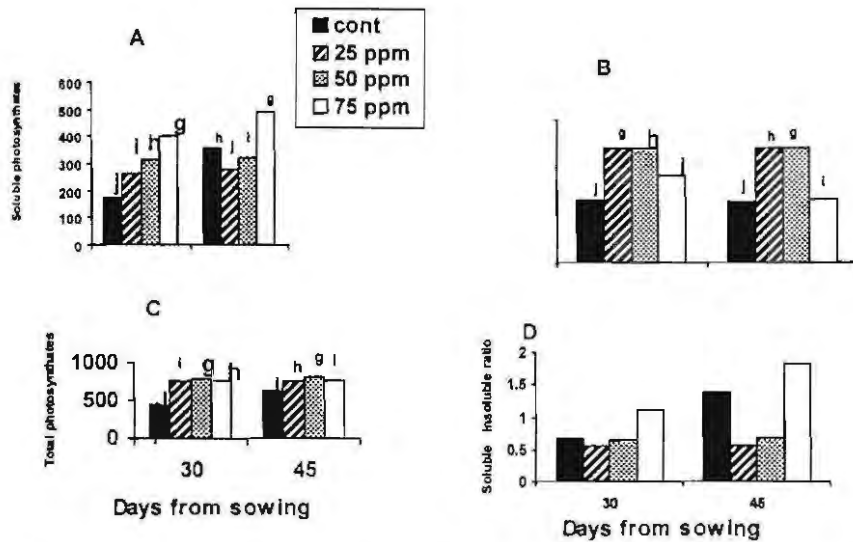


Fig. (7): Effect of different concentrations of gibberellic acid (GA₃) on photosynthetic activity (Cpm g⁻¹ f wt) (A, B, C & D) of cowpea leaves. Bars in a grouping labeled with the same letter are not significant as indicated by LSD at (P < 0.05).

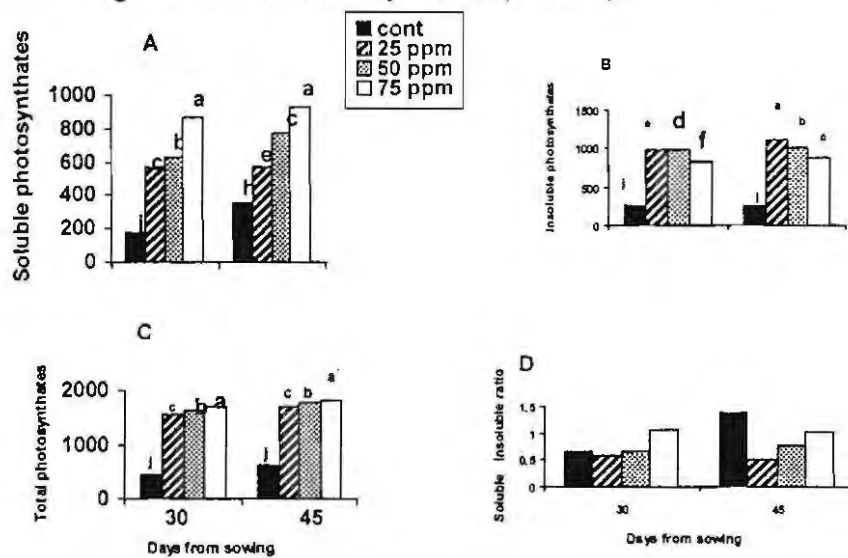


Fig. (8): Effect of different concentrations of kinetin on photosynthetic activity (Cpm g⁻¹ f wt) (A, B, C & D) of cowpea leaves. Bars in a grouping labeled with the same letter are not significant as indicated by LSD at (P < 0.05).

The pattern of changes in soluble, insoluble and consequently total photosynthates as well as the ratio of soluble/insoluble in response to the different kinetin treatments was more or less similar to that observed in GA₃-treated plants during the experimental period (see figures 15 and 16).

Changes in carbohydrates content

The changes in the mean carbohydrate fractions (total soluble sugars, glucose, sucrose and polysaccharides) of the variously growth regulators-treated plants throughout plant growth and development are shown in figures 9,10 and 11 for indole acetic acid, gibberellic acid and kinetin respectively.

Above the level which was detected at the first harvest of vegetative stage (30 days old) marked increases ($P \leq 0.05$) in the different carbohydrate fractions were apparent at second harvest of vegetative stage (45 days old) in the variously IAA-treated plants. Afterwards, slight increase was obtained at flowering stage (60 days old) followed by sharp decline at fruiting stage (75 days old), except for total soluble sugars, which showed a slight increase at fruiting stage. In comparing with control plants, seed pretreatment with IAA at all used concentrations (100,150 and 200ppm) caused a marked increase ($P \leq 0.05$) in total soluble sugars, sucrose, glucose and polysaccharides of cowpea leaves all over the growth stages (30,45,60 and 75 days old) except at the first vegetative stage (30 days old) when the level of sucrose content of control was higher than that of different IAA-treated plants and for glucose when the lower concentrations (100 and 150ppm) of IAA induced the lowest amount of glucose (Fig.9).

In control and various GA₃-treated plants, the carbohydrate fractions increased progressively from the first harvest of vegetative stage to flowering stage. At fruiting stage (75 days old), further increases were observed in the values of total soluble sugars whereas marked decrease set in for the values of glucose, sucrose and polysaccharides (Fig. 10).

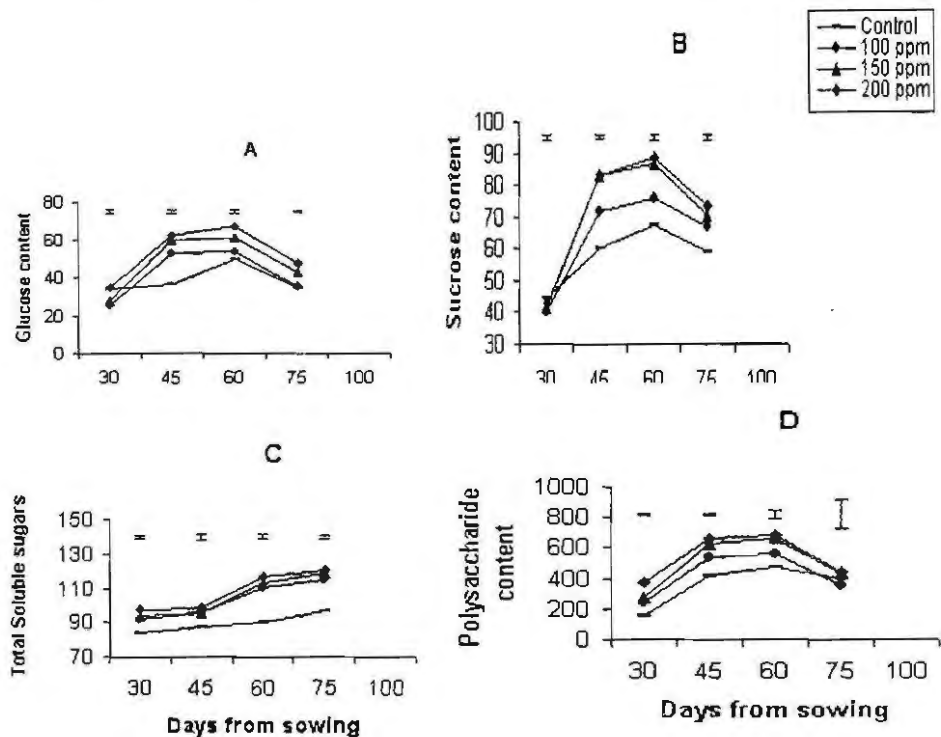


Fig. (9): Effect of seeds presoaking in different concentrations of indole acetic acid (IAA) on carbohydrate contents (A,B,C & D) (mg. g⁻¹ D. wt.) of cowpea plants. Vertical bars represent LSD values at P < 0.05.

Seed presoaking in GA₃ at different concentrations (25, 50 and 75ppm) caused significant increase (P ≤ 0.05) in total soluble sugars and polysaccharides during the successive stages of growth, while glucose content appeared to decrease (P ≤ 0.05) in response to GA₃ treatments particularly at 25 and 50ppm on the days 30 and with all concentrations on the days 75 from sowing (Fig. 17). The available results represented in figure 17 showed that the relative high concentrations of GA₃ (50 and 75ppm) caused significant increase in sucrose content of cowpea leaves all over the growth period. On the other hand, the lowest concentration (25ppm) decreased the values of sucrose content below the control value during vegetative and flowering stages.

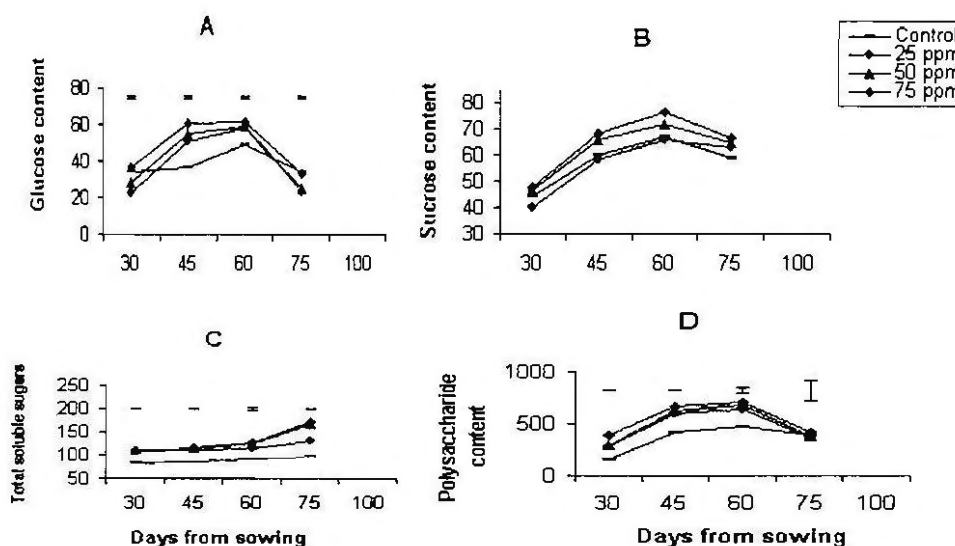


Fig. (10): Effect of seeds presoaking in different concentrations of gibberellic acid (GA₃) on carbohydrate contents (A,B,C & D) (mg. g⁻¹, D. wt.) of cowpea plants. Vertical bars represent LSD values at P < 0.05.

As compared with the values of carbohydrate fractions detected in the leaves at the first harvest of vegetative stage, these fractions showed a marked progressive increase throughout second harvest of vegetative and flowering stages in the variously kinetin-treated plants. The values of these fractions showed an additional increase in the leaves of control plants whereas at fruiting stage, a sharp decline was observed in these fractions in response to the different concentrations of kinetin (Fig. 11). In relation to the control values, presoaking of the seeds with kinetin at all concentrations (25, 50 and 75) significantly increased ($P \leq 0.05$) sucrose and glucose content throughout the experimental stages except at first harvest of vegetative stage for sucrose and glucose and at fruiting stage for glucose when the values of control were higher than that of treated plants with lowest concentration of kinetin (Fig. 11). Again, the perusal of the data represented in figure 19 showed that the application of kinetin at different concentrations (25, 50 and 75 ppm) increased ($P \leq 0.05$) total soluble sugars and polysaccharides content at all stages of plant growth. It is interesting to mention that the increase in kinetin

concentration increase the values of total soluble sugars and polysaccharides.

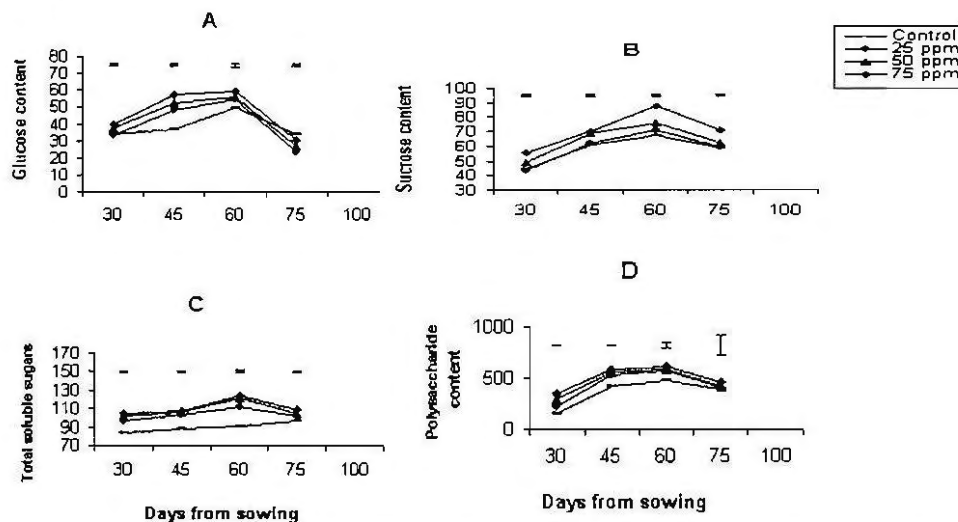


Fig. (11): Effect of seeds presoaking in different concentrations of kinetin (K) on carbohydrate contents (A,B,C & D) (mg. g⁻¹ D. wt.) of cowpea plants. Vertical bars represent LSD values at P < 0.05.

DISCUSSION AND CONCLUSION

Seed priming with IAA at 100,150 and 200ppm enhanced the production of total chlorophylls and carotenoids during the development of cowpea leaves which in turn increased Hill reaction rate as well as ¹⁴CO₂ fixation (see figures 13 and 14) in response to different IAA treatments in cowpea leaves. The promotive effect of IAA on photosynthetic activity of cowpea leaves probably resulted from the massive increase in leaf area production, pigment production as well as rate of transpiration by inducing stomatal opening caused by IAA. These results are in agreement with the finding of the others [Narwadkar & Anserwandekar (1989) and Aldesuquy (2000)]. In this connection, IAA may also directly affect the photosynthetic machinery by increasing the plastide biogenesis in cowpea leaves and subsequently increases the number of proplastides or newly development chloroplast [Aldesuquy & Baka (1998)]. Also, several workers have reported about plant hormones stimulation of development of chloroplast and the whole photosynthetic machinery [Wenshan *et al.*, (1994); Nanda & Nanda (1995) and Aldesuquy (2000)].

Generally the changes in total soluble sugars, glucose, sucrose and polysaccharides during cowpea leaf development are consistent with the changes in leaf area, pigments content as well as Hill reaction activity and $^{14}\text{CO}_2$ fixation. The observed decline in glucose, sucrose and polysaccharide fractions after 75 days from sowing in control and IAA treated plants was probably due to their mobilization to the developing seeds within the emerged legume. Polysaccharides mobilization includes the hydrolysis to soluble sugars and this may be the reason for the observed increase in total soluble sugars in cowpea leaves during this period. A considerable increase was found in pigments content of some plants when sprayed with IAA accompanied with an increase in saccharides synthesis and translocation [Ahmad *et al.*, (1989) Shadad & El-Tayeb (1990); Darusalam *et al.*, (1998) and Aldesuquy (2000)]. In this respect, [Wenshan *et al.*, (1994)], found that translocation of photosynthates from wheat flag leaves was increased by the application of IAA to spikes. [Kiseleva *et al.*, (1998)], found that the high sink activity and fast dry matter production in barley panicle were related to IAA which determined photosynthetic demand and their transport, partitioning and metabolism particularly during milk ripeness stage.

Gibberellic acid at all concentrations was found to stimulate the production of total chlorophylls, carotenoids and Hill reaction activity and as well as $^{14}\text{CO}_2$ fixation. The increase in pigment production in response to seed pretreatment with GA_3 appeared to be in agreement with the finding of [Younis *et al.*, (1991)]. Furthermore, the general increase in chlorophylls and total pigments in response to GA_3 treatment is in accord with the results obtained by [Shadad & Heikal (1982); Misera (1995) and Ramadan (1998)] who gave an evidence for the involvement of exogenously applied GA_3 in the production of pigments in *Phaseolus vulgaris*, *Pogostemon cablin* and *Vicia faba*, respectively.

The promotive effect induced by GA_3 treatments on $^{14}\text{CO}_2$ assimilation may probably due to its stimulative effect on leaf expansion and photosynthetic pigments of cowpea plants all over growth periods. These results are in agreement with those obtained by [Jordi *et al.*, (1994) and Naidu & Swamy (1995)] who stated that photosynthesis in various plant types was found to be mostly increased by GA_3 treatments. In this respect, [Hansen *et al.*, (1996)] found that the rate of photosynthesis increased by 20-30% after application of GA_3 (4%) in cotton plants. Also, [Khan (1996)] indicated that photosynthetic rate,

leaf area and dry mass of *Brassica juncea* mostly increased by GA₃ treatment. Moreover, [Bishoi & Krishnamoorthy (1993)] showed that 10 and 100 mg GA₃/litter increased leaf area and net photosynthesis in peanut plants. Furthermore, [Pedhadiya *et al.*, (1987)] found that both photosystem I (PSI) and photosystem II (PSII) were significantly promoted and the ratio of PSII/PSI did not change significantly in response to GA₃ treatments.

The increase in carbohydrates pools in leaves of cowpea plants in response to GA₃ application as induced by seed pretreatment is supported by the observed stimulation in photosynthetic activity and the accumulation of the dry matter in shoot of GA₃ treated plants. In agreement with these results [Dawh (1989)] reported that GA₃ significantly increased the total carbohydrate and soluble sugars in the leaves of *Cineraria hyprida* and this increases might come through enhancing leaf growth. Also, [Hegazy *et al.*, (1990)] found that GA₃ applications either by soaking or spraying increased carbohydrate fractions (reducing sugars sucrose and polysaccharides) in different stages of gladiolus corms and corms formation. Moreover, [Mahgoub (1992)] on rose plants and [Abdalla *et al.*, (1992)] on red radish plants showed also noticeable increases in the total carbohydrates and total soluble sugar contents in response to GA₃ applications.

Application of kinetin at a various concentrations (25, 50 and 75) induced marked increase in pigments content of developing cowpea leaves. These results were in consistent with those obtained by [Aldesuquy & Gaber (1993) and Wu *et al.*, (1998)] who found that kinetin increased chlorophyll level in different plant species. Moreover on the day 75 where leaves of control plants undergo senescence indole acetic acid, gibberellic acid or kinetin may delay the senescence of cowpea leaves by retaining the chlorophyll. Kinetin appears to be the most active growth bioregulator in this target. The stimulative effect exerted by kinetin on pigment biosynthesis might presumably due to the fact that kinetin increases the rate of transpiration and this will possibly increase the rate of translocation of mineral and cytokinin from root to the developing shoot. Thus, [Richmond & Lang (1975)] have shown that kinetin prevented chlorophyll loss in detached *Xanthium* leaves. Moreover, [Uheda & Kureishi (1978)] found that kinetin increased transpiration and chlorophyll synthesis. Furthermore, [Mullr & Leopold (1966)] reported that the concept of mass flow in the phloem is stimulated by kinetin induced mobilizing center which act as suction

pump through the changes in osmotic potential.

Seed priming with kinetin stimulates the accumulation of soluble, insoluble photosynthates as well as Hill reaction activity. These promotive effects induced by kinetin may be probably due to its stimulative effect on leaf expansion and photosynthetic pigments as well as transpiration rate of cowpea plants. Furthermore, kinetin may exert its effect on photosynthetic machinery at the chlorophyll and chloroplast level by increasing plastids biogenesis and consequently increased the number of proplastids or newly developed chloroplasts [Aldesuquy & Baka (1998)]. In this connection, there are many reports on plant hormone stimulation of development of chloroplasts and the whole photosynthetic machinery [Sakr (1985) and Baka & Al desuquy (1991)].

In the majority of cases, seed priming with kinetin induced noticeable increases in glucose, sucrose, total soluble sugars and polysaccharide contents in leaves as compared with the contents detected in control plants. Thus, the obtained results show that kinetin stimulates the production of carbohydrates in cowpea leaves and these may be explained on the fact that kinetin increases the leaf expansion, production of photosynthetic pigments as well as its biogenesis and consequently stimulates photosynthetic activity. The pronounced increases in total soluble sugars by kinetin treatment in cowpea plants may be probably due to an increase in invertase activity which led to a simultaneous increase in soluble carbohydrates [Howard & Witham (1983)]. In contrast to these results [Erisman & Wegner (1967)] found that, there was a massive accumulation of starch in case of *Lemna minor* plants as a result of kinetin treatments.

Generally when the leaves of cowpea plants started senescence there was a loss of chlorophyll and carbohydrate fractions. Application of kinetin played an important role in delaying senescence of cowpea leaves by retaining its chlorophyll and enhancing the formation of carbohydrate fractions. Therefore, the increase in $^{14}\text{CO}_2$ fixation induced by kinetin could be due to the increase in chlorophyll content and the increase in enzymes responsible for $^{14}\text{CO}_2$ fixation. As a result of such changes by kinetin, there may be a rapid movement of assimilates from the leaves (source) to the developing seeds (sink) resulting in improving yield quality of cowpea plants.

Seed presoaking in different doses of IAA, GA₃ or kinetin appeared to increase the different yield parameters (pod length, pod

weight, number of pods/plant, number of seeds/pod, 100-seeds weight and relative growth yield) of cowpea plants if compared with untreated plants (see figures 27, 28 and 29). Concerning the harvest index, except for the different concentrations of GA₃ (25, 50 and 75ppm) which induced a significant decrease in this yield component, the different concentrations of IAA (100,150 and 200ppm) and kinetin (25, 50 and 75) significantly increased the harvest index of cowpea plants above the control values, the magnitude of response was more pronounced with 100 and 75ppm of IAA or kinetin respectively. The increase in cowpea productivity is probably due to the increase in rate of translocation of photosynthates from leaves to developing seeds caused by hormone treatments [Ray & Choudhuri (1981)]. Thus the enhancement of yield attributes in response to the used growth regulators was correlated to the stimulatory effect of these growth regulators on expansion of leaf area, pigment production and photosynthetic activity which in turn increased carbohydrates content in cowpea leaves and consequently increased the yield capacity of cowpea plants. In this respect [Li *et al.*, (1994)] indicated that GA₃ increased photosynthetic rate and promoted the translocation of assimilates from leaves to pod, thus increasing the pod number/plant and 100 pod weight in *Arachis hypogea*. Also, [Zhang (1988)] showed that GA₃ increased fruit firmness which had stimulatory effect on leaf photosynthesis of *Persimmon*. In agreement with these results, the application of GA₃ on *Vicia faba* plants increased the number, of pods, number of seeds/pod, 100-seed weight and seed yield per plant [Khan (1996)]. In such situation, fruit set, fruit weight, fruit length and fruit diameter have been improved by GA₃ application for different plant species [Abou-Aziz *et al.*, (1987)] on apricot; [El-Fakharany *et al.*, (1995)] on apple and [Mohammed & Tylar (1990)] on peach fruit [Nowak & Czaplá (1991)] on soybean; [Abd-El Fatah (1997)] on broad bean [Khan (1997)] on Indian mustard and [Bhai & Singh (1998)] on okra). Kinetin application appeared the most effective growth bioregulator in improving yield and yield components of cowpea plants. In this connection. [Herzog (1982)] found that a higher level of endogenous cytokinin improves yield of wheat plants. Also [Ray & Choudhuri (1980)] found that, the increase in grain yield of rice by kinetin treatment, as indicated by higher mobilization index and harvest index might mediated through increased longevity of leaves particularly the flag leaf which perhaps contributed to grain filling by enhancing duration of photosynthetic supply to grains. These results are in accord

with those obtained by [Hussein et al., (1998)].

All used doses of growth regulators led to an accumulation in glucose, sucrose and consequently total soluble sugars in the yielded cowpea seeds. It is of interest to note that the increase in total soluble sugars in response to the lowest concentrations of the used growth regulators (100, 25 and 25ppm for IAA, GA₃ or kinetin respectively) was accompanied by significant decrease in polysaccharides in relation to control. The obtained data led us to conclude that IAA at 100 and 150ppm, GA₃ at 25ppm or kinetin at 25ppm stimulate the degradation of polysaccharides and consequently decrease it significantly ($P \leq 0.05$) in the developing seeds. In this connection, [Luis et al., (1986)] found that GA₃ increased the accumulation of reducing sugars during natural maturation of *Satuma mandarin* fruits. Also, [Samara et al., (1989)] added that GA₃ at 250 and 500ppm reduced the starch percentage in banana fruits. [Hegazy et al., (1990)], reported that reducing sugars and sucrose contents were increased as a result of kinetin application. In radish cotyledons, cytokinin application can cause increases in soluble carbohydrates content and acid invertase activity [Howard & Witham (1983)]. In other systems [Erismann & Wegnaer (1967)] found an excessive accumulation of starch in case of *lemna minor* as a result of kinetin treatment. [Mothes (1964)] suggested that kinetin may be a model of substances which cause storage organs (Seed and tubers) to fill up according to its influence upon attraction and retention.

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تأثير بعض منظمات النمو على المحتوى الصبغى ، الكفاءة التمثيلية والمحتوى
الكربوهيدراتى لأوراق نباتات اللوبيا

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أجريت هذه الدراسة لبيان تأثير نقع بذور اللوبيا فى تركيزات مختلفه من منظمات
النمو الممثلة فى اندول حمض الخليك، حمض الجبريلليك أو الكينيتين على نمو وأيض
النباتات أثناء مراحل نموها وتطورها وكذلك إنتاجيتها. وأيضا ركزت هذه الدراسة على
دراسة تأثير هذه المنظمات على المحتوى الصبغى ، الكفاءة التمثيلية، تفاعل هل والمحتوى
الكربوهيدراتى لأوراق نباتات اللوبيا المستخدمة، وقد أسفرت هذه الدراسة عن نتائج يمكن
إيجازها فيما يلى:

لوحظ أن نقع بذور اللوبيا فى تركيزات متباينه من منظمات النمو السابقه أدت إلى
زيادة واضحه فى المحتوى الصبغى والكفاءة التمثيليه وتفاعل هل وكذلك المحتوى
الكربوهيدراتى لأوراق نباتات اللوبيا.