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# EFFECT O MOISTURE STRESS AND GROWTH REGULATORS ON CHLOROPHYL CONTENT AND CARBOHYDRATE METABOLISM IN *PISUM SATIVUM* (L.)

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## ABSTRACT

*The aim of the present investigation is to find out how far the application of growht regulators namely CCC and $GA_3$ affects the photosynthetic pigments and carbohyrates metabolism of one of the most important vetgetables (Pisum sativum) under shortage of irrigation water.*

*Under all conditions of water supply, the chlorophyll content was greater in treated plants with growth regulators in the young and mature stages of development. In the mature stage of development, the chlorophyll content was greater in $GA_3$ treated than in CCC treated plants.*

*With regard to the photosynthetic activity it was greater in the young than in the mature stage. The photosynthetic activity in treated plants was higher than in untreated ones. In case of $GA_3$ treated the activity was greater than in CCC treated plants.*

*The greater chlorophyll content as well as the highest photosynthetic activity of $GA_3$ treated plants referred to the higher rate of photosynthesis in comparison with that of CCC treated plants.*

*The carbohydrate content as well as the total soluble sugars of the shoot are greater in $GA_3$ treated than in CCC treated plants. The untreated plants contain less total carbohyrates and soluble sugars than the treated one with growth regulators. The application of growth regulators has resulted in remarkable increase in activity of $\alpha$ and $\beta$ amylase.*

## INTRODUCTION

The study of the water economy of cultivated plants is of prime importance in Egyt since the water resources are limited. The cultivated lands do not exceed 6% of the total land area of Egypt. The present investigation deals with one of the important vegetables namely *Pisum sativum* L. Variety Victory Freezer. In a previous investigation the study included the effect of water supply and growth regulatars on vegetative growth and yield of the plant. The present study includes the study of chlorophyll content and carbohydrates content of the same plant. Also the stuyd aims at evaluation of the effect of application of growth regulators namely chlorocholine chloride (CCC) and gibberellic acid ($GA_3$) on chlorophyll content and carbohydrates.

## MATERIALS AND METHODS

### 1-Chloroplast pigments.

For the estimation of chlorophylla,b and carotenoids, the spectrophotometric method recommended by Metzner et al., (1965) was used. To determine the concenteration of the pigmments (chlorophyll a, chlorophyll b and carotenoids) as μ /ml, the following equations were adopted:

Chlorophyll a = 10.3 $E_{664}$ - 0.918 $E_{645}$

Chlorophyll b = 19.7 $E_{645}$- 3.87 $E_{664}$

Carotenoids = 4.3 $E_{452}$ (0.0265 Chl.a + 0.426 Chl.b)

Then, the fractions were calculated as mg/g. dry weight of leaves. The interrelationships between these pigment fractions as well as their rate of biosynthesis as influenced by different treatments could aslo be clasified and represented as ratio of chlorophyll a/chorophyll b (a/b) and chlorophyll a+chlorophyll b/carotenoids (a+b/c)(Khodary, 1973).

**2-Photosynthetic Effeciency.**

The photosynthetic activity (photosynthetic electron transport) of the treated and untreated plants was measured by using the isolated chloroplasts which were prepared by a procedure similar to that described by Aronoff (1946), Osman et al., (1982), Mackinney (1941) and Arnon & Shavit (1963).

**3- Carbohyrate Estimation**

300 mg of oven-dry plant material was extracted with 5 ml. of borate buffer (28.63 g. boric acid+29.8 g. Kcl + 3.5 g . NaOH in a litter of hot distilled water), left for 24 h then centrifuged and filtered. The filtrate was used for the

determination of the direct reducing value (DRV) and total reducing value (TRV), while the residue was dried at 80°C for the determination of polysaccharides (Naguib, 1963 & 1964). and Nelson (1944).

**4-Enzyme Activity (α and β amylases):**

The enzyme activity was assayed according to Rick and Stegbauer (1974).

## RESULTS AND DISCUSSION

**1-Chloroplast Pigments.**

Chlorophyll "a" content diminshed with age in the treated and untreated plants with growth regulators. In the early stage of (50 days), the values were higher than in the late stage (80 days). Examination of figs. 1 & 2 reveals that chlorophyll "a" decreased also with deficiency in water supply in all treatments.

Application of growth regulators has resulted in rise in chlorophyll content particularly $GA_3$. Chlorophyll "b" was much less than chlorophyll "a" under the different conditions. In response to deficiency in water supply and application of growht regulators, it followed the same trend as chlorophyll

"a". It decresed with decrease in water supply and increased with application of growth regulators particulary $GA_3$.

The carotenoids content is less than that of chlorophyll "a" and more than chlorophyll "b" in the different treatments. The response of carotenoid content to water supply and application of growth regulators was more or less similar to that of chlorophyll "a" and "b". The response of total pigments to variations in water supply and treatments with growth regulators was more or less similar to those of individual pigments.

The ratio of chlorophyll "a" to "b" was about twice or even more in the different treatments and in both stages of development. The ratio of chlorophyll a+b to carotenoids varied between 2.26 and 4.72 in the various treatments and in the two stages of development. This may refer to the fact that production of chlorophyll a+b was greater than that of carotenoids.

The above mentioned results concerning the chlorophyll content are general agreement with the findings of the several workers (Bokhari, 1976; El-Sharkawi and Salama, 1977; Dwivedi et al., 1979) reported a decrease in chloroplast pigment content under drought stress conditions. Virgin (1965) showed that a small water deficit (16%) of maize leaves caused a strong inhibition of chlorophyll "a" synthesis. Maranville and Paulsen (1970) cleared that chlorophyll content decreased with increasing water stress in maize.

Chlorophyll "a" were affected than chlorophyll "b". Omar et al., (1986) found that the content of chlorphylls and carotenoids were decreased, in general in the leaf tissues of *Vicia faba* plants as the soil moisture available before irrigation decreased. Nagwan (1988) reported that the increase in moisture stress resulted in decrease in chlorophyll content of soybean leaves. Mahmoud (1990) also observed in soybean that there is a general decrease in chlorophyll "a" chlorophyll "b" and carotenoids with increase in water stress.

The increase in chlorophyll content due to application of $GA_3$ was obtained by some investigators. El-Tahawi et al., (1982) found that application of $GA_3$ to 30-days-old phaseolus plants increased chlorophyl "a" and "b" and El-Sweify (1989) found that $GA_3$ application (50 ppm.) increased chlorophyll "a", chlorophyll "b", carotenoids and total pigments in flax.

With regard to the effect of CCC appliaction on chlorophyll content, Gabr et al.,(1979) demonstrated in the content of chlorophyll "a", chlorophyll "b", chlorophyll (a+b), carotenoids and total chlorophyll to carotenoids ratios in cotton in response to CCC treatment. El-Tahawi et al. (1982) showed that CCC application increased the leaf content of chlorophyl "a" and "b" in 70-days-old *Phaseolus vulgaris* plants. Again Nagwan (1988) observed increase in chlorophyll content of soybean plants sprayed with CCC.

**2-Photosynthetic Activity**

In addition to the chlorophyll and carotenoid contents, the photosynthetic activity is very important in the process of photosynthesis. The photosynthetic activity exhibited remarkable changes with age water supply and application of growth regulators. It decreased with age and deficiency in water supply, (fig. 3).

Application of growth regulators, particularly $GA_3$ resulted in a rise in photosynthetic activity. The above results are in agreement with those of other investigators, including Hatt (1967), Hesse and Lenz (1982), Boyer and Bower (1970), and Goyal (1989). Hart (1967) reported that water stress may affect photosynthesis directly, by affecting various biochemical processes involved in photosynthesis, and indirectly by reducing the intake of $CO_2$ as a result of stomatal closure. Boyer and Bower (1970) recorded that reduction in photosynthetic rate as a result of water stress may be attributed to a decrease in the capacity of electron transport.

**3-Carbohydrate Content.**

**a) Monosaccharides.**

Examination of Figs. 4&5 demonstrates that generally the content of monosaccharides diminished with age and increased with the increase in moisture stress or deficiency in

water supply. Application of growth regulators particularly in the early vegetative stage (50 days) has resulted in a remarkable increase in monosaccharide content. The effect of $GA_3$ was more pronounced than that of CCC in the early vegetative stage.

**b)Sucrose.**

There was a constant increase in sucrose content with decrease in water supply or the increase in moisture stress in all treatments and in each stages of development. This phenomenon may be referrd to the importance of increase in soluble sugars in osmotic regulation under conditions of high moiture stress. Generally, the application of growth regulators is accompanied by rise in sucrose content which is a soluble sugar (Fig. 4&5). The effect of CCC is more prominent in the early stage of development and that of $GA_3$ in the late stage of development.

**c) Total soluble sugars.**

As mentioned before, the soluble sugars content is very important for osmotic regulation since it increase the capacity of root to absorb water from the soil, particularly under conditions of high soil moisture sress. The decrease in water supply in the different treatments was assoicated with a rise in the total soluble sugars content. Spraying plants with either

CCC and $GA_3$ increased the total soluble sugars content, particularly in the early stage of development (50 days).

It is evident that soluble sugars, including monosaccharides, sucrose and the total soluble sugars increased with deficiency in water supply accompanied by increased in moisture stress. Under such conditions, the hydrolysis of carbohydrates being activated and soluble sugars increased for osmotic adjustment. In contrast, the insoluble carbohydrates including polysaccharides as well as the total carbohyrates diminished with increase in moisture stress fro active hyrolysis. This phenomenon is important for osmotic regulation.

The role of the growth regulators would be the acitvation of hydrolysis and accumulation of more soluble sugars for osmotic adjustment and improvement of the water balance as shwon later by the relaive turgidity of plant tissues.

The results concerning the effect of moisture stress and application of growth regulators as recorded in the present investigation are in agreement with those obtained by. Hussein and kandil (1979) who showed that increasing depletion of available water before irrigation from 40 to 80% significantly decreased carbohydrates content in leaves and stems. Hussein and Mandour (1980) and Batanouny et al., (1988) with corn and Hussein et al., (1988) with soybean are in agreement with this finding.

Nagwan (1988) observed a reduction in total carbohydrate content and increases in soluble sugars in soybean plants with elongation of irrigation interval . Mahmoud (1990) found that the increase in moisture stress had resulted in a decrease in toeal available carbohyrates in soybean levaes and seeds, while the soluble sugars increased.

Results of the presnt investigation dealing with the effec of growth regulators on total carbohydrates and soluble sugars are in agreement with thoes obtianed by Litvinova and Yuldashev (1971) who observed that foliar spraying of CCC on cotton increased the content of saccharide, dextrins, lignin, starch and hemicellulose in leaves and stems. Nagwan (1988) reported a rise in both total and soluble sugar contents in soybean plants as a result of spraying with CCC.

Application of $GA_3$ has resulted in a rise in carbohydrate content and soluble sugars as shown by El-Shihy (1979) who recorded a high increase in reducing, non-reducing as well as total sugar contents of stems, leaves and grains of Zea mays as a result of $GA_3$ treatement. El-Tahawi et al., (1982) found that the application of $GA_3$ to 30-days-old phaseolus plants increased the total carbohydrate content. Farghal and El-Tantawy (1990) pointed out that gibberellic acid exhibited a stimulatory effect on carbohydrate contents (total-soluble, reducing and non-reducing sugars) in cucumber seedlings.

**4-Enzymatic Activity.**

Almost all metabolic reactions are affected by plant water deficits. Severe water deficits, generally cause an overall decrease in enzymatic activity. Howevr, these effects are selective; as the cytoplasm is dehydrated, the activity of some enzymes involved in synthsis is reduced before that of others, while levels of others increase as a result of water deficit.

In the studied enzymes namely α-amylase and β-amylase and protease, the activity tends to decrease with age and increase with the rise in moisture stress (Figs 6&7). The reverse change occurred in case of nitrate reductase activity.

**α- and β-Amylases.**

The increase in moisture stres due to shortage in water supply was accompanied by increas in activity of α & β-amylases and in turn the accumulation of soluble sugars. Such behaviour serves for osmotic adjustment and in turn increased in the capacity for water absorption under adverse conditions.

Application of growth regulators (CCC & $GA_3$) has resulted in a remarkable increase in the activity of α- and β-amylases. The rise in the activity of both enzymes was greater in case of $GA_3$ compared with CCC.

There was a decline in the activity of α- and B- amylases with age. In the early stage (50 days), the activity of both enzymes was greated than in the late stage (80 days). Comparison of the activity of enzymes under the differnt conditions with accumulation of soluble sugare previously discussed reveals that the greater the acitivyt of the enzymes, the greater the accumulation of soluble sugars. This may indicate that soluble sugars increased with rise in moisture stress and application of growth regulators. Also the soluble sugars diminished with age .

The activity of some enzymes involved in synthesis could be reduced before that of others, while the levels of others increase as a result of water deficit. Joun Jacobsen et al., (1986) found that water stress enhances expression of an α-amylase gene in barley leaves.

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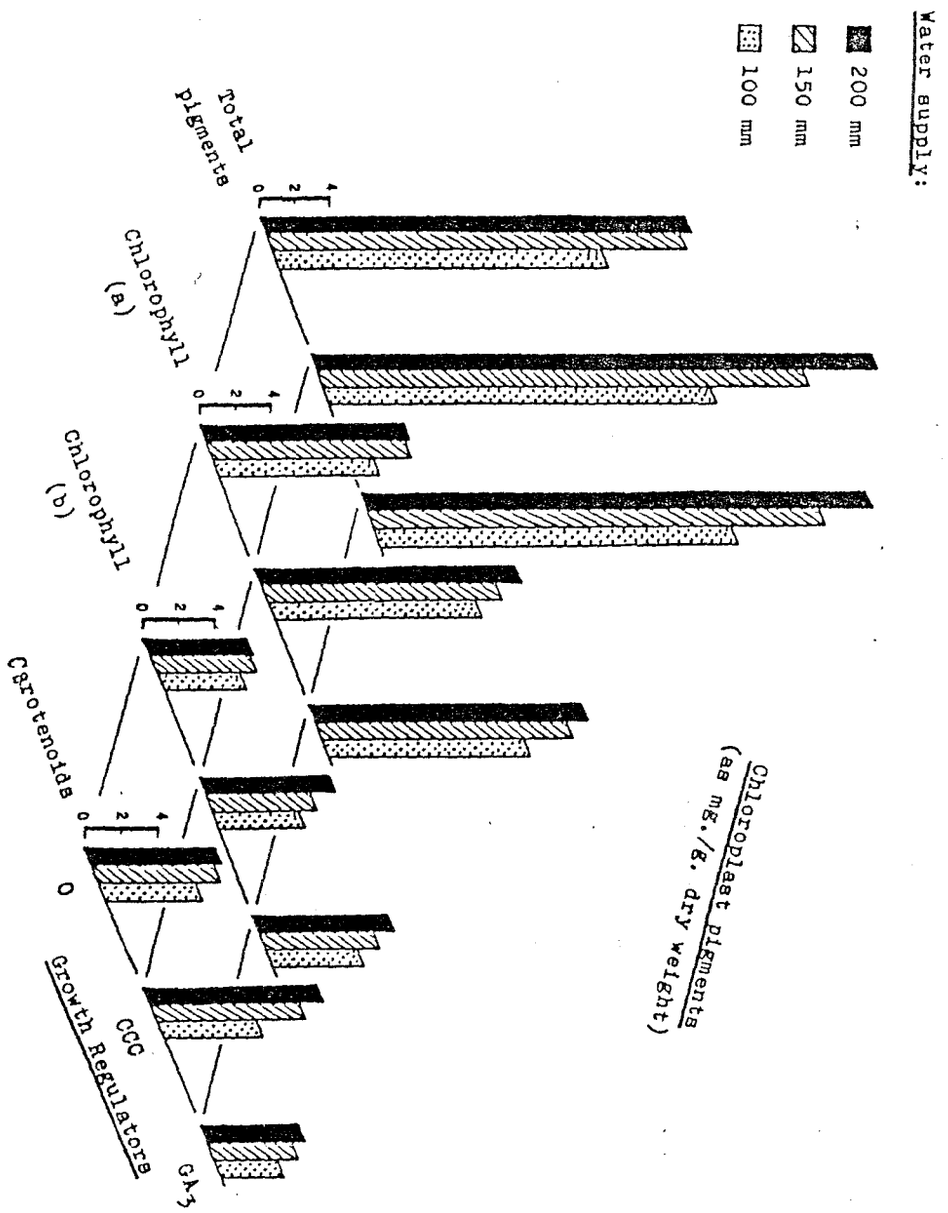


**Fig. (1).** Effect of water supply and growth regulators on chloroplast pigments of pea leaves after 50 days of growth.

plants under drought stress. This agress with Katoch et al. (1983) and Rose (1988).

Konopska and Wiszniewska (1970) and Osman and Ahmed (1983) reported increased protein content as a result of CCC application.

Shady et al. (1984) recorded a reduction in total soluble N and amino N cotents in the shoots of *Vicia faba* during growth, especially in the presence of 50 ppm. $GA_3$. Protein N content increased during seed maturation, while total soluble N and amino N contents decreased.

**Proline Content.**

Examination of Table 2 and Fig. 3 reveals that proline content showed remakable variations under different conditions including:

a- Stage of development.

b- Moisture stress.

c- Treatment with the growth regulators (CCC&$GA_3$)

The proline conent exhibited appreciable decrease with age particularly when subjected to severe moisture stress. The content was much greater in the early stages of 50 days compared with that in the old stage of 80 days.

The effect of moisture stress on proline content is highly significant. The results demonstrated that there is appreciable increase in proline content with rise in (a) a contribution to the osmotic balance when electrolytes are lower in the cytoplasm and in the vacuole, (b) a

protective effect on enzymes in the presence of high electrolytes in the cytoplasm, and (c) increased solubility of proteins by proline.

**Nucleic Acids.**

**The** content of DNA and RNA exhibitd remarkable variations with age of plant, deficincy in water supply and application of growth regulators. Under all conditions, the RNA content wes much greater than the DNA content (Table 3 and Fig. 4).

The DNA showed a tendency towards increase in content with application of growth regulators particularly in the case of $GA_3$. The effect of moisture stress on DNA contect was irregular and insignificant.

The RNA content demonstrated considerable change with increase in moisture stress and treatment with growth regulators. Increase in moisure stress resulted in considerable decrease in RNA content in treated and untreated plants. Application of growth regulators particularly $GA_3$ resulted in remarkable increase in RNA content.

May and Milthorpe (1962) reported that water deficits impair the nucleic acid system as a result of increased RNAase activity, the degradation of RNA proceeds rapidly than its synthesis and this in turn, affects enzyme production and growth.

Cell division seems to be affected less by water deficit than cell enlargement. Kramer ( 1980) reported that the DNA content of cotyledonary leaves of radish is reduced to about 40 percent of the control leaves at a leaf water potential of - 2 bars and to 20 percent at -

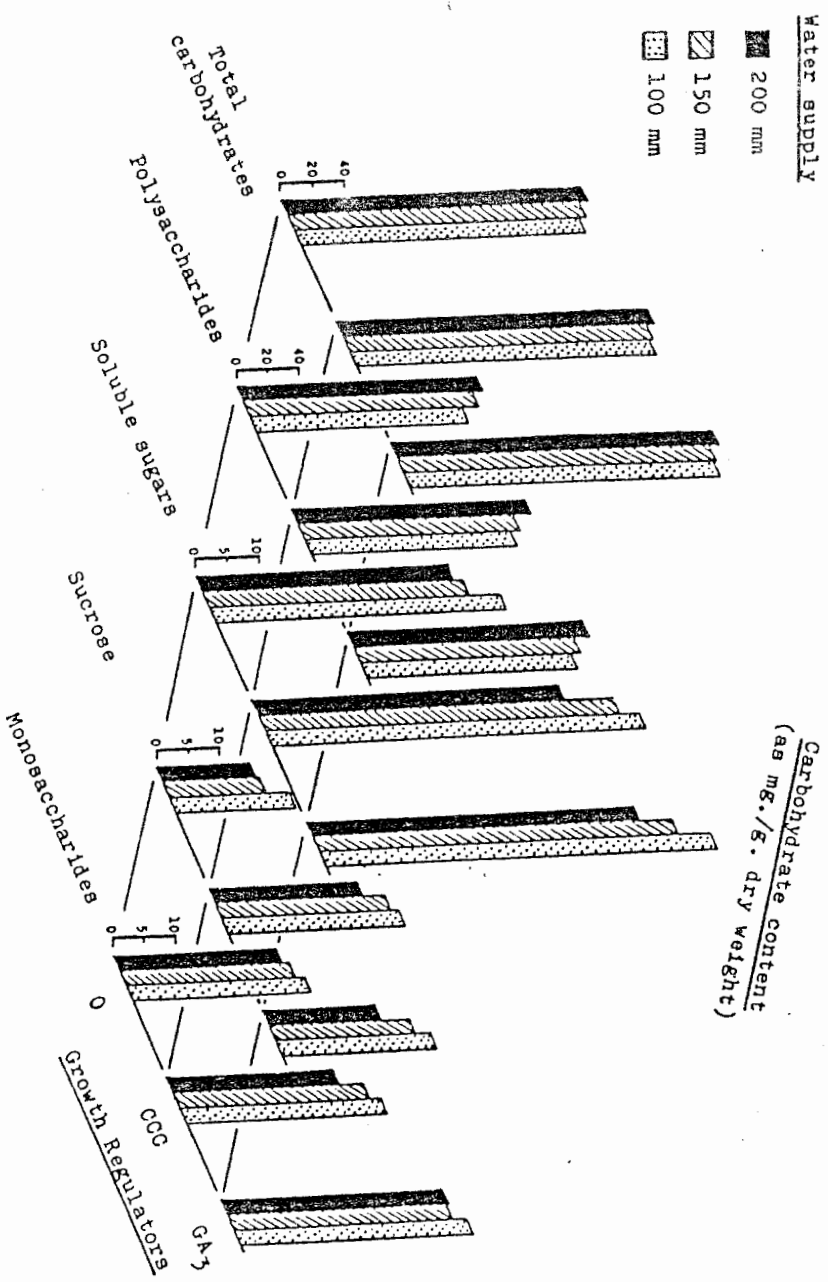


**Fig. (4).** Effect of water supply and growth regulators on carobohydrate content of pea shoots after 50 days of growth.

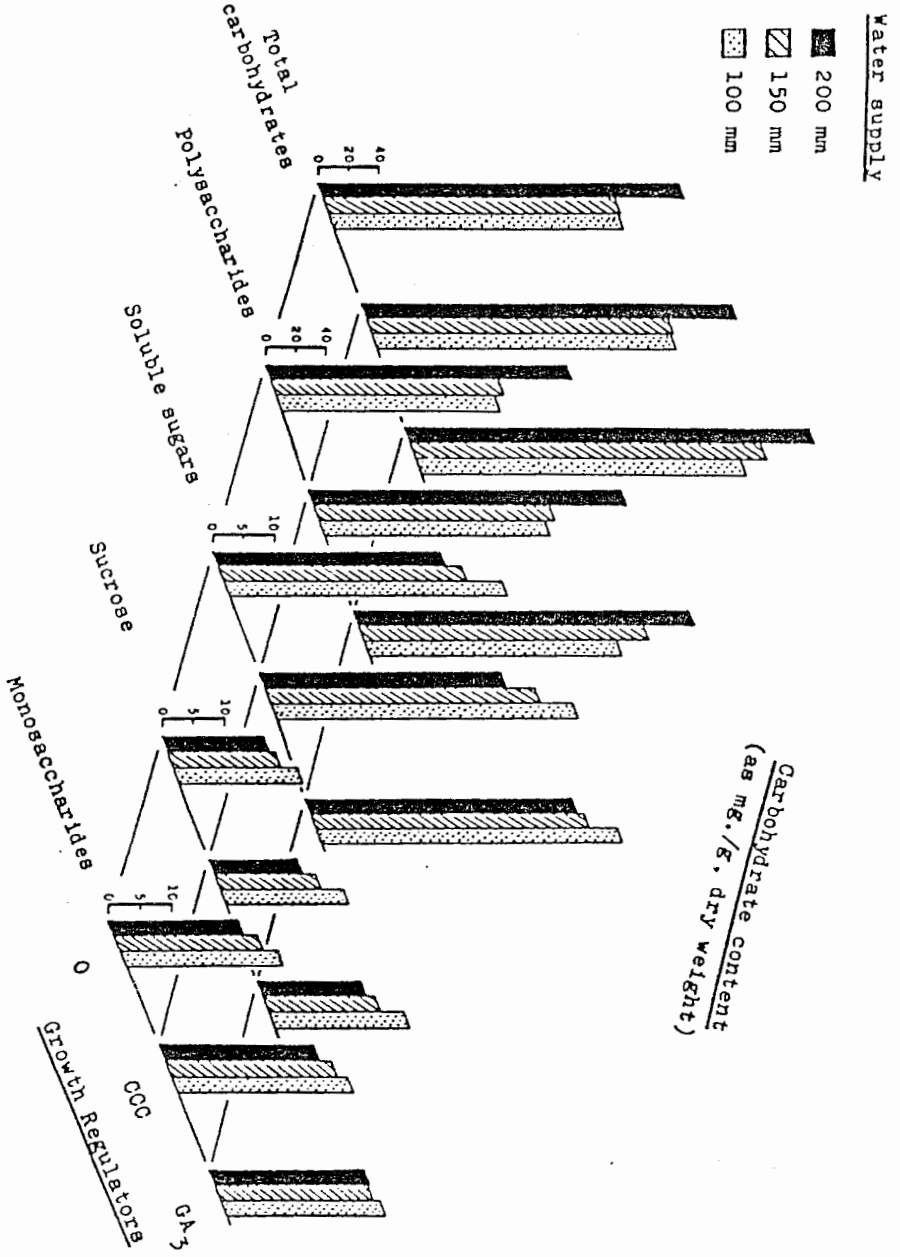


Fig. (5). Effect of water supply and growth regulators on carbohydrate content of pea shoots after 80 days of growth.

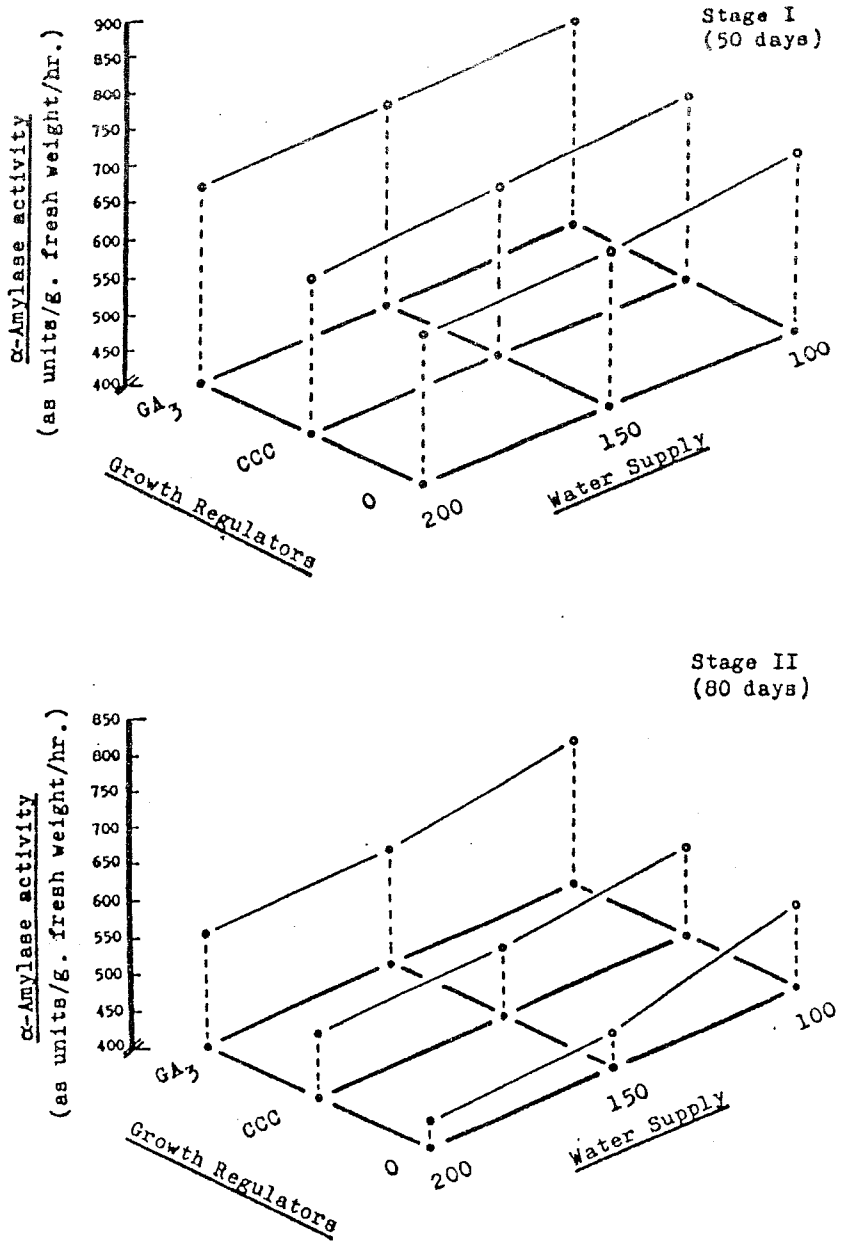


**Fig. (6).** Effect of water supply and growth regulators on the activity of $\alpha$-amylase enzyme of pea plants.

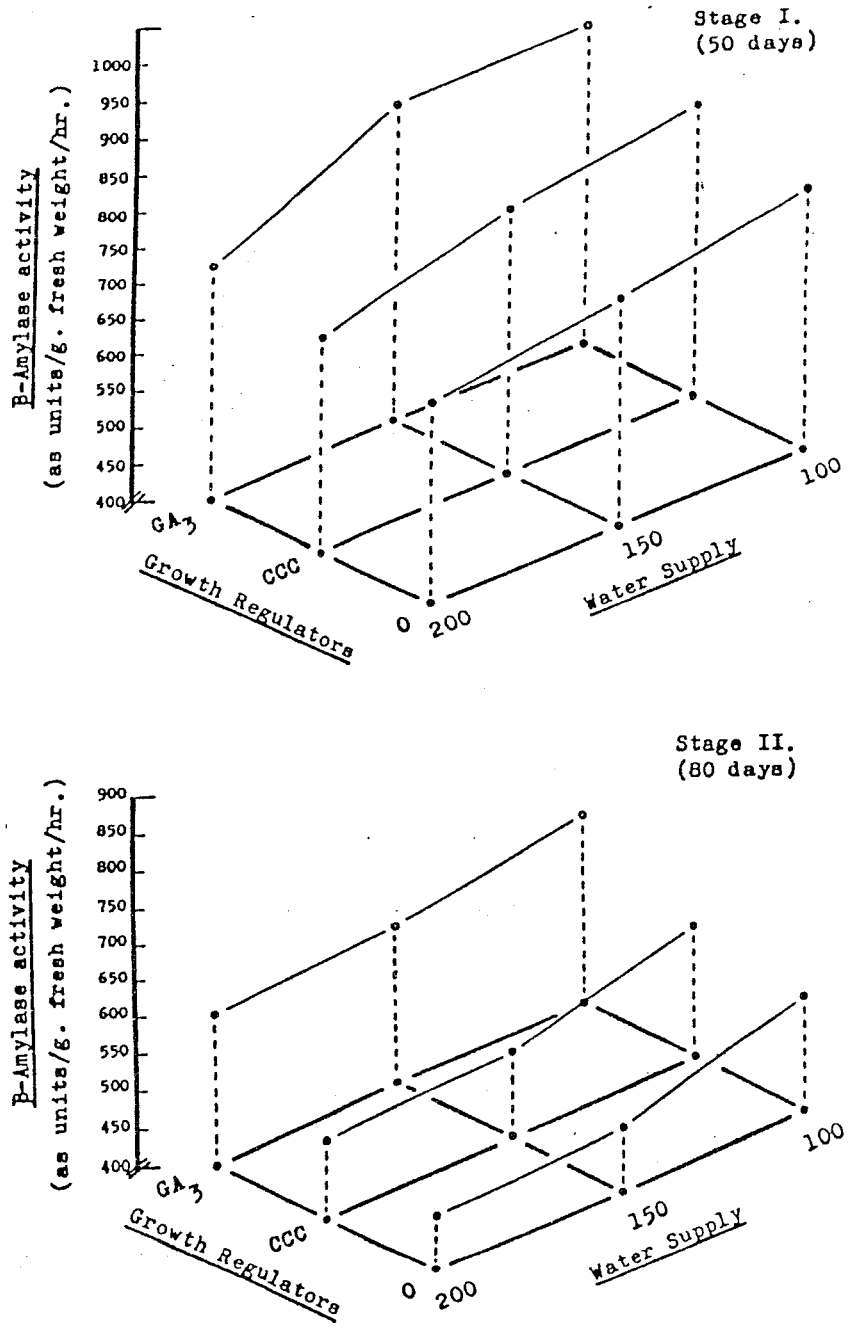


Fig. (7). Effect of water supply and growth regulators on the activity of ß-amylase enzyme of pea plants

# تأثير نقص الرطوبة ومنظمات النمو على المحتوى الكلوروفيللى والأيض الكاربوهيدراتى لنبات البسلة

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يهدف البحث معرفة تأثير الجبربللين والسيكوسيل على صبغات التمثيل الضوئى والأيض الكربوهيدراتى لأحد المحاصيل الهامة وهو نبات البسلة تحت ظروف النقص فى الإمداد المائى.

أدت المعاملة بمنظمات النمو إلى زيادة المحتوى الكلوروفيللى، إلا أن المعاملة بالجبريللين أدت إلى زيادة اكبر من المعاملة بالسيكوسيل فى مرحلة النضج.

المعاملة بمنظمات النمو أدت إلى زيادة كفاءة البناء الضوئى وكانت الزيادة باستعمال الجبريللين اكبر منها فى حالة السيكوسيل.

المحتوى الكربوهيدراتى وكذلك السكريات الذائبة فى المجموع الخضرى فى النباتات المعاملة بالجبريللين كانت أعلى منها فى النباتات المعاملة بالسيكوسيل وكذلك أدت المعاملة بمنظمات النمو إلى زيادة نشاط انزيمى ألفا وبيتا أميليز.