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INFLUENCE OF 2,4-D*- AND CCC**- TREATMENT ON THE PIGMENT CONTENTS AND PHOTOSYNTHETIC ACTIVITY OF HORDEUM VULGARE AND SORGHUM BICOLOR

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ABSTRACT

The influence of 2,4-D and CCC-treatment on the pigment contents and photosynthetic activity of the isolated thylakoids of two crop plants (H. vulgare and S. bicolor) through different growth stages has been investigated. The data presented indicate that both chlorophylls a and b of H. vulgare showed an increase with CCC treatment at the seedling and fruiting stages, and a decrease in the other stages, except at the low concentrations which maintained an increase. In S. bicolor, most of the CCC treatments resulted in an increase in chlorophyll contents at all growth stages, except the flowering stage. On the other hand, chlorophyll content in H. vulgare increased in all 2,4-D levels at the seedling stage, but decreased with the two lower levels (5×10^{-6} and 5×10^{-5} M) later with development. S. bicolor exhibited an increase in chlorophyll content at all stages of growth, except at

* 2,4-Dichlorophenoxy acetic acid
** 2-Chloroethyl trimethyl ammonium chloride (Chlorcholine chloride).
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the fruiting stage, in response to 2,4-D treatment. Carotenoids showed a decrease with CCC at all growth stages, except the fruiting stage, in H. vulgare and the seedling and preflowering stages in S. bicolor. On the other hand, leaf carotenoids increased in H. vulgare with the two higher levels of 2,4-D, but decreased in S. bicolor with all levels at all stages of development .

Treatment of H. vulgar and S. bicolor with different concentrations of CCC decreased the photosynthetic activity of the isolated thylakoids, but an increase was noticed, with most levels, later during the fruiting stage. The photosynthetic activity of both H. vulgare and S. bicolor showed a reduction with 2,4-D at the preflowering and flowering stages, but an increase early in the seedling and later at the prefuiting and fruiting stages. It has been noticed that 2,4-D could cause an initial stimulatory effect on photosynthesis during the seedling stage, but this effect was counteracted later during the flowering stage.

INTRODUCTION

Plant growth regulators are known to affect plant metabolism and function, and may cause either benificial or injurious effect on growth and yield. CCC and 2,4-D are among the plant growth regulators which may modify plant characters.

The effect of CCC on plant growth was extensively studied by many workers [1, 4, 12] who reported shortening effect on wheat sprayed with CCC. On the other hand, many authors reported that treatment of clover plants with high concentrations of CCC caused significant increase in the photosynthetic pigments content [2, 8].

Also, the effect of 2,4-D on several crop plants has been discussed by many workers. A slight increase was recorded in the yield of barley, *Sorghum*, oats and wheat plants treated with 2,4-D [15,18]. Singh and Adlakha [20] found that the highest yield of wheat grain and straw was obtained with amino 2,4-D. Similar results were obtained by singh and Moolani [19] who noticed that foliage spraying with 2,4-D at 6-week stage was effective for weed control and increased the yield of wheat. A reduction in the pigment content of the leaves of 7-day-old barley seedlings was recorded in response to 2,4-D treatment [5].

The present study has been devoted to examine the influence of certain concentrations of CCC and 2,4-D on the chloroplasts pigments content and photosynthetic activity of the isolated chloroplasts of two crop plants, *Hordeum vulgare* and *Sorghum bicolor*, through different developmental growth stages.

MATERIALS AND METHODS

Hordeum vulgare variety Giza 119 and *Sorghum bicolor* variety Giza 3 were germinated under glasshouse conditions (temperature $28 \pm 2^{o}C$ and relative humidity 60%). Seeds of the two plants were germinated in porcelain glazed pots (35 cm deep x 25 cm diameter) filled with HCl-treated sand which was thoroughly washed with deionized water. The sand culture technique and nutrient solution described by Hoagland and Arnon (7) were used in this investigation. Each of the two plant species was represented by 50 pots, each having 20 similar-sized seeds, sown 1 cm below the surface and irrigated with the base nutrient solution. The pots were arranged in such design so that each pot would receive similar light condition. The 50 pots were divided into 2 lots of 25 pots each. The first lot was treated with chlorcholine chloride (CCC) while the other with 2,4-dichlorophenoxy acetic acid (2,4-D). Again, each lot was divided into five groups covering the control and four treatments. Each of the control and four treatments was represented by five replicates. After seedling emergence, the plants were sprayed with different concentrations (5×10^{-6}, 5×10^{-5}, 5×10^{-4} and 5×10^{-3} M) of the growth regulators after 1st, 7th, 10th, 12th and 14th week for H. vulgare and after 1st, 6th, 8th and 12th for S. bicolor. The concentrations outlined above follow more or less the phenological stages represented by the seedling, preflowering, flowering, prefruiting and fruiting stages.

Pigment analysis

The different pigment fractions (chlorophyll a, chlorophyll b and carotenoids) were estimated using the method described by Metzner et al. [11].

Photosynthetic electron transport

The photosynthetic activity of the treated and untreated plants was measured by using the isolated thylakoids which was obtained from the osmotically shocked chloroplasts. Chloroplasts were isolated by a procedure similar to that described by Osman et al. [14]. Leaves were detached from plants grown at different growth stages, rinsed in cold tap water, wiped thoroughly and preserved in moisture-proof pliofilm bags for 2 hours in a refrigerator at 4°C.

For chloroplast isolation about 20 g of the fresh leaves were shredded, mixed for 1 minute in a blender with a buffered combined solution of 0.4 M sucrose, 20 mM HEPES-KOH (pH 7.8), 3 mM $MgCl_2$, 4 mM sodium ascorbate and 0.1 % bovine serum albumin. The mash was strained through cheese-cloth and filtered immediately through small plug. The suspension was centrifuged for 1 minute at 8,ooo g, the pellet was resuspended in the isolation medium and centrifuged for 5 min at 300 g, the supernatant was then centrifuged for 10 min at 1,000g. The sediment which contained chloroplasts was osmotically shocked in 20 ml cold distilled water for 20 min then centrifuged for 2 min at 10,000g. The obtained thylakoids were resuspended in 2 ml buffer solution and the lumps were dispersed with the aid of a glass stirring rod. The chlorophyll content (chlorophyll a and b) of the obtained thylakoids was determined according to the method described by Mackinney [9].

The photosynthetic activity of the isolated thylakoids was measured by following the reduction of potassium ferricyanide spectrophotometrically at 420 nm at 28 °C. The sample contained 0.2 ml of the thylakoid suspension, 3.8 ml HEPES buffer (pH 7.8) and 5×10^{-4}M potassium ferricyanide. The mixture was illuminated before the spectrophotometric measurement for 5 minutes by means of a slide projector provided with heat protection filter. The light intensity at the sample level was 300 W m^{-2}.

Statistical analysis

The obtained data were treated statistically in order to assess the significant difference between the effects of different growth stages, treatments and their interaction. The method of two-way analysis of variance was followed [13].

RESULTS AND DISCUSSION

The data presented in Tables 1 and 2 show that chlorophylls (a and b) content of *H. vulgare* showed an increase with CCC at the seedling and fruiting stages but a decrease in the other stages, except the lower concentrations (5×10^{-6} and 5×10^{-5}) which maintained a decrease compared to the untreated control. In S. bicolor most CCC treatments resulted in an increase in chlorophyll content at all growth stages, except at the flowering stage. In this regard, the photosynthetic pigment content Chl a and Chl b and carotenoids increased in clover with higher CCC concentrations [2, 8] . The treatment of Sorghum seeds with CCC at concentration of 0.025 mg/ml brought about an increase in chlorophyll content of seedling leaves by 10% compared to untreated control [17]. On the other hand, chlorophylls content was found in H. vulgare to increase with all 2,4-D levels only at the seedling stage, but later with development the two higher levels resulted in an increase, while the two lower ones (5 X 10^{-6} and 5 x 10^{-5} M) in a decrease. With 2,4-D, again S. bicolor maintained an increase in chlorophylls at all stages of growth, except at flowering stage. In contrast, carotenoids showed a decrease with CCC at all stages of growth, except at fruiting in H. vulgare and seedling and preflowering stages in S. bicolor (Table 3). In H. vulgare, however, the two lower 2,4-D levels decreased leaf carotenoids, whereas the two higher ones induced an increase in carotenoid content. On the other hand, all the tesed concentrations of CCC brought about reduction in the carotenoid content of S. bicolor at all stages of development. Such results are in agreement with the data obtained by Harvath and Lontai [5] who observed that application of 2,4-D to leaves of 7-days-old barley seedlings caused a decrease in pigment production. Although chlorophylls continued to increase, carotenoids did not increase between 8-11 days-old in control plants but decreased in treated plants.

The data presented in Table 4 show that the tested CCC levels resulted in an increase in Chl a/Chl b ratios in H. vulgare at the prefruiting and fruiting stages. Its higher levels brought about an increase, while the lower ones a decrease in such ratios in S. bicolor. Moreover, Chl a + Chl b/carotenoids ratio tended to increase in both crop species treated with each growth regulator throughout growth stages, except at the preflowering with CCC and at the flowering with 2,4-D. This result indicates that the various pigment fractions could be differently affected in response to treatment with growth regulator and the induced effect depends upon the stage of development in each plant.

The results present in Table 5 indicate that CCC decreased the photosynthetic activity of the isolated thylakoids in both crop species, though an increase was observed with most levels later during the fruiting stage. In this respect, Hegazi and Kaush [6] proposed a high specific effect of CCC in delaying the synthesis of structural chloroplast protein, while Devay et al. [3] detected an increase in photosynthetic activity of *Phaseolus vulgaris* leaves with CCC. The photosynthetic activity of both *H. vulgare* and *S. bicolor*, however, decreased also with 2,4-D at the preflowering and flowering stages but increased early in the seedling and later at the prefruiting and fruiting stages. It could be suggested that 2,4-D having an initial stimulatory effect on photosynthesis during the seedling stage, but later during the flowering stage this effect was counteracted. Such result could be compared with the work conducted by Pronina and Ladonin [16] who found that the double phosphorus level in the nutrient solution and 2,4-D at concentration of 5×10^{-4}M had increased Hill reaction in barley chloroplasts by 10-15%. They believed that high phosphorus level overcomed the inhibitory effect of 2,4-D which was connected with the activation of non-cyclic phosphorylation. It was found that in isolated chloroplast lamellar membranes of radish the number of chlorophyll molecules attendant to each electron transport chain was 25 % lower in chloroplasts from 10^{-3} M 2,4-D- treated than in chloroplasts from untreated plant, but the efficiency of coupling between electron flux and ATP formation was not significantly different in the two chloroplast types [10].

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Table (1) : Chlorophyll a content (ug./mg dry weight) of leaves at different growth stages of each of *Hordeum vulgare* and *Sorghum bicolor* as affected by CCC and 2,4-D.

Treatments (M)	Seed.	Prefl.	Fl.	Prefr.	Fr.	Seed.	Prefl.	Fl.	Prefr.	Fr.
	Hordeum vulgare					*Sorghum bicolor*				
CCC										
0.0	10.3	8.1	6.7	6.4	5.5	10.7	6.6	6.2	5.8	5.4
$5x10^{-6}$	9.8	8.0	7.0	7.0	6.5	9.9	10.5	4.9	6.4	5.5
$5x10^{-5}$	9.3	6.6	5.8	6.4	6.5	12.0	7.7	5.5	5.1	5.2
$5x10^{-4}$	11.8	6.9	6.2	6.2	7.7	10.0	6.0	4.9	6.6	6.3
$5x10^{-3}$	10.8	7.2	5.0	5.0	7.0	12.7	6.6	4.0	6.2	6.6
2,4-D										
0.0	10.3	8.1	6.7	6.4	5.5	10.7	6.6	6.2	5.8	5.4
$5x10^{-6}$	12.2	7.1	4.6	4.6	5.1	11.2	7.9	5.1	7.1	5.2
$5x10^{-5}$	12.4	6.8	5.7	7.7	5.1	12.9	6.6	5.9	6.8	6.1
$5x10^{-4}$	11.0	8.5	8.6	6.6	7.6	9.9	8.6	5.6	5.6	7.7
$5x10^{-3}$	12.3	8.9	5.7	7.1	8.7	8.3	---	---	---	---

Statistical analysis

	CCC *H. vulgare*	CCC *S. bicolor*	2,4-D *H. vulgare*	2,4-D *S. bicolor*
Growth stages	**	**	**	**
Treatments	*	ns	ns	ns
Interaction	**	**	**	**

Table (2) : Chlorophyll b content (ug./mg dry weight) of leaves at different growth stages of each of *Hordeum vulgare* and *Sorghum bicolor* as affected by CCC and 2,4-D.

Treatments (M)	Seed.	Prefl.	Fl.	Prefr.	Fr.	Seed.	Prefl.	Fl.	Prefr.	Fr.
	Hordeum vulgare					*Sorghum bicolor*				
CCC										
0.0	3.5	3.1	2.9	2.6	2.3	4.3	2.3	2.4	2.3	2.1
$5x10^{-6}$	3.7	3.2	2.8	2.9	2.5	4.3	3.7	2.0	2.5	2.5
$5x10^{-5}$	4.3	2.7	2.2	2.7	2.7	4.7	2.5	2.2	2.0	2.0
$5x10^{-4}$	4.7	3.0	2.8	2.8	3.2	3.3	2.2	2.0	2.7	2.3
$5x10^{-3}$	4.7	2.8	2.1	2.1	2.9	5.3	2.6	1.7	2.4	2.4
2,4-D										
0.0	3.5	3.1	2.9	2.6	2.3	4.3	2.3	2.4	2.3	2.1
$5x10^{-6}$	4.8	2.6	1.9	2.0	2.1	4.8	2.9	1.9	2.7	2.0
$5x10^{-5}$	4.6	2.8	2.3	3.4	2.3	5.0	2.5	2.3	2.7	2.3
$5x10^{-4}$	4.8	3.4	2.5	2.8	3.4	3.9	3.2	2.2	2.8	2.4
$5x10^{-3}$	4.8	3.3	2.4	3.2	3.3	2.7	---	---	---	---

Statistical analysis

	CCC *H. vulgare*	CCC *S. bicolor*	2,4-D *H. vulgare*	2,4-D *S. bicolor*
Growth stages	**	*	**	**
Treatments	ns	ns	**	ns
Interaction	**	ns	**	ns

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Table (3): Carotenoids content (ug /mg dry weight) of leaves at different growth stages of each of *Hordeum vulgare* and *Sorghum bicolor* as affected by CCC and 2,4-D.

Treatments (M)	Plant stages									
	Seed.	Prefl.	Fl.	Prefr.	Fr.	Seed.	Prefl.	Fl.	Prefr.	Fr.
	Hordeum vulgare					*Sorghum bicolor*				
CCC										
0.0	3.7	2.3	1.6	1.8	1.7	2.4	1.3	1.5	2.0	2.4
$5x10^{-6}$	2.8	1.9	1.9	1.5	1.7	2.4	2.3	1.3	1.7	1.5
$5x10^{-5}$	2.8	2.0	1.9	1.3	1.8	2.5	1.7	1.3	1.3	1.5
$5x10^{-4}$	3.7	2.2	1.6	1.6	2.0	2.3	1.7	1.2	1.6	1.7
$5x10^{-3}$	4.9	2.2	1.3	1.3	1.9	2.8	1.5	0.9	3.6	3.6
2,4-D										
0.0	3.7	2.3	1.6	1.8	1.7	2.4	1.3	1.5	2.0	2.4
$5x10^{-6}$	2.6	2.0	1.3	1.2	1.4	2.4	1.7	1.6	1.7	2.3
$5x10^{-5}$	3.6	2.0	1.6	1.7	1.5	2.8	1.4	1.4	1.7	1.7
$5x10^{-4}$	3.6	2.3	2.6	1.4	2.0	2.1	0.6	1.4	2.2	0.4
$5x10^{-3}$	3.9	2.6	1.5	1.6	2.4	2.0	---	---	---	---

Statistical analysis

	CCC		2,4-D	
	H. vulgare	*S. bicolor*	*H. vulgare*	*S. bicolor*
Growth stages	**	**	**	**
Treatments	ns	**	**	**
Interaction	**	**	ns	**

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Table(4): Chlorophyll a/Chlorophyll b (a/b) and chlorophyll a+ chlorophyll b/ carotenoids (a+b/c) ratios at different growth stages of each of *Hordeum vulgare* and *Sorghum bicolor* as affected by CCC and 2,4-D.

Treatments (M)	Plant stages									
	Seed.	Prefl.	Fl.	Prefr.	Fr.	Seed.	Prefl.	Fl.	Prefr.	Fr.
	Hordeum vulgare					*Sorghum bicolor*				
(a/b) CCC										
0.0	2.9	2.6	2.4	2.5	2.4	2.5	3.1	2.6	2.6	2.6
5x10^{-6}	2.7	2.6	2.5	2.4	2.6	2.3	2.8	2.4	2.6	2.2
5x10^{-5}	2.2	2.4	2.6	2.4	2.5	2.5	3.1	2.4	2.5	2.6
5x10^{-4}	2.5	2.4	2.4	2.2	2.4	2.7	2.7	2.4	2.5	2.8
5x10^{-3}	2.3	2.7	2.4	2.4	2.5	2.2	2.5	2.3	2.8	2.8
2,4-D										
0.0	2.9	2.6	2.4	2.5	2.4	2.5	3.1	2.6	2.6	2.6
5x10^{-6}	2.5	2.7	2.5	2.3	2.5	2.4	2.7	2.7	3.0	2.6
5x10^{-5}	2.7	2.5	2.6	2.3	2.4	2.6	2.6	2.5	2.5	2.7
5x10^{-4}	2.3	2.5	2.5	2.3	2.3	2.6	2.7	2.5	2.4	3.1
5x10^{-3}	2.6	2.7	2.4	2.4	2.7	2.1	---	---	---	---
(a+b/c) CCC										
0.0	3.7	4.9	6.0	5.2	4.5	6.4	7.3	5.7	4.2	3.2
5x10^{-6}	5.1	5.9	5.3	6.5	5.2	5.9	[illegible]	5.4	5.2	5.5
5x10^{-5}	5.2	5.6	4.3	6.8	5.1	6.8	4.2	6.2	5.6	4.9
5x10^{-4}	4.6	4.5	5.0	5.8	5.6	5.9	6.4	5.6	5.9	5.0
5x10^{-3}	3.2	4.6	4.1	5.7	5.2	6.7	6.3	6.1	2.6	2.9
2,4-D										
0.0	3.7	4.9	6.0	5.2	4.5	6.4	7.3	5.7	4.2	3.2
5x10^{-6}	6.4	4.9	5.2	5.5	5.1	6.7	4.6	4.4	5.6	3.2
5x10^{-5}	4.8	4.8	5.0	6.6	5.3	6.4	3.9	6.0	5.6	5.0
5x10^{-4}	4.5	5.2	4.6	6.5	5.4	6.7	10.7	5.7	3.8	5.0
5x10^{-3}	4.5	4.8	5.4	6.7	5.0	6.1	---	---	-	---

Statistical analysis

	CCC *H. vulgare*	CCC *S. bicolor*	2,4-D *H. vulgare*	2,4-D *S. bicolor*
(a/b)				
wth stages	**	*	**	**
Treatments	*	**	**	**
Interaction	**	**	**	**
(a+b/c)				
Growth stages	**	*	ns	*
Treatments	**	**	**	**
Interaction	ns	**	**	**

Table(5): Photosynthetic electron transport measured as a function of ferricyanide redution of the isolated thylakoids (umol. ferricyanide / mg. chlorophyll / hour) of leaves at different growth stages of each of *Hordeum vulgare* and *Sorghum bicolor* as affected by CCC and 2,4-D.

Treatments (M)	Seed.	Prefl.	Fl.	Prefr.	Fr.	Seed.	Prefl.	Fl.	Prefr.	Fr.
	Hordeum vulgare					*Sorghum bicolor*				
CCC										
0.0	16.4	30.8	48.7	30.0	8.0	9.0	29.7	33.6	12.0	10.8
$5x10^{-6}$	13.2	27.9	24.3	9.2	36.4	5.0	10.7	19.2	9.2	49.6
$5x10^{-5}$	12.3	14.7	17.6	25.3	6.2	8.4	13.0	17.8	28.5	9.2
$5x10^{-4}$	9.4	16.2	17.5	27.0	11.4	7.1	9.7	7.5	10.2	26.3
$5x10^{-3}$	8.8	8.8	21.9	20.1	10.0	9.4	8.8	6.6	33.9	11.5
2,4-D										
0.0	16.4	30.8	48.7	30.0	8.0	9.0	29.7	33.6	12.0	10.8
$5x10^{-6}$	19.1	15.8	16.7	20.9	17.6	3.8	5.2	4.0	21.0	12.8
$5x10^{-5}$	18.2	16.2	48.1	75.1	16.9	13.4	27.3	18.0	68.2	12.8
$5x10^{-4}$	20.4	25.8	62.9	36.9	10.3	12.6	38.7	25.2	33.2	7.9
$5x10^{-3}$	21.9	17.7	16.0	32.8	17.2	2.7	----	----	----	----

Statistical analysis

	CCC *H. vulgare*	CCC *S. bicolor*	2,4-D *H. vulgare*	2,4-D *S. bicolor*
Growth stages	ns	**	**	**
Treatments	ns	*	*	**
Interaction	ns	**	**	**

تاثيـر معاملات ثنائى كلوروفينوكسى حمض الخليك ، كلوركولين كلوريد على محتوى الاصباغ والبناء الضوئى فى نباتى الشـعير والذرة الرفيعــة

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تم استقصاء تاثير معاملات ٢ , ٤ـ ثنائى كلوروفينكسى حمض الخليك ، كلوركولين كلوريد على محتوى الاصباغ ونشاط البناء الضوئى للسيلاكويدات المعزولة فى نباتى المحاصيل (الشعير والذرة الرفيعة) خلال فترات النمو المختلفة.

اوضحت النتائج زيادة فى كل من كلوروفيل ا ،ب فى نبات الشعير عند استخدام كلوركولين كلوريد وذلك فى مرحلتى البادرات والاثمار بينما نقصت فى المراحل الاخرى .
وبالنسبة لنبات الذرة الرفيعة نجد ان معظم معاملات كلوركولين كلوريد ادت الى زيادة فى محتوى الكلوروفيل فى كل مراحل النمو عدا مرحلة الازهار.

وعلى الجانب الاخر فقد زاد المحتوى الكلورفيللى لنبات الشعير مع كل تركيزات ٢ , ٤ـ ثنائى كلوروفينوكسى حمض الخليك فى مرحلة البادرات ولكنه نقص مع التركيزين المنخفضين فيما بعد . وقد اظهر نبات الذرة الرفيعة زيادة فى محتوى الكلوروفيل فى كل مراحل النمو فيما عدا مرحلة الاثمارـ كرد فعل لمعاملة ٢ , ٤ـ ثنائى كلوروفينكسى حمض الخليك .

ولقد ابدت الكاروتينويدات نقصا باستخدام كلوركولين فى كل مراحل النمو ، فيما عدا مرحلة الاثمار فى نبات الشعير وكذلك مرحلتى البادرات وما قبل الازهار فى نبات الذرة الرفيعة ومن جهة اخرى زادت الكاروتينويدات لورقة نبات الشعير مع التركيزين المرتفعين من ٢ , ٤ـ ثنائى كلوروفينكسى حمض الخليك ، ولكنها نقصت فى نبات الذرة الرفيعة وذلك فى كل مراحل النمو .

ولقد ادت المعاملات المختلفة من كلوركولين كلوريد الى نقص فى نشاط البناء الضوئى للسيلاكويدات المعزولة من نباتى الشعير والذرة الرفيعة بينما لوحظت زيادة معظم التركيزات اثناء مرحلة الاثمار .

واظهر استخدام ٢ , ٤ـ ثنائى كلوروفينوكس حمض الخليك على نباتى الشعير والذرة الرفيعة اختزالا لنشاط البناء الضوئى فى مرحلتى ما قبل الازهار والازهار ولكن كانت هناك زيادة مبكرة فى مرحلة البادرات وأخيرا فى مرحلتى ما قبل الاثمار والاثمار وعليه فان من الواضح ان للمركب ٢ , ٤ـ ثنائى كلوروفينوكسى حمض الخليك تأثير محفز على البناء الضوئى فى مرحلة البادرات ولكن هذا التأثير يضمحل فى مرحلة الازهار .