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Effect of growth regulators on *Vicia faba* plants irrigated by sea water

Leaf area, pigment content and photosynthetic activity

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Abstract

The antagonistic effects of some growth regulators [*i.e.* indol-3-yl-acetic acid (IAA), gibberellic acid (GA_3) or kinetin] on stress imposed by sea water on leaf area, pigment and photosynthetic activity in leaves of broad bean plants at different stages of development were investigated. Seed priming with GA_3 alleviated either partially or completely the effects induced by the two levels of sea water (10 and 25 %) used on leaf area at all experimental stages. However, IAA, GA_3 and kinetin inhibited leaf growth by themselves in almost all measurements. Seed pretreatment with kinetin alleviated the inhibition of pigment production in sea water-irrigated plants. Furthermore, GA_3 or kinetin nullified the deleterious effects imposed by irrigation with sea water particularly the high level (25 %) on photosynthetic $^{14}CO_2$ fixation.

Introduction

Photosynthesis in various plant types is generally reduced by salinity, irrespective of the types of salinizing agent used. The salt concentration which bring about severe reduction in photosynthesis varies greatly between plant types (Hoffman and Phene 1971, Udevenko *et al.* 1971, Lippina and Bikmulkhametova 1972, Reed *et al.* 1980, Kaiser 1984, Gaber 1985, Kalaji and Nalborczyk 1991). The pigment content was markedly activated by salt stress in maize but it was reduced in safflower (El-Deep 1984). GA_3 and IAA increased the pigment content of salinized plants in grasses (Varshnay and Baijal 1979), kidney bean (Shaddad and Heikal 1982), broad bean (Gaber 1985), wheat flag leaf (Aldesuqy 1991) or in *Pisum* (Younis *et al.* 1991). On the other hand, Salama *et al.* (1980) demonstrated that chlorophyll (Chl) content was not significantly influenced by hormonal treatments in salinized sugar beet. Richmond and Lang (1957) found that kinetin prevented Chl loss in detached

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Xanthium leaves. Similar results were obtained with soybean by Lindoo (1979) and with oats by Dumbroff and Walker (1980) and Hsiao and Kao (1980).

The present investigation was undertaken to add more information to the role of IAA, GA_3 and kinetin in alleviating stress induced by irrigation with sea water on leaf area, pigment content, Hill activity of isolated chloroplasts and photosynthetic activity in leaves of *V. faba* which is an economically important crop in Egypt.

Materials and methods

Plants: Homogeneous *V. faba* L. (cv. Giza 2) seeds were surface sterilized by soaking in 0.01 M $HgCl_2$ solution for 3 min, washed thoroughly with distilled water, and divided into four sets, which were soaked in distilled water, 50 mM IAA, 10 mM GA_3 or 100 mM kinetin, respectively, for about six hours. After soaking, thoroughly washed seeds were planted (15 per pot) on 23 February 1991 in earthenware pots (30 cm in diameter) filled with 3 kg soil (sand, clay 2/1 v/v). The pots were kept in greenhouse under a normal day/night regime, and irrigated with tap water when required. After two weeks only five uniform seedlings were left in each pot. The plants of each set were divided into three groups, which were irrigated with 0, 10 or 25 % sea water, respectively. The standard sea water contained [kg m^{-3}]: Cl^- - 21.6, Na^+ - 11.1, SO_4^{2-} - 2.85, K^+ - 0.49, PO_3^-- 16.6; salinity was 38.5 g kg^{-1}; pH = 8.1; its electrical conductivity 62 mmhos cm^{-1}. After thinning, the plants received 35 g(N) m^{-2} as ammonium nitrate and 35 g(P) m^{-2} as superphosphate.

Samples from the 3rd leaf of main shoot (numbered from the base) were taken after 29, 39 and 49 d after sowing. The samples of each treatment were ten replicates for measuring leaf area and triplicates for pigment content, photosynthetic and Hill activities.

Leaf area was measured by weighing the image of the leaf and comparing that mass with the mass of a known area.

Chl *a*, Chl *b* and carotenoid contents were measured by the spectrophotometric method as recommended by Metzner *et al.* (1965).

$^{14}CO_2$ uptake: As described by Gaber (1985) a definite fresh mass of leaf discs was introduced into the fixation apparatus (Fig. 1). An aqueous solution of ^{14}C-sodium carbonate of known activity (3.7 MBq cm^{-3}) was pipetted into the apparatus followed by H_2SO_4 (10 %). The evolved $^{14}CO_2$ passed over and radioactivity of the green leaf discs was measured using a *Packard Scintillation Counter.*

Hill reaction assay: As described by Arnon (1949), detached leaves were ground, using a chilled blender, in 50 mM Na-Tricine (pH = 7.8), 0.3 M sucrose and 2 mM $MgCl_2$ and the resulting homogenate was filtered. A chloroplast pellet was obtained by centrifugation at 2000 *g*. The pellets were suspended in 0.1 M NaCl and centrifuged again. The resulting pellets were resuspended in 1 mM Na-Tricine, 10 mM NaCl and 10 mM $MgCl_2$ and kept at - 4.0 °C.

Photosystem 2 activity as indicated by the rate of 2,6-dichlorophenol indophenol (2,6-DCPIP) photoreduction (Trebst 1972) was obtained using a *Spekol* spectrocolorimeter.

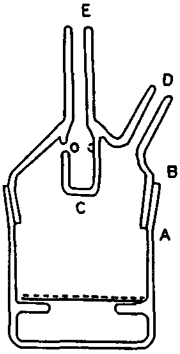

Fig. 1. $^{14}CO_2$-fixation apparatus. *A*: main container; *B*: lid; *C*: inner container (1 cm^3 capacity); *D*, *E*: side arms.

The results were first subjected to an analysis of variance (*ANOVA*). If *ANOVA* showed a significant ($P \leq 0.05$) effect, the least significant difference was used to compare treatments (Snedecor and Cochran 1967).

Results

Table 1. Effect of seed presoaking in indol-3-yl-acetic acid (IAA), gibberellic acid (GA_3) or kinetin on leaf area [cm^2 per leaf] of bean plants irrigated by two concentrations of sea water.

Presoaking treatment	Sea water [%]	Time after sowing [d] 29	39	49
None	0	42.11	50.07	40.10
	10	26.11	45.08	26.00
	25	22.28	33.64	25.86
IAA	0	29.71	53.42	33.86
	10	26.73	45.17	37.75
	25	23.21	35.27	29.07
GA_3	0	29.19	41.03	24.64
	10	44.93	50.06	38.93
	25	24.09	44.93	42.44.
Kinetin	0	34.73	43.27	46.14
	10	28.49	34.75	37.07
	25	26.55.	30.80	30.25
L.S.D.	$P \leq 0.05$	4.571	5.099	5.246
	$P \leq 0.01$	5.417	7.157	7.363

Leaf area: As compared with control, 10 and 25 % sea water caused generally a significant decrease ($P < 0.01$) in the area of the 3rd leaf of bean plants during the overall growth period (Table 1). The inhibition of leaf area growth caused by sea water was not alleviated when the seeds were treated with IAA or kinetin; leaf growth only recovered in GA_3-treated plants. In addition, IAA, GA_3 and kinetin inhibited leaf growth by themselves in almost all measurements.

Chlorophyll: 10 % sea water caused a marked increase ($P < 0.01$) and 25 % sea water caused a marked decrease in Chl *a* content of the 3rd leaf after 29 d from sowing (Table 2). Ten days later, the two levels of sea water decreased Chl *a* content whereupon a non-significant effect was obtained during the subsequent growth period (49 d old). Presoaking of bean seeds in kinetin induced an increase in Chl *a* content in the leaves of bean plants above the control level. Seed pretreatment with IAA or GA_3 had no similar effect. The differences in Chl *b* content were mostly non-significant, with the exception of the negative effect of IAA, evident especially on the day 29.

Carotenoids: Carotenoids content was non-significantly affected by the two levels of sea water till the 49th d from sowing (Table 2). The changes induced by presoaking of seeds in aqueous solutions of kinetin or GA_3 were small and significant only in the case of IAA pretreatment on the day 49.

Table 2. Effect of seed presoaking in indol-3-yl-acetic acid (IAA), gibberellic acid (GA_3) or kinetin on chlorophyll (Chl) and carotenoids (Car) content [mg m^{-2}] in developing leaves of bean plants irrigated by two concentrations of sea water.

Presoaking treatment	Sea water [%]	Time from sowing [d] 29			39			49		
		Chl *a*	Chl *b*	Car	Chl *a*	Chl *b*	Car	Chl *a*	Chl *b*	Car
No	0	0.47	0.24	0.14	0.63	0.23	0.22	0.55	0.21	0.20
	10	0.54	0.23	0.16	0.56	0.26	0.20	0.54	0.20	0.21
	25	0.41	0.21	0.13	0.52	0.26	0.22	0.56	0.21	0.20
IAA	0	0.41	0.18	0.14	0.53	0.27	0.20	0.45	0.19	0.16
	10	0.39	0.17	0.13	0.46	0.22	0.17	0.55	0.20	0.21
	25	0.39	0.18	0.12.	0.59	0.27	0.23	0.49	0.20	0.18
GA_3	0	0.43	0.19	0.15	0.60	0.31	0.22	0.46	0.19	0.18
	10	0.53	0.23	0.17	0.65	0.28	0.26	0.58	0.23	0.20
	25	0.50	0.22	0.17	0.47	0.23	0.17	0.53	0.20	0.21
Kinetin	0	0.53	0.24	0.18	0.61	0.31	0.25	0.53	0.21	0.21
	10	0.57	0.21	0.17	0.77	0.34	0.33	0.64	0.23	0.25
	25	0.50	0.23	0.18	0.68	0.32	0.28	0.67	0.29	0.26
L.S.D.	$P \leq 0.05$	0.010	0.028	0.028	0.028	0.039	0.015	0.028	0.014	0.008
	0.01	0.015	0.039	0.039	0.039	0.055	0.021	0.039	0.019	0.012

Hill reaction: Throughout the experimental stages, there was a significant increase or decrease in the Hill reaction rate under the influence of 10 or 25 % sea water, respectively, as compared with the levels in the control chloroplasts (Table 3). Seed priming with IAA or GA_3 and especially with kinetin induced an increase in photoreduction of DCPIP throughout the experimental stages.

Table 3. Effect of seed presoaking in indol-3-yl-acetic acid (IAA), gibberelic acid (GA_3) or kinetin on the Hill reaction ($H_2O \rightarrow$ DCPIP) [mmol (DCPIP) kg^{-1} (Chl) s^{-1}] by chloroplasts of bean plants irrigated by two concentrations of sea water.

Presoaking treatments	Sea water [%]	Time from sowing [d] 29	39	49
No	0	56.85	87.50	72.96
	10	64.36	103.06	82.04
	25	40.28	54.63	51.39
IAA	0	58.52	105.83	80.83
	10	82.31	114.82	96.30
	25	94.91	56.85	51.39
GA_3	0	63.43	112.96	100.28
	10	81.67	137.04	120.00
	25	51.39	73.15	58.43
Kinetin	0	85.18	115.37	101.20
	10	90.19	171.67	135.95
	25	56.21	88.43	80.74
L.S.D.	$P \leq 0.05$	3.77	4.85	2.87
	$P \leq 0.01$	5.09	6.54	3.87

Changes in photosynthetic $^{14}CO_2$ fixation: The low level of sea water (10 %), in general, induced a slight increase in soluble, insoluble and total photosynthates at all growth stages (Table 4). On the other hand, the higher level of sea water (25 %) induced a marked reduction in photosynthates. The sea water concentration reduced the ratio of soluble to insoluble photosynthates more than the 10 % treatment. The

Table 5. Ratio of parameters measured at 25 % of sea water and control after 29, 39 and 49 d and the same ratio on the 39th day as affected by growth regulators.

Parameter	Day 29	39	49	Ratio on 39th day Treatments IAA	GA_3	Kinetin
Leaf area	0.53	0.67	0.65	0.70	0.90	0.62
^{14}C fixation	0.49	0.51	0.47	0.56	0.97	1.12
Soluble/insoluble photosynth.	0.68	0.63	0.59	0.50	0.68	0.61
Chl *a*	0.87	0.82	1.02	0.94	0.75	1.08
Hill reaction	0.71	0.62	0.71	0.65	0.85	1.01

Table 4. Effect of seed presoaking in indol-3-yl-acetic acid (IAA), gibberelic acid (GA_3) or kinetin on photosynthetic activity [s^{-1} kg^{-1}(f.m.)] of bean plants irrigated by two concentrations of sea water.

Presoaking treatment	Sea water [%]	Time from sowing [d]											
		29				39				49			
		Soluble	Insoluble	Soluble/ insoluble	Total	Soluble	Insoluble	Soluble/ insoluble	Total	Soluble	Insoluble	Soluble/ insoluble	Total
No	0	0.454	0.057	0.134	0.511	0.535	0.069	0.128	0.604	0.420	0.055	0.129	0.475
	10	0.537	0.070	0.118	0.607	0.595	0.090	0.111	0.685	0.515	0.075	0.115	0.591
	25	0.229	0.041	0.092	0.260	0.256	0.053	0.081	0.309	0.183	0.041	0.076	0.223
IAA	0	0.477	0.071	0.113	0.548	0.612	0.089	0.115	0.701	0.489	0.078	0.105	0.566
	10	0.515	0.105	0.082	0.620	0.678	0.119	0.095	0.797	0.608	0.092	0.111	0.699
	25	0.265	0.060	0.074	0.324	0.269	0.070	0.065	0.338	0.253	0.062	0.068	0.313
GA_3	0	0.518	0.127	0.068	0.645	0.619	0.104	0.100	0.723	0.520	0.097	0.090	0.616
	10	0.609	0.157	0.065	0.766	0.720	0.145	0.084	0.865	0.692	0.126	0.092	0.817
	25	0.363	0.136	0.060	0.463	0.494	0.094	0.088	0.588	0.455	0.073	0.104	0.528
Kinetin	0	0.630	0.188	0.083	0.766	0.837	0.152	0.092	0.989	0.709	0.145	0.084	0.853
	10	0.725	0.121	0.065	0.913	0.910	0.242	0.063	1.152	0.788	0.183	0.760	0.970
	25	0.541	0.004	0.075	0.662	0.559	0.124	0.078	0.678	0.526	0.105	0.083	0.630
L.D.S.	$P \leq 0.05$	0.026	0.004	0.014	0.011	0.010	0.012	0.009	0.028	0.023	0.009	0.011	0.029
	$P \leq 0.01$	0.035	0.005	0.018	0.015	0.014	0.016	0.012	0.038	0.031	0.012	0.015	0.039

interaction of sea water salinity and growth hormones induced an additional increase in photosynthates content. Kinetin was the most effective hormone in stimulating the accumulation of photosynthates. The presoaking of bean seeds in IAA, GA_3 or kinetin, in general, reduced the ratios of soluble to insoluble intermediates.

Interrelationship among results (Table 5): The salinity of sea water at 25 % caused reduction in the leaf area due to restricted $^{14}CO_2$ assimilation which was the result of changes in the pigment levels which might in turn affect Hill reaction rate. The growth regulators alleviated the stress imposed by sea water on these parameters with varied degrees: kinetin or GA_3 had a pronounced effect, but IAA only a slight one.

Discussion

The cumulative leaf area decreased significantly in response to stress induced by sea water (Table 1), probably due to the higher ratios of mesophyll surface area to leaf area as indicated for *Phaseolus vulgaris* (Longdtreth and Park 1979), see also results of Wignarajah *et al.* (1975), El-Shahaby (1981), Gaber (1985), Aldesuquy (1991), Kalaji and Nalborczyk (1991) for various plant species. The alleviating effect of seed priming was more pronounced with kinetin and GA_3 than with IAA. In accord with these results, Naito *et al.* (1980), Sakr (1991), Younis *et al.* (1991) reported beneficial effect of GA_3 and IAA growth of salinized plant where in most cases an increase in water uptake of the treated plants was presumably the consequence rather than cell expansion.

The mostly non-significant stimulative effect of sea water stress on pigment content (Table 2) of bean leaves was in accord with the results of Chavan and Karadge (1980) using *Arachis hypogea* and might be due to the fact that salinity led to an increase in chloroplast number (Aldesuquy 1991). Seed priming with kinetin stimulated the pigment content of bean leaves while IAA or GA_3 treatment had only a little effect. The stimulative effect of growth hormones on pigments in leaves of sea water irrigated plants is in accord with the results obtained by Varshney and Baijal (1979), Shaddad and Heikal (1982), Aldesuquy (1991) and Younis *et al.* (1991) who worked with grasses and kidney bean.

The rate of transformation of soluble photosynthates to the insoluble ones and the Hill activity were drastically reduced in sea water stressed plants (Tables 3 and 4). This inhibition of photosynthesis may be due to the decline in Chl content in consequence to reduction in leaf area. In addition, water stress inhibits photosynthesis either by inducing stomatal closure or by directly affecting the photosynthetic machinery at the mesophyll and chloroplast level (Berkowitz and Gibbs 1981, Kaiser and Heber 1981, Gaber 1985). The positive of seed pretreatment on salt water stressed plants was more pronounced with kinetin or GA_3 than IAA. In this connection, there are many reports on plant hormone stimulation of chloroplast developmant as well as the whole photosynthetic machinery (*e.g.*, Hayashi 1961, Turner and Bidwell 1965, Wild *et al.* 1981, Sakr 1985).

Generally, the inhibition of photosynthesis by sea water (Table 5) was alleviated and even promoted over the control value by GA_3 and especially kinetin. However, the leaves were inhibited in growth by sea water and might undergo changes in chemical composition that would affect the photosynthesis. A very important point was that the plants at salinity might respond as plants under water stress, and thus the reduction in photosynthesis could well be the result of more closed stomata, which may be wider opened by GA_3 and kinetin.

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