Pak. J. Bot., 11(1) 79-84, 1979.

ISOLATION AND CHARACTERIZATION OF GROWTH INHIBITORS IN THE LEAVES OF *MESEMBRYANTHEMUM* SPECIES.

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Absttsct

Chaıcoal adsorbed fraction of methanolic extracts of *Mesembryanthemum forskahlei* and *M. crystallinum* leaves contained inhibitors of root and hypocotyl growth of lettuce. Gibberellic acid-induced growth of root and hypocotyl was also inhibited by the crude inhibitor extract. The presence of high concentration of Gibberellic acid (10 ppm) not only neutralized the growth inhibiting activity of the inhibitor (s) from the two species but also gave higher growth promotion of the hypocotyl as compared to water control. Paper chromatographic seperation of the crude extracts of the two species of *Mesembryanthemum* leaves revealed the presence of two distinct inhibitory zones (Inhibitor-A and Inhibitor-B). These two inhibitors from both the species were found to resist high temperatures (50 - 80°C).

Introduction

Mesembryanthemum crystallinum and other species of the family Mesembryanthemaceae grow luxuriantly in high salt conditions. Willert et al (1976) reported that mature leaves of *M. crystallinum* showed Crassulancean acid metabolism (CAM) after 37 days of growth in saline condition. They concluded that salt-treated, CAM-exibiting *Mesembryanthemum* plants are under environmental (NaCl) control.

Two species of *Mesembryanthemum* i.e. *M. forskahlei* and *M. crystallinum* were found to grow luxuriantly in saline soil of Benghazi, Libya. Preliminary anatomical study of these plants revealed the presence of abundant of salt crystals in the leaves and stems of both the species. This communication gives an account of the level and nature of growth inhibiting substances in the leaves of *Mesembryanthemum* species growing in saline soil.

Materials and Methods

Mature leaves of *M. forskahlei* Hochst., and *M. crystallinum* L. covered with glittering, water-filled papillas on both surfaces of the leaves were collected randomly from El Litham area (about 8 kilometers from Benghazi City centre, Libya). The leaves were

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brought to the laboratory in seperate polythene bags, washed throughly several times with tap water, and used immediately for the extraction and estimation of growth inhibitors. For the extraction of growth inhibitors, 650 g of mature leaves were selected randomly and sliced to about 1 cm pieces and extracted with three changes of absolute methanol at 5°C for 48 hr giving a total of 3.5 litres of extract. After filteration, 300 ml of distilled water was added and the volume reduced to water phase in a forced-air oven maintained at 40°C. Active charcoal (1g/10 g fresh tissue) was added to the water fraction, shaken and filtered. The charcoal residue was washed with 200 ml of acetone each time with three changes of acetone. The pooled acetone extracts were reduced to dryness at 35°C and redissolved in 26 ml of ethanol. This crude inhibitor extract was subjected to lettuce hypocotyl and root growth test before and after seperating the extract through paper chromatography.

For testing the crude inhibitor extract, volumes of ethanol equivalent to 10 g fresh weight of the leaves were applied on two disc of Whatman paper No 5 kept in 9 cm Petri dishes. Ethanol was evaporated with a hair dryer and 3.0 ml of distilled water with or without GA_3 in it were added. After standing for 24 hr at room temperature (22 ± 2°C), ten 24 hr dark-germinated seeds of *Lactuca sativa* (cv. Great Lakes) were transferred. Length of roots and hypocotyls were measured after 3 days of further growth in the dark. The crude inhibitor extracts were streaked on 15 cm wide strips of Whatman paper No 1 and developed at 22 ± 2°C by descending chromatography employing the solvent n-butanol-acetic acid-water (4 : 1 : 5 V/V, upper layer). Paper corresponding to each Rf zone was excised and placed in 9 cm Petri dishes together with two discs of Whatman paper No 5, 3 ml of distilled water was added to every dish and left for 24 hr at room temperature. The effect upon the growth of lettuce seedlings was studied as described for the crude extract.

To study the effect of high temperatures on the activity of growth inhibiting zones, paper chromatograms containing the inhibitors were cut into small pieces and extracted with 80% ethanol for 24 hr at 5°C with occasional shaking. After filteration, alcohol was removed at 40°C and the aqueous fraction diluted with distilled water to obtain 1 ml/ 5 g fresh tissue. Test tubes containing 3 ml of these extracts were held at 22, 50, 70, 80°C for exactly 20 minutes and brought immediately to room temperature by plunging the tubes in cold water for few minutes. These extracts were then subjected to root and hypocotyl growth test as described above.

Results and Discussion

The charcoal adsorbed fraction of the methanolic extracts of *M. forskahlei* Hochst, and *M. crystallinum* L. leaves contained compound (s) inhibitory to root and hypocotyl growth of lettuce *(Lactuca sativa* L. cv. Great Lakes) in complete darkness (Table 1). 1 and 10 ppm GA_3-induced growth of the root and hypocotyl of lettuce was also inhibited considerably by the crude extract. However the presence of high concentration of GA_3 (10 ppm) not only neutralized the growth-inhibiting activity of the inhibitor (s) from the two species but also gave higher growth promotion of the hypocotyls.

TABLE 1. Inhibition of root and hypocotyl growth of lettuce by the charcoal adsorbed fraction of methanolic extracts of *M. forskahlei* and *M. crystallinum* leaves in the presence and absence of gibberellic acid. ±, Standard error of mean.

	M. forskahlei						*M. crystallinum*					
	Hypocotyl length (mm)	% Inhibition[+]	% Reversal[++]	Root length (mm)	% Inhibition[+]	% Reversal[++]	Hypocotyl length (mm)	% Inhibition[+]	% Reversal[++]	Root length (mm)	% Inhibition[+]	% Reversal[++]
Control (Water)	21.2 ± 0.71	–	–	19.4 ± 0.49	–	–	20.3 ± 0.54	–	–	18.6 ± 0.78	–	–
1 ppm GA_3	24.9 ± 0.96	–	–	21.3 ± 0.63	–	–	22.1 ± 0.94	–	–	23.1 ± 0.90	–	–
10 ppm GA_3	28.3 ± 1.2	–	–	24.1 ± 0.92	–	–	25.8 ± 0.81	–	–	27.4 ± 0.66	–	–
Ihnibitor Ext. (10 g F. Wt equivalent)	11.5 ± 0.83	45.75	–	10.3 ± 1.11	46.90	–	12.5 ± 0.53	38.42	–	8.3 ± 1.10	50.73	–
Inhibitor Ext. + 1 ppm GA_3	19.7 ± 1.12	–	84.53	11.3 ± 0.98	–	10.98	20.3 ± 1.00	–	100.0	11.4 ± 0.93	–	30.69
Inhibitor Ext. + 10 ppm GA_3	22.6 ± 0.88	–	114.43	12.6 ± 1.32	–	25.27	22.0 ± 0.74	–	121.79	12.5 ± 1.20		41.58

+ % Inhibition = $\frac{Xc - Xt}{Xc} \times 100$

Xc = Coutrot Xt = Treated

++ % Reversal = $\frac{(Xi - Ra) - Xi}{Xc - Xi} \times 100$

(Xi - Ra) = Inhibitor + GA_3 Xi = Inhibitor only.

Root growth inhibition was also reverted by GA_3 but to a lesser extent than that of the hypocotyls. Similar to the results presented in Table 1, increasing concentration of GA_3 (0.01 – 10 ppm) were found to increase significantly the root length of dark and light grown seedlings of lettuce and *Amaranthus caudatus* (Unpublished results). Promotion of root formation by GA_3 in a number of regeneration systems have been reported by Nanda *et al* (1072), Ericksen (1971), Varga & Humphries (1974), Kochba *et al* (1974), Hansen (1975, 1976) and Coleman & Greyson (1977).

When the crude inhibitory extracts were subjected to paper chromatographic seperation in n-butanol-acetic acid-water (4 : 1: 5 v/v, upper layer), the inhibitory zones of root and hypocotyl growth of lettuce present in *M. forskahlei* was found at Rf 0.0 – 0.2 (Inhibitor-A) and 0.8 – 1.0 (Inhibitor-B) and in *M. crystallinum* at Rf 0.0 – 0.2 (Inhibitor-A) and 0.4 – 1.0 (Inhibitor-B) as shown in Fig. 1.

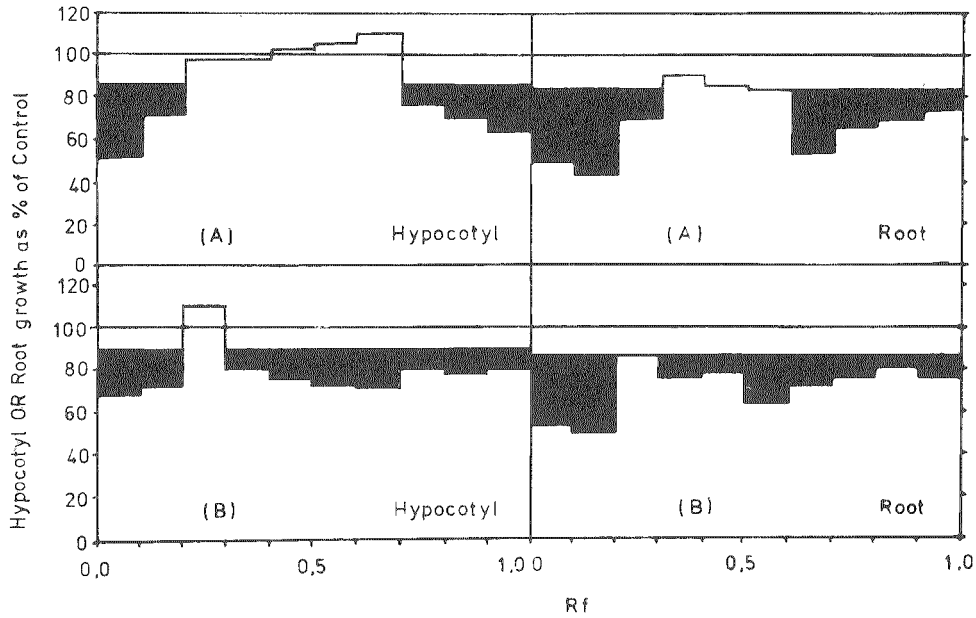

Fig. 1 Histograms of charcoal adsorbed fraction of *M. forskahlei* (A) and *M. crystallinum* (B) leaves chromatographed on Whatman paper No. 1 and developed in the solvent n-butanolacetic acid-water (4 : 1 ; 5 v/v, upper layer). *Dark* areas are significantly different (P 0.05) from the control.

TABLE 2. **Effect of high temperatures on the activity of growth inhibitors (A and B) isolated from the leaves of *Mesembryanthemum* species. ±, Standard error of mean.**

Plant species	Treatment (Tem. °C)	Inhibitor-A	Hypocotyl length (mm)	Root length (mm)	Inhibitor-B	Hypocotyl length (mm)	Root length (mm)
	(Rf 0.0-0.2					(Rf 0.4-1.0	
M. forskahlei	22 (Control)	-	22.4 ± 0.83	25.4 ± 1.16		22.4 ± 0.83	25.4 ± 1.16
	22	+	12.5 ± 1.31	7.2 ± 0.36	+	16.3 ± 1.00	24.3 ± 0.82
	50	+	13.1 ± 1.43	8.1 ± 0.39	+	17.1 ± 1.32	29.1 ± 1.98
	70		13.0 ± 0.86	8.6 ± 0.50	+	16.3 ± 0.98	24.8 ± 0.86
	80	+	12.0 ± 0.73	8.6 ± 0.48	+	16.9 ± 1.26	23.0 ± 1.60
	(Rf 0.0-0.2)					(Rf 0.8-1.0)	
M. crystallinum	22	+	15.8 6.65	12.8 0.49	+	19.0 0.73	20.3 0.59
	50	+	17.6 0.89	12.2 0.69	+	18.1 0.51	21.1 0.31
	70	+	14.0 0.80	11 3 0.72	+	17.7 0.98	23.1 1.26
	80	+	15.0 0.95	11.2 1.21	+	16.9 0.59	21.6 0.86

Effect of high temperatures on the activity of growth retardants from both the species of *Mesembryanthemum* was also investigated. The result presented in Table -2 indicated that the activity of Inhibitor-A and B of both the species on root and hypocotyl growth of lettuce remained unaltered at high temperatures (50 - 80°C) as compared to the inhibitors kept at room temperature (22°C). Similar temperature-resistant growth inhibitor (Inhibitor-A) has been reported earlier in the mature leaves of *Suaeda fructicosa* growing in saline soil (Khizar & Khan, 1977).

Acknowledgements:

We extend our thanks to Dr. M.A. Mokahel, Head, Department of Botany and Dr. Awad El Hossadi, Dean, Faculty of Science, University of Gharyounis for encouragement and providing the facilities to work.

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