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The effect of humic acids on root growth and endogenous levels of auxin and inhibitory-like substances

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ABSTRACT

The present paper reports on the effect of complete humic acid extract and medium molecular mass fraction thereof on the growth of sunflower root sections and on the endogenous level of plant growth regulators. Humic acids in nutrient solution opposed to humic acids or nutrient solution alone caused the growth rate in root sections to diminish. Higher concentrations of humic acids proved to have and even stronger inhibitory effect. Root sections treated with 285 mg l^{-1} of medium molecular mass fraction showed improved growth and higher endogenous levels of growth promoting substances than the control. Concentrations as high as 2000 mg l^{-1} of complete humic acid extract caused an increase in the endogenous levels of inhibitors. It is suggested that humic acids affect root growth either by altering the metabolism of endogenous plant regulators or by acting as growth-regulating substances themselves.

RESUMEN

En el presente trabajo se informa el efecto de un extracto completo de ácidos húmicos y su fracción de medio peso molecular sobre el crecimiento y el nivel endógeno de reguladores del crecimiento en raíces de girasol. Los ácidos húmicos en solución nutritiva disminuyen el crecimiento de secciones radicales, a diferencia de lo observado al utilizar ácidos húmicos o solución nutritiva sola. Cuanto mayor es la concentración, mayor el efecto inhibidor. Las secciones radicales tratadas con 285 mg l^{-1} de la fracción de medio peso molecular, mostraron aumento en el crecimiento y en el contenido endógeno de sustancias promotoras del crecimiento. Concentraciones tan altas como 2000 mg l^{-1} del extracto completo causaron un aumento en los niveles endógenos de inhibidores. Estos resultados sugieren que los ácidos húmicos afectan el crecimiento radical, ya sea por alterar el metabolismo de los reguladores del crecimiento o por actuar *per se* como sustancias reguladoras.

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INTRODUCTION

Humic acids (HA) are known to affect various aspects of plant metabolism, including mineral nutrition. A growing body of evidence suggests that in addition to their effect on soil, HA can also affect vital processes. There are several studies on the effect of HA either alone or in nutrient solution on root growth (Fialová, 1969; Bystřická and Sladký, 1972; Hernando, Ortega y Fortún, 1975; Mylonas and McCants, 1980). The promotion of root growth has been reported in wheat (Fialová, 1969), root apex of *Solanum dulcamara* (Bystřická and Sladký, 1972) and corn, at concentrations higher than 640 ppm (Tan and Nopamornbodi,

1979). The presence of HA alone (Fialová, 1969) or in nutrient solution (Hernando *et al*, 1969) causes the growth rate of root sections to diminish. The possible hormonal character or auxin-like nature of HA has been investigated (O'Donnell, 1973; Ortega *et al*, 1979), but despite the relevance of endogenous levels of plant regulators to root growth, no attempt has been made to date to correlate the effects of HA with this two parameters. The present paper therefore seeks to shed further light on this subject.

MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L., Morgan hybrid) fruits ranging from 6 to 6.5 mm in size were treated with 1% Captan for 5 min, rinsed with distilled water, and sown in an inert medium under 12 h light at 27°C and 12 h dark at 15°C. Straight radicles of equal lenght were decapitated after four days and cut into sections of 5.4 mm. These latter were surface sterilized for 10 min. in Triton X-100 (0.3%) and 10 sec. in 95% ethanol, and then rinsed with sterile water.

Extraction and purification of humic acids: HA were extracted from an A1 horizon of a Typic Argiudoll (5.5% organic matter, loam texture, pH 5.9). Extraction and purification were carried out essentially as described by Lakatos et al. (1977).

Fractionation of HA: HA were separated into three fractions of nominal molecular mass 500-10,000; 10,000-100,000; and greater than 100,000 by membrane filtration as follows: 500 mg freeze-dried HA were dissolved in 20 ml 0.1 N NaOH and the solution eluted through strong ion exchange resins (Dowex 1x8-50 in OH^- form and Amberlite IR-120 in H^+ form). The eluted solution (pH 2.7) was brought to pH 6 with Na OH and completed to 50 ml with water. The ultrafiltration procedure was carried out in an Amicon pressurized ultrafiltration cell under stirring. The solution was filtered through an Amicon XM 100 A membrane (nominal molecular mass cut-off 100,000). The cell was pressurized with nitrogen. The solution was washed through with water and filtration was continued until the eluted solution was colorless. The solution remaining in the cell was called the high molecular mass fractions ($\underline{M}_r$>100,000). This procedure was repeated with Amicon PM 10 and UM 0.05 membranes (nominal molecular mass cut-off 10,000 and 500 respectively), and the solutions remaining in the cell were called medium (10,000-100,000) and low (500-10,000) molecular mass fractions respectively. Only the complete extract and medium molecular mass fraction were used for the purposes of the present experiments.

Incubation medium: the concentrations of medium molecular mass fraction were prepared according to the percentage of fractionation (i.e. 285 ppm represent medium molecular-mass fraction content in 1000 ppm of complete extract). All solutions were adjusted to pH 5.3 and then sterilized by filtration through Millipore 0.2 µm filters. Radicles sections were incubated in HA solutions with or without half-strength low Ca^{+2} Steimberg solution (Steimberg, 1941), and kept in the dark at 20°C under periodical shaking. Growth was measured after 48 h using a stereo microscope fitted with an ocular micrometer.

Extraction of plant growth substances: sunflower fruits were incubated in HA solutions in the dark at 25°C until the radicles reached a length of 7-10 cm. The solution was changed every 48 h. For each treatment 5 g of material were ground and then extracted with methanol overnight at 4°C. After evaporation *in vacuo* the extract was resuspended with 0.1 M $NaHCO_3$ (pH 8) and peroxide-free diethyl ether. The aqueous medium was acidified to 2.7 with 1 N HCl and partitioned five times with diethyl ether. The ether-soluble acidic fraction was dried, diluted with 80% aqueous methanol, and applied to 20x20 platic silica gel 60 F_{254} thin layer chromatography plates (Merk). Samples of ABA and IAA were chromatographed using isopropanol: ammonia: water (100:14:6, by vol.) as solvent. UV-visible spots and 1.5 cm sections were carefully scraped off and assayed by means of the wheat straight-growth coleoptile test (Eliasson, 1969). Sections of plates without samples were used as control.

Statistical analysis: the statistical analysis involved an analysis of the variations in three fixed factors, subsequent comparison of the average of different treatments with the control according to the Dunnett test (Dunnett, 1955), and *a priori* orthogonal contrasts (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Fractionation of HA yielded 70% high, 28% medium and 2% low molecular mass fractions. The fact that only latter two possibly penetrate the cell may indicate that these are the physiologically active fractions (Führ

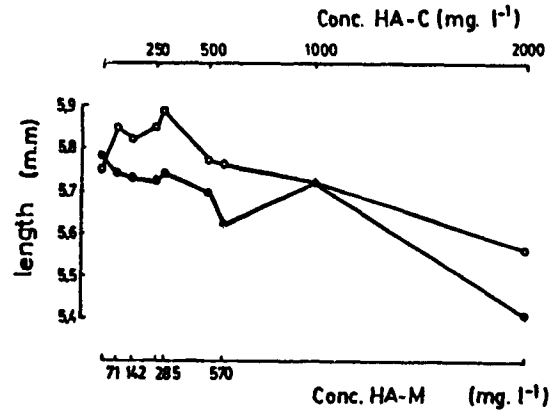

Fig. 1: Effect of concentrations of complete HA (HA-C) and medium molecular mass fraction (HA-M) on the growth of sunflower radicles. The results shown are average of 20 experiments. (o) without nutrients, (•) with nutrients.

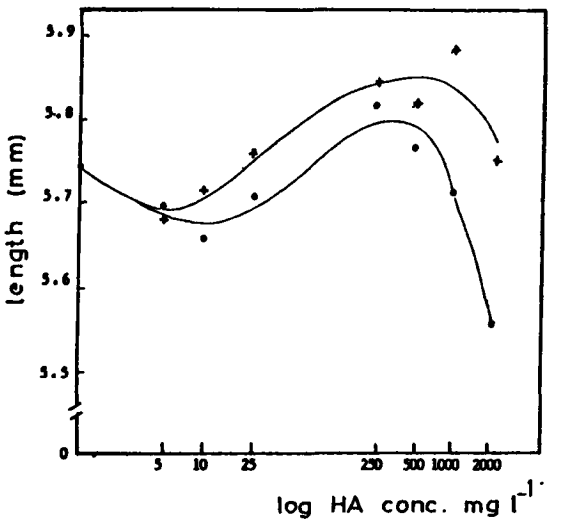

Fig. 2: Effect of concentrations of complete (•) and medium molecular mass fraction (+) corresponding to each concentration of complete HA, on growth of radical sections of sunflower. The results are average of 20 experiments.

and Sauerbeck, 1966; Härtel and Hinteregger, 1978).

The effect of concentrations of complete HA extract and medium molecular mass fraction on the growth of sunflower root sections is shown in Fig. 1. Controls were floated in distilled water or nutrient solution alone. The presence of HA in nutrient solution caused a reduction in the growth of root sections as compared with the effect of nutrient solution alone ($P \leq 0.05$). Similar results have been reported by other authors (Fialová, 1969; Hernando *et al*, 1969). This growth-inhibiting effect could result from the blockage of functional groups involved in root growth, brought about by mineral nutrient-HA reactions (Schnitzer and Poapst, 1967; Ortega *et al*, 1982), thus reducing the accesibility of HA and mineral nutrition (Wallace and Wallace, 1982) to the plant. In fact, on the basis of the Dunnett test, HA inhibited root growth only at 2000 mg l^{-1}. This inhibitory effect observed at high concentrations is in agreement with the findings of Mylonas and McCants (1980).

Eventhough the improved growth observed in roots treated with 285 mg l^{-1} medium molecular mass fraction was less significant ($P \leq 0.1$) (Fig. 2), in general this fraction was more effective than the complete extract in promoting root elongation. There are two possible explanation for the inhibition of root growth by complete HA extract: either it is due to the antagonistic effect of high and low molecular mass fractions or it is induced by high concentration itself (Tan and Nopamornbodi, 1979).

The discrepancy among published results regarding the effect of HA on root growth could well derive from the fact that the source of HA is differente in each case, it is also possible that the various procedures used give rise to different results (Hernando *et al*, 1975; Ortega *et al*, 1979; Ortega *et al*, 1982). For example, in this appear the Ca^{2+} present in the Hoagland nutrient solution was found to precipitate HA; however, there has been no indication of this effect in the reports of other authors.

Endogenous growth-regulators content in sunflower roots with and without HA are shown in Fig. 3. Roots treated with 2000 mg l^{-1} HA show a large inhibitory zone corresponding to Rfs of 0.6-0.9, with maximum activity at the Rf 0.8 (Fig. 3 A). The small inhibition zones with Rfs of 0.1 and 0.2 may be a result of the presence of naringenine glucoside (Bottini, *et al*, 1978). In this case the level of growth-promoting substances does not differ significantly from those in the control (Fig. 3 C). By contrast, the endogenous level of inhibitors in roots treated with medium molecular mass HA fraction is low (Fig. 3 B), whereas the level of growth-promoting substances is higher than in the control.

It is suggested that HA-induced promotion or inhibition of root growth occurs either because of an

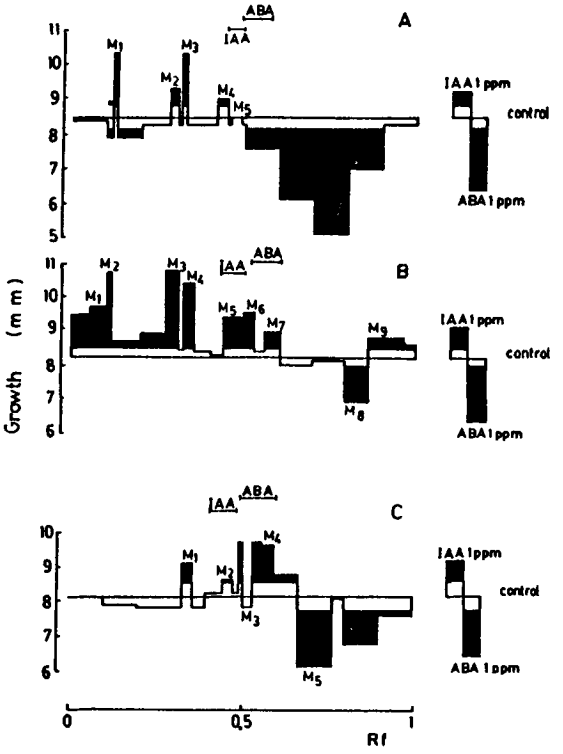

Fig. 3: Levels of auxin- and inhibitor-like substances in extracts of sunflower roots. A: treated with 2000 mg l-1 of complete extract; B: treated with 285 mg l-1 of medium molecular mass fraction; C: treated with distilled water. Abscissa: Rfs in TLC plates.

increase or decrease in the synthesis or because of a decrease or increase in the catabolism of endogenous growth regulators, or both. Whichever the case, the presence of growth-regulating substances in HA can not be disregarded.

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