Uptake of Humic Substances by Plants: a Study Using Tritium Autoradiography and FTICR MS Analysis

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1. INTRODUCTION

Bioavailability of humic substances (HS) to plants is still questionable due to difficulties in HS determination in plant tissues. The most convenient way is a usage of labeled compounds, and the availability of isotope-labeled HS is an important prerequisite to elucidate the fate of the heterogeneous organic matter in complex environments. On the other hand, presence of the isotope does not allow identifying specific compounds of HS that entered a target object. To solve this problem, isotope-labeled HS together with Fourier Transformed Ion Cyclotron Resonance mass spectrometry (FTICR MS) approach could be applied. This study was aimed (1) to find out uptake and distribution of HS in wheat seedlings and (2) to identify humic-derived compounds that can found in plants.

2. MATERIALS AND METHODS

For this study humic acids (HA) derived from leonardite was used. To synthesize isotope-labeled HA, an approach developed in (1) for the preparation of tritium-labeled HS was used. The obtained ^{3}H-HA sample was dissolved in a phosphate buffer (0.028 M, pH 6.8) and purified by dialysis during a month at 4ºC. It allowed eliminating exchangeable tritium of OH^-, $COOH^-$, and NH_n groups of HA. Plants of wheat *Triticum aestivum* L. were used for the experiments. Wheat seeds were germinated at 24°C in the dark for 72 h followed by transferring seedlings into the 0.5 l tanks containing Knopp nutrition solution. After another 72 h plants were transferred into the vials containing HA at concentration 50 mg·l^{-1} with specific radioactivity 0.02 Curie·l^{-1}. After 24 h plants were harvested and subjected to autoradiography. To perform FTICR MS analysis, shoots' tips were colleted and subjected to methanol extraction followed by FTICR MS. FTICR was conducted at

Apex Qe (Bruker Daltonics, Bremen, Germany) equipped with Apollo II electrospray ionization (ESI) source and 12 Tesla actively shielded superconductive magnet. Negative ionization mode was used with spray shield set to 3 kV and capillary voltage 3.5 kV. Flow rate was set to 120 mkl/hour. 100 scans in mass range 150…2000 Da were collected for each sample. To find tritium traces, FIRAN software (Kunenkov, 2006-2008) was used under the following considerations: a) there is only one tritium atom in the labeled ion; b) for each labeled ion there is ion without the label which consists only of ^{12}C, ^{1}H, ^{16}O and ^{14}N; c) for each labeled ion there is ion without the label but with one of its ^{12}C replaced by ^{13}C (this consideration were used to find ion charge by mass difference between two unlabeled ions); d) labeled ion present in mass spectrum of extract from shoots subjected to ^{3}H-HA must be absent in mass spectrum of extract from shoots subjected to non-modified HA.

3. RESULTS AND DISCUSSION

The obtained results revealed that coal HA were intensively adsorbed by wheat plants. The roots of the plants subjected to ^{3}H-HA were characterized with homogeneous distribution of signal intensity. Distribution of HA within the shoots was also relatively homogeneous except for the tips of the leaves where local increase of signal density was observed. That finding was evident for the fact that HA could be taken up through plant roots and moved al least in the xylem with transpiration stream to areas of new growth.

FTICR MS analysis of extract demonstrated presence of tritium-labelled fatty acids in the shoots of wheat plants treated with ^{3}H-HA. As fatty acids are important moieties for plant lipids biosynthesis, the obtained data demonstrated clearly that HA-derived compounds can be used for membrane formation.

4. CONCLUSIONS

Distribution of HA in plants was demonstrated to be relatively homogeneous except for the tips of both roots and leaves where increase in ^{3}H-HA concentration was observed. FTICR analysis of extract from tips leaves revealed presence of tritium-labelled fatty acids what was evident that HA-derived compounds could be used for membrane formation.

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REFERENCES

1. Badun, G. A. et al.: // Bulgarian J. of Ecol. Sci. 2(3-4), 26-27 (2003).