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Chemical composition and potential bioactivity of volatile from fast pyrolysis of rice husk

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ABSTRACT

Volatile components remaining on the surface of biochar may contribute to its effects on plant growth. To identify the potential bioactivity of these substances, the chemical composition of volatiles from fast pyrolysis of rice husks was analyzed by Py-GC/MS and verified in the Plant Metabolic Network database. A total of 81 compounds, primarily aromatic (38.60%) and ether (6.53%), were identified among the volatiles produced using various pyrolysis conditions. Moreover, among the 13 compounds identified to play important roles in plant metabolic processes, three (eugenol, 4-hydroxybenzoate, and salicyl alcohol) are known to be involved in plant defense mechanisms; these were analyzed in further detail. Analysis using AutoDock 4.2 showed that eugenol and salicyl alcohol interact with 3SOE and 3VIL, which are important proteins in plant defense against herbivores. Thus, biochar may influence plant growth and development through volatile components that remain on its surface.

1. Introduction

Biochar is the carbon-rich solid product obtained from thermal decomposition or pyrolysis of biomass, such as corncobs, straw, peanut shells, and rice husks [1,2]. Because of its high carbon content, resistance to erosion and biodecomposition, porous structure, high surface area, and pH, biochar has often been used for carbon sequestration and soil amelioration [3–6], and has gradually been accepted as a strategy to guarantee food security under climate change and soil degradation, especially in China, which has a huge population [7–13].

Although the effects of biochar for improvement of plant biomass or yield are well-known, its functional mechanisms remain obscure. Several possible mechanisms of biochar which affect plant growth have been proposed, including: (1) providing nutrition, which can be directly absorbed by plants; (2) increasing cation exchange capability via its large surface area and multiple functional groups, which may enhance nutritional efficiency; (3) decreasing soil bulk density and increasing soil pH; and (4) providing habitat to promote microbial growth via its porous structure. Because it can be produced from various feedstocks and under many different pyrolysis conditions, biochar can exhibit different physical and chemical properties [14] and produce different plant responses [7,8].

In addition to its physicochemical properties, biochar contains small organic molecules in volatile or tarry vapors [15] that might also contribute to plant growth. Most of these small molecules are present in the liquid byproducts (e.g., wood vinegar) of biomass pyrolysis, and many of their functions (e.g., stimulating plant growth, improving fruit quality, accelerating seed germination, disinfecting soil, and serving as herbicides) have previously been reported [16–18]. During the process of biochar production, some volatiles may condense on the surface of the biochar. Therefore, biochar may also affect plant growth via these small molecules.

In the present study, fast pyrolysis of rice husks was performed via Py-GC/MS to qualitatively and semi-quantitatively investigate the distribution of products in a gaseous state. Moreover, these small molecules were further investigated using the Plant Metabolic Network (PMN: http://www.plantcyc.org) database, to evaluate their possible effects on plant metabolic pathways based on documented experimental results. Through a combination of experimental research and bioinformatics analysis, the possible mechanisms underlying the effects of biochar on plant growth via absorbed volatiles were examined.

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2. Materials and methods

2.1. Biochar preparation

The rice husks (variety Shennong 1001) used in this study were obtained from the Rice Research Institute of Shenyang Agricultural University in china. The rice husks were washed in deionized water (Milli-Q) for 5 min to remove dust.

After air-seasoning, 0.5 mg rice husk were placed in the quartz-tube micro-reactor with quartz wool, and the quartz wool was placed in the analytical pyrolyzer (CDS5200, Agilent) for fast pyrolysis. At an initial temperature of 40 °C, a platinum filament was used to heat the quartz tube at a rate of 20 °C/s. The highest treatment temperature (HTT) was set at 300 °C, 400 °C, 500 °C, 600 °C, 700 °C, and 800 °C to obtain different types of rice husk biochar and volatiles. Each treatment was held for 15 s when the temperature reached the HTT.

The volatiles obtained were immediately analyzed by GC/MS (7890A GC and 240-MS, Agilent). The carrier gas was helium (99.999%) and the flow rate was 1 mL/min. The injector temperature was kept at 280 °C. A VF-5MS capillary column (30 m × 0.25 mm) was used for chromatographic separation. A split ratio of 75:1 was used to inject the pyrolysis volatiles into the GC. The oven temperature was programmed to increase from 40 °C for 1 min to 295 °C for 20 min. An ion trap mass spectrometer was employed to analyze the pyrolysis products. Mass spectra were obtained from m/z 50 to 1000. The heating rate of the GC was 8 °C/min. For each HTT fast pyrolysis, three replicates were performed in parallel under identical conditions.

The identified compounds were individually examined using the PMN to investigate their potential effects on plant growth. The PMN matched mass spectra and authentic standards. An AMDIS deconvulsion program was used to separate co-elution of peaks. The compounds were identified using the Wiley 6.0 (Wiley, New York, NY, USA) and Mass Spectral Library (Version 2.0, National Institute of Standards and Technology, NIST/EPA/NIH, USA), Mass-Bank, and by mass fragmentation pattern. The relative amount of each compound was determined by analysis of the total integrated area (1000) per sample. Compounds with a probability >80% in the library search program were identified as being likely hits. Some compounds present in small quantities were not included in our analysis because they could not readily be identified due to insufficient mass spectrum quality or inadequate evaluation of their relative concentrations. All experiments were repeated six times, and the electron impact ionization mass spectra obtained are reproducible and suitable for library matching.

2.2. Docking analysis

Proteins involved in metabolic pathways affected by the volatile compounds of interest were obtained from the PMN. The structures of the target proteins were obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb). The small molecular 3D structures were built using Chemoffice 2010 (ChemOffice 2010: CambridgeSoft Corporation). The docking analyses for volatiles and target proteins were performed using the AutoDock tools (ADT) v. 1.5.6 and the AutoDock program v. 4.2; (AutoDock, AutoGrid, and AutoTors) from the Scripps Research Institute (http://www.scripps.edu/mb/olson/doc/autodock).

3. Results and discussion

3.1. Compound distribution in volatiles from rice husk pyrolysis

The electron impact ionization mass spectra obtained from the experiment were reproducible and suitable for library matching. The total ion chromatograms produced under the six pyrolysis conditions were similar (Fig. 1), showing similarity in compound distribution.

In total, 81 compounds were identified as the products of rice husk pyrolysis (Table 1). These compounds could be divided into ten groups according to their functional groups: alcoholic hydroxyls, ester group, carboxylic group, alkyls, ether bond, aldehyde group, carbonyls, and phenolic hydroxyls.

Multiple isomerides are present at different temperatures, such as $C_7H_8O_2$, $C_7H_8O_3$, $C_8H_{10}O_2$ and $C_9H_{10}O_3$ (Fig. 2). The compounds had varying tautomer counts. This result implies that the production of compounds changes with temperature. The volatiles of rice husk exhibit various functional groups and structures. The functional groups were produced from the feedstock during the thermal degradation process used to create the biochar. The functional groups and structures of compounds are important to organisms. Therefore, we researched the function of the pyrolysis products using PMN and PubChem. However, the yield of pyrolysis volatiles could be increased, because the sweeping gas removed pyrolysis products from the reaction zone to minimize secondary reactions such as thermal cracking, repolymerization, and recondensation of char residue [19–21].

3.2. Analysis of pyrolysis products using the PMN

The compounds identified were individually examined using the PMN to determine their potential bioactivity. A total of 14 types of compounds were identified as candidates with activity in plant metabolic pathways. Some of the volatiles with potential bioactivity are shown in Table 2. The choice to query only the PMN databank was based on the fact that these organic compounds activities had been experimentally tested in previous studies. PMN analysis indicated that the compounds play important roles in plant growth, and most are involved in biosynthesis. For example, salicyl alcohol, eugenol, and 4-hydroxybenzaldehyde play roles in plant defense against herbivores. Salicin makes an important contribution to plant defense against herbivores in *Populus* and *Salix* species [22–24]. In general, a number of different phenolic glycosides can accumulate to extremely high levels in leaves of *Populus* and *Salix* species. The salicyl alcohol might play an important positive role in the salicin biosynthetic pathway. The precursor of salicin synthesis is benzoate, which can also inhibit the growth of mold, yeasts, and some bacteria [25]. Therefore, as an intermediate product, the exogenous salicyl alcohol contained in biochar may contribute to increase benzoate and salicin contents through feedback inhibition of benzoate decomposition, and provide substrate for salicin biosynthesis, which in turn increase plant resistance. Eugenol, which is related to some components of floral scents, is a low-molecular-weight organic compound [26]. It is involved in defense mechanisms, acting as an airborne signal to attract natural predators of herbivores, directly repelling herbivores or activating defense-related genes in neighboring plants or healthy tissue in infected plants. 4-Hydroxybenzoate is one of the major cell wall-bound phenolic acids and plays a major role in plant defensive response against pathogens. In addition, 4-hydroxybenzoate is an important intermediate in the biosynthesis of ubiquinone-9 and several plant secondary metabolites.

All three candidate compounds were all included in plant defense mechanism. If absorbed by plants, they may reduce the need for precursors and decrease the energy demand to maintain these metabolic pathways. These might represent a potential contribution of biochar to plant response, directly or indirectly.