PLANT BIOSTIMULANTS FROM INTEGRATED BIOREFINERY WASTES

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INTRODUCTION

Intensive agricultural practices

Monoculture

Over-fertilization

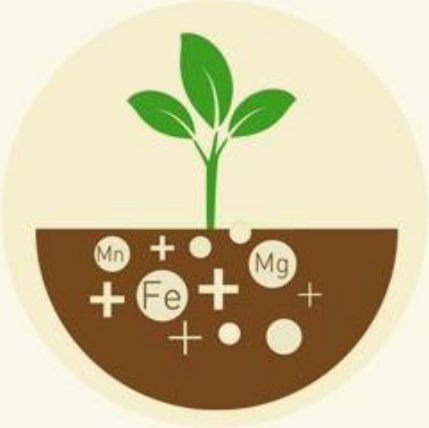
Decrease in soil quality!

Intensive sustainable agriculture

Plant biostimulants

Biostimulants are able to:

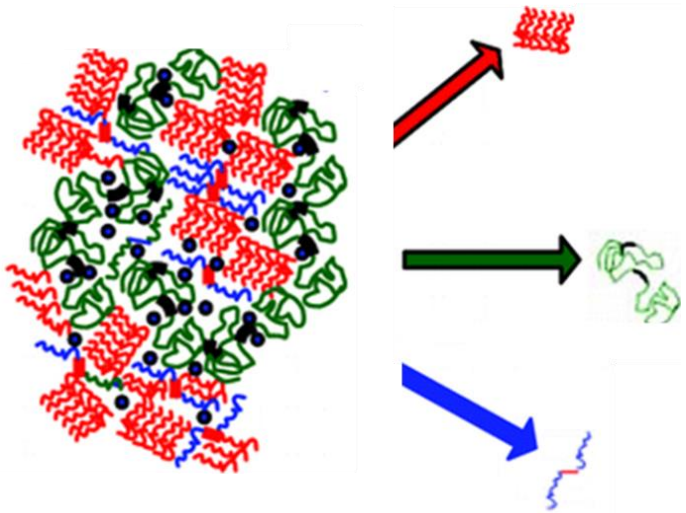
Improve **tolerance to abiotic stress**



Improve **nutrient use efficiency**

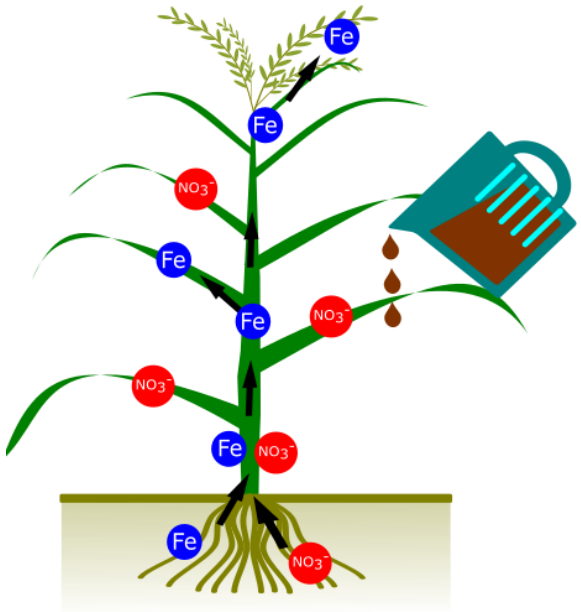
Improve **crop quality**

WHAT ARE HUMIC SUBSTANCES?



Humic Substances are supramolecular associations of relatively small and heterogeneous molecules held together by weak and dispersive forces (van der Waals), produced during the biotic and abiotic decay of biological tissues

Humic Substances application may promote plant growth at different levels by stimulating nutrient uptake, by affecting the respiration processes, by modifying internal hormonal balance



In the context of recycling/reusing agro-industrial residues (EEA report No 2, 2013), we isolated humic-like lignins from

***Non*-food, energy crops**

Biorefinery wastes

THE BIOREFINERY CONCEPT

CELLULOSE

PRODUCTION OF

ETHANOL

POLYMERS

RESIDUE (LIGNIN)

CARDED

BURNT

HIGH VALUE

PRODUCTS

In the context of recycling/reusing agro-industrial residues (EEA report No 2, 2013), we isolated humic-like lignins from

***Non*-food, energy crops**

Biorefinery wastes

Humic-like biostimulants from energy crops

Why giant reed (*Arundo donax* L., 1753) – **AD**?

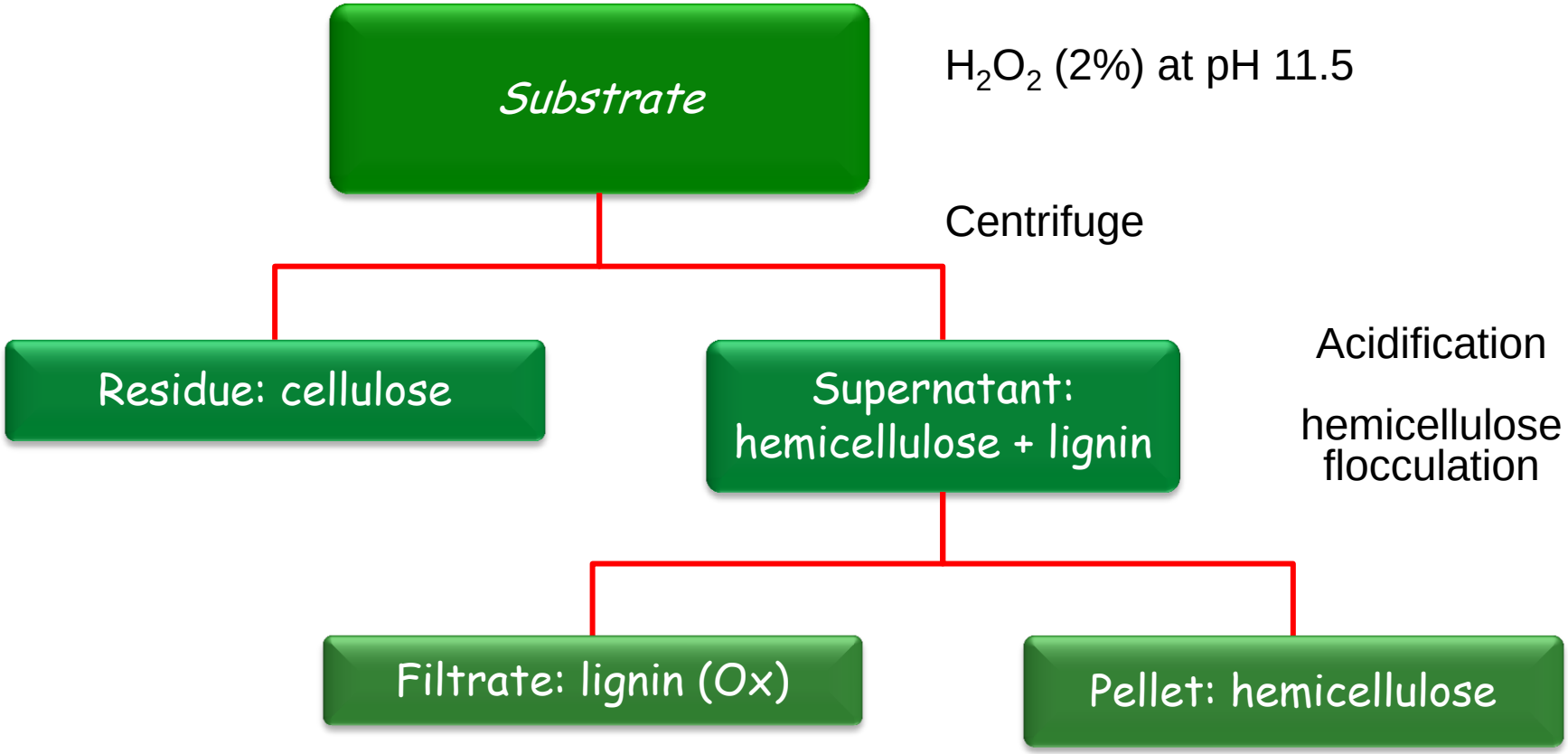
Large amount of dry biomass ha^{-1}

It grows in different habitats

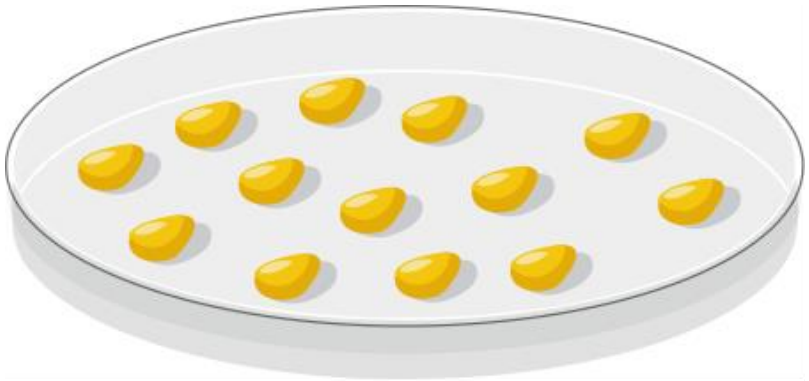
Very resistant to biotic and abiotic avversitites

Low fertilizer inputs

MATERIALS & METHODS

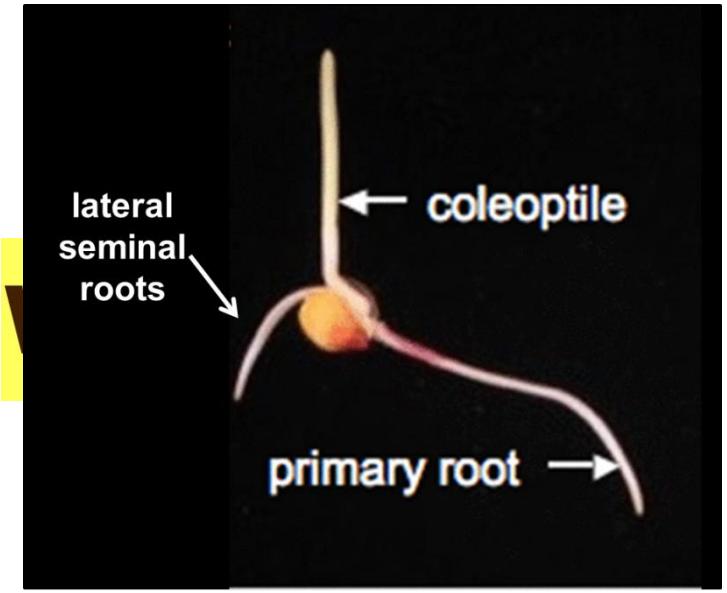


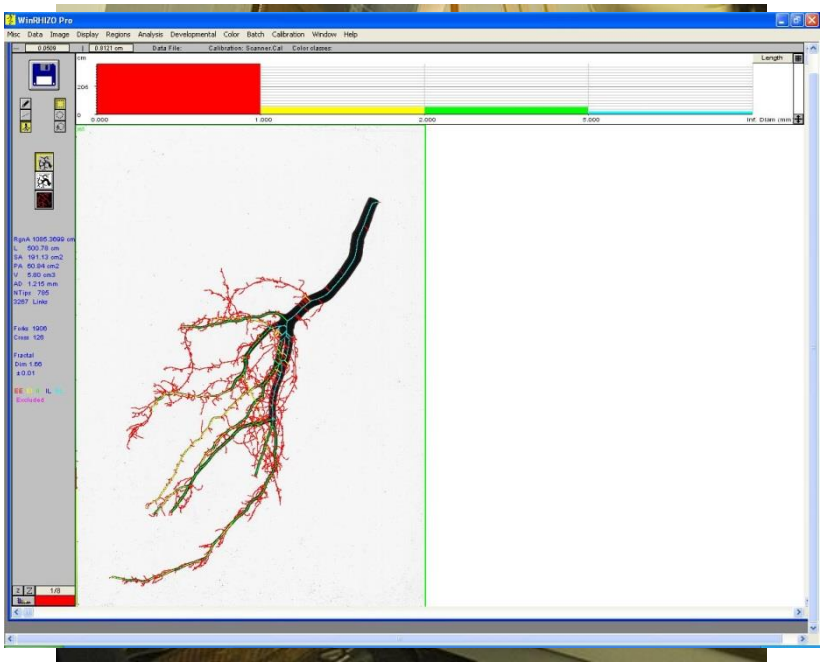
GERMINATION TEST



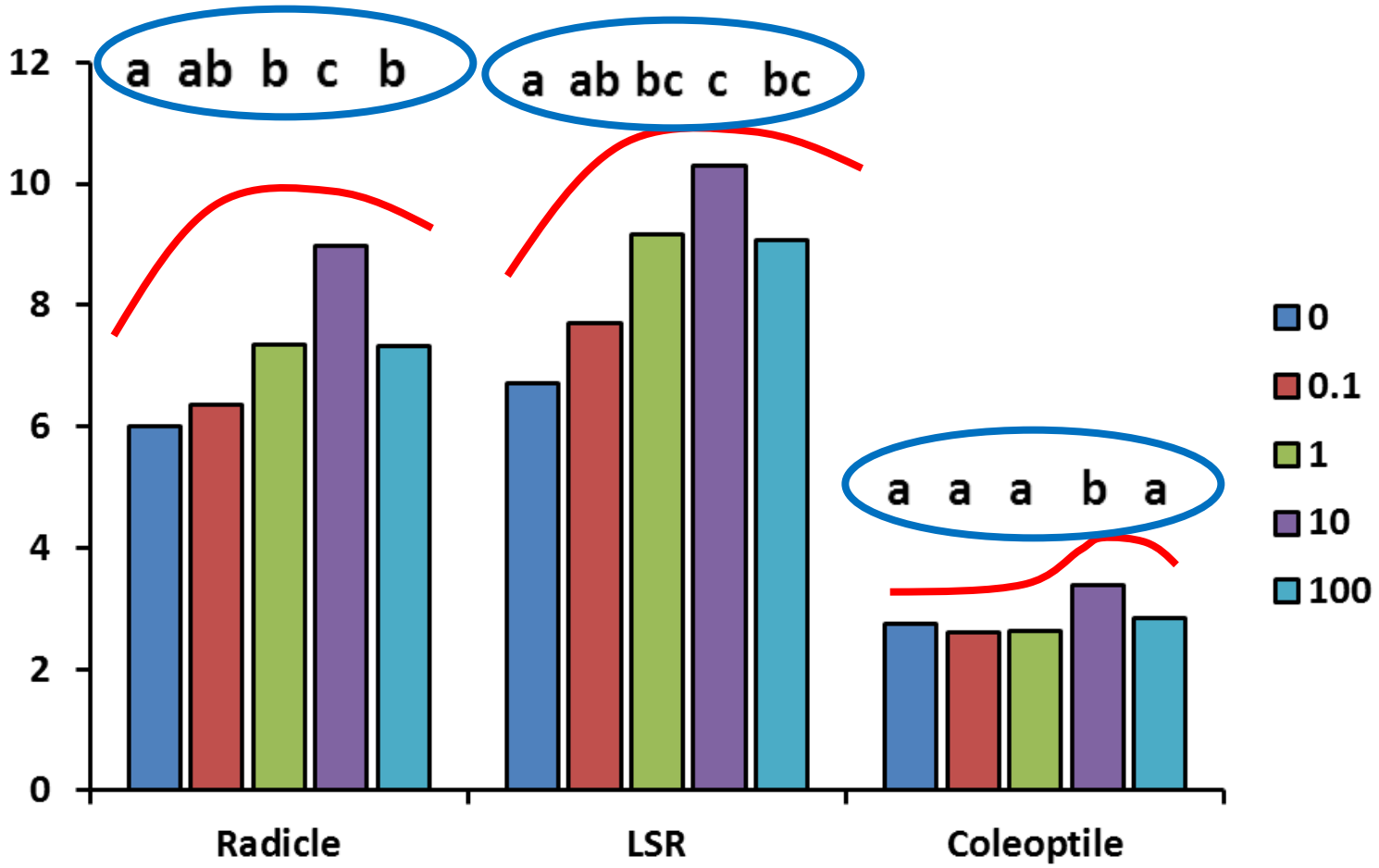
Concentrations (ppm of Organic Carbon)

0
0.1
1
10
100

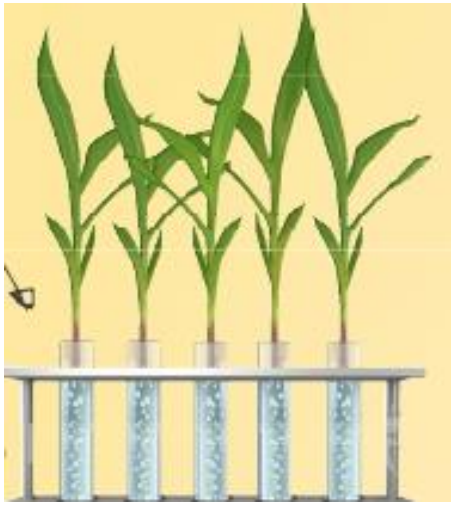




Length (cm) of radicle root, lateral seminal root (LSR), and coleoptile for maize seedlings treated with different concentrations (ppm OC) of AD water-soluble lignins.

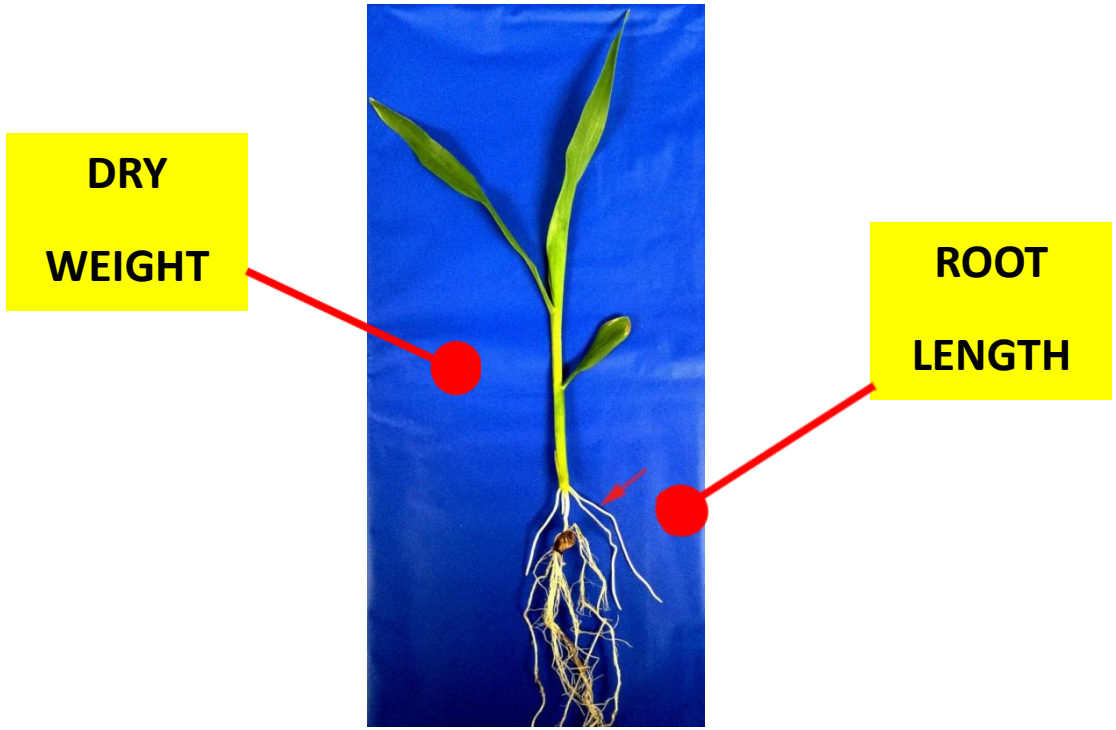


Test their bioactivity towards maize plantlet early growth

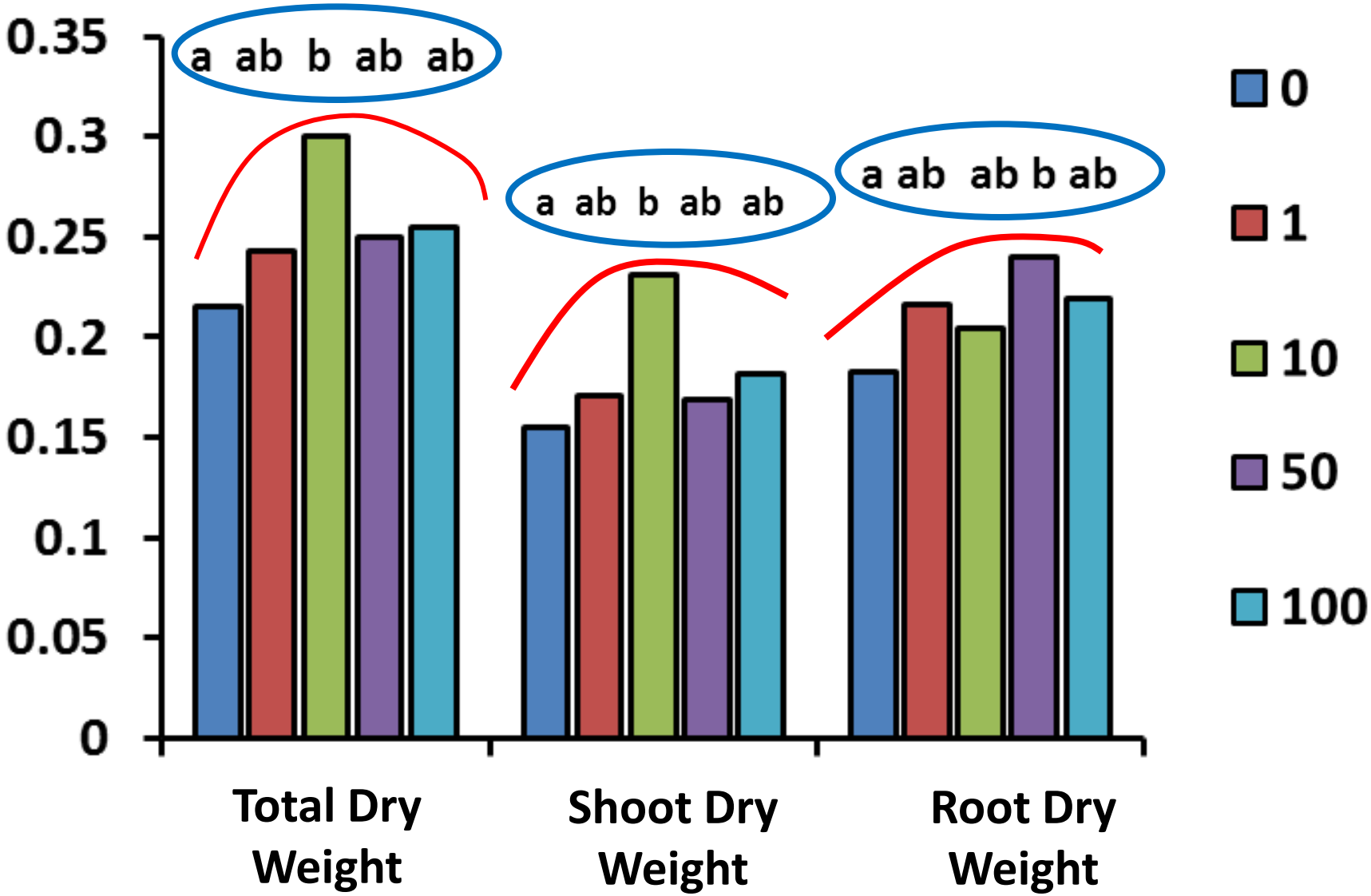


Concentrations (ppm of Organic Carbon)

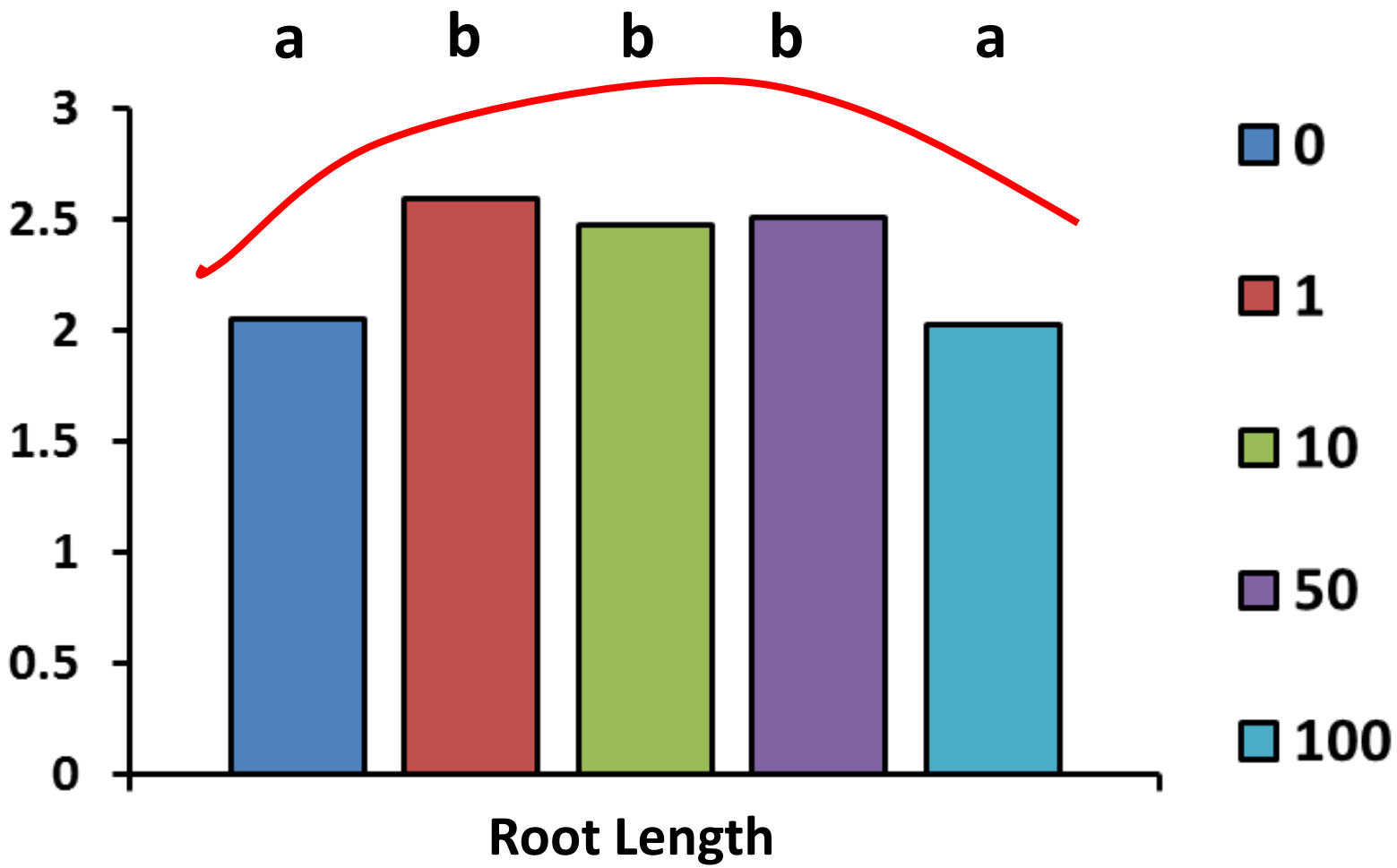
0
1
10
50
100



Total Dry Weight (g), Shoot Dry Weight (g) and Root Dry Weight (g x 3) of maize seedlings treated with AD water-soluble lignins.

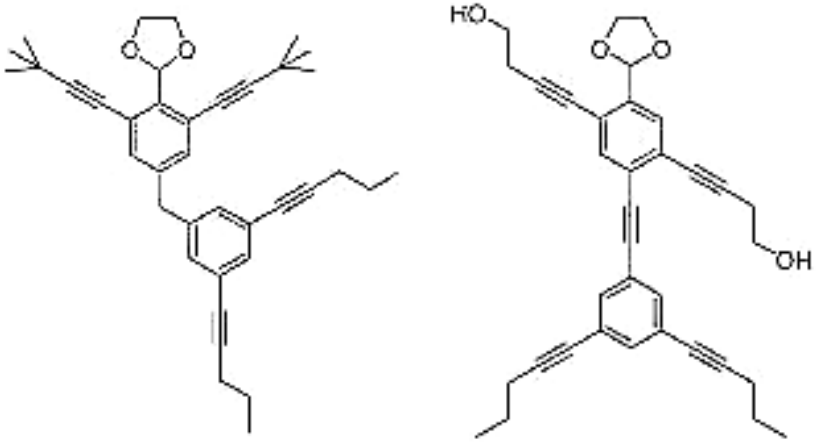


Root length (m) of maize seedlings treated with AD water-soluble humic-like lignins



HOW DOES LIGNIN AFFECT PLANT DEVELOPOMENT?

WHAT IS ITS CHEMICAL STRUCTURE?



- **Analytical techniques**

- **^{13}C CP MAS NMR Spectroscopy**
- **^{31}P NMR Spectroscopy**
- **HPSEC**

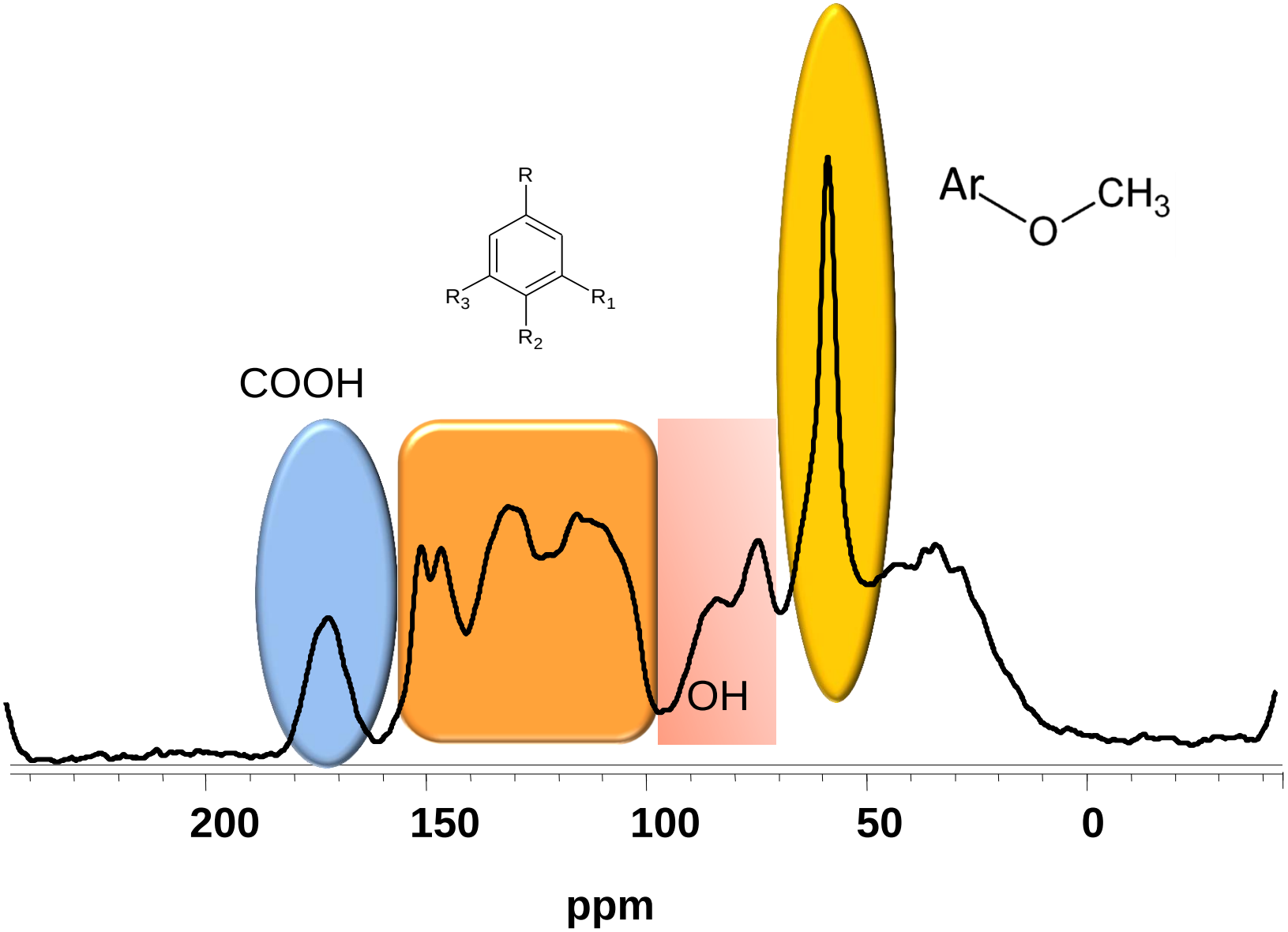
NMR SPECTROSCOPY

Nuclear Magnetic Resonance (NMR) Spectroscopy allows to obtain detailed information on the molecular structure of the examined compounds.

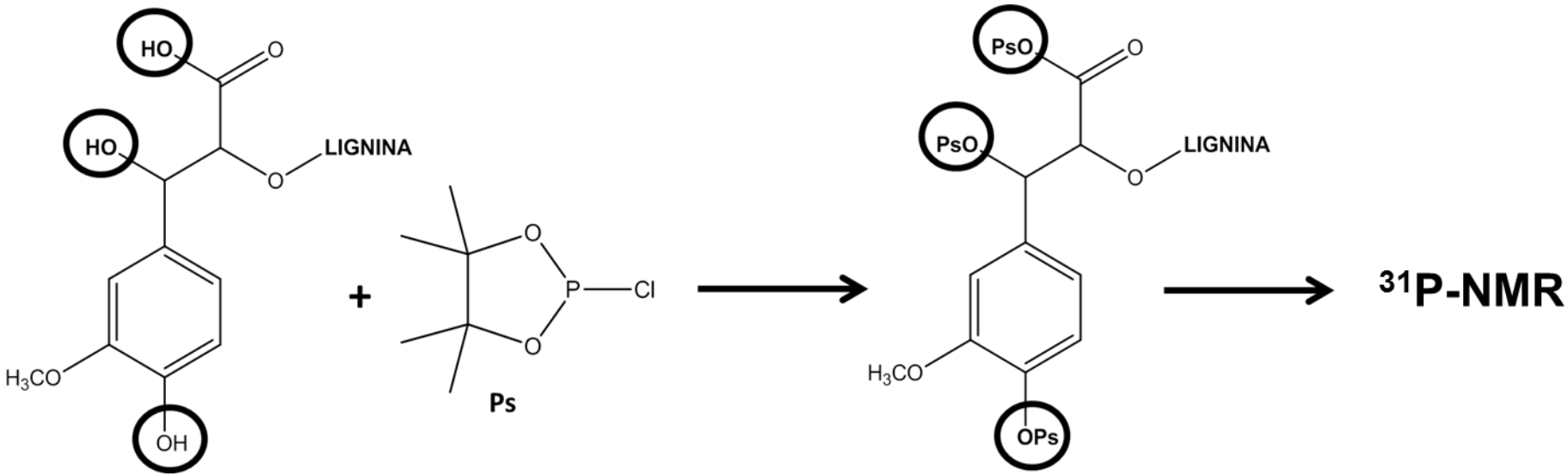
NMR experiments rely on the adsorption of electromagnetic radiation from molecules placed in a strong magnetic field. Only particular nuclei can be studied by NMR spectroscopy (^{1}H, ^{13}C, ^{31}P).

- **^{13}C CP MAS NMR Spectroscopy**
- **^{31}P NMR Spectroscopy**

^{13}C CP-MAS Spectrum of AD.

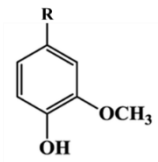


^{31}P –NMR: sample derivatization

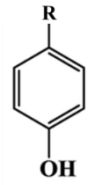


Reaction of lignin with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxa-phospholane (Ps)

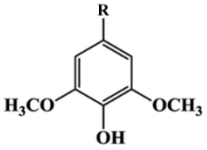
^{31}P-NMR spectrum of humic-like substrate from AD



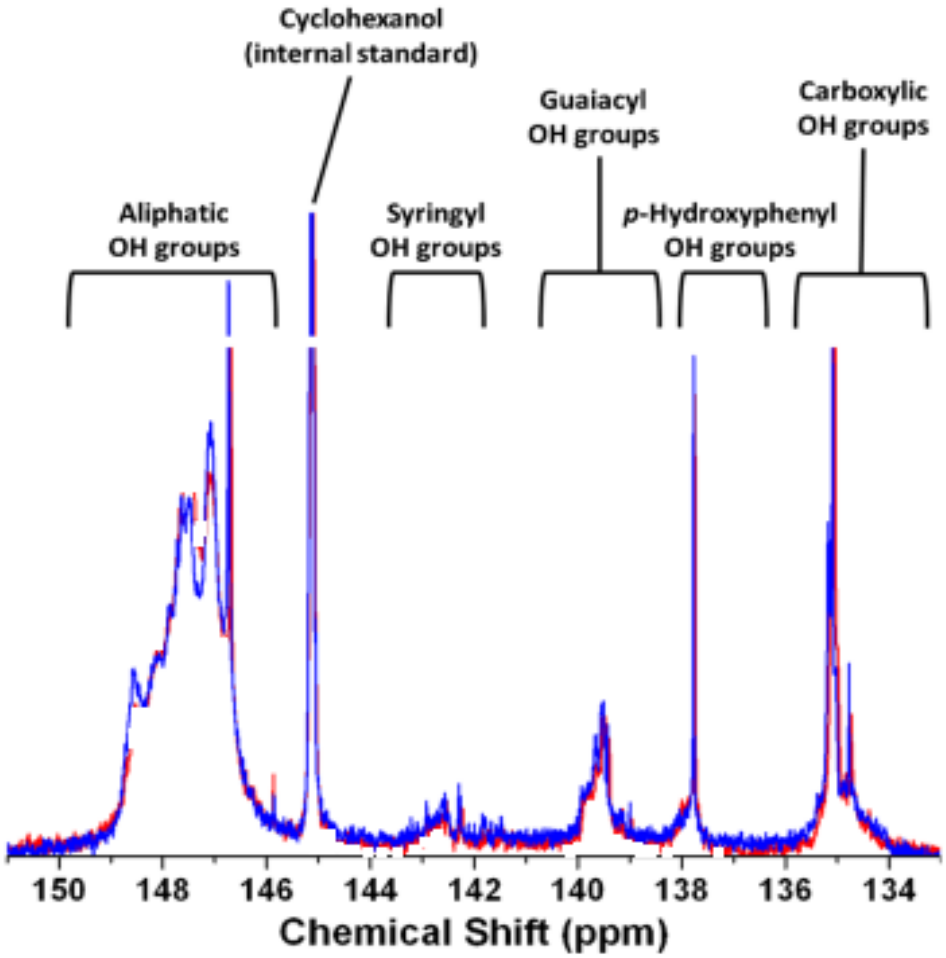
Guaiacyl (G)



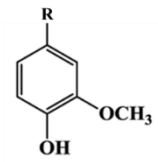
***p*-Hydroxyphenyl (H)**



Siringyl (S)



Amount of OH groups (mmol g^{-1} of lignin)



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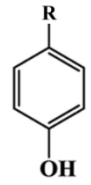

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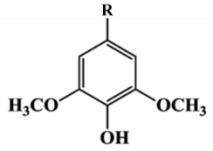
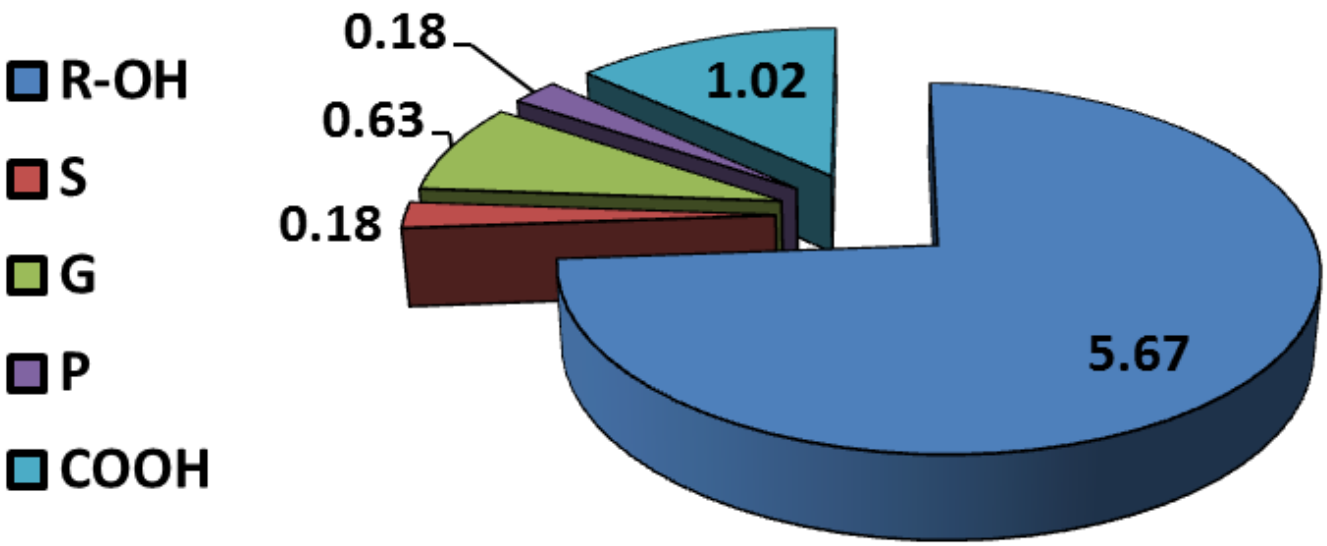
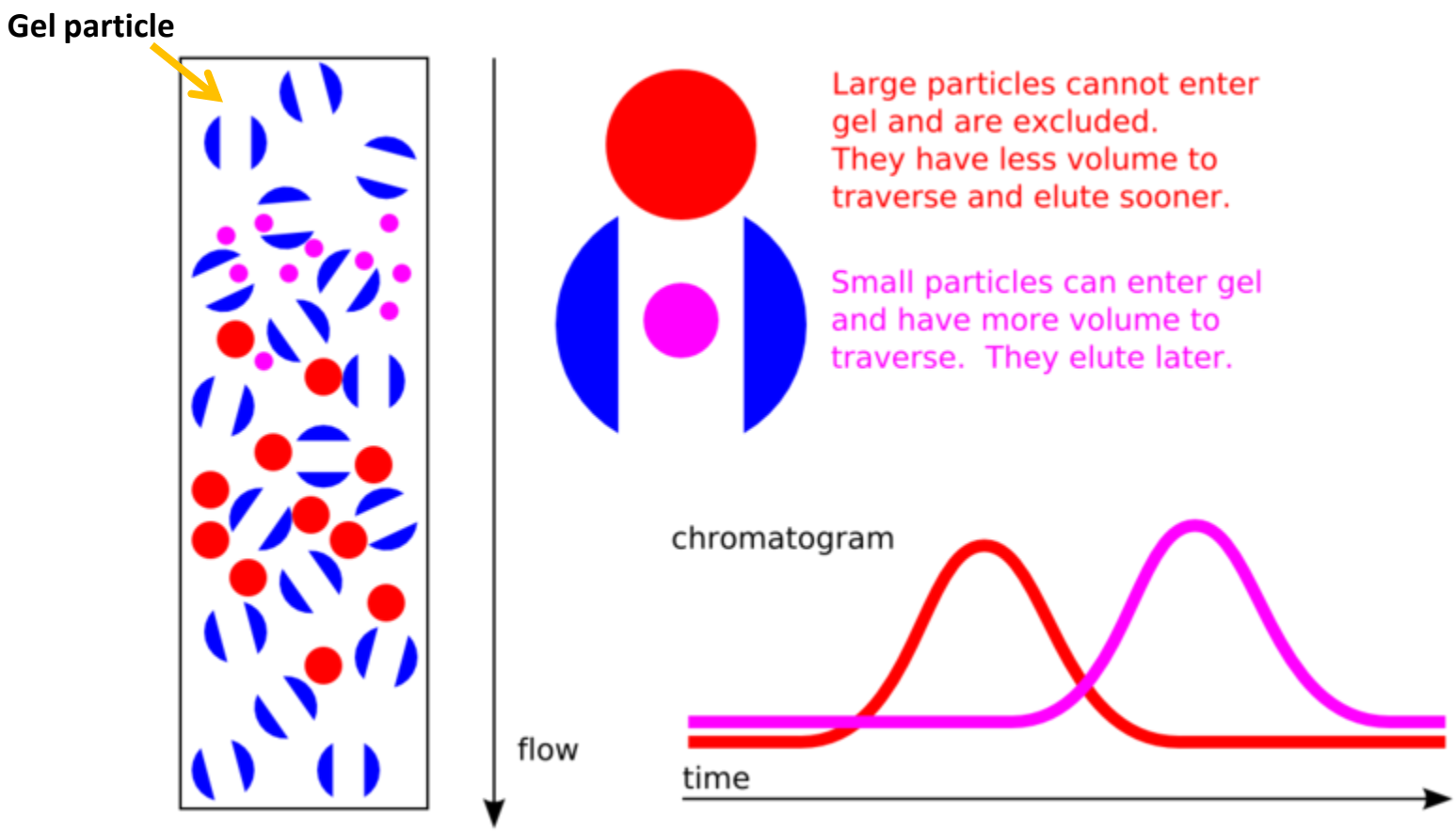


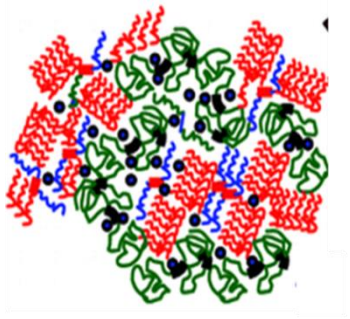
Siringyl (S)

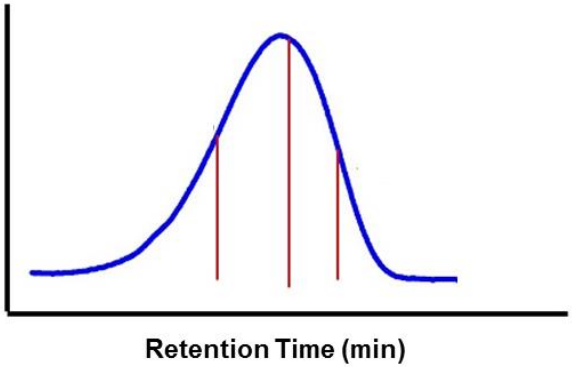


Principles of High Performance Size Exclusion Chromatography (HPSEC)



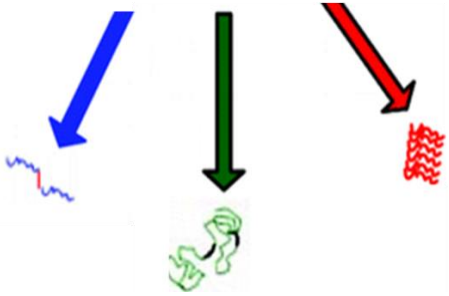
The supramolecular nature of Humic Substances

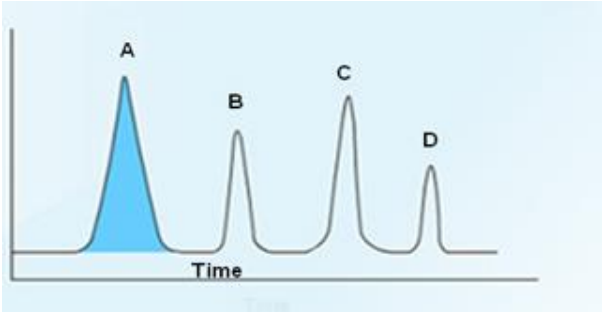




+

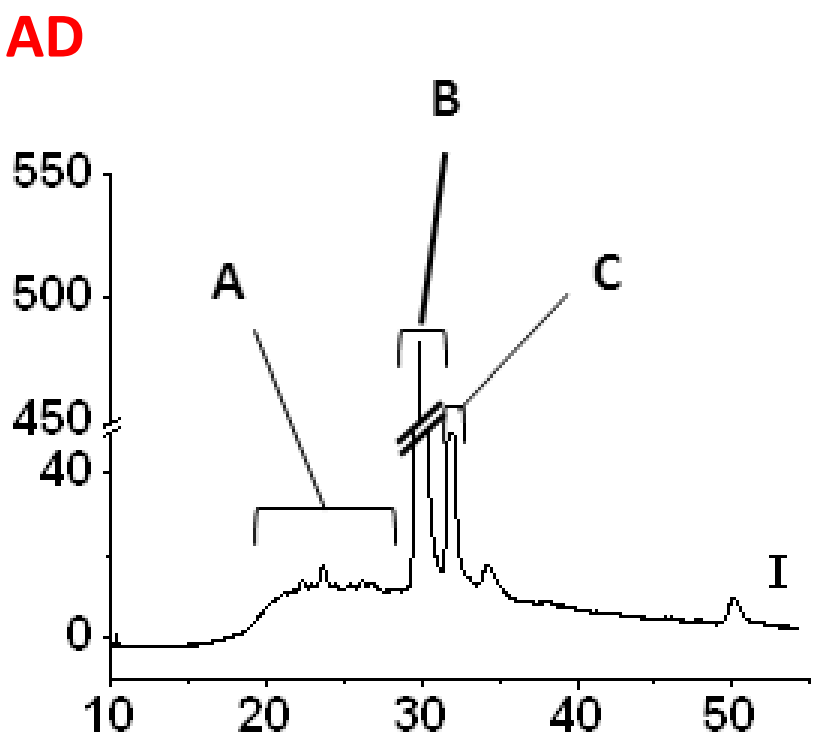
Acetic acid

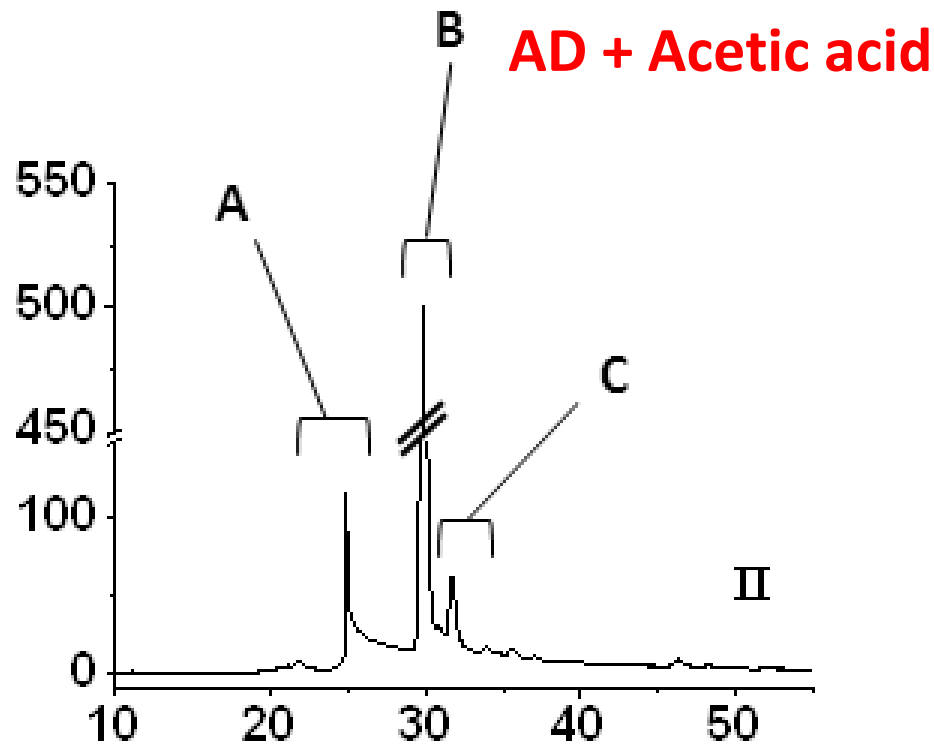




Adding AcOH to the studied solutions allows us to understand the polymeric/supramolecular nature of the extracts.

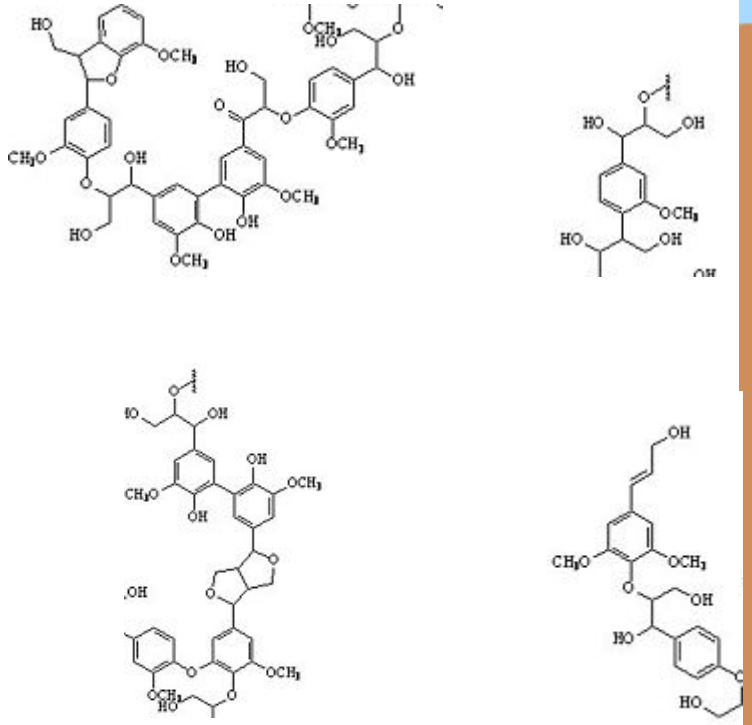
HPSEC chromatogram for lignin from giant reed (AD) without or with acetic acid

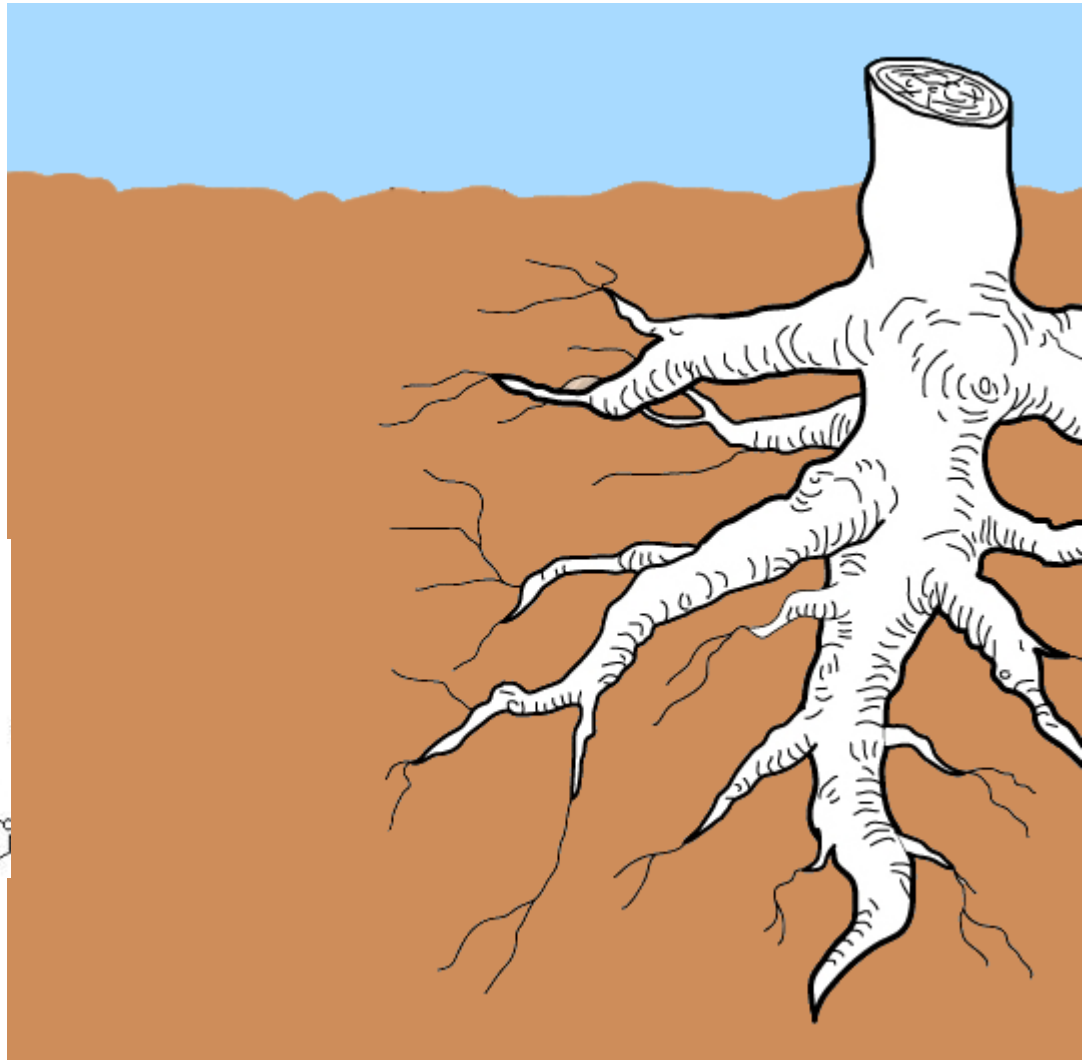




LIGNINS ISOLATED BY H_2O_2 ARE A MIXTURE OF SMALL MOLECULES (less than 900 Da) ARRANGED IN APPARENTLY LARGE SUPRAMOLECULAR CLUSTERS

The humic-like lignin adheres to the root surfaces, its conformation is disrupted by root exudates, thus releasing bioactive phenolics with plant promoting activity.



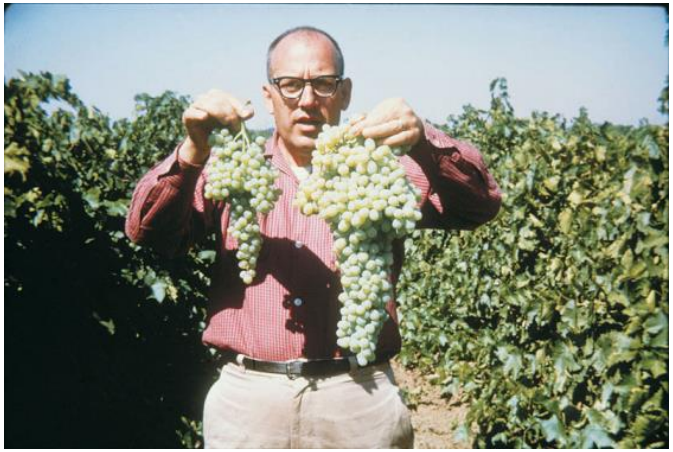


HUMIC SUBSTANCES DISPLAY HORMONE-LIKE EFFECT (AUXIN- AND GIBBERELLIN-MIMETIC) TOWARDS PLANTS DEVELOPMENT

AUXIN

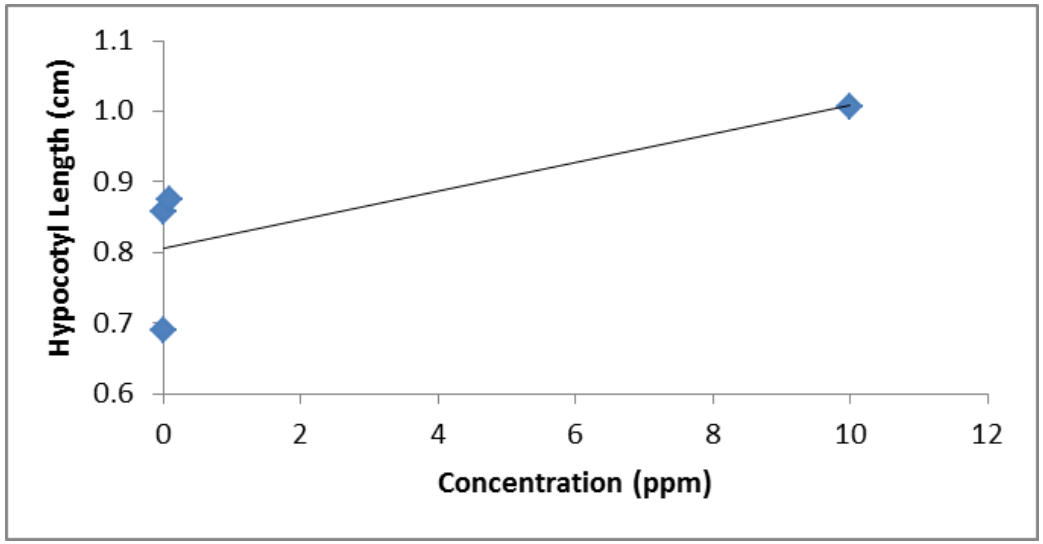
DR5::GUS assay:
negative outcome

GIBBERELLIN



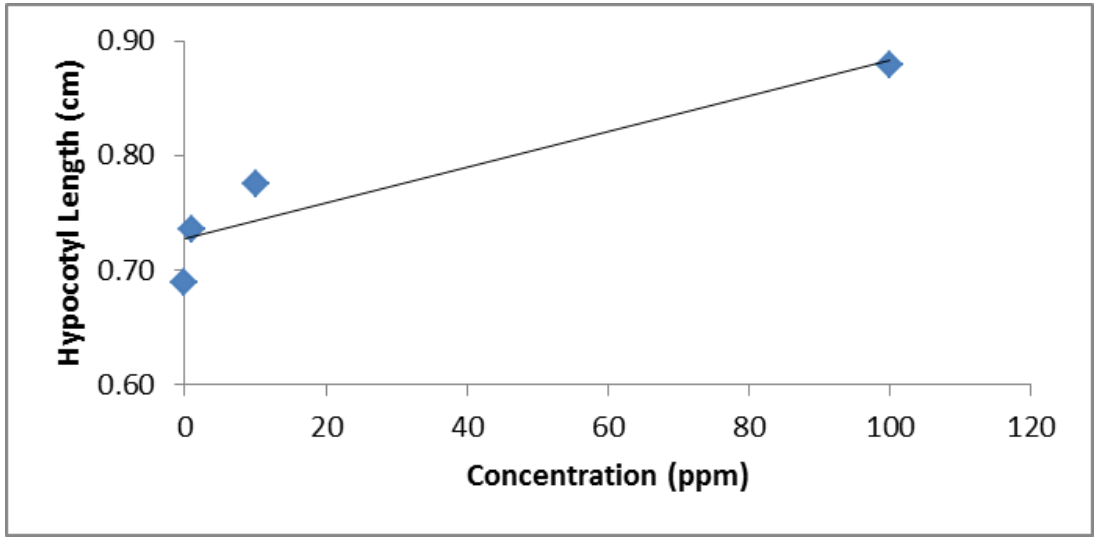
Audus test:
chicory hypocotyl elongation

Audus test – Gibberellin-like activity assay (length of chicory hypocotyl)

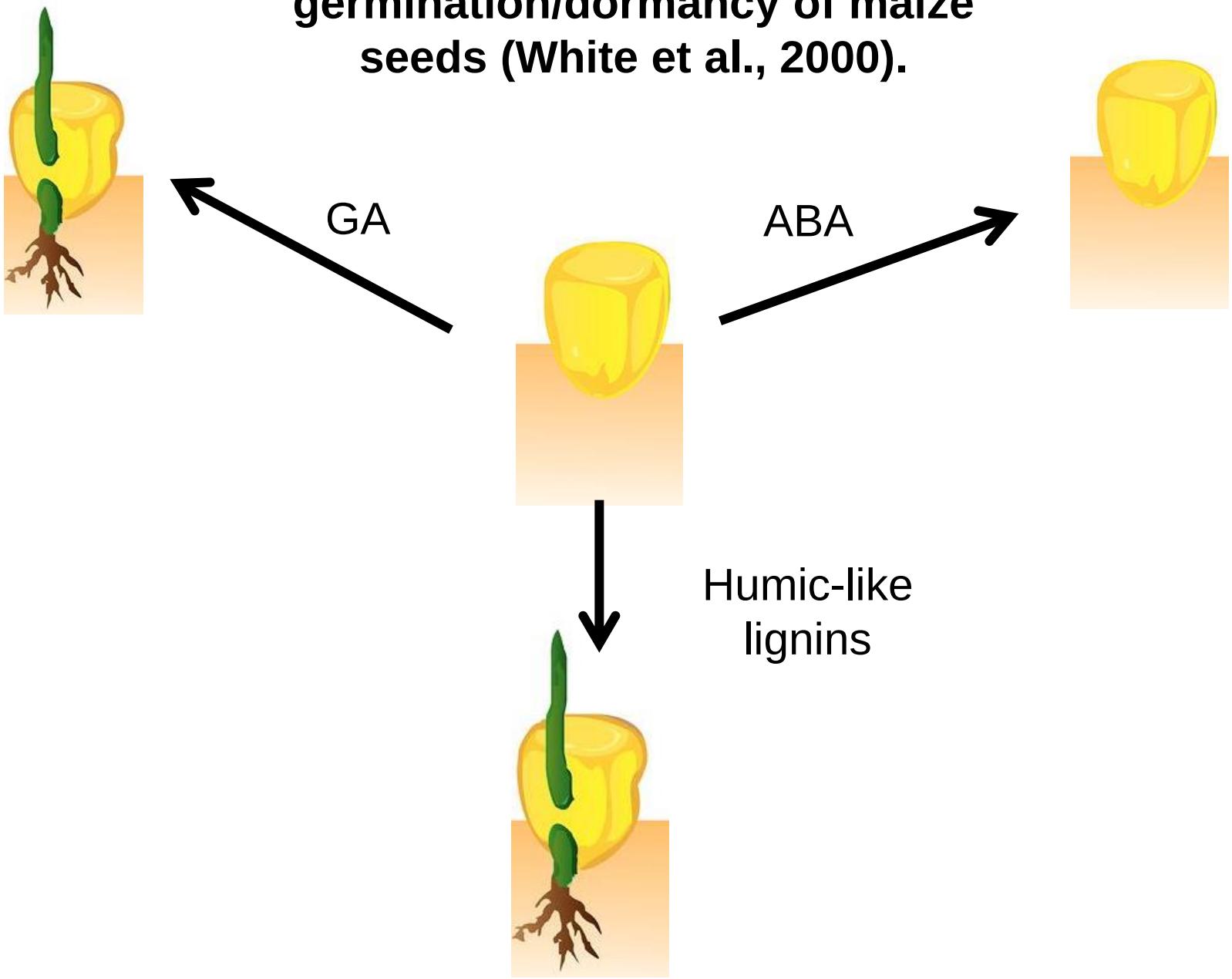


Gibberellic Acid application

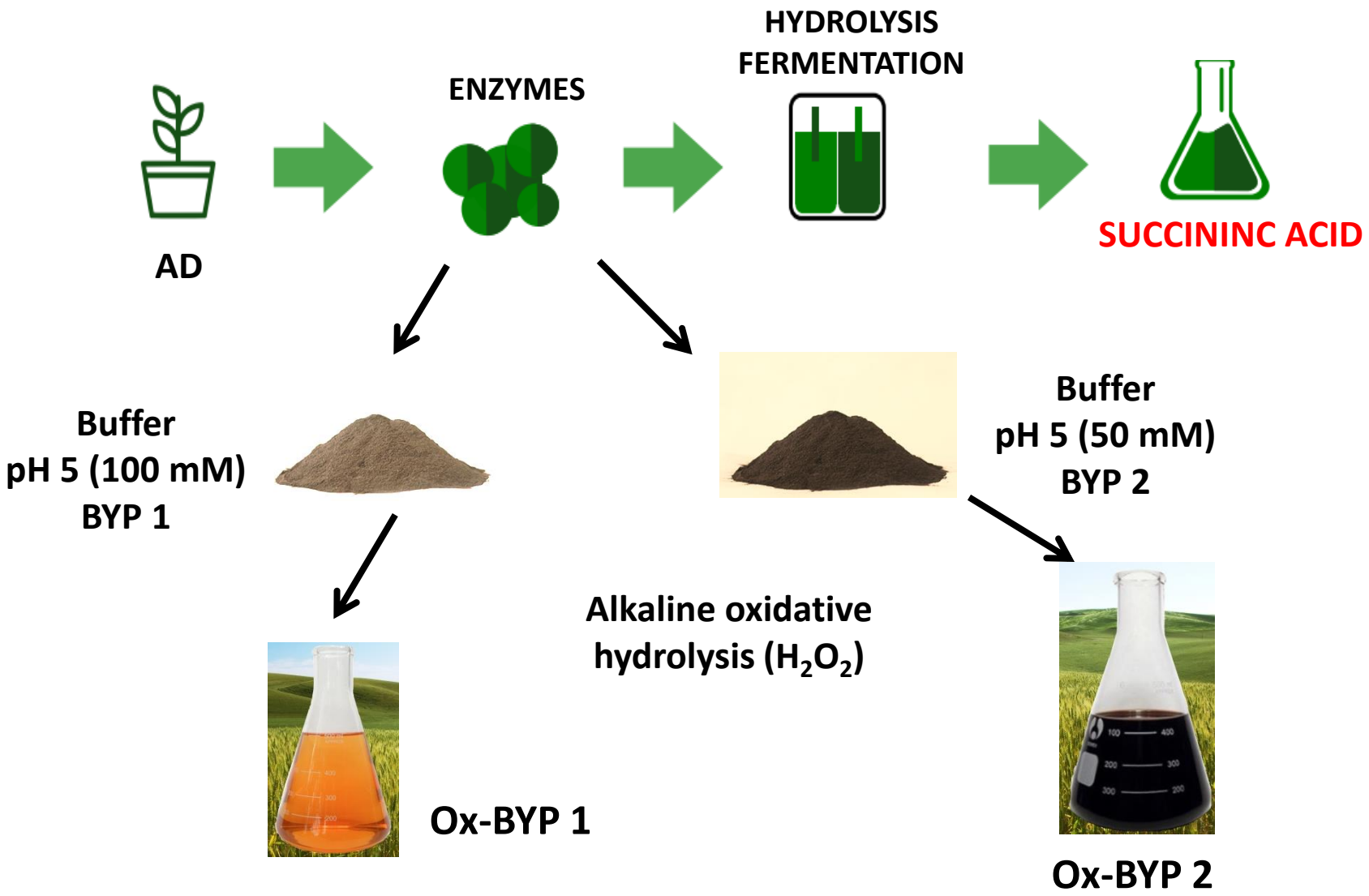
AD lignin application



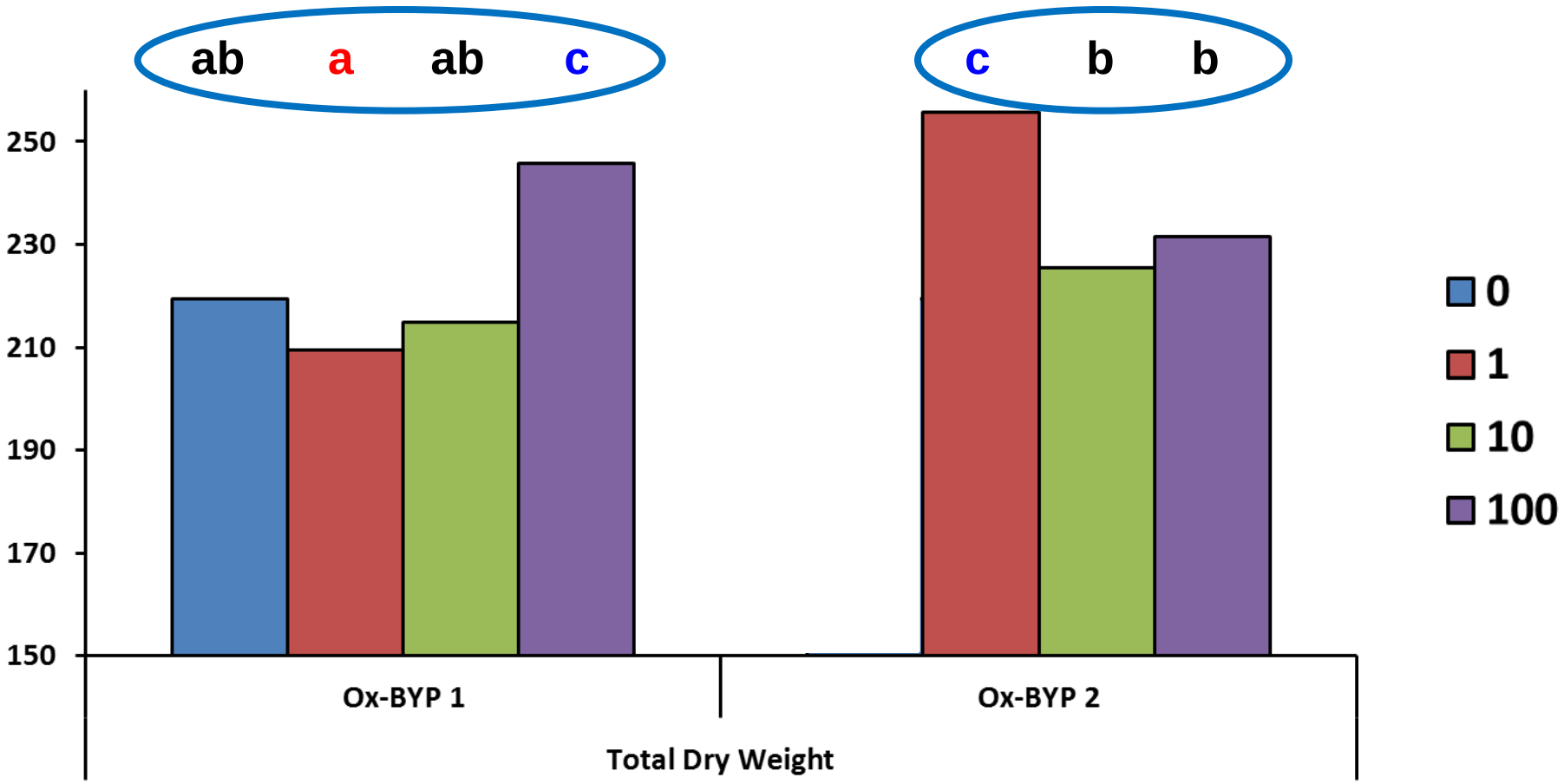
The GA/ABA ratio regulates the germination/dormancy of maize seeds (White et al., 2000).



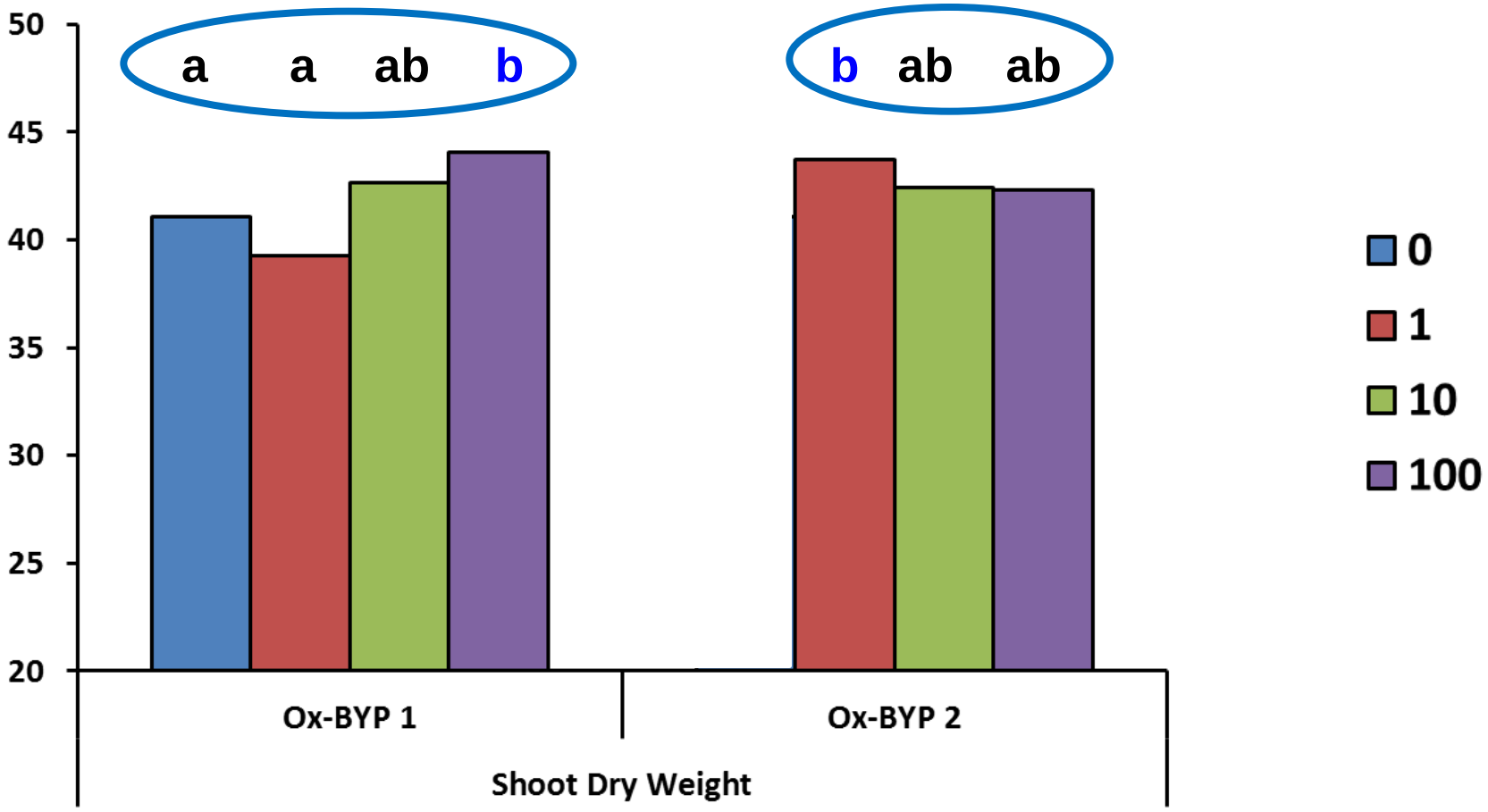
PLANT BIOSTIMULANTS FROM BIOREFINERY WASTES



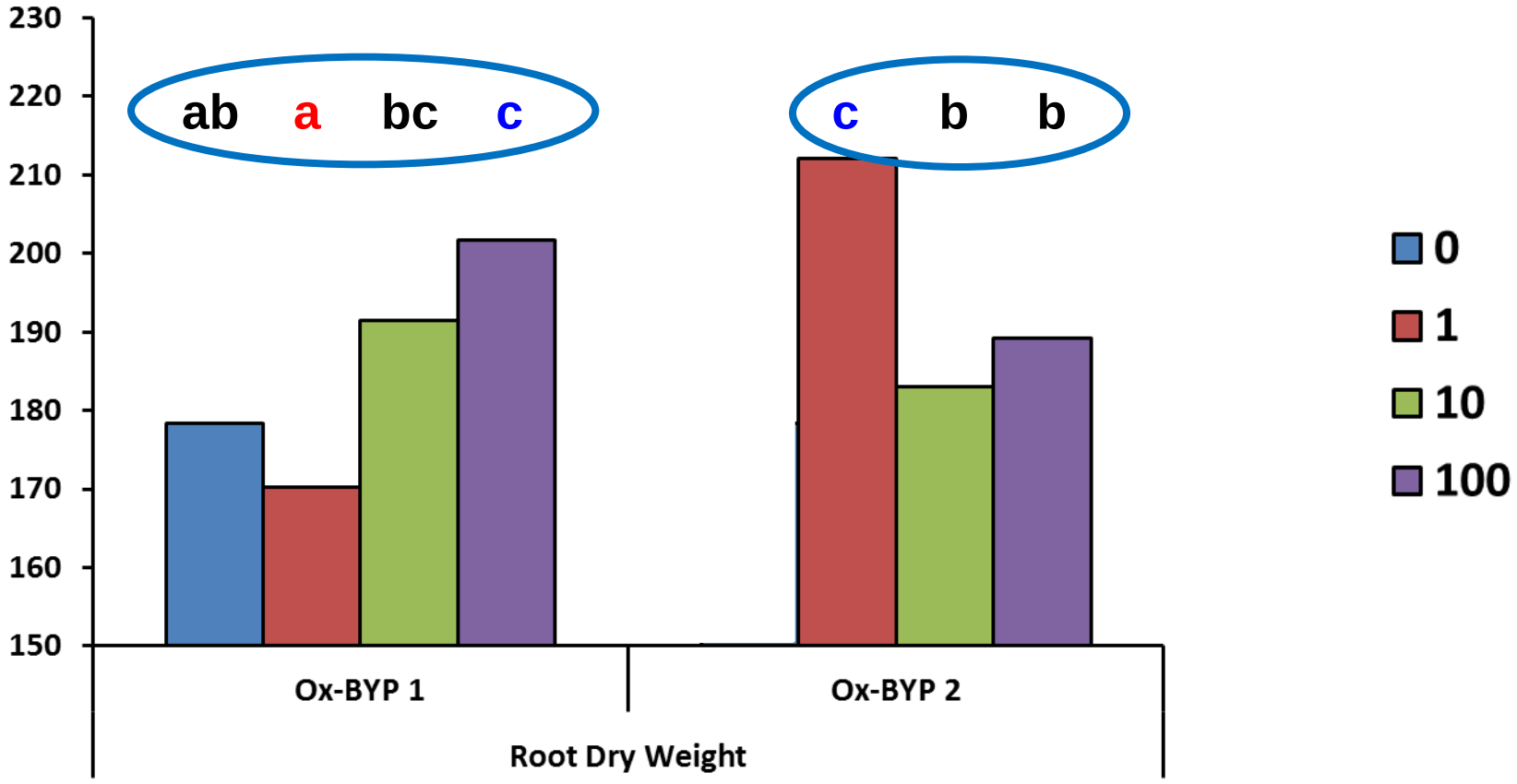
Total Dry Weight (mg) of maize seedlings treated with plant biostimulants from two oxidized biorefinery residues (Ox-BYP 1 and Ox-BYP 2).



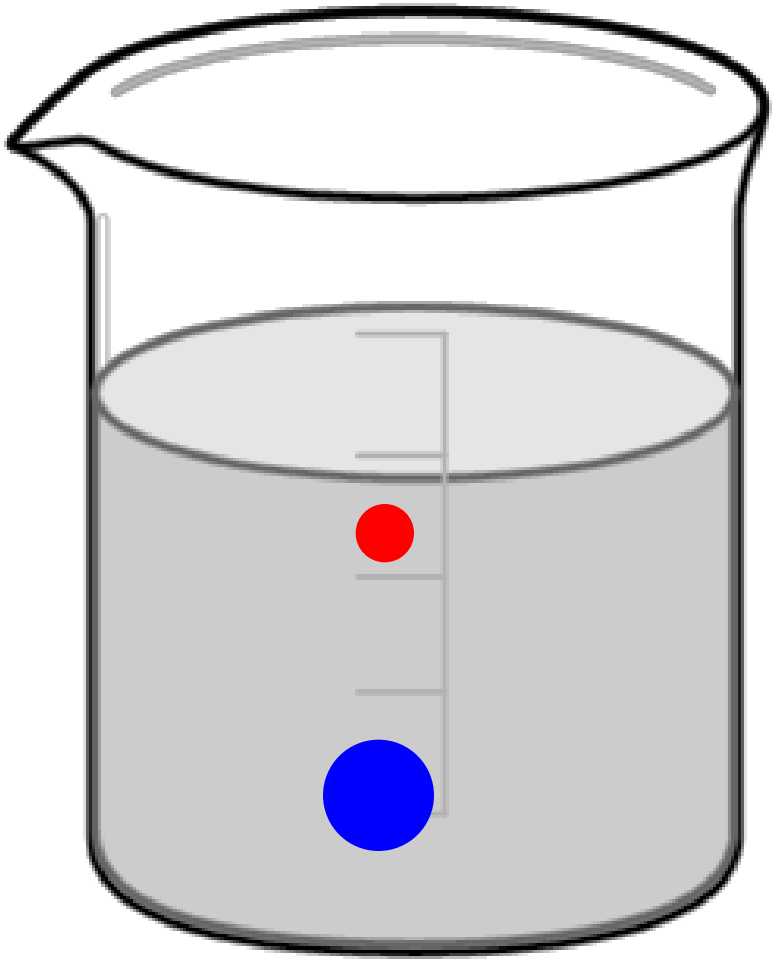
Shoot Dry Weight (mg) of maize seedlings treated with plant biostimulants from two oxidized biorefinery residues (Ox-BYP 1 and Ox-BYP 2).



Root Dry Weight (mg) of maize seedlings treated with plant biostimulants from two oxidized biorefinery residues (Ox-BYP 1 and Ox-BYP 2).



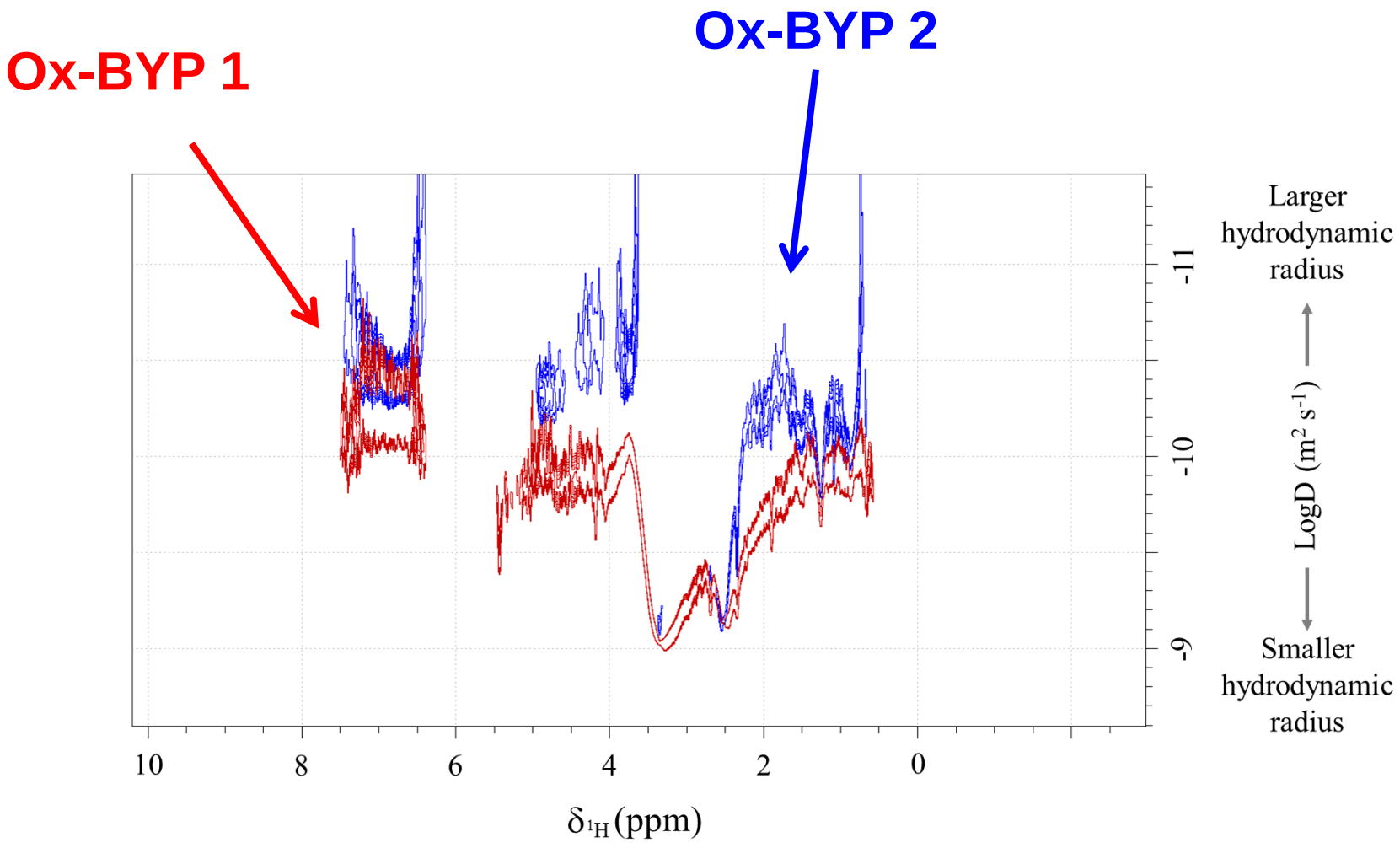
DIFFUSION ORDERED NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY



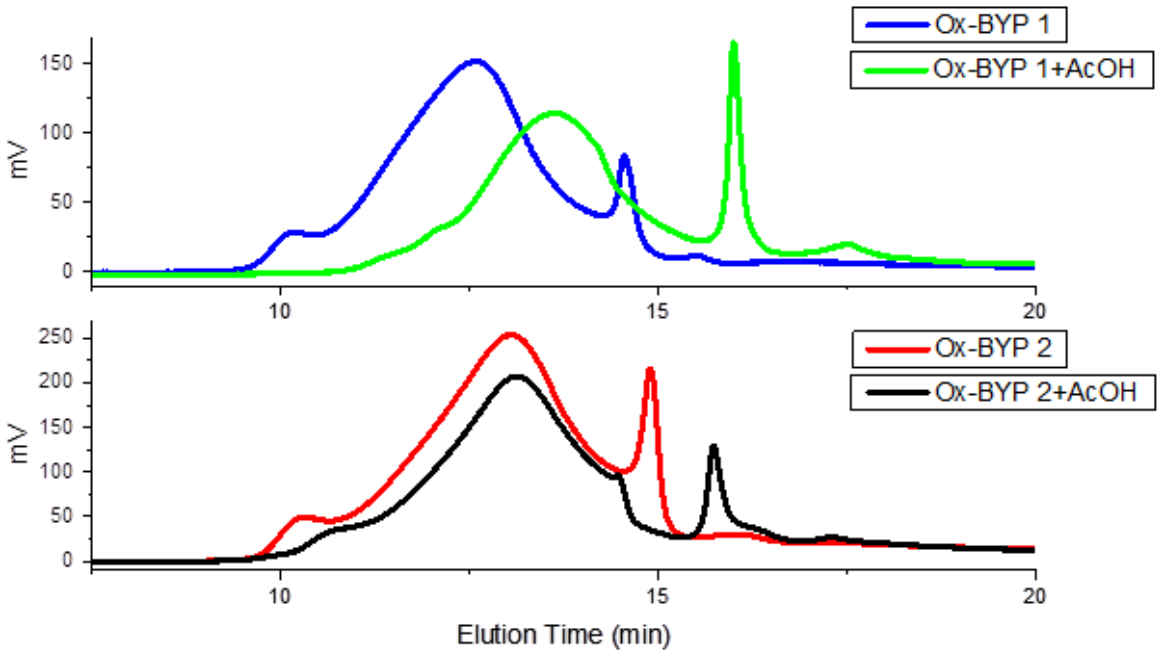
The application of particular radiofrequencies to the sample is able to provide information on the diffusivity of molecules, and on their hydrodynamic *radius*

$$D = \frac{kT}{6\pi\eta R}$$

Two-Dimensional ^{1}H-DOSY NMR projections for water-soluble materials after alkaline hydrolysis



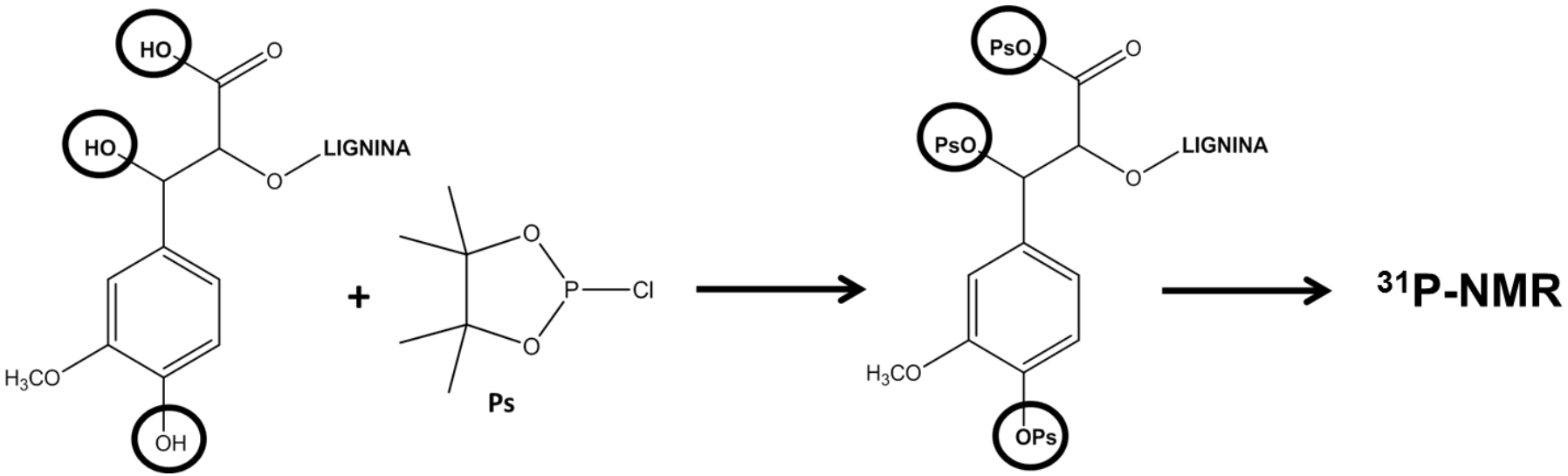
HPSEC profiles before and after AcOH addition for Ox-BYP 1 and Ox-BYP 2



Ox-BYP 1 is composed of a polymeric core and some *non*-covalently bound molecules

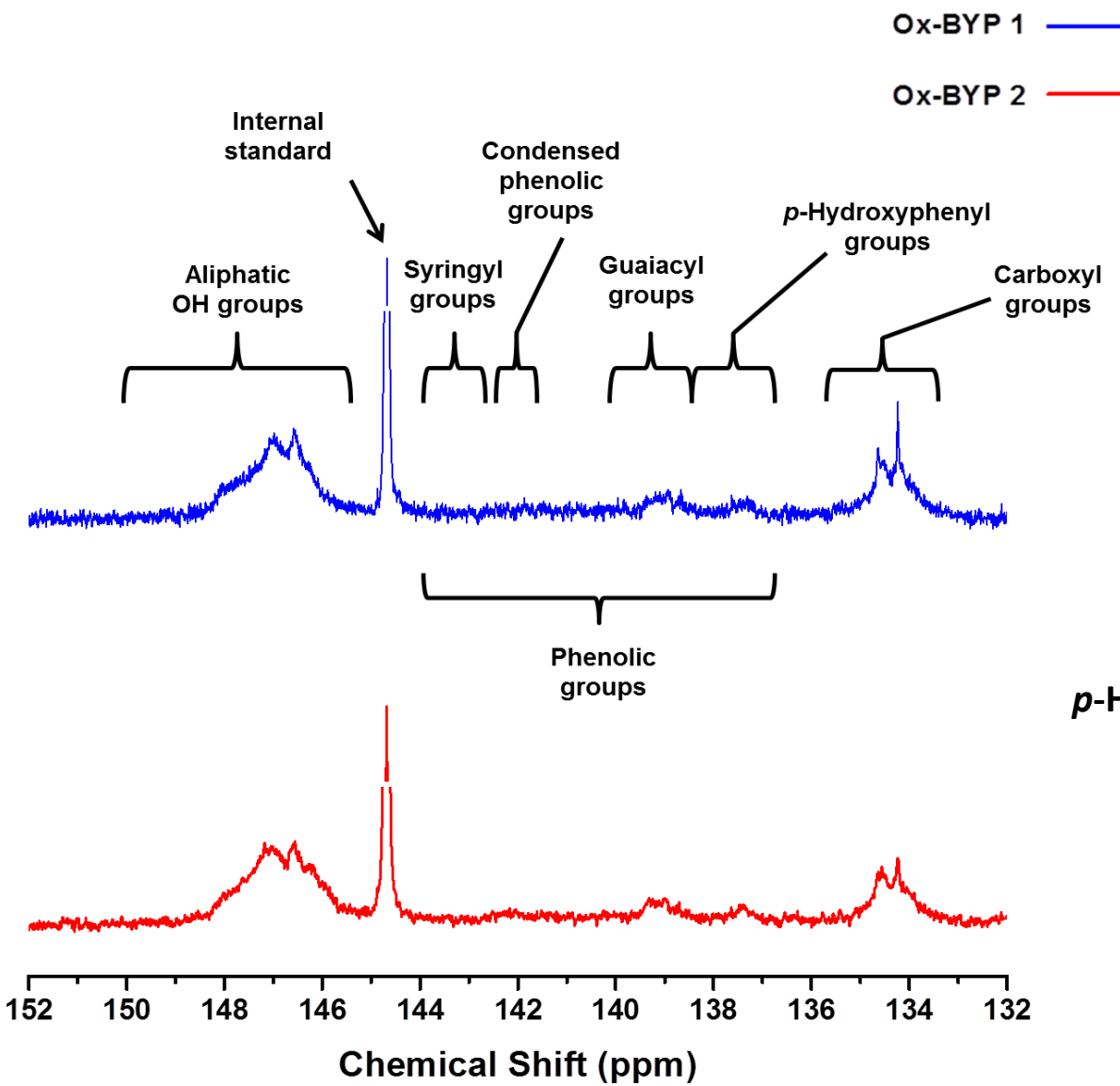
Ox-BYP 2 conformational structure more resembles that of a polymer

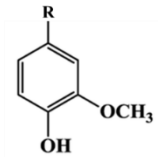
^{31}P-NMR spectroscopy



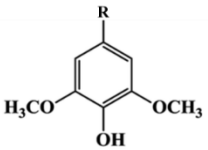
Ps derivatization

^{31}P-NMR spectra of Ox-BYP 1 and Ox-BYP 2

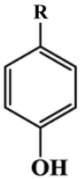




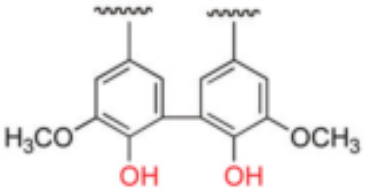
Guaiacyl



Siringyl

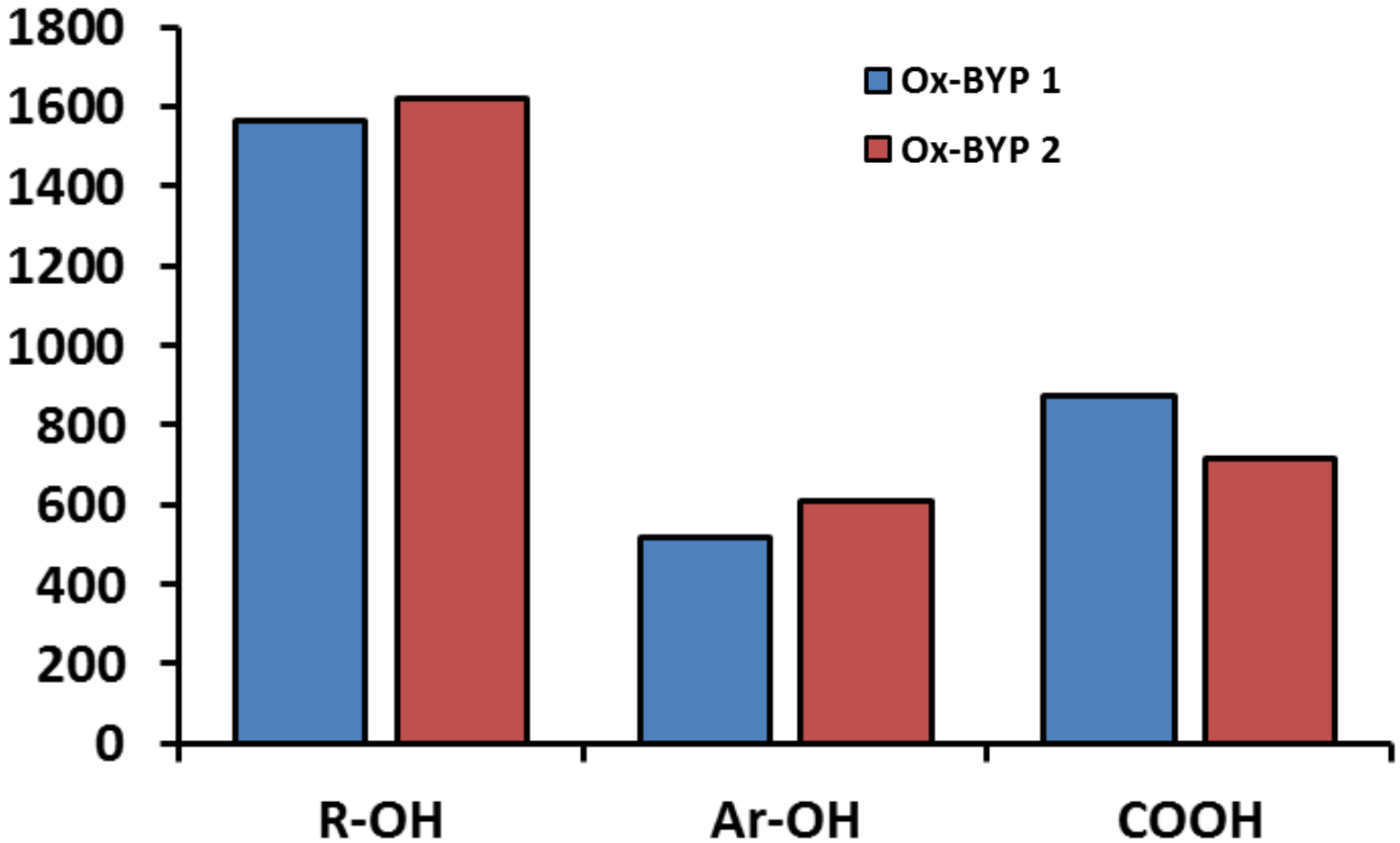


p-Hydroxyphenyl



Condensed phenols

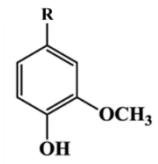
Amount (μmol g^{-1} of substrate) of different OH groups



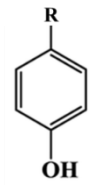
16.7 % more in Ox-BYP 2

18.1 % more in Ox-BYP 1

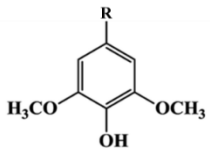
Amount (μmol g^{-1} of substrate) of different phenolic groups



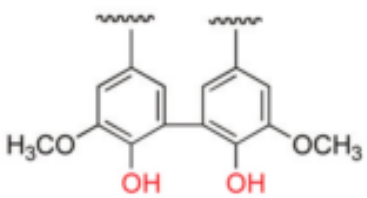
Guaiacyl (G)



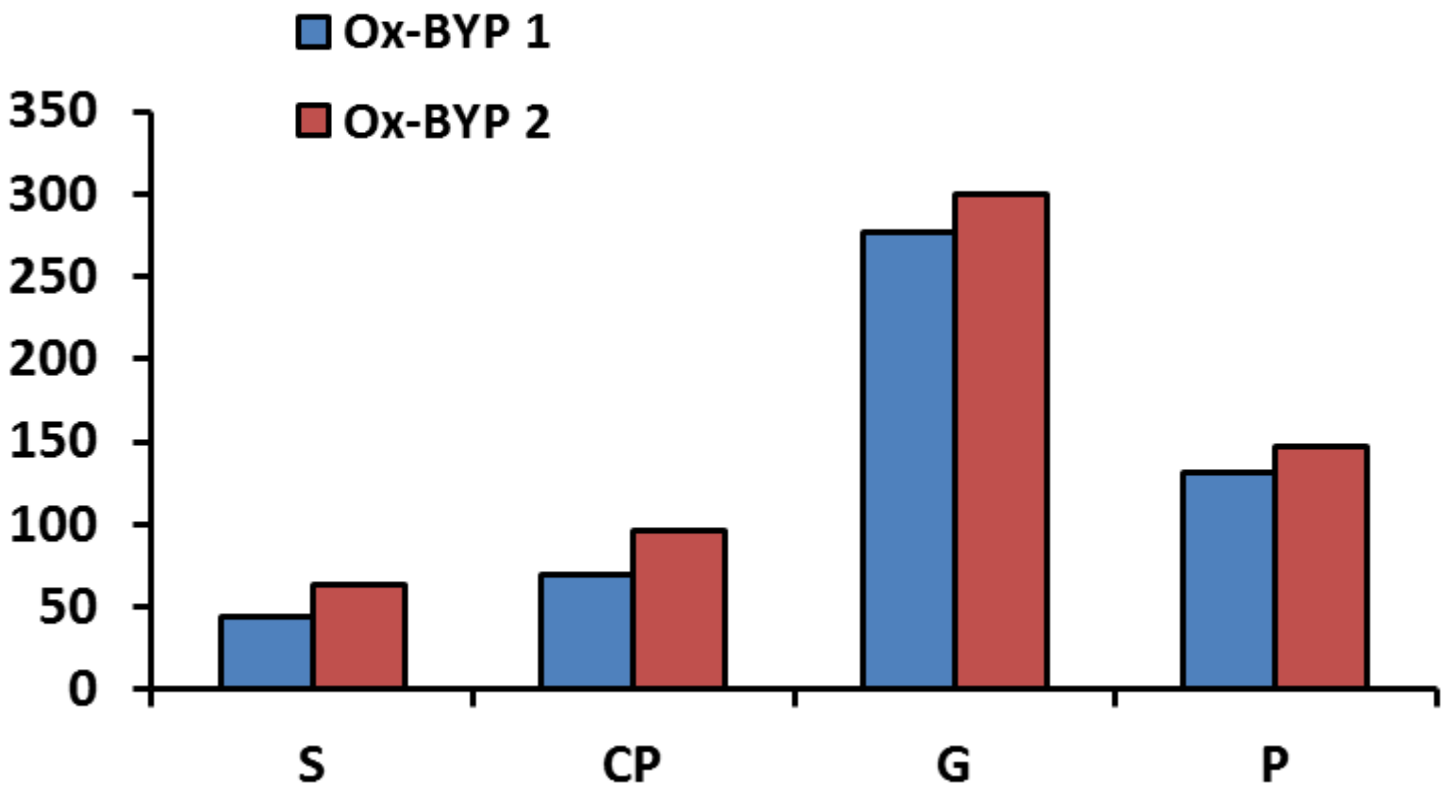
p-Hydroxyphenyl(H)



Siringyl (S)



Condensed phenols (CP)



45 % more in Ox-BYP 2

39 % more in Ox-BYP 2

1 ppm OC Ox-BYP 1: inhibitory due to the occurrence of toxic compounds

Such noxious effect is overcome at greater concentrations

The greater hydrophobicity of Ox-BYP 2 may have determined a strong adsorption of the material onto the root surface, thus allowing a closer interaction with root cells and the stimulation of plant growth processes

Similar effect at different concentrations: similar mechanism of plant bio-stimulation

CONCLUSIONS

Humic-like lignins with plant growth-promoting activity could be successfully isolated from integrated biorefinery residues.

Their molecular and conformational structure account for their biological effect.

Exploiting biorefinery wastes to produce plant biostimulants could increase the sustainability of agricultural productions by providing a new material for intensified sustainable agriculture.

THANK YOU
FOR
YOUR ATTENTION