

Supplementary data for the article:

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Supplementary material

Interaction of carbohydrate coated cerium-oxide nanoparticles with wheat and pea: stress induction potential and effect on development

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1. INTRODUCTION

Supplementary Table S1. Literature data about the effect of different coated and uncoated nCeO₂ on various plant species

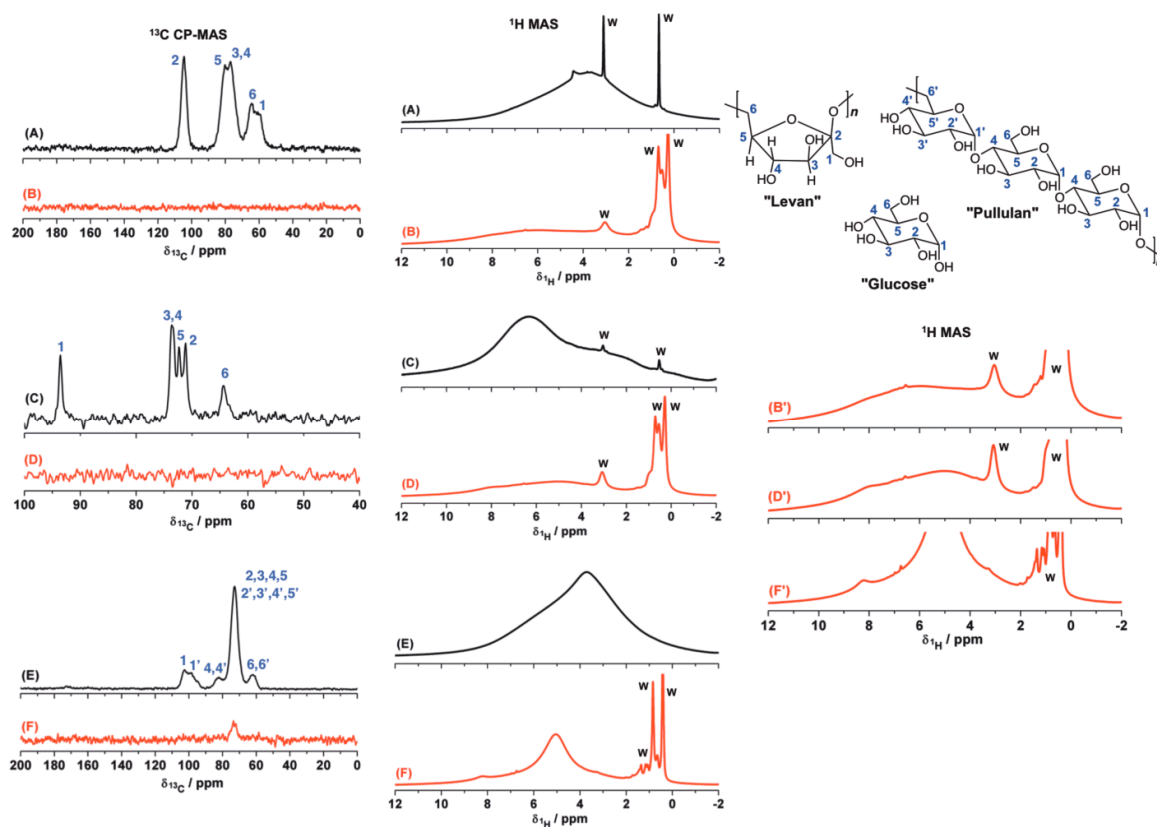
Nanoparticle	Species	Dose	Treatment duration	Effects	References
Citric acid-CeO ₂	<i>Solanum lycopersicum</i>	62.5 and 500 mg kg ⁻¹	210 days	Decrease dry weight, total sugars, starch and reducing sugars	[1]
		125 mg kg ⁻¹		Decrease starch and reducing sugars	
		~250 mg kg ⁻¹		Reduce dry weight and total sugars	
	<i>Raphanus sativus</i>	200 mg L ⁻¹	5 days	Increase root biomass	[2]
CeO ₂	<i>Medicago arborea</i>	100 - 400 mg L ⁻¹	4 weeks	Increase root length	[3]
		200 mg L ⁻¹		Decrease root dry weight	

CeO ₂	<i>Lactuca sativa</i>	250 - 1000 μg mL ⁻¹	Few days	Decrease root length	[4]
	<i>Lolium Perenne</i>	500 - 1000 μg mL ⁻¹		Increase root length	
	<i>Solanum lycopersicum</i>	500 μg mL ⁻¹		Decrease root length	
CeO ₂	<i>Cucumis sativus</i>	2000 mg L ⁻¹	9 days	Decrease seed germination	[5]
		500 - 4000 mg L ⁻¹		Increase root growth	
		500 - 4000 mg L ⁻¹		Increase shoot elongation	
	<i>Solanum lycopersicum</i>	2000 mg L ⁻¹	6 days	Decrease seed germination	
		1000 and 4000 mg L ⁻¹		Decrease root growth	
	<i>Zey mays</i>	500 - 2000 mg L ⁻¹	8 days	Decrease seed germination	
		4000 mg L ⁻¹		Increase root growth	
		2000 and 4000 mg L ⁻¹		Decrease shoot elongation	

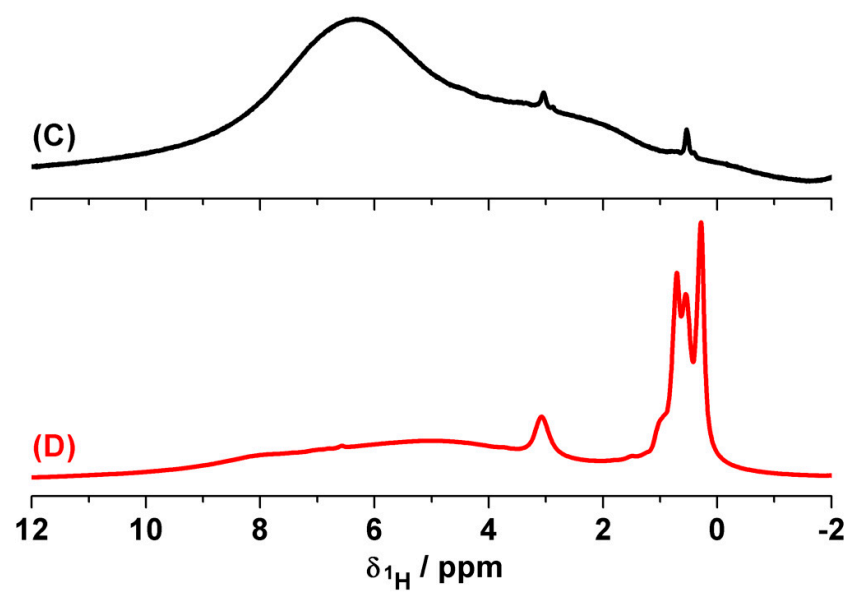
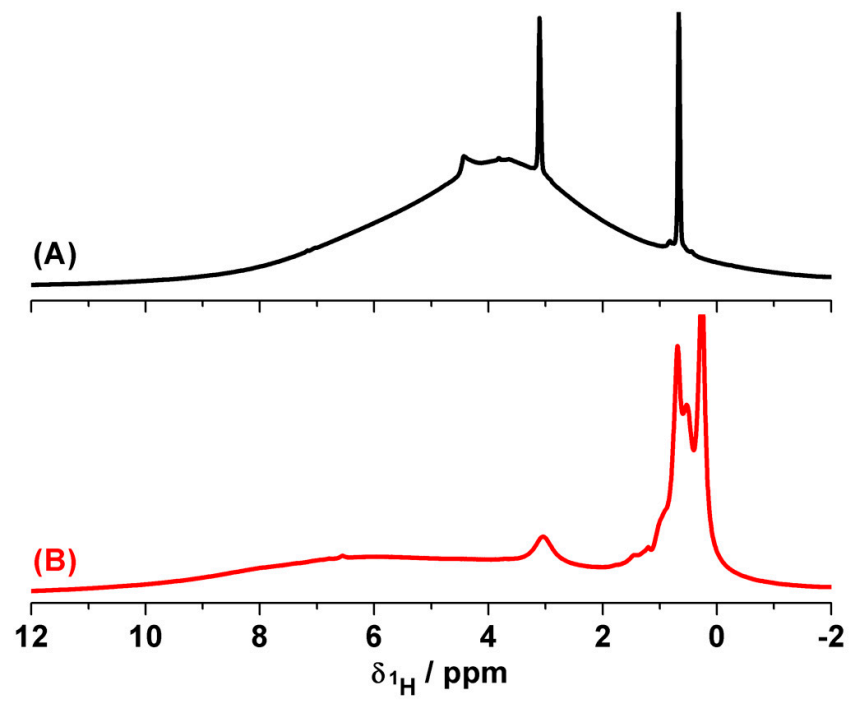
	<i>Medicago sativa</i>	2000 and 4000 mg L ⁻¹	9 days	Decrease root growth	
		500 and 1000 mg L ⁻¹		Increase shoot elongation	
CeO ₂	<i>Glycine max</i>	500 - 4000 mg L ⁻¹	5 days	Decrease rooth growth	[6]
CeO ₂	<i>Oryza sativa</i>	62.5 - 500 mg L ⁻¹	10 days	Change enzyme activity in shoots and roots	[7]
CeO ₂	<i>Oryza sativa</i> grains	500 mg kg ⁻¹	135 days	Decrease antioxidant activity and TPC	[8]
CeO ₂	<i>Raphanus sativus</i>	500 mg kg ⁻¹	12 days	Decrease seed germination	[9]
		125 mg kg ⁻¹	40 days	Increase TAA in leaves	
CeO ₂	<i>Cucumis sativus</i>	800 mg kg ⁻¹	53 days	Decrease TPC	[10]

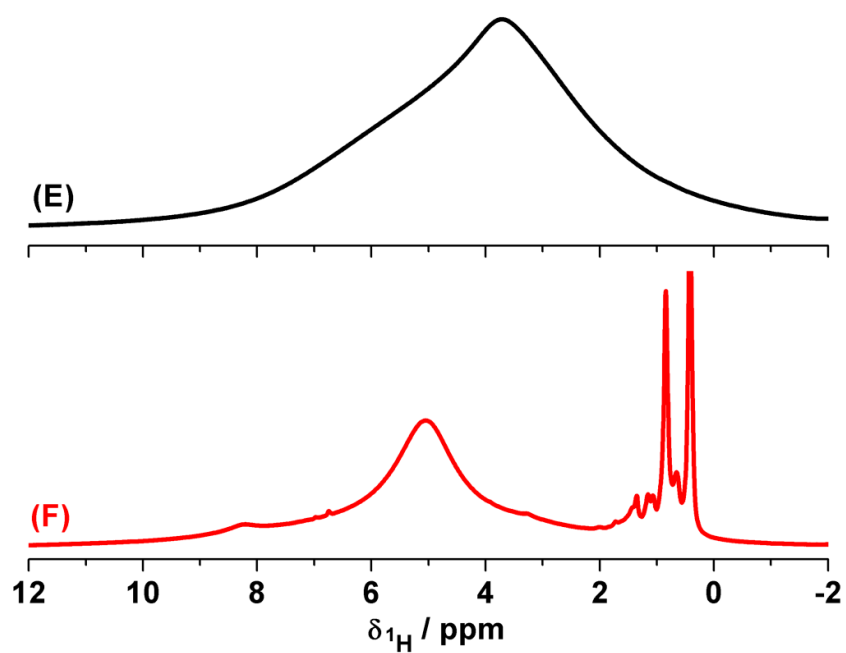
2. RESULTS AND DISSCUSSION

In the present work, the Ce ions induced a strong relaxation for the different ^{13}C nuclei present in the starting materials avoiding the detection of resonance signals, even when not all the carbons were interacting or in the surrounding of the $n\text{CeO}_2$. In this sense, the Ce ions were well dispersed in the entire materials, taking into account that practically all the carbon signals were vanished due to the interaction or proximity with Ce. In the case of P- CeO_2 , the one remained ^{13}C resonance signal was still present at 73.4 ppm, which was the most intense signal in the ^{13}C CP-MAS spectrum of the pullulan sample (Figure. 4 upper panel). In the rest of the sample containing Ce ions, the use of ^{13}C direct polarization or ^{13}C CP-MAS using short contact times (50-100 μs) strategies gave rise to the same results.

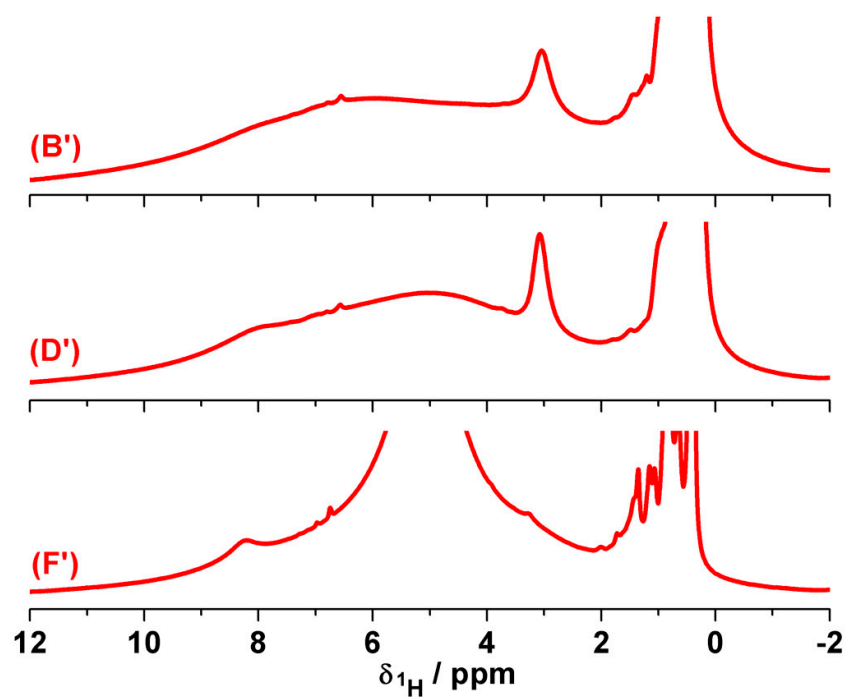


Supplementary Figure S1. Left panel: ^{13}C CP-MAS ss-NMR spectra (15 kHz) and middle panel: ^1H -MAS ss-NMR spectra (30 kHz) for the levan (A), L- CeO_2 (B), glucose (C), G- CeO_2 (D), pullulan (E) and P- CeO_2 (F) samples. Right panel: magnification of the ^1H -MAS regions is shown in spectra B', D' and F'.

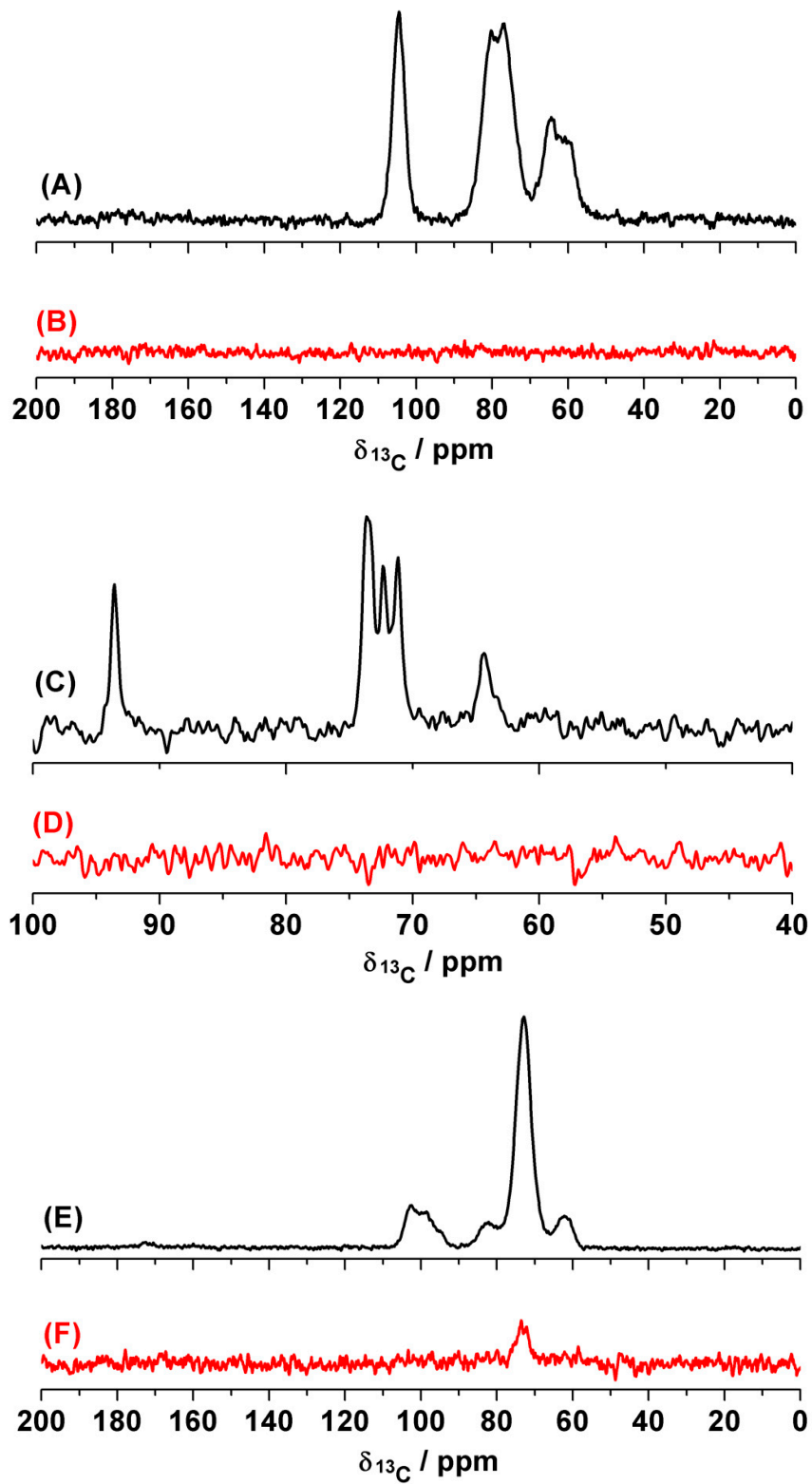




Supplementary Figure S1 A, B, C, D, E and F. ^1H -MAS ss-NMR spectra (30 kHz) for the levan (A), L-CeO₂ (B), glucose (C), G-CeO₂ (D), pullulan (E) and P-CeO₂ (F)



Supplementary Figure S1 B', D' and F'. Magnification of the ^1H -MAS regions is shown in spectra B' (L-CeO₂), D' (CeO₂) and F' (P-CeO₂).



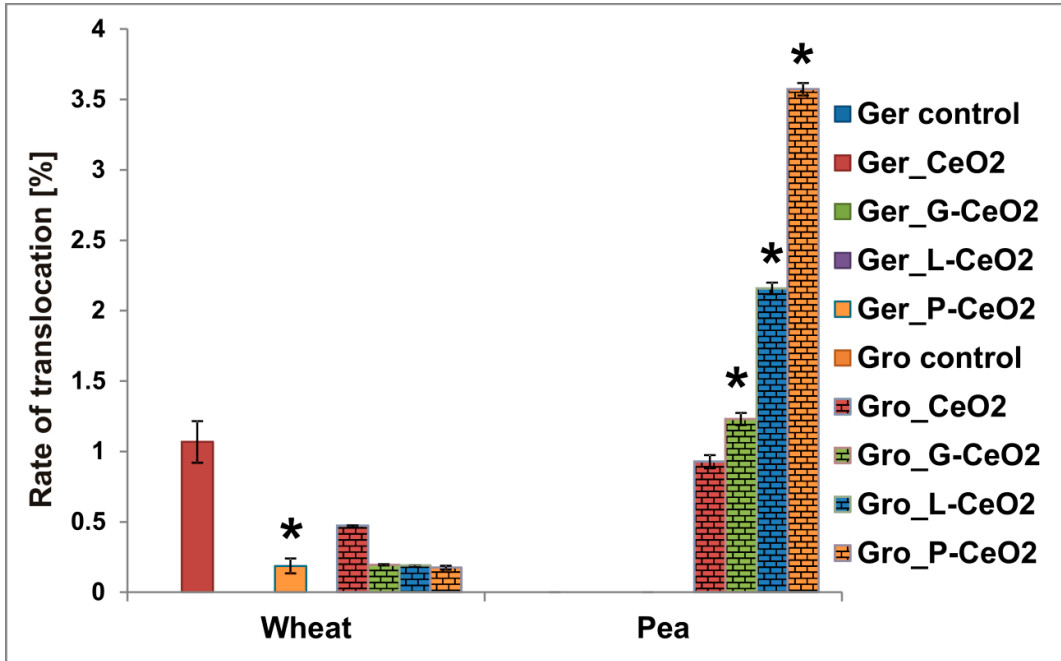
Supplementary Figure S1 A, B, C, D, E and F. ^{13}C CP-MAS *ss*-NMR spectra (15 kHz) for the levan (A), L-CeO₂ (B), glucose (C), G-CeO₂ (D), pullulan (E) and P-CeO₂ (F) samples.

The assignment of the ^{13}C NMR signals was done in comparison with previous results in the *ss*-NMR for glucose and in the solution-state NMR for levan and pullulan materials. To the best of our knowledge, this is the first work which represents *ss*-NMR for levan and pullulan compounds. Related to pullulan material, the anomeric carbons ($\text{C}_{1-1'}$) were differentiated from the rest of the hydrocarbon chain corresponding to the different environments of $\alpha_{1\rightarrow4}$ (C_1) and $\alpha_{1\rightarrow6}$ (C_1') bounds where these carbons were involved (Table 2).

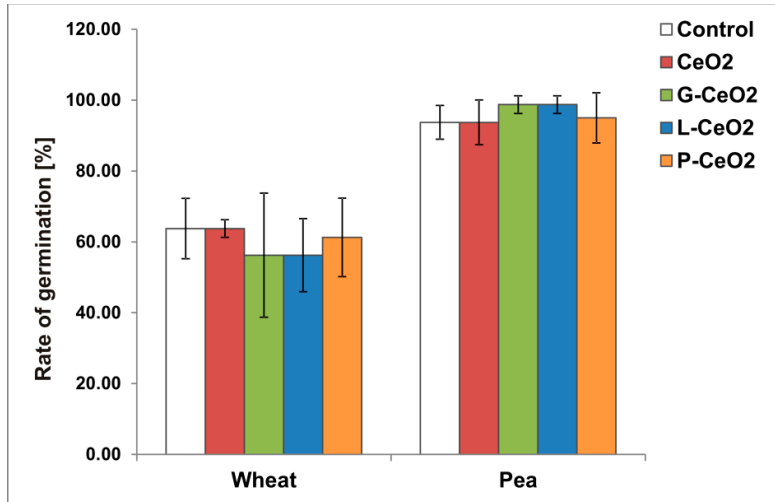
Supplementary Table S2. ^{13}C CP-MAS chemical shifts (ppm) for glucose, levan and pullulan materials.

CarbonAtom ^a	Glucose	Levan	CarbonAtom ^a	Pullulan
C ₁	93.5	60.7	C ₁ / C _{1'}	102.5 / 98.3
C ₂	71.1	104.6	C ₂ / C _{2'}	73.0
C ₃	73.5	76.8	C ₃ / C _{3'}	73.0
C ₄	73.5	76.8	C ₄ / C _{4'}	82.5
C ₅	72.2	80.6	C ₅ / C _{5'}	73.0
C ₆	64.3	64.6	C ₆ / C _{6'}	62.1

With the aim to get more structural information related to the nanocomposites with Ce ions, ^1H -MAS *ss*-NMR experiments were done and the results are shown in Figure. 4. The ^1H -MAS spectrum for the P-CeO₂ shows two ^1H resonance signals at 5.1 and 8.2 ppm ascribed to -CH-OH and -OH hydrogens, respectively. However, the rest of the ^1H signals in G-CeO₂ and L-CeO₂ were highly affected and vanished by the paramagnetic effect of Ce ions with the exception of the water populations. These results indicated that in P-CeO₂ sample there are some biopolymer regions that did not interact with the nCeO₂, explaining that a ^{13}C signal at 73.4 ppm among the ^1H signals at 5.1 and 8.2 ppm are still present in the NMR spectra. Even when the linewidth of the ^1H -MAS spectra was broad and contained some water signals indicated as 'W' in the starting materials without Ce ions, the interaction with Ce vanished the main proton signal in each sample (Figure. 4, middle and bottom panel). Interestingly, the ^1H -MAS spectra of any of the nCeO₂ present similar patterns of water structuration at a proton chemical shift ($\delta^1\text{H}$) of 0-2 ppm. These water populations were related to weakly interactions among water molecules. In the starting materials without Ce ions, some water clusters are present at a $\delta^1\text{H} = 1$ and 3 ppm [11]. However, weakly associated water (WAW) molecules interact with the nanostructure surface through the hydrogen bonds or electrostatically. The nano-structuration of the different materials with Ce ions involved also the organization of water molecules with a new, similar and well-resolved pattern of ^1H signals in the samples with CeO₂. In this sense, the nCeO₂ particles were highly homogenous dispersed in the G-CeO₂ and L-CeO₂ samples due to the vanishing of all ^1H and ^{13}C -NMR signals considering that the *ss*-NMR experiments were done in the same conditions.



Supplementary Figure S2. Rate of translocation, expressed as a percentage, in wheat and pea after Ger treatment and Gro treatment. Values are shown as mean \pm SE; * indicates a statistically significant difference in comparison with the corresponding control, $p < 0.05$



Supplementary Figure S3. Rate of germination, expressed as a percentage, in wheat and pea. Values are shown as mean \pm SE; * indicates a statistically significant difference in comparison with the corresponding control, $p < 0.05$

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