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Biological conversion of residual lignin from wheat straw into phytohormones prevents the release of greenhouse gases

Abstract

In Mexico, the cultivation of *Triticum aestivum* (wheat) generates straw waste, which is incinerated and pollutes the atmosphere. An ecological alternative for the final disposal of this residue is the residual lignin of wheat straw or RELIWS. Which is hydrolyzed into aromatics by the laccase of *Penicillium chrysogenum* and later converted by *Rhizobium etli* into in some type of gibberellins or GIT. Therefore, the objectives of this work were: a) to depolymerize RELIWS by *P. chrysogenum,* b) to transform the aromatics of the depolymerization of RELIWS into GIT, c) to demonstrate the effect of GIT from *R. etli* on *Phaseolus vulgaris* and *Triticum aestivum.* In that sense RELIWS was depolymerized by *P. chrysogenum* laccase into aromatics, then *R. etli* transformed into GIT in *P. vulgaris* and *T. aestivum* with the response variables: germination percentage, phenology: plant height (PH) and root length (RL); biomass: quantified in fresh and dry weight of the aerial part and the root (AFW/RFW and ADW/RDW). The experimental data were analyzed by ANOVA/Tukey $\alpha = 0.05$, using the Statgraphic Centurion 16.103 ® program. The results registered laccase activity with 15.74 UL^{-1}, and generation of aromatics converted into GIT by *R. etli* in *P. vulgaris* in a seedling with a ADW of 0.3 g and a RDW of 0.12 g with 0.01 mL; statistically different numerical values compared to the 0.2 g ADW and 0.1 g RDW in *P. vulgaris* treated with pure gibberellin (GI-std); In *T. aestivum* the GIT of *R. etli* caused a 0.022 g in the ADW and 0.012 g of RDW; statistically different numerical values with the 0.016 g of ADW and 0.008 g of RDW. in *T. aestivum* fed with mineral solution used as relative control (RC). The above demonstrated that it is possible to have an intelligent disposal of the RELIWS through a double biological action and to avoid an environmental contamination problem, as well as production of greenhouse gases for global warming.

Keywords: Lignin, atmospheric pollution, plant growth promoting microorganisms, monooxygenases, phytohormones, climate change mitigation

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Introduction

Triticum aestivum (wheat) is an agricultural crop of greater commercial demand in México, with an annual production of approximately 3.5 million tons,[1,2] with this around 1105 million tons of straw are generated per year.[3] Hydrocarbons and PM10 with particles smaller than 10 microns.[4,5] An alternative to avoid the burning of wheat straw is to extract the residual lignin from wheat;[6–9] since contains 17% lignin, which makes it difficult to degrade, therefore it is commonly calcined[4,10–12] that generates emissions of greenhouse gases causing global warming and also pollutants into the air such as: CH_4 (methane), CO (carbon monoxide), NO_2 or nitrogen dioxide.[2,13,14] RELIWS by treating it with acetic acid (CH_3-COOH) and then autoclaving it; and can be depolymerized by chemical methods that are expensive and polluting[15,16] instead, an ecological alternative is the depolymerization of RELIWS by *P. chrysogenum* that synthesizes laccase to break the lignin bonds to obtain aromatics[17–21] and then the conversion of aromatics by *Rhizobium etli,* that has the capacity to transform aromatics into type of gibberellins or GIT or GITs.[5,8,22,23] The hypothesis of this research was: the aromatics obtained from the depolymerization of RELIWS can be transformed by *R. etli* into a possible GIT that applied to *P. vulgaris* and *T. aestivum* will promote plant growth. Therefore, the objectives of this work were: a) to depolymerize RELIWS by *P. chrysogenum,* b) to transform the aromatics of the depolymerization of RELIWS into GIT, c) to demonstrate the effect of GIT from *R. etli* on phenology and biomass of *Phaseolus vulgaris* and *Triticum aestivum.*

Material and methods

From the collection of the Environmental Microbiology Laboratory of the Chemical-Biological Research Institute of the UMSNH, *P. chrysogenum* was selected based on its high capacity to depolymerize RELIWS of mitosporic fungi isolated from pine and oak forest soil in the city of Morelia, in the state of Michoacán, Mexico at pH 6.0. *P. chrysogenum* was activated on RELIWS agar (g/L) 10; casein peptone 5; yeast extract 1.3; bromothymol blue 10 ppm; 2.5 mL of 10% (w/v) detergent solution, and 1 mL/L of trace element solution; 18 g/L agar was added, the pH was adjusted to 5.5 and sterilized at 121°C/20 min.[24,25]

Inoculation of *P. chrysogenum* in RELIWS broth

To separate the *P. chrysogenum* mycelium from the RELIWS agar, 15 mL of sterile saline-detergent solution: 12 mL of 0.85% NaCl and 3.0 mL of 0.01% detergent (Roma ^{MR}) was poured into the Petri dish. The solution was removed with a bacteriological loop and recovered with a sterile 10 mL pipette. 12.5 mL of *P. chrysogenum* was then inoculated into a 500 mL Erlenmeyer flask with 250 mL of RELIWS broth and incubated on a rotary shaker for 19 days at 30°C and 150 rpm. 10 mL samples were taken in sterile screw-top test tubes every third day, day zero was the start of the experiment for the measurement of laccase activity as an indirect measure of RELIWS depolymerization[26–29] of different concentrations of RELIWS were used to optimize the generation of aromatics, the RELIWS broth was modified to two concentrations of 10 g/L and 30 g/L of RELIWS and

the concentrations of casein peptone, yeast extract and $CuSO_4$ were adjusted (Table 1). Both RELIWS were given the name RELIWS medium std.[6,30]

Table 1 Changes in the concentration of RELIWS: casein peptone, yeast extract and $CuSO_4$ to achieve maximum depolymerization of RELIWS

RELIWS concentration	Casein peptone		Yeast extract		$CuSO_4$		Code
	10g/L	2.5g/L	2.6g/L	0.65g/L	0.1g/L	0.025g/L	
30 g/L	X*	-	X	-	X	-	RELIWS-1
	-	X	-	X	-	X	RELIWS-2
10 g/L	X	-	X	-	X	-	RELIWS-3
	-	X	-	X	-	X	RELIWS-4
	5g/L		1.3g/L		0.05g/L		RELIWS- medium std

*Added= (X), Not added= (-.)

Determination of Laccase Activity

The RELIWS broth depolymerized by *P. chrysogenum* was centrifuged at 8000 rpm at 4°C/15 et. to eliminate the mycelium. Laccase activity was measured in a spectrophotometer by the oxidation of 2,2'-acyno-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) at 420 nm, 25°C at 0 minute, at 3 minutes and at 5 minutes, with a molar extinction coefficient of ε420=3600 M-1 cm-1; A reaction mixture was prepared with 2.4 mL. of 25 mM sodium acetate buffer, pH 3.0, 300.0 µL of 10 mM ABTS and 300.0 µL of the sample.[6,12] To determine the laccase units, the following equation was used.[10,27]

$$UL^{-1} = \frac{A(1x106)(Vt)(C)}{t(\varepsilon)(Vm)}$$

C = Cell size (1.0 cm)

Ɛ = Molar extinction coefficient of ABTS

Vm = Sample volume (mL.)

Vt = Total volume of the reaction (mL.)

t = Reaction time (min.)

A= Δ Abs 420 nm = Final Abs - Initial Abs

Adjustment of the RELIWS depolymerization filtrate after depolymerization.

RELIWS with the chemical composition: casein peptone, yeast extract and CuSO4 concentrations were filtered to remove *P. chrysogenum* mycelium. The aromatic-rich residue was adjusted with 4.0 g/L of casein peptone, 2.0 g/L of yeast extract, 2.0 g/L of glucose and 160 ppm of $CuSO_4$ $5H_2O$. The pH was adjusted to 7.0 and sterilized at 121°C for 20 min. Conversion of RELIWS aromatics by *R. etli* isolated from *Melilotus indicus* infective and effective to nodulate *Phaseolus vulgaris* and *Melilotus indicus*, according to biochemical and molecular identification has the ability to convert aromatics into possible GIT in this case, the genus *R. etli* is part of the collection of the Environmental Microbiology Laboratory IIQB-UMSNH, was activated in agar-yeast extract mannitol Congo red (g / L): Mannitol 10; K_2HPO_4 0.5; $MgSO_4$ 0.2; NaCl 0.1; yeast extract 10; Congo red 10mL / L; Bacteriological agar 18 g / L. The supernatant of each broth was inoculated with *R. etli* for 16 days at 30 °C, and 10 mL samples were taken on days 9, 12, and 16. Aromatic transformations in a RELIWS GIT were frozen to eliminate *R. etli* the effect of GITs was demonstrated by a bioassay of *P. vulgaris* and *T. aestivum* seeds.[31–33]

Effect of transforming *R. etli* into a gibberellins-type (GIT) on the phenology and biomass of *P. vulgaris* and *T. aestivum*. Seeds of *P. vulgaris* and *T. aestivum* were disinfected with 1% NaClO and 70% C_2H_5OH, then rinsed six times with sterile distilled water. Seed beds were sanitized and filled with solarized soil moistened to 80% field capacity; each treatment was assigned a key based on the change in the concentrations of RELIWS, casein peptone, yeast extract, and $CuSO_4$,[1,19,34] these are listed in Table 2.

Table 2 Transformation code of aromatics in GITs by *R. etli* according to the concentration of: casein peptone, yeast extract and $CuSO_4$

Concentrations	Code
Giberellin standard 10 mg/L	GI-std
RELIWS-1 30g/L, casein peptone 10g/L, yeast extract 2.6g/L, $CuSO_4$ 0.1g/L.	TGIt-1
RELIWS-2 30g/L, casein peptone 2.5 g/L, yeast extract 0.65g/L, CuSO4 0.025g/L.	TGIt-2
REWLIWS-3 1 0g/L, casein peptone 10g/L, yeast extract 2.6g/L, $CuSO_4$ 0.1g/L.	TGIt-3
RELIWS-4 10g/L casein peptone 2.5 g/L, yeast extract 0.65g/L, $CuSO_4$ 0.025g/L.	TGIt-4
RELIWS-5 10g/L casein peptone 5 g/L yeast extract 1.3g/L, $CuSO_4$ 0.05g/L.	TG1t-medium std

GIT was applied at a dose of 0.01 mL to *P. vulgaris* and *T. aestivum* seeds; seeds used as absolute controls (AC) were irrigated only with distilled water; seeds used as relative controls (RC) were fed with 100% mineral solution; seeds used as references from GIT medium std were treated with analytical grade gibberellic acid (GI std) (Sigma) at a concentration of 10 mg/L and fed with 50% mineral solution; seeds treated with the transformed aromatics (GIT-1,2,3,4 and TGIt-medium std) were given a dose of 0.01 mL of crude extract and fed with 50% mineral solution. The seeds were placed in darkness for 3 days, and after germination, they were left in a solarium for another 4 days, and then they were taken to a greenhouse until seeds reached the flowering stage. The following were measured: phenology: plant height (PH) and root length (RL); biomass: aerial fresh weight (AFW) and radical weight (RWF), then the aerial and radical parts were dried at 80°C/24h, to obtain the aerial dry weight (AWD) and radical dry weight or RDW[31,35] the experimental data were analyzed by ANOVA/ Tukey with a significance level α of 0.05 using the Statgraphic Centurion 16.103 ® program.[36]

Figure 1 shows the laccase activity of *P. chrysogenum* at 19 days of RELIWS depolymerization. On day 9, the laccase activity was found to be 15.74 UL^{-1} in the culture medium coded as RELIWS-2, which is registered in Table 3; compared to the laccase activity of

7.84 UL^{-1} in the RELIWS-medium std culture medium. While on day 9 in the RELIWS-1 culture medium, the laccase activity of *P. chrysogenum* was 11.54 UL-1, a statistically different numerical value from the *P. chrysogenum* laccase activity of 7.78 UL^{-1} in the RELIWS-3 culture medium; different from the numerical value of the *P. chrysogenum* laccase activity with 9.38 UL^{-1} in RELIWS-4. These results indicate that the laccase activity of *P. chrysogenum* was dependent on a specific concentration of RELIWS as reported by Baltierra-Trejo et al.,[27] who recorded the highest percentage of depolymerization was 32.4% with 30 g/L of RELIWS, compared to 20, 40 and 50 g/L of RELIWS, indicating that the genetic capacity of the RELIWS enzymatic depolymerization system in some genera and species of basidiomycetes of the type *Pleurotus ostreatus*[6], in contrast to the results registered in the present investigation. When *P. chrysogenum* was grown in RELIWS-2 and RELIWS-4, the casein peptone concentration was reduced to 2.5 g/L, yeast extract to 0.65 g/L, and CuSO4 to 0.025 g/L to induce the highest depolymerization of RELIWS to aromatics in the shortest time, thus an increase in laccase activity was observed in RELIWS depolymerization. The maximum RELIWS concentration was determined to be 30 g/L to induce measurable laccase activity; while sterile control was no inoculated with *P. chrysogenum.*

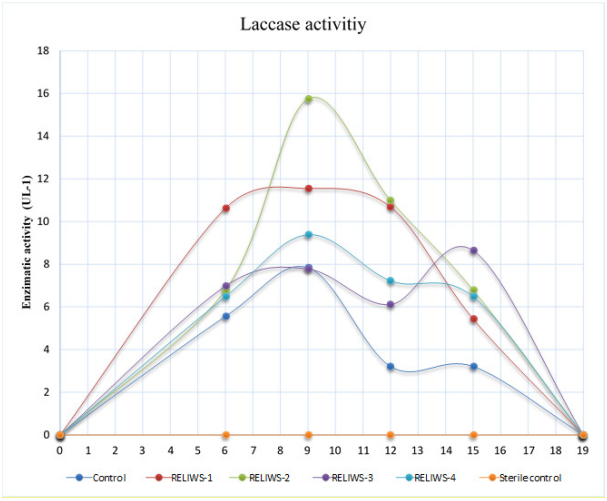

Figure 1 *Penicillium chrysogenum* laccase activity at different RELIWS concentrations.

For any explanation see the text related to Figure 1, the reference culture medium RELIWS or std was used as a control, sterile control was not inoculate

Table 3 Experimental design the effect of crude GIT extracts from *Rhizobium etli* compared to pure gibberellin (GIT std) on the phenology and biomass of *P. vulgaris* and *T. aestivum*

P. vulgaris*/*T. aestivum	**water**	**Mineral solution at 100 %**	**mineral solution at 50%**	**Pure (GIT std) Giberellin 0.01 mL**	**Crude extract GIT of R. etli 0.01 mL**
Absolute control irrigated water only (AC)	+	-	-	-	-
Relative control fed mineral solution at 10%%(RC)	-	+	-	-	-
Giberellin-std (GI-std) fed mineral solution at 50%	-	-	+	+	-
Transformed crude extract of gibberellins-like (GIT) of *R. etli* from RELIWS-2 fed mineral solution at 50%	-	-	+	-	+

(+): applied (-) non applied

Figure 1 shows the laccase activity of *P. chrysogenum* during the 19 days of RELIWS depolymerization. It was detected that on day 9, the maximum laccase activity was reached with 15.74 LU^{-1} in the RELIWS-2 culture medium, recorded in Table 1; compared to the laccase activity of 7.84 UL^{-1} in the RELIWS-medium std. culture medium. While on day 9 in the RELIWS-1 culture medium, the laccase activity was 11.54 UL^{-1}, a numerical value statistically different from the laccase activity of *P. chrysogenum* of 7.78 UL^{-1} in the RELIWS-3 culture medium; different from the numerical value of the laccase activity of *P. chrysogenum* with 9.38 UL^{-1} in RELIWS-4. These results indicate that the laccase activity of *P. chrysogenum* was dependent on a specific concentration of RELIWS as reported by Baltierra-Trejo et al.,[27] who registered the highest percentage of depolymerization 32.4% with 30 g/L of RELIWS, compared to 20, 40 and 50 g/L of RELIWS in this research, which supports that in *P. chrysogenum* the genetic capacity of the RELIWS enzymatic depolymerization system is inducible faster than that of some genera and species of basidiomycetes such as *Pleurotus ostreatus*[6]. While when *P. chrysogenum* was grown in RELIWS-2 and RELIWS-4, the concentration of casein peptone was reduced to 2.5 g/L, yeast extract to 0.65 g/L and $COSO_4$ to 0.025 g/L that induced the highest depolymerization of RELIWS into aromatics in a shorter time, by increasing the laccase activity.[3,30,37,38]

Table 4 shows the effect of crude extract transformed with a gibberellin-like compound from *R. etli* of aromatics RELIWS-2 on germination of *P. vulgaris* seeds with 95.3%, a numerical value statistically different from that registered in *P. vulgaris* seeds used as relative control (RC) only fed with 100% mineral solution with 83.3%. While *T. aestivum* treated with the same crude extract potentially transformed with gibberellins by *R. etli* or GIT-2 reached a germination percentage of 95.8%, a numerical value statistically different from the germination percentage registered in *T. aestivum* used as relative control (RC) only fed with 100% mineral solution with 84.6%. The literature indicates that *R. etli* has the genetic ability to convert plant aromatics into potential gibberellins that stimulate the synthesis of α-amylase, in the endosperm of the seed they convert starch into glucose increasing and accelerating the germination of the seeds of *P. vulgaris* and *T. aestivum*;[31,32,38] also glucose is transported to the growth sites of the embryo, initiating the growth of the seedling of both plants.[5,39]

Table 5 shows the effect of gibberellin-like transformed crude extract (GIT) by *R. etli* from RELIWS-2, on the phenology of *P. vulgaris* with a PH of 61.5 cm, a RL of 21 cm both numerical values were statistically different from those registered in *P. vulgaris* fed only with a 100% mineral solution or relative control (RC) with a PH of 43.7 cm, a RL of 19 cm. Regarding the biomass of *P. vulgaris,* at the seedling level treated with the crude gibberellin-like transformed extract (GIT) of *R. etl*i from RELIWS-2 it reached 2.9 g of AFW and 1.3 g in RFW, these results show that *P. vulgaris* treated with the crude gibberellin-like transformed extract (GIT) of *R. etli* from RELIWS-2 had no statistical difference compared to *P. vulgaris* treated with the standard GIT. The effect of GIT on *P. vulgaris* was as expected by

gibberellins that induce stem growth;[23,40] as well as the generation of apices growth and cell elongation, consequently the accelerated increase in the height of *P. vulgaris*.[29] The numerical values of the effect of *R. etli* GIT on the phenology and biomass of *P. vulgaris* were also statistically different from those registered for *P. vulgaris* or RC, with 2.9 g in AFW and 1.0 g in RFW. While *P. vulgaris* treated with the crude gibberellins transformed extract (GIT) of *R. etli* from RELIWS-2 registered an ADW of 0.3 g, an RDW of 0.12 g, numerical values statistically different from those registered for *P. vulgaris* used as a RC, with 0.18 g in ADW and 0.07 g of RDW. The crude gibberellins transformed extract (GIT) of *R. etli* from RELIWS-2, shows that it is possible to have an intelligent disposition of RELIWS through a double biological action that avoids an environmental pollution problem, as well as the production of greenhouse gases and global warming.[5,9,15,16,24]

Table 4 Germination percentage of *Phaseolus vulgaris* and *Triticum aestivum* seeds treated with the RELIWS-2 transformed by *Rhizobium etli* into a type of gibberellins (GIT)

	Germination percentage (%)
P. vulgaris[1]	
Absolute control irrigated water only (AC)	87.5[b*]
Relative control fed mineral solution at 100% (RC)	83.3[b]
[2]Standard Giberellin fed mineral solution at 50% (GIT-std)	91.6[a]
[3]Transformed crude extract of a gibberellin-like (GIT) of *R. etli* from RELIWS-2 aromatics fed mineral solution at 50%	95.3[a]
T. aestivum[1]	
Absolute control irrigated water only (AC)	80.1[b]
Relative control fed mineral solution at 100% (RC)	84.6[b]
[2]Standard Giberellin (GI-std) fed mineral solution at 50%	93.8[a]
[3]Transformed crude extract of a gibberellin-like (GIT) of *R. etli* from RELIWS-2 aromatics fed mineral solution at 50%	95.8[a]

*Different letters indicate statistical differences based on Tukey ANOVA α=0.05. [1]n= 4 replicates. [2]Dose: 0.01 mL/seed equivalent to concentration 10 ppm. [3]Dose: 0.01 mL/seed.

Table 5 Effect of transformed crude extract of a gibberellin-like (GIT) compound of *Rhizobium etli* from RELIWS-2 aromatics on the phenology and biomass of *Phaseolus vulgaris* seedling

P. vulgaris1	**Plant height (cm)**	**Radical length (cm)**	**Fresh weight (g)**		**Dry weight (g)**	
			aerial	**radical**	**aerial**	**radical**
Absolute control fed water (AC)	40.3[b*]	19[b]	1.7[c]	0.8[c]	0.15[c]	0.07[c]
Relative control fed mineral solution at 100%(CR)	43.7[b]	19[b]	1.9[c]	1.0[b]	0.18[c]	0.07[c]
[2]Giberellin std (GI-std)	52.8[a]	20.3[a]	2.3[b]	1.2[b]	0.20[b]	0.10[b]
[3]Transformed crude extract of a gibberellin-like of *R. etli* from RELIWS-2 aromatics fed mineral solution at 50%	61.5[a]	21[a]	2.9[a]	1.3[a]	0.3[a]	0.12[a]

*Different letters indicate statistical differences based on Tukey's ANOVA test α=0.05.

[1]n= number of replicates = 4 replicates. [2]Dose: 0.01 mL/seed, equivalent to concentration 10 ppm. [3]Dose: 0.01 mL/seed.

Table 6 shows the effect of gibberellin-like transformed (GIT) crude extract of *R. etli* from aromatics of RELIWS-2 on the phenology of *T. aestivum* 21.92 registered of PH, 19.58cm of RL, these numerical values were statistically different from those registered in *T. aestivum* used as RC with 19.75cm of PH and 15.83cm of LR. In the biomass of *T. aestivum* treated with the transformed gibberellin-like (GIT) crude extract of *R. etli* from RELIWS-2 registered ADW of 0.022 g and RDW of 0.012 g. Both numerical values were statistically different from those registered in *T. aestivum* used as RC with 0.016 g of DAW and 0.008 g of DRW based on these facts the literature indicates that gibberellins are phytohormones that accelerate and increase cell division in the stem in subapical meristems of the roots. The GIT generated by *R. etli* induced a greater increase and length of the roots, with a higher density of root hairs that improve the mineral uptake capacity of this plant tissue.[22,38]

Table 6 Effect of the aromatic transformation from RELIWS-2 by *Rhizobium etli* into gibberellins (GIT) on the phenology and biomass of *T. aestivum* in seedlings

T. aestivum1	**Plant height (cm)**	**Radical (cm)**	**Fresh weight (g)**		**Dry weight (g)**	
			Aerial	**radical**	**Aerial**	**radical**
Absolute control irrigated water only (CA)	19.17[c*]	15.92[b]	0.12[b]	0.010[b]	0.015[b]	0.007[b]
Relative control fed mineral solution at 100%	19.75[b]	15.83[b]	0.14[b]	0.016[a]	0.016[b]	0.008[b]
[2]Standard gibberellin (GIT-std) fed mineral solution at 50%	20.00[a]	16.33[b]	0.13[b]	0.015[a]	0.018[ab]	0.010[ab]
[3]Transformed crude extract of gibberellins-like (GIT) of *R. etli* from RELIWS-2 fed mineral solution at 50%	21.92[a]	19.58[a]	0.15[a]	0.018[a]	0.022[a]	0.012[a]

*Different letters indicate statistical differences based on Tukey's ANOVA test α=0.05. [1]n= number of replicates = 4 replicates. [2]Dose: 0.01 mL/seed, equivalent to concentration 10 ppm. [3]Dose: 0.01 mL/seed.

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The effect of the gibberellin-like transformed (GIT) crude extract of *R. etli* from RELIWS-2 on the phenology and biomass of *P. vulgaris* is shown in Table 7. Initially with a PH of 146.25 cm, with a RL of 22.25 cm, in relation to the biomass 5.0 of AFW, a RFW of 4.2g, 1.1g of ADW and 0.3 of RDW these results support that the GITs of *R. etli* accelerate and improve the healthy life cycle of *P. vulgaris* with a dose of 50% of the mineral solution, which avoids the excessive use of chemical fertilization, at the same time that the natural origin of this type of gibberellins of RELIWS-2, reduces the generation of greenhouse gases by the burning of agricultural residues rich in lignin.[5,12,19,23] While the numerical values of phenology and biomass with the crude gibberleins extract of *R. etli* were statistically similar and different from those recorded in *P. vulgaris* used as RC fed with the 100% mineral solution with 108.75 g of PH, 16.7 g of RL, 4.6 g of AFW, 2.3 g of RFW, 1.1 g of ADW and 0.9 g of DRW. The transformed crude gibberellins extract (GIT) of *R. etl*i from RELIWS-2 represents an added value and intelligent use of agricultural waste, whose main chemical composition is lignin, to generate aromatic polymers that are precursors of substances that promote plant growth in favor of sustainable agriculture.[6,8,18,26]

Table 7 Effect of crude transformed extract of gibberellins-like (GIT) of *Rhizobium etli* from RELIWS-2 aromatics on the phenology and biomass of *Phaseolus vulgaris* at flowering.

P. vulgaris I	**Plant height (cm)**	**Radical length (cm)**	**Fresh weight (g)**		**Dry weight (g)**	
			aerial	**radical**	**aerial**	**radical**
Absolute control irrigated water only (AC)	124.25^{c*}	17.5^{b}	3.8^{c}	2.6^{b}	0.8^{b}	0.2^{a}
Relative control fed mineral solution (RC)	108.75^{c}	16.7^{c}	4.6^{a}	2.3^{b}	0.9^{b}	0.2^{a}
[2]Standard giberellin fed mineral solution at 50% (GI-std	149^{a}	21.62^{a}	5.4^{a}	3.7^{a}	1.1^{a}	0.3^{a}
[3]Transformed crude extract of gibberellins-like (GIT) of *R. etli* from RELIWS-2, fed mineral solution at 50%	146.25^{b}	22.25^{a}	5.0^{a}	4.2^{a}	1.1^{a}	0.3^{a}

*Different letters indicate statistical differences based on Tukey's ANOVA test α=0.05. [1]n= number of replicates = 4 replicates. [2]Dose: 0.01 mL/seed, equivalent to concentration 10 ppm. [3]Dose: 0.01 mL/seed.

Table 8 shows the effect of the crude extract transformed into a gibberellin type (GIT) of *R. etli* from RELIWS-2 on the phenology and biomass of *T. aestivum* with 50% of the mineral solution at flowering level, with PH of 61.40 cm, a RL of 20.80 cm, an AFW of 2.52g, a RFW of 2.76g and a DAW of 0.49g, a DRW of 0.24g, these results confirm that the effect observed in *T.aestivum* was in response to the gibberellins of the double fermentation depolymerization of RELIWS then *R. etli,* that induced a faster and better flowering of *T. aestivum*, for the formation of leaves equal quality to those generated by *T. aestivum* with the 100% mineral solution.[22] The numerical values of *T. aestivum* with 50% mineral solution with *R. etli* gibberellins had statistically different or similar values to those registered in *T. aestivum* used as RC with the 100% mineral solution: with 51.6 cm of PH, with 19.00 cm of RL, with 1.43 g of AFW, 1.39 g of RFW, 0.30 g of ADW and 0.18 g of RDW. This shows that the intelligent use of RELIWS-2 in gibberellins-type plant growth promoting substances gives added value to the lignin that is normally burned and avoids the generation of greenhouse gases, a way to mitigate the carbon footprint of agriculture and climate change.[3,9,22,24]

Table 8 Effect of the transformed crude extract of gibberellins-like (GIT) of *Rhizobium etli* from RELIWS-2 on the phenology and biomass of *T. aestivum*

***T. aestivum*[1]**	**Plant height (cm) radical length (cm)**		**Fresh weight (g)**		**Dry weight (g)**	
			aerial	**radical**	**aerial**	**radical**
Absolute control irrigated water only (AC)	39.60^{c*}	18.00^{b}	0.92^{d}	1.27^{c}	0.19^{c}	0.10^{b}
elative control fed mineral solution (RC)	51.60^{b}	19.0^{a}	1.43^{c}	1.39^{b}	0.30^{b}	0.18^{b}
[2]Standard giberellin fed mineral solution at 50% (GI-std)	57.60^{ab}	19.80^{a}	1.78^{b}	1.98^{b}	0.39^{b}	0.26^{a}
[3]Transformed crude extract of gibberellins-like (GIT) of *R. etli* from RELIWS-2 fed mineral solution at 50%	61.40^{a}	20.80^{a}	2.52^{a}	2.76^{a}	0.49^{a}	0.24a

L.*Different letters indicate statistical differences based on Tukey's ANOVA test α=0.05. [1]n= number of replicates = 4 replicates. [2]Dose: 0.01 mL/seed, equivalent to concentration 10 ppm. [3]Dose: 0.01 mL/seed.

Conclusion

Wheat straw, with its high lignin content, is a polymer of plant origin. Due to its complexity, it is difficult to recycle in the soil to sustain soil fertility. Therefore, farmers few times look for ways to reintegrate it into the soil. It is generally left unused or burned, which causes a serious problem of air pollution and the generation of greenhouse gases CO, CO_2, etc., that contribute to global warming. This is due to conventional agricultural activities responsible for a considerable carbon footprint that complicates agricultural production and also negatively affects all life forms on planet Earth. Therefore, any viable form of reuse of RELIWS recycling gives added value to lignin as a raw material with agricultural and industrial biotechnological potential, a way to mitigate the impact of climate change. This is why the strategy of converting the complex liginin polymer by a microscopic mitosporic fungus with nutritional needs and a shorter generation time than basidiomycetes, to convert RELIWS into aromatic monomers, precursors of gibberellins by a common genus and species of growth-promoting bacteria, is an alternative to minimize the problem of air pollution with particles derived from the burning of RELIWS while reducing the generation

of greenhouse gases, decreasing the carbon footprint of agriculture in the different modalities, added value to the regulation of plant growth with low-cost natural phytohormones that allow regulating excessive doses of chemical fertilizers, in favor of sustainable agriculture for future generations that does not compromise natural resources such as soil, water and air.

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Conflicts of interest

The authors declare no conflicts of interest.

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