

SEED TREATMENTS WITH LIPO-CHITOOLOGOSACCHARIDES ENHANCE MAIZE PRODUCTIVITY IN BRAZILEAN ENVIRONMENTS

WILSON STORY VENANCIO¹, PEDRO HENRIQUE DE MEDEIROS BUSO² AND MARTIN DIAZ-ZORITA³

¹CWR Pesquisa Agrícola, Rua Theodoro Kluppel, No 30, Bairro Olarias, Ponta Grossa, Paraná, Brazil.

²Private consultant at Curitiba, Paraná, Brazil.

³Facultad de Agronomía, Universidad Nacional de La Pampa, RN 35 km 334, 6300, Santa Rosa, La Pampa, Argentina.

(Received 17 February, 2021; Accepted 15 April, 2021)

Key words: Soil microbiology, Dryland production, Integrated seed treatments, Signal molecules.

Abstract – This work aimed to establish the contribution on maize (*Zea mays* L.) productivity of seed treated with a formulation containing lipo-chitooligosaccharides (LCOs) under field conditions in regions of Brazil. Five corn field trials were conducted in the Brazilian States of Paraná and Goiás. In each site, three seed treatments were established in combination with four chemical products (i) seeds without inoculation; ii) seeds treated with LCOs at planting; iii) seeds treated with LCOs 192 days before planting. Shoot and root vegetative growth and grain yield components were evaluated, and the data analyzed based on ANOVA and regression procedures. Averaged over 20 field trials, the use of maize seeds treated with LCOs promoted greater plant growth and grain production not only in response to more vigorous establishment of the plants but also keeping uniform and more active growth during their development. The mean grain yield response (390 kg ha⁻¹), equivalent to 4.7 % more grain production, resulted from the combined effect of the LCOs on the formation and filling of the grains.

INTRODUCTION

Maize (*Zea mays* L.) grain yield gaps under current Brazilian production conditions, independently of the planting season, are attributed to water deficit and mainly to differences in the use recommended crop management practices (Andrea *et al.*, 2018). The moderate use of intensified crop production models, and particularly those practices that provides better nutrition and growth conditions for the crops, partially explains the gaps in maize production. The use of biofertilizers is one of the pillars practices to develop high productive and sustainable modern agricultural providing tools for combined processes frequently observed in natural systems (Raffi, 2018).

The symbiosis between rhizobia and legumes and between mycorrhizae and other plants and the contribution of the use of agricultural inoculants to favor these processes is broadly documented. Diverse studies worldwide show natural benefits of these symbiosis not only related with plant nutrition

but also promoting its growth (Parniske, 2008, Hungria *et al.*, 2006). The lipochitooligosaccharides (LCOs) are compounds naturally produced by many soil microorganisms being functional active supporting the initiation of symbiosis relationships with plants. Several of these molecules have been also described as nod-factors when delivered from rhizobia and as myc-factors produced from mycorrhizae (Gough and Cullimore, 2011). Thus, the LCOs compounds can promote the primary actions on the plant roots of microorganisms like rhizobia and mycorrhizae. Rhizobia species will form active nitrogen fixing nodules in legumes and mycorrhizae readily form symbiotic associations also with legumes and other plant species. The symbiotic value of mycorrhizae to plants is also very well established and includes an increase in effective root surface area for nutrient uptake, nutrient access beyond the root surface, and enhanced efficiency of roots to uptake water and nutrients from the soil including nitrogen and phosphorus thereby

enhancing plant growth and health (Jansa *et al.*, 2003; Tanaka and Yano, 2005; Gringera *et al.*, 2007).

The activity of rhizobia-produced LCOs and myc factors are closely related and parallel in activity because they evolved from common origins, with mycorrhizae developing the plant symbiotic system before rhizobia obtained the ability from mycorrhizae (Gough and Cullimore, 2011). As non-leguminous plants evolved (e.g. maize), they retained some of the molecule-detection genetics and machinery from ancestral origins that are present in legumes for association with rhizobia and LCOs. Both nod-factor LCOs and myc-factor LCOs are chemically similar and share similar recognition and response mechanisms that lead to early germination, root branching, nodulation and mycorrhization (Miransari and Smith, 2009; Gough and Cullimore, 2011; Schwinghamer *et al.*, 2015). Further, LCOs produced by rhizobia can stimulate germination of mycorrhizal spores and subsequent root colonization by mycorrhizal hyphae and increase mycorrhizal colonization (Xie *et al.*, 1995). The combination of LCOs to mycorrhiza allows earlier and greater pre-symbiotic activity of these organisms with the plant than mycorrhiza alone (Olah *et al.*, 2005). This indirect effect can be mimicked or enhanced by coating maize seeds with both flavonoids, like genistein and deizine, and with LCOs (Smith and Osburn, 2013). Maize and soybean [*Glycine max* (L.) Merrill] field trials performed in Wisconsin (USA) showed that treating the seeds with flavonoids or LCOs increased approximately 5 % the grain productivity while the combined application of these molecules almost doubled the crop responses (Smith and Osburn, 2013).

The soils under current agricultural production conditions, mostly rotated with maize, soybean, and other crops, contain abundant rhizobia and mycorrhizal fungi in their microbiomes (Karasawa *et al.*, 2002). The plants and microbes can use

molecular signals to communicate (Smith *et al.*, 2015a). Thus, treating maize seeds with LCOs can facilitate the initiation of symbiotic interactions during germination and the early plant growth stages. Consequently, the treated crops with this signal molecule could lead in vigorous crop establishment supporting active vegetative growth and grain production. Our objective was to measure changes in the productivity of maize crops using seeds treated with a formulation containing LCOs molecules under current Brazilian production practices.

MATERIALS AND METHODS

During the 2017/18 growing season, five maize field trials were performed along two Brazilian States (Paraná and Goiás) covering a wide range of agricultural production environments (Table 1). In all locations, the crops were grown following best recommended practices for high productivity (Table 1) and the use of NPK(S) fertilizers (Table 2), and the preventive protection against weeds, pests, and diseases (Aguiar *et al.*, 2014). The selected sites were all under long-term agriculture crop rotation, including soybean crops, and the soils mostly classified as Cambisols and Ferralsols (designated Latossolo-type soil) (Table 2). During the studied season, the mean rainfall between December to May varied between 843 and 893 mm and no limitations for normal maize growth were noted (Table 3).

In each location, the studied treatments combined three biological seed treatments [*i*) seeds without the LCOs treatment; *ii*) seeds treated with LCOs at planting; *iii*) seeds treated with LCOs before planting] with four chemical seed treatments (Table 4). The biological formulation contained 8.8×10^{-7} % in mass of LCOs in an aqueous carrier (Novozymes Inc., WI, USA) applied at a dose of 0.33 ml kg^{-1} of seeds. All the chemical seed treatments were

Table 1. Location and main crop management practices of 5 maize field trials in the States of Paraná (PR) and Goiás (GO), Brazil.

Sites	City, State	Latitude	Longitude	Previous crop	Hybrid	Planting date	Harvesting date
1	Rio Verde, GO	17°32'35"S	51°02'42"W	<i>Glycine max</i>	DK290PRO3	Feb/09/2018	Jun/30/2018
2	Montividiu, GO	17°15'54"S	51°09'38"W	<i>Glycine max</i>	DK290PRO3	Feb/09/2018	Jun/27/2018
3	Sta. Helena de Goiás, GO	18°00'26"S	50°31'02"W	<i>Glycine max</i>	DK290PRO3	Feb/10/2018	Jun/28/2018
4	Aparecida do Rio Doce, GO	18°12'11"S	51°24'61"W	<i>Glycine max</i>	DK290PRO3	Feb/10/2018	Jun/28/2018
5	Palmeira, PR	25°25'44"S	50°03'15"W	<i>Triticum aestivum</i>	DK290PRO3	Dec/02/2017	Apr/30/2018

applied 192 days before planting while the LCOs formulation was sequentially applied on top of the chemically treated seeds at planting or 192 days before the planting date. The treated seeds were packed in paper bags and stored at 20° to 24°C in a dark and dry chamber with air humidity lesser than 70 % until planting.

Approximately at 30 to 35 days after emergence, at the v6 growing stage (50 % of the plants with 6 leaves completely developed, Ritchie *et al.* 1986), the stand of plants was measured counting the

established plants in 3 m of the 2 central rows of each plot. Also, 5 adjacent plants located in the external rows of each plot were collected for measuring the shoot and the root dry matter. The variation in height and in shoot dry matter of the plants from the three biological seed treatments were estimated using the coefficient of variability per location calculated from 24 observations (6 field replicates and 4 chemical seed treatments).

At physiological maturity, the shoot height from the soil surface to the top of the male inflorescence

Table 2. Soil type and main properties (0 to 20 cm top layer) and applied fertilization treatments in 5 maize field trials in the States of Paraná and Goiás, Brazil. Soil type based on Brazilian Soil Classification System (EMBRAPA, 2018), SOM = soil organic matter, Pe = soil extractable phosphorus. Base NPK: in furrow fertilization at planting, Crop NPK(S): broadcasted fertilization during early crop growth.

Sites	Soil type	SOM %	pH	Pe mg kg ⁻¹	Sand ---	Silt %	Clay ---	Fertilizer			
								Base N-P-K	Crop kg ha ⁻¹ N-P-K	S kg ha ⁻¹ (S)	
1	Latossolo roxo argiloso	4.32	5.92	16.9	58	12	30	10-30-20	400	46-00-00	300
2	Latossolo roxo muito argiloso	4.12	5.32	18.6	30	7	63	10-30-20	400	46-00-00	300
3	Latossolo vermelho muito argiloso	4.20	6.1	22.2	36	26	38	10-30-20	400	46-00-00	300
4	Neosolo arenoso	2.7	5.8	19.3	87	10	3	10-30-20	400	46-00-00	300
5	Cambissolo franco	3.17	4.7	53	41.8	34	24.2	04-16-08	350	27-00-00 (22)	150

Table 3. Monthly rainfall in 5 maize field experimental sites in the States of Paraná and Goiás, Brazil.

Sites	Planting date	Rainfall (mm month ⁻¹)					
		December	January	February	March	April	May
1	Feb/09/2018	-	270	180	155	180	97
2	Feb/09/2018	-	265	198	169	136	109
3	Feb/10/2018	-	287	163	193	133	67
4	Feb/10/2018	-	291	193	158	149	66
5	Dec/02/2017	232	330	79	250	2	-

Table 4. Chemical seed treatments in 5 maize field experimental sites in the States of Paraná and Goiás, Brazil.

Seed Treatment	Fungicide		Insecticide	
	Active ingredient (g.L ⁻¹)	Dose (mL.Kg de seed ⁻¹)	Active ingredient (g.L ⁻¹)	Dose (mL. Kg de seed ⁻¹)
A	Metalaxil-M (20), Tiabendazol (150), Fludioxonil (25)	1.5	-	-
B	Metalaxil-M (20), Tiabendazol (150), Fludioxonil (25)	1.5	Clotianidin (600)	3.5
C	Metalaxil-M (20), Tiabendazol (150), Fludioxonil (25)	1.5	Thiamethoxam (350)	6.0
D	Metalaxil-M (20), Tiabendazol (150), Fludioxonil (25)	1.5	Clotianidin (600), Clorantraniliprole (635)	3.52.4

and the perimeter of the fourth internode were measured. The grain production and the yield components (plant stand, single grain weight) were measured from the complete harvest of 8 m from the 5 central rows of each of the plots. The number of grains per unit area was estimated from the ratio between the grain yield and the single grain weight. All the grain productivity parameters were calculated at 13.5 % moisture content.

The studies were configured using a factorial (3 biological seed treatments x 4 chemical seed treatments) randomized complete block design with 6 replicates per location in 60 m² plots (10 rows with 0.5 m row spacing and 10 m long) with a separation of 1.0 m between plots.

In each of the locations the established plant stands were independent of the applied seed treatments and in none of them there were differences between the seed chemical treatments (Table 5). These results supported the use of 20 replications (5 locations x 4 seed chemical treatments) for the analysis of variance (ANOVA) of the LCOs effects on crop growth parameters integrating the diverse production and environmental conditions. Also, it favors the analysis per location based on 24 replications (6 field

replicates x 4 seed chemical treatments). The differences between means were determined using the LSD test at a significance level of 0.01 (Di Rienzo *et al.* 2020). Correlation and regression analysis were calculated between selected variables and the comparison of the regression fitted lines between the mean productivity of each site (location x chemical seed treatment) and the grain yields for the biological seed treatments (Analytical Software, 2008).

RESULTS

Crop establishment and growth

The established populations of maize crops varied between 56,000 and 66,000 plants ha⁻¹ and were independent of the use of LCOs molecules in combination with the different chemical seed treatments combinations (Table 5). The application of the LCOs, in almost all the locations, did not modified the efficacy of the crop establishment (Table 5). Only in the site from Parana State the use of LCOs averaged among the 4 chemical seed treatments reduced the population in approximately 3,500 plants ha⁻¹ (Table 5).

Table 5. Summary of the ANOVA results and mean population of maize crops from seed treatments (ST) with LCOs combined with different chemistries in 5 field experimental sites in the States of Paraná and Goiás, Brazil. F values followed by * indicates p<0.05, df = degrees of freedom

		Experimental sites				
		1	2	3	4	5
		Mean population (plants ha ⁻¹)				
		66111	66361	66222	66222	56584
Source of variation	df	----- F-value -----				
Chemical ST	3	0.06	0.25	0.30	0.12	0.44
LCOs ST	2	0.17	0.03	0.02	0.15	6.25 *
Chemical ST x LCOs ST	6	0.23	0.14	0.14	0.09	1.00

Table 6. Summary of the ANOVA results and mean parameters of vegetative growth of maize crops developed from seed treatments with LCOs applied at planting or 192 days before planting (dbp) combined with 4 chemical seed treatments in 5 sites in the States of Paraná and Goiás, Brazil. CV: mean coefficient of variability among plots, DM: dry matter, P(x): statistical probability level of the LSD mean comparison test. In each column, different letters show significant differences (p<0.05) treatments.

Treatment	Height				Shoot DM				Root DM		SDM:RDM	
	cm plant ⁻¹		CV		kg ha ⁻¹		CV		kg ha ⁻¹			
Without LCOs	77	B	4,2	A	79	B	2,3	A	19	B	3,2	A
LCO (at planting)	79	A	3,5	AB	106	A	2,1	A	24	A	3,2	A
LCO (192 dbp)	78	A	3,2	B	93	AB	2,2	A	22	AB	3,2	A
p(x)	0.016		0.093		0.0125		0.900		0.0129		0.545	

DISCUSSION

The mean productivity of maize in these studies showed the frequent results observed under normal Brazilian production and environmental conditions. The grain production was strongly related with the formation and grains, however differences in the single grain weight suggested the potential limitation for crop production due to stressful conditions during the seed filling period. We observed that, under similar plant populations and environmental growing conditions, the production of maize grains was reduced when the plant growth since early stages of development was limited.

The plants developed after the application of the microbial signal molecules showed better growth since early stages supporting greater formation and filling of grains (Tables 6 and 7). These results agree with the observations that microbial driven process promote the growth of the plants and validate the contribution of LCOs also in non-legumes (Smith *et al.*, 2015b). The crops treated with LCOs showed more shoot and root growth keeping a similar ratio between both parameters compared with the control

without the application of this molecules. Ramos and Díaz-Zorita (2019) described more lateral root growth suggesting a difference in the resources allocation within the root systems but keeping balanced with the growth above ground. This behavior suggests that the treatments were beneficial for the overall growth of the plants and not in response to changes in the internal allocation of resources that could lead in unbalanced growth between roots and shoots. The LCOs could favored the germination (Prithiviraj *et al.*, 2003) and the initiation of plant symbiosis with mycorrhizae (Gough and Cullimore, 2011; Xie *et al.*, 1995) increasing and maintaining a more effective root exploration of the soil and the uptake of nutrients and other resources during the growth of the crops (Bolan, 1991). Achieving a greater number of grains per square meter in response to the application of LCOs and under similar plant populations supports a better and uniform vegetative growth conditions of the crops. While the greater single grain weight in the crops treated with LCOs, without the observation in changes in the duration of the seed filling period, also supports the fact of more active

Table 8. Mean grain yield of maize crops developed from seed treatments with LCOs applied at planting or 192 days before planting (dbp) in 20 field trials from the combination of 4 chemical seed treatments planted in 5 sites in the States of Paraná and Goiás, Brazil. In each row, different letters show significant differences ($p < 0.10$) between treatments. Note: The identification code for the field trials within each location was randomly allocated to avoid further interpretations between the chemical seed treatments.

Field trial	Seed treatment					
	Without LCOs		LCOs (at planting)		LCOs (192 dbp)	
	----- Grain yield (kg ha ⁻¹) -----					
101	8599	A	9165	B	9088	B
102	8652	A	9173	B	9128	B
103	8699	A	9186	C	9087	B
104	8689	A	9163	B	9099	B
201	8837	A	9320	C	9174	B
202	8902	A	9369	B	9397	B
203	8868	A	9319	B	9051	A
204	8816	A	9336	B	9365	B
301	7963	A	8418	C	8281	B
302	7994	A	8524	C	8228	B
303	7971	A	8476	B	8378	B
304	7998	A	8492	C	8255	B
401	9013	A	9152	A	9062	A
402	8818	A	9240	C	9128	B
403	8827	A	9225	B	9143	B
404	8836	A	9222	C	9119	B
501	7403	A	7919	B	7583	AB
502	7384	A	7905	B	7709	AB
503	7335	A	7987	B	7811	B
504	7699	A	7792	A	7746	A

growth during reproductive stages. The combined result of both grain yield components leads to a greater production when the crops developed from LCOs treated seeds and agreed with the observations of Smith and Osburn (2013) and Díaz-Zorita *et al.* (2010) from field studies in Wisconsin (USA) and the pampas region (Argentina), respectively. The mean grain yield contribution, approximately 5 % of the attainable grain production, was like the mean responses to the enhanced presence of microbial plant growth promoters applied to the seeds of maize crops under dryland production conditions (Cassán and Díaz-Zorita, 2016). Adding the LCOs molecules in the maize seed treatments enhanced its productivity independently of the attained mean yields (Fig.1) suggesting that the overall crop growth limitation was a regular condition along the studied environments. The mean grain yield response to LCOs tend to diminish when reducing the productivity of the field trial mostly due to less production when the treatments were applied several months before planting. The site with the lowest mean grain yields was planted late in spring and although achieved greater number of grains per square meter it showed lighter ($344 \text{ mg grain}^{-1}$) single grain weight than the rest of the sites ($458 \text{ mg grain}^{-1}$). These differences suggest that in this location the grain filling period happens mostly under stressful conditions with potential limitations in the capture and transformation of photosynthetic energy. We speculate that the activity of the applied molecules diminishes during their aging on the seeds due to diverse processes including oxidation and biological degradation. The projection of the daily rate of reduction on the response to the application of the LCOs (0.66 kg ha^{-1}) calculated from the data shown in the table 8 is expected when the molecules are applied almost until 680 days before planting.

Our results support that maize crops treated with LCOs promoted greater plant growth and grain production not only in response to more vigorous establishment of the plants but also keeping uniform and more active growth during their development. In Brazil, within the frequent dryland management production practices.

CONCLUSION

Under current Brazilian maize production practices, the biological treatment of seeds with LCOs applied

in combination with chemical compounds contributes to reduce the grain yield gap to attain high productivity. Averaged over 20 field trials, the use of maize seeds treated with LCOs promoted greater plant growth and grain production not only in response to more vigorous establishment of the plants but also keeping uniform and more active growth during their development. The mean grain yield response (390 kg ha^{-1}), equivalent to 4.7 % more grain production, resulted from the combined effect of the LCOs on the formation and filling of the grains.

These results support, under the tropical and subtropical regions of Brazil, the feasibility of effectively implementing seed treatment of maize with this novel biological seed treatment containing LCOs molecules providing integral benefits for the current crop production systems.

Declaration of Conflict of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Authors' Contributions

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final

REFERENCES

- Aguiar, A.T., Gonçalves, C., Paterniani, M.E., Tucci, M. and Castro, C.E. 2014. *Instruções agrícolas para as principais culturas econômicas*. Boletim IAC no 200, 7^a Ed. Campinas: Instituto Agronômico., 452 pp.
- Analytical Software. 2008. Statistix 9. User's manual. Tallahassee, FL (USA), 454 pp.
- Andrea, M.C. da S., Boote, K.J., Sentelhas, P.C. and Romanelli, T.L. 2018. Variability and limitations of maize production in Brazil: Potential yield, water-limited yield and yield gaps. *Agricultural Systems*. 165. 264-273. 10.1016/j.agsy.2018.07.004.
- Bolan, N.S. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil*. 134 : 189-207.
- Cassán, F. and Diaz-Zorita, M. 2016. *Azospirillum sp.* in current agriculture: From the laboratory to the field. *Soil Biology and Biochemistry*. 103 : 117-130. <http://dx.doi.org/10.1016/j.soilbio.2016.08.020>.
- Di Rienzo, J.A., Casanoves, F., Balzarini, M., Gonzalez, L., Tablada, M. and Robledo, C.W. 2020. InfoStat versión 2020. Centro de Transferencia InfoStat, FCA,

- Universidad Nacional de Córdoba, Argentina. URL <http://www.infostat.com.ar>
- Díaz-Zorita, M., Micucci, F. G., Baliña, R. M. and Fernández-Canigia, M. V. 2010. Los lipoquitooligosacáridos en la producción de maíz (*Zea mays*). In: *IX Congreso Nacional de Maíz. Trabajos presentados y Resumen de Conferencias*. AIANBA Agroactiva (ed.), Rosario, Santa Fe, 17-19 Nov. 2010. Argentina., pp. 266-268.
- Gough, C. and Cullimore, J. 2011. Lipochitooligosaccharide signaling in endosymbiotic plant-microbe interactions. *MPMI*. 24(8) : 867-878.
- Gringera, M.S., Drijber, R.A. and Weinhold, B. 2007. Increased abundance of arbuscular mycorrhizae fungi in soil coincides with the reproductive stages of maize. *Soil Biology and Biochemistry*. 39 : 1401-1409.
- Hungria, M., Campo, R.J., Mendes, I.C. and Graham, P.H. 2006. Contribution of biological nitrogen fixation to the N nutrition of grain crops in the tropics: the success of soybean (*Glycine max* L. Merr.) in South America. In: Singh, R. P., Shankar, N., Jaiwal, P. K. (eds.). *Nitrogen Nutrition and Sustainable Plant Productivity*. Studium Press, Houston, p. 43-93.
- Jansa, J., Mozafar, A. and Frossard, E. 2003. Long-distance transport of P and Zn through the hyphae of an arbuscular mycorrhizal fungus in symbiosis with maize. *Agronomie*. 23 : 481-488.
- Karasawa, T., Kasahara, Y. and Takebe, M. 2002. Differences in growth responses of maize to preceding cropping caused by fluctuation in the population of indigenous arbuscular mycorrhizal fungi. *Soil and Biochemistry*. 34 : 851-857.
- Miransari, M. and Smith, D. 2009. Rhizobial lipochitooligosaccharides and giberellins enhance barley (*Horeum vulgare* L.) seed germination. *Biotechnology* 8 : 270-275.
- Olah, B., Briere, C., Becard, G., Denarie, J. and Gough, C. 2005. Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago trunculata* via the DMI1/DMI2 signalling pathway. *The Plant Journal*. 44 : 195-207.
- Parniske, M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology*. 6 : 763-775.
- Prithiviraj, B., Zhou, X., Souleimanov, A., Kahn, W.M. and Smith, D.L. 2003. A host-specific bacteria-to-plant signal molecule (Nod factor) enhances germination and early growth of diverse crop plants. *Planta*. 216: 437-445.
- Raffi, M.M. 2018. Sustainable agriculture and the role of biofertilizers. *Journal of Academia and Industrial Research*. 7 : 51-59
- Ramos, M.L. and Díaz-Zorita, M. 2019. Mejoradores biológicos del crecimiento aplicados a la producción de maíz. AAPRESID. Red de Innovadores 45-54. Rosario. Santa Fe. Argentina. www.aapresid.org.ar
- Ritchie, S.W., Hanway, J.J. and Benson, G.O. 1986. How a corn plant develops, Special Report No. 48, Iowa State University. Ames, IA (USA) 22 pp.
- Schwinghamer, A., Souleimanov, P., Dutilleul and Smith, D. 2015. The plant growth regulator Lipochitooligosaccharide (LCO) enhances the germination of canola (*Brassica napus* [L.]). *J. Plant Growth Regul.* 34 : 183-195.
- Smith, D.L., Subramanian, S., Lamont, J.R. and Bywater-Ekergård, M. 2015a. Signaling in phytomicrobiome breadth and potential. *Frontiers in Plant Science*. 709. Doi 10.389/fpls.2015.00709
- Smith, S., Habib, A., Kang, Y., Legget, M. and Díaz-Zorita, M. 2015b. LCO applications provide improved responses with legumes and nonlegumes. In: de Bruijn F (ed) *Biological Nitrogen Fixation*, v.2, chapter 107. John Wiley and Sons, Inc, Hoboken, NJ, USA, pp 1077-1086. doi:10.1002/9781119053095.ch107
- Smith, R.S. and Osburn, R.M. 2013. Lipochitooligosaccharide combination compositions for enhanced plant growth and yield. US 8,357,631 assigned to Novozymes Bioag Inc.
- Tanaka, Y. and Yano, K. 2005. Nitrogen delivery to maize via mycorrhizal hyphae depends on the form of N supplied. *Plant, Cell and Environment*. 28 : 1247-1254.
- Xie, Z.P., Staehelin, C., Vierheilig, H., Wiemken, A., Jabbouri, S., Broughton, W.J., Vogeli-Lange, R. and Boller, T. 1995. Rhizobial nodulation factors stimulate mycorrhizal colonization of nodulating and nonnodulating soybeans. *Plant Physiol*. 108 : 1519-1525.
-
-