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RESEARCH ARTICLE

Effect of Lipo-chitooligosaccharides on seed germination, growth, vigour and biochemical changes in soybean seedling

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SUMMARY

The bacterium-to-plant signal, lipo-chitooligosaccharides (LCOs) or Nod factor induces cell division and enhances seed germination. The experiment was conducted to test the efficacy of LCO on soybean seed germination and seedling growth under room temperature by roll towel method. Different concentrations of LCO were used for seed treatment along with microbial inoculum and biocontrol agents as a control. Among the treatments, concentration of LCO @ 1.8 and 3.6 ml/kg of seed performed better compared to other treatments. The results revealed that treating with LCO could influence soybean seed germination and growth of seedlings.

Key Words : Lipo-chitooligosaccharides (LCOs), Soybean, Seed germination

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Soybean (*Glycine max* L.), called as a 'miracle crop' with over 40 per cent protein and 20 per cent oil and possess nutritional superiority since it contains essential amino acids, unsaturated fatty acids, carbohydrates, vitamins and minerals. Soybean protein constitutes about 5 per cent amino acid lycine which is deficient in most of the cereals. In addition, it contains a good amount of minerals, salts and vitamins (thiamine and riboflavin) and its sprouting grains contain a considerable amount of vitamin C. Soybean cultivation in India was negligible until 1970, but it gained importance thereafter, with a production of 6 million tonns in 2003 and this has made India the 5th largest producer of soybean in the world today.

Lipo-chitooligosaccharies are called as Nod factors, which are signaling molecules having chitin β-1-4-linked N-acetyl-D-glucosamine backbone, varying in length between three to six sugar units, and a fatty acyl chain on the C-2 position of the nonreducing sugar. Three of the nod genes- *nod*A, *nod*B, and *nod*C – are required for synthesizing this basic structure. NodA is an *N*-acyltransferase that catalyzesthe addition of a fatty acyl chain. NodB is a chitin-oligosaccharide deacetylase that

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removes the acetyl group from the terminal nonreducing sugar, and NodC is a chitin-oligosaccharide synthase that links *N*-acetyl-D-glucosamine monomers (Stokkermans *et al*., 1995).

Symbiosis of *Rhizobium* (*Bradyrhizobium japonicum*) with soybean roots begins with a rhizobia-to-plant signal molecule, Lipo-chitooligosaccharide (LCOs), or Nod factor (Bai *et al*., 2002). Morphogenesis and nodule formation in roots are the process induced by Lipo-chitooligosaccharide molecules. Low concentration of LCOs causes some physiological process in legume and non-legumes such as root hair deformation ontogeny of compete nodule structures cortical cell division Defense related enzymes have been activated by the LCO. LCO enhances cell division and embryo development which in turn increases germination in barley (Miransari and Smith, 2009) and cauliflower (Supanjani *et al*., 2009). The objective of this experiment was to assess the impact of Lipo-chitooligosaccharides on germination of soybean in comparison with microbial inoculums such as *Rhizobium* and phosphobacteria along with biocontrol agents like pseudomonas and trichoderma.

MATERIAL AND METHODS

The experiment was conducted in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, India. Soybean seeds were surface sterilized with 0.1 per cent mercuric chloride. Further, the seeds were treated with microbial inoculums, biocontrol agents and different concentration of LCO *viz.,* 0.9, 1.8 and 3.6 ml/kg as seed treatment and it was compared with recommended seed treatment of *Pseudomonas fluorescens* 10g/kg of seed, *Trichoderma viride* 4g/kg of seed, *Rhizobium* 600g/kg of seed, *Rhizobium* + *Phosphobacteria* 600g/kg of seed and control without any treatment. Fifteen treated seeds were placed in germination paper (Roll towel method) with three replications. The data gathered were analysed in a Complete Randomised Block Design as per the method of Gomez and Gomez (1984).

Vigour index :

Vigour index (VI) was computed using the formula adopted by Abdul-Baki and Anderson (1973) :

VI = Germination percentage × DMP of single seedling (g)

Biochemical parameters :

Chlorophyll content :

The total chlorophyll content of was estimated by adopting the procedure of Yoshida *et al*. (1971) and the contents were expressed as mg g^{-1} of fresh weight.

Soluble protein :

Leaf soluble protein content was estimated in fully expanded young leaf at specified time interval by following procedure of Lowry *et al*. (1951) and expressed as mg g^{-1} of fresh weight basis.

Chlorophyll index :

SPAD chlorophyll meter (Minolta model 502, Japan) was used to measure chlorophyll index. The measurements were taken from physiologically fully expanded leaf in five plants from each replication and the mean values were computed using the method described by Peng *et al.* (1993).

Indole acetic acid oxidase activity (IAA oxidase) :

IAA oxidase activity of leaf was estimated by the method proposed by Paratharasarathy *et al*. (1970) and the enzyme activity was expressed as µg unoxidised auxin g^{-1} h^{-1}.

Leaf area :

Leaf area was measured in both rice and tomato using a Leaf Area Meter (LICOR, Model LI 3000) and expressed as cm^2 $plant^{-1}$.

Photosynthetic rate :

The photosynthetic rate was measured using portable photosynthesis system (LI-6400XT, Licor Inc, Nebraska,USA) of IRGA.and expressed as µmol m^{-2} s^{-1}.

RESULTS AND DISCUSSION

The results of the study are presented in Table 1-5. All the treatments could significantly increase the seed germination over control. Among the seed treatments T_2- *Pseudomonas fluorescens*, T_4- *Rhizobium* and T_8- LCO @ 3.6 ml/kg recorded 100 per cent germination and excluding the control and T_5- *Rhizobium* + *Phosphobacteria*. All other treatments were at par with each other. LCO seed treatment @ 1.8 ml/kg influenced plant height and increased 58.5 per cent over control. Dry weight and vigour index were significantly influenced

by seed treatment of LCO at concentration of 3.6 ml/kg of seed.LCO, signaling molecules when applied as seed treatment to either leguminous or non-leguminous increases the seedling emergence (Prithiviraj *et al*., 2003) which give additional strength to the present experiment. Cell division was induced by nod factor was reported in non –leguminous plants (Daychok *et al*., 2000). Increase in cell cycle may be the reason for better germination

Table 1 : Impact of LCO, microbial inoculums and biocontrol agentsas seed treatment on seedling growth of soybean (± S.E.)

Treatments	No. of seedling	Shoot length (cm)	Root length (cm)
T_1-Control	12.7 ±0.33	13.85±2.07	6.60±0.97
T_2-*Pseudomonas fluorescens* @ 10g/kg	15.0 ±0.00	11.26±0.34	8.57±1.17
T_3-*Trichoderma viride* @ 4g/kg	14.3 ±0.33	17.59±1.28	14.44±1.75
T_4-*Rhizobium* @ 600g/ha	15.0 ±0.00	18.70±0.85	12.90±1.15
T_5-*Rhizobium* + *Phosphobacteria* @ 600g/ha	13.3 ±0.67	17.40±0.95	11.20±1.23
T_6-LCO @ 0.9 ml/kg;	14.3 ±0.33	16.93±0.83	13.53±1.37
T_7-LCO @ 1.8 ml/kg	14.3 ±0.33	17.60±1.01	14.93±1.29
T_8-LCO @ 3.6 ml/kg	15.0 ±0.00	17.27±0.37	10.20±0.35

Table 2 : Impact of LCO, microbial inoculums and biocontrol agents as seed treatmenton seed germination and vigour index of soybean (± S.E.)

Treatments	Germination (%)	Dry weight (g)	Vigour index
T_1-Control	84.5 ± 2.23	0.065 ±0.004	5.53 ±0.44
T_2-*Pseudomonas fluorescens* @ 10g/kg	100.0 ± 0.00	0.086 ±0.009	8.58 ±0.89
T_3-*Trichoderma viride* @ 4g/kg	95.5 ± 2.23	0.076 ±0.004	7.26 ±0.40
T_4-*Rhizobium* @ 600g/ha	100.0 ±0.00	0.082 ±0.007	8.24 ±0.66
T_5-*Rhizobium* + *Phosphobacteria* @ 600g/ha	88.9 ±4.43	0.087 ±0.013	7.78 ±1.46
T_6-LCO @ 0.9 ml/kg;	95.5 ±2.23	0.068 ±0.002	6.52 ±0.23
T_7-LCO @ 1.8 ml/kg	97.8 ±2.23	0.071 ±0.004	6.98 ±0.53
T_8-LCO @ 3.6 ml/kg	100.0 ±0.00	0.110 ±0.016	10.96 ±1.65

Table 3 : Impact of LCO, microbial inoculums and biocontrol agents as seed treatment on biochemical changes in soybean

Treatments	Soluble protein (mg g^{-1})	Chlorophyll content (mg g^{-1})	Chlorophyll index
T_1-Control	5.01±0.095	0.665±0.0126	24.15±0.29
T_2-*Pseudomonas fluorescens* @ 10g/kg	5.12±0.097	0.720±0.0137	24.31±0.33
T_3-*Trichoderma viride* @ 4g/kg	5.28±0.100	0.728±0.0138	24.30±0.31
T_4-*Rhizobium* @ 600g/ha	5.26±0.100	0.782±0.0148	25.29±0.36
T_5-*Rhizobium* + *Phosphobacteria* @ 600g/ha	5.69±0.108	0.799±0.0152	26.30±0.34
T_6-LCO @ 0.9 ml/kg;	5.58±0.106	0.874±0.0166	26.20±0.38
T_7-LCO @ 1.8 ml/kg	6.06±0.115	0.832±0.0158	28.23±0.47
T_8-LCO @ 3.6 ml/kg	5.97±0.113	0.806±0.0153	26.69±0.40

Table 4 : Impact of LCO, microbial inoculums and biocontrol agents as seed treatment on photosynthetic rate and leaf area in soybean

Treatments	Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	Leaf area (cm^2)	IAAO activity (µg of unoxidisedauxin g^{-1} hr^{-1})
T_1-Control	20.52±0.366	75.78±1.641	290.76±5.58
T_2-*Pseudomonas fluorescens* @ 10g/kg	20.83±0.372	76.06±1.647	294.42±5.65
T_3-*Trichoderma viride* @ 4g/kg	21.00 ±0.375	76.55±1.658	298.52±5.73
T_4-*Rhizobium* @ 600g/ha	21.17±0.378	76.70 ±1.658	302.02±5.80
T_5-*Rhizobium* + *Phosphobacteria* @ 600g/ha	21.48±0.383	77.47±1.661	297.38±5.71
T_6-LCO @ 0.9 ml/kg;	21.86±0.390	77.99±1.678	299.41±5.75
T_7-LCO @ 1.8 ml/kg	22.10 ±0.394	78.12±1.689	304.67±5.85
T_8-LCO @ 3.6 ml/kg	21.43±0.382	77.31±1.692	294.67±5.66

Table 5 : Correlation analysis for Shoot length, physiological and biochemical characters with seedling vigour of soybean

	Shoot length (cm)	Chlorophyll index	Soluble protein (mg g^{-1})	Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	Leaf area (cm^2)	Dry weight (g)	Germination (%)	Vigour index
Shoot length (cm)	1.000							
Chlorophyll index	0.560**	1.000						
Soluble protein (mg g^{-1})	0.771**	0.832**	1.000					
Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	0.580**	0.921**	0.724**	1.000				
Leaf area (cm^2)	0.622**	0.918**	0.730**	0.988**	1.000			
Dry weight (g)	0.094	0.263	0.490**	0.020	0.052	1.000		
Germination (%)	0.153	0.296	0.488**	0.337*	0.251	0.467**	1.000	
Vigour index	0.112	0.294	0.540**	0.091	0.095	0.973**	0.656**	1.000

and initiated growth at early stage of soybean.LCO increased plant growth which may be caused by 'hormone like' effect of Nod factor (Souleimanow *et al*., 2002).

The response of LCO on biochemical characters was studied and found that there was significant increase in soluble protein and chlorophyll content. T_7 – LCO @1.8 ml/kg performed reported higher value of soluble protein (6.06 mg g^{-1}) where as in chlorophyll content T_6 – LCO @ 0.9 ml/kg reposed better (0.874 mg g^{-1}). Physiological parameters such as photosynthetic rate and leaf area seem to be not influenced by the LCO seed treatments.Plant growth was enhanced through inducing seed germination (Zhang and Smith, 2001), enhancing plant growth, development and yield for both legume and non-legumes, increasing the photosynthetic rates when sprayed onto the leaves (Almaraz *et al*., 2006).

LCO application mimics modification in cytokinin-auxin balance provoked by application of cytokinins during pseudonodules formation on root in *Macroptilium atropurpureum* (Relic *et al*., 1993). Nod factor activity was reduced at low pH condition. Root hair curling and nod factor production both are related to the interaction of rhizobia and plant root system (Staley, 2003). Biomass accumulation is stimulated by Nod factor which changes the plant structure and morphology (Souleimanov *et al*., 2002). Present result report that LCO, 3.6 ml/kg of soybean was effective to enhance the seed germination which was supported by Supanjani*et al*. (2005). Prithiviraj *et al*. (2003) suggested that, in the case of LCO application, the increase in stomatal aperture was the result of greater photosynthetic CO_2 uptake by the chloroplasts, and not the primary cause of increased photosynthesis rate.

The present result of this experiment clearly shows that LCO enhanced the germination more effectively than microbial inoculum and biocontrol agent through morphogenesis and physiological changes. This indicates that the production of secondary metabolites enhances the soybean seed germination and growth.

REFERENCES

Abdul-Baki, A.A. and Anderson, J.D. (1973). Vigor determination in soybean seed by multiple criteria. *Crop Sci*., **13** : 630-633.

Almaraz, J., Zhou, X., Souleimanov, A. and Smith, D.L. (2006). Gas exchange characteristaics and dry matter accumulation of soybean treated with Nod factors. *J. Plant Physiol*., **164** : 1391-1393.

Bai, Y., Souleimanov, A. and Smith, D.L. (2002). An inducible activator produced by a *Serratia proteamaculans* strain and its soybean growth-promoting activity under greenhouse condition. *J.Exp. Bot.*, **53** : 1495-1502.

Daychok, J.V., Tobin, A.E., Price, N.P.J. and Von Arnold, S. (2000). Rhizobial Nod factors stimulate somatic embryo development in *Piceaabies*. *Plant Cell Rep*., **19** : 290-297.

Evans, L.S., Lewin, K.F. and Vella, F.A. (1980). Effect of nutrient medium pH on symbiotic nitrogen fixation by *Rhizobium leguminosarum* and *Pisumsativum*. *Plant & Soil*, **56** : 71-80.

Gomez, K.A. and Gomez, A.A.(1984). *Statistical procedures for agricultural research*. An IRRI book, Wiley Interscience Publication, John Wile and Sons, New York, USA. pp. 680.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**: 265-275.

McKay, I.A. and Djordjevic, M.A. (1993). Production and

excretaion of nod metabolites by *Rhizobium leguminosarum* bv *trifolli* are disrupted by the same environmental factors that reduce nodulation in the field. *Appl. & Environ. Microbiol.,* **59** : 3385-3392.

Miransari, M. and Smith, D. (2009). RhizobialLipo-chitooligosaccharides and gibberellins enhance barley (*Hordeum vulgare* L.) seed germination. *Biotechnol*., **8**(2) : 270-275.

Parthasarathy, K., Balu, D.R.C. and Rao, P.S. (1970). Studies on sandal spur. VII. Polyphenol oxidase activity and metabolism of sandal (*Santalum album*) in healthy and diseased. *Proc. Indian Acad. Sci.,* **72**: 277-284.

Peng, S., Garcia, F.V., Laza, R.C. and Cassman, K.G. (1993). Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration. *Agron J.*, **85** : 987–990.

Prithiviraj, B., Zhou, X., Souleimanov, A., Khan, 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*, **21**: 437-445.

Relic, B., Talmont, F., Korsinska, J., Golonowski, W., Prome, J.C. and Broughton, W.J. (1993). Biological activity of *Rhizobium* sp. NGR234 Nod factors on *Macroptiliumatro purpureum*. *Mol. Pl. Microbe Interact*., **6** : 764-774.

Richardson, A.E., Djordjevic, M.A., Rolfe, B.G. and Simpson, R.J. (1988). Effect of pH, Ca, and Al on the exudation from clover seedling of compounds that induce the expression of nodulation genes in *Rhizobium trifolii*. *Plant & Soil*, **109** : 37-47.

Smith, D.L., Prithiviraj, B. and Zhang, F. (2002). Rhizobial signals and control of plant growth. In: Nitrogrn Fixation: Global Perspectives, Finan, T.M., M.R. O'Brain, D.B. Layzell, K. Vessey and W.E. Newton (Eds.). CABI Piblishing, Wallingford, UK., pp: 327-330.

Souleimanow, A., Prithivirajand, B. and Smith, D.L. (2002). The major Nod factor of *Bradyrhizobium japonicum* promotes early growth of soybean and corn. *J. Exp. Bot*., **53** : 1929-1934.

Staley, T.E. (2003). Initial white clover nodulation under saturation levels of rhizobia relative to low-level liming of an acidic soil. *Soil Sci.*, **168** : 540-551.

Stokkermans, T.J.W., Ikeshita, S., Cohn, J., Carlson, R.W., Stacey, G., Ogawa, T. and Peters, N.K. (1995). Structural requirements of synthetic and natural product lipo-chitin oligosaccharides for induction of nodule primordial on *Glycine soja*. *Pl. Physiol*., **108**: 1587-1595.

Supanjani, F., Lee, K.D., Duzan, H. and Smith, D.L. (2009). Effect of Lipo-chitooligosaccharide on germination and seedling growth of cauliflower. *J. Akta Agrosia*., **12** (1) : 75-82.

Supanjani, F., Maboob, A. Souleimanov, K.D., Lee and Smith, D.L. (2005). Stability and activity of the major nod factor produced by *Bradyrhizobium japonicum* following purification, sterilization and storage. *Crop Sci*., **45** : 1281-1285.

Yoshida, S., Forno, D.A. and Cock, J.H. (1971). Laboratory manual for physiological studies of rice. IRRI Publication, Philippines, 36-37pp.

Zhang, F. and Smith, D.L. (2001). Interorganismal signaling in suboptimum environments: The legume-rhizobia symbiosis. *Adv. Agron*., **76** : 125-161.