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(54) Title: LIPO-CHITOOOLIGOSACCHARIDE AND FLAVONOID COMBINATION FOR ENHANCED PLANT GROWTH AND YIELD

(57) Abstract: Compositions and methods for enhancing plant growth and crop yield are described. The compositions include lipo-chitooligosaccharides in combination with flavonoid compounds. The method includes applying the compositions to seeds and/or plants.



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**LIPO-CHITOLIGOSACCHARIDE AND FLAVONOID COMBINATION
FOR ENHANCED PLANT GROWTH AND YIELD****BACKGROUND OF THE INVENTION**

5 The present invention relates to compositions and methods for enhancing plant growth and crop yield. The compositions include lipo-chitooligosaccharides in combination with flavonoid compounds. The method includes applying the compositions to seeds and/or plants.

10 Nitrogen fixation plays a vital role in agricultural production by making atmospheric nitrogen available in a form that can be used by plants. In plants of the Leguminosae family, the symbiotic interaction between the plants and nitrogen-fixing bacteria of the Rhizobiaceae family ("rhizobia") enhances plant growth and crop yield. The symbiotic interaction is initiated when a plant releases flavonoid compounds that
15 stimulate rhizobial bacteria in the soil to produce "Nod-factors." Nod-factors are signaling compounds that induce the early stages of nodulation in plant roots, which lead to the formation of nodules of nitrogen-fixing bacterial colonies within the roots. Although this process occurs naturally over time in legumes, agricultural procedures have been developed to begin the process earlier. These procedures include providing
20 nitrogen-fixing bacteria to seeds or soil and applying Nod factors directly to seeds prior to planting.

 Nod factors have recently been shown to also enhance the germination, growth and yield of legumes and non-legumes through processes other than nodulation
25 (US6,979,664; Prithivaraj *et al.*, *Planta* 216: 437-445, 2003). Although the effects of Nod factors on nodulation have been widely studied and reviewed, *e.g.*, Ferguson and Mathesius, J. *Plant Growth Regulation* 22: 47-72, 2003, the mechanisms for Nod factor effects independent of nodulation are not well understood. Application of Nod factors to seeds of legumes and non-legumes stimulates
30 germination, seedling emergence, plant growth and yield in crop and horticultural plant species, *e.g.*, as described in US6,979,664 and US5,922,316. Foliar application of Nod factors has also been demonstrated to increase photosynthesis (US7,250,068), fruiting and flowering (WO 04/093,542) in crop and horticultural plant species.

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Nod factors are lipo-chitooligosaccharide compounds (LCO's). They consist of an oligomeric backbone of β -1,4-linked *N*-acetyl-D-glucosamine ("GlcNAc") residues with an N-linked fatty acyl chain at the nonreducing end. LCO's differ in the number of GlcNAc residues in the backbone, in the length and degree of saturation of the fatty acyl chain, and in the substitutions of reducing and nonreducing sugar residues. LCO structure is characteristic for each rhizobial species, and each strain may produce multiple LCO's with different structures. LCO's are the primary determinants of host specificity in legume symbiosis (Diaz, Spaink, and Kijne, *Mol. Plant-Microbe Interactions* 13: 268-276, 2000).

LCO synthesis can be stimulated by adding the appropriate flavonoid during bacterial fermentation with a given genus and species of rhizobium. The flavonoid molecules bind to the rhizobium and turn on bacterial genes for the production of specific LCO's which are released into the fermentation medium. In nature, leguminous plants release the appropriate flavonoid, which binds to soil rhizobia, turning on genes for LCO production. These LCO's are released by bacteria into the soil, bind to the roots of leguminous plants, and initiate a cascade of plant gene expression that stimulates formation of nitrogen-fixing nodule structures on legume roots. Alternatively, modified and synthetic LCO molecules can be produced through genetic engineering or chemical synthesis. Synthetic LCO's interact with plants and stimulate nodulation in the same manner as naturally produced molecules.

Flavonoids are phenolic compounds having the general structure of two aromatic rings connected by a three carbon bridge. Flavonoids are produced by plants and have many functions, *e.g.*, as signaling molecules and as protection against insects, animals, fungi and bacteria. Classes of flavonoids include chalcones, anthocyanidins, coumarins, flavones, flavanols, flavonols, flavanones, and isoflavones. (Jain and Nainawatee, *J. Plant Biochem. & Biotechnol.* 11: 1-10, 2002; Shaw, *et al.*, *Environmental Microbiol.* 11: 1867-1880, 2006.)

Therefore it is an object of the present invention to provide compositions and special methods enhancing plant growth as well as flowering of the plants but also the crop yield of legumes and non-legumes.

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SUMMARY OF THE INVENTION

An exemplary composition comprises at least one lipo-chitooligosaccharide and at least one flavonoid compound selected from the group consisting of flavones, flavanols, flavonols, flavanones, and isoflavones. An exemplary method comprises administering a composition according to the invention to a plant or seed in an effective amount for enhancing plant growth or crop yield. In another embodiment, the method comprises sequentially treating a plant or a seed with at least one lipo-chitooligosaccharide and at least one flavonoid compound selected from the group consisting of flavones, flavanols, flavonols, flavanones, and isoflavones.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides compositions and methods for enhancing plant growth and crop yield, and arises from the results of experiments, reported herein, that reveal unexpected, synergistic effects of a combination of lipo-chitooligosaccharide and flavonoid compounds on plant growth and crop yield when applied to seeds and/or foliage. Thus, a composition for enhancing plant growth and crop yield is provided which comprises at least one lipo-chitooligosaccharide and at least one flavonoid compound selected from the group consisting of flavones, flavanols, flavonols, flavanones, and isoflavones. Preferably the flavonoid compound is selected from the group consisting of genistein, daidzein, formononetin, naringenin, hesperetin, luteolin, and apigenin.

The lipo-chitooligosaccharide, which is used for the preparation of the composition, consists of a lipo-chitooligosaccharide produced by bacterial genera selected from the group consisting of *Bradyrhizobium*, *Rhizobium*, *Sinorhizobium*, and *Mesorhizobium*. Said lipo-chitooligosaccharide may be produced at least in part by chemical synthesis. In a special embodiment of the present invention the lipo-chitooligosaccharide is produced at least in part by a genetically modified cell or organism. Compositions according to the invention comprise the lipo-chitooligosaccharide at a concentration of between about 10^{-5} M to about 10^{-14} M, preferably at a concentration of between about 10^{-6} to 10^{-10} M. In addition to the lipo-chitooligosaccharide the composition comprises a bacterium that produces this compound. In comparison to the concentration of the comprising LCOs the effective

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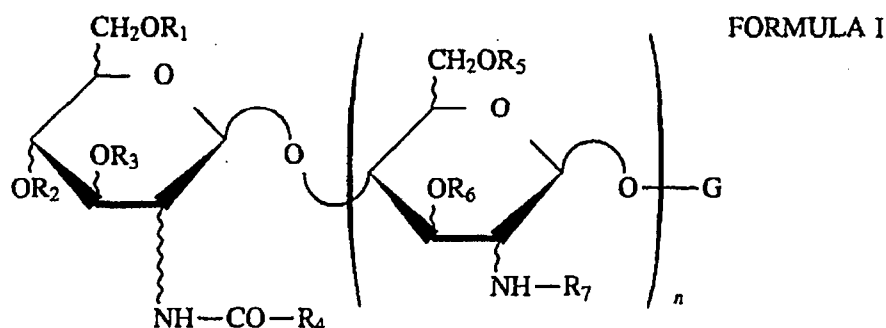
concentration of flavonoids has to be higher. Hence in compositions according to the invention the flavonoid compound is present at a concentration of between about 20 μM to about 800 μM . If the concentration of the flavonoid compound is in the range of a concentration between about 100 μM to about 500 μM , very favorable growth rates
5 are achieved.

A further embodiment of the present invention is a method for enhancing plant growth or crop yield comprising administering to a plant or a seed said composition in an effective amount for enhancing plant growth or crop yield. The treated plant may be a legume or a non-legume. Preferably if the treated plant is selected from the
10 group consisting of soybeans, peas, chickpeas, drybeans, peanuts, clover, alfalfa, corn, cotton, rice, tomatoes, canola, wheat, barley, sugar beet, and grass, increased growth and harvest yields are achieved. In order to process the method described here, the composition comprising at least one lipo-chitooligosaccharide and at least one flavonoid compound selected from the group consisting of flavones, flavanols,
15 flavonols, flavanones, and isoflavones, is administered by applying the composition to one or more of foliage, seeds, and soil that is in the immediate vicinity of the plant or seed. The composition, which is applied to a plant or a seed in this method preferably comprises at least one lipo-chitooligosaccharide and at least one flavonoid compound selected from the group consisting of flavones, flavanols, flavonols, flavanones, and
20 isoflavones, in an effective amount for enhancing plant growth or crop yield. In the composition, which is used in this method, the comprising flavonoid compound may be selected from the group consisting of genistein, daidzein, formononetin, naringenin, hesperetin, luteolin, and apigenin.

A special embodiment of the method of the present invention for enhancing
25 plant growth or crop yield comprises a sequential administration to a plant or a seed, in either order, a first composition comprising at least one lipo-chitooligosaccharide in an effective amount for enhancing plant growth or crop yield, and a second composition comprising at least one flavonoid compound, selected from the group consisting of flavones, flavanols, flavonols, flavanones, and isoflavones, in an effective
30 amount for enhancing plant growth or crop yield. In this combination flavonoid compounds selected from the group consisting of genistein, daidzein, formononetin, naringenin, hesperetin, luteolin, and apigenin are especially preferred.

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For the purposes of this invention, a "lipo-chitooligosaccharide" ("LCO") is a compound having the general LCO structure, *i.e.*, an oligomeric backbone of β -1,4-linked *N*-acetyl-D-glucosamine residues with an N-linked fatty acyl chain at the non-reducing end, as described in U.S. Pat No. 5,549,718; U.S. Pat No. 5,646,018; U.S. Pat No. 5,175,149; and U.S. Pat No. 5,321,011.



in which

G is a hexosamine which is variously substituted, for example, by an acetyl group on the nitrogen, a sulfate group, an acetyl group and /or an ether group on an oxygen,

$R_1, R_2, R_3, R_5, R_6,$ and R_7 , which may be identical or different, represent H, CH_3CO-, C_xH_yCO- where x is an integer between 0 and 17, and y is an integer between 1 and 35, or any other acyl group such as for example a carbamyl,

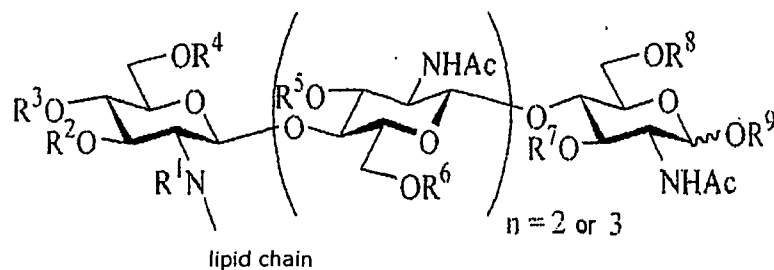
R_4 represents a mono-, di- or triunsaturated aliphatic chain containing at least 12 carbon atoms and as such represents a lipid chain,

n is an integer between 1 and 4.

According to an advantageous form of this embodiment the lipo-oligosaccharide conforming to the invention is characterized in that it is of the formula (II) below.

This basic structure may contain modifications or substitutions found in naturally occurring LCO's, such as those described in Spaink, *Annual Review in Microbiology* 54: 257-288, 2000; D'Haeze and Holsters, *Glycobiology* 12: 79R-105R, 2002. Also encompassed by the invention are synthetic compounds having the basic structure of an LCO, such as those described in WO2005/063784:

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Formula II

in which

- n represents 1, 2 or 3;
- 5 R_1 represents a substituent chosen from H, C_{1-6} -alkyl, $C(O)H$ and $C(O)CH_3$;
 R_2, R_3, R_6 represent independently of each other a substituent chosen from H, C_{1-6} -alkyl, $C(O)C_{1-6}$ -alkyl, $-C(S)C_{1-6}$ -alkyl, $-C(O)OC_{1-6}$ -alkyl, $-C(O)NH_2$, $-C(S)NH_2$, $-C(NH)NH_2$, $-(C(O)NHC_{1-6}$ -alkyl, $-C(S)NHC_{1-6}$ -alkyl, and $-C(NH)NHC_{1-6}$ -alkyl;
- R_4 represents a substituent chosen from H, C_{1-6} -alkyl, and R_{21}
- 10 R_5 represents a substituent chosen from H, C_{1-6} -alkyl, fucosyl and R_{22}
 R_7 represents a substituent chosen from H, C_{1-6} -alkyl, arabinosyl and R_{23}
 R_8 represents a substituent chosen from H, C_{1-6} -alkyl, fucosyl, methylfucosyl, sulfofucosyl, acetylfucosyl, arabinosyl, SO_3H , SO_3Li , SO_3Na , SO_3K , $SO_3N(C_{1-8}$ -alkyl) $_4$ and R_{24} ,
- 15 R_9 represents a substituent chosen from H, C_{1-6} -alkyl, mannose, glycerol, and R_{25} ;
 $R_{21}, R_{22}, R_{23}, R_{24}, R_{25}$ represent, independently of each other, a substituent chosen from $C(O)C_{1-6}$ -alkyl, $-C(S)C_{1-6}$ -alkyl, $-C(O)OC_{1-6}$ -alkyl, $-C(O)NH_2$, $-C(S)NH_2$, $-C(NH)NH_2$, $-C(O)NHC_{1-6}$ -alkyl, $-C(S)NHC_{1-6}$ -alkyl, and $-C(NH)NHC_{1-6}$ -alkyl,

20 These lipochito-oligosaccharides (LCO) either may be isolated directly from a particular culture of rhizobia, synthesized chemically, or obtained chemo-enzymatically. Via the latter method, the oligosaccharide skeleton may be formed by culturing of recombinant Echerichia coli bacterial strains in a fermenter, and the lipid chain may then be attached chemically.

25

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This means, that modified LCO's may be produced through genetic engineering and precursor oligosaccharide molecules for the construction of LCOs may be synthesized by genetically engineered organisms, *e.g.*, as disclosed in Samain *et al.*, *Carbohydrate Research* 302: 35-42, 1997.

5 LCO's used in embodiments of the present invention may also be recovered from the culture medium of fermentation cultures of Rhizobiaceae bacterial strains that produce LCO's, such as strains of *Azorhizobium*, *Bradyrhizobium* (including *B. japonicum*), *Mesorhizobium*, *Rhizobium* (including *R. leguminosarum*), *Sinorhizobium* (including *S. meliloti*), and bacterial strains genetically engineered to produce LCO's.
10 These methods are known in the art and have been described, for example, in U.S. Pat. Nos. 5,549,718 and 5,646,018, which are incorporated herein by reference. Commercial products containing LCO's are available, such as OPTIMIZE® (EMD Crop BioScience).

LCO's may be utilized in various forms of purity and may be used alone or with
15 rhizobia. Methods include simply removing the rhizobial cells from the mixture or continuing to isolate and purify the LCO molecules through LCO solvent phase separation followed by HPLC chromatography as described by Lerouge, *et.al* (US 5,549,718). Purification can be enhanced by repeated HPLC, and the purified LCO molecules can be freeze-dried for long-term storage. This method is acceptable for
20 the production of LCO's from all genera and species of the Rhizobiaceae.

Within the legume family, specific genera and species of rhizobium develop a symbiotic nitrogen-fixing relationship with a specific legume host. These plant host:rhizobia combinations are described in Hungria and Stacey, *Soil Biol. Biochem.* 29: 819-830, 1997, which also lists the effective flavonoid *Nod* gene inducers of the
25 rhizobial species, and the specific LCO structures that are produced by the different rhizobial species. However, LCO specificity is only required to establish nodulation in legumes. It is not necessary to match LCO's and plant species to stimulate plant growth and/or crop yield when treating seeds or foliage of a legume or non-legume with LCO's.

30 Appropriate flavonoids include compounds from the classes of flavones, flavanols, flavonols, flavanones, and isoflavones. Such compounds may include, but are not limited to, genistein, daidzein, formononetin, naringenin, hesperetin, luteolin, and apigenin. Flavonoid compounds are commercially available, *e.g.*, from Natland

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International Corp., Research Triangle Park, NC; MP Biomedicals, Irvine, CA; LC Laboratories, Woburn MA. Flavonoid compounds may be isolated from plants or seeds, *e.g.*, as described in US5,702,752; US5,990,291; US6,146,668, which are incorporated herein by reference. Flavonoid compounds may also be produced by
5 genetically engineered organisms, such as yeast, as described in Ralston, *et al.*, *Plant Physiology* 137: 1375-1388, 2005, and which is incorporated herein by reference.

In one embodiment of the invention, the composition may be prepared by combining one or more flavonoid(s) and one or more LCO(s) in an agriculturally appropriate solvent. An "effective amount" of the composition is an amount that
10 increases plant growth or crop yield when compared with the growth or crop yield of plants or seeds that have not been treated with the composition. For example, flavonoid concentration in the composition may range from 20-800 μM , preferably 100-500 μM . LCO concentration in the composition according to the present invention may range from 10^{-5} M to 10^{-14} M, preferably from 10^{-6} M to 10^{-10} M. The LCO
15 component may consist of purified or partly purified LCO, or a mixture of the LCO and the rhizobia that produce the LCO. The agriculturally appropriate solvent is preferably an aqueous solvent, such as water.

Although it is convenient to combine and apply the flavonoid and LCO components in a single mixture, in one embodiment of the invention the flavonoid component and the LCO component may be applied separately and sequentially in
20 either order. The term "composition" herein applies to both combined and sequentially applied mixtures of flavonoid and LCO components. Other additives that may be applied either simultaneously or sequentially include fertilizers (*e.g.*, calcium, nitrogen, potassium, phosphorous), micronutrients (*e.g.*, metal ions), and pesticides
25 (*e.g.*, fungicides, insecticides, nematicides, and herbicides).

The term "plant" as used herein includes tubers, roots, stems, leaves, flowers, and fruits. The composition may be applied directly to seeds or plants or may be placed in soil in the vicinity of a seed or plant prior to or at the time of planting. In a preferred embodiment, the composition is sprayed on seeds, tubers, or foliage.
30 Seedlings, as well as more mature plants, may be treated. Flowers and fruits may also be treated by spraying. Roots of transplants may be sprayed or dipped in the composition prior to planting.

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The composition may be applied to monocot or dicot plants, and to legumes and non-legumes. In one embodiment, the composition is applied to field-grown plants. In another embodiment, the composition is applied to greenhouse-grown plants. For example, the composition may be applied to seeds or foliage of legumes, such as soybeans, peas, chickpeas, dry beans, peanuts, clover, alfalfa, and of non-
5 legumes such as corn, cotton, rice, tomatoes, canola, wheat, barley, sugar beet, and grass. In general, the composition is applied to seeds in a single application, and the seeds may be planted immediately or stored before planting. The composition also may be applied to soil that is in the immediate vicinity of the plant or the seed.
10 Advantageously the composition may also be applied to foliage. Foliar application generally consists of spraying the composition on the plant foliage one or more times during the growing period. In addition, if the flavonoid compound and LCO are applied sequentially, the flavonoid compound may be applied to seeds and the LCO to foliage.

Good results and increased grain yields may be achieved when liquid
15 formulations of LCO and flavonoid are applied on seed. As a side-effect it was found, that the administration of the compositions according to the present invention the treated plants are more resistant against harm caused by fungi and bacteria, but also against that caused by insects and animals. In a special embodiment of the present invention for the preparation of the liquid formulation a LCO product may be used,
20 which can be obtained for example from *Rhizobium leguminosarum bv viceae*. This LCO product may be used in concentration of about 1×10^{-8} M. The flavonoid product may have a concentration of about 10 mM and may be consisting of genistein and daidzein. Such a flavonoid product may be a customary product like ReVV® (EMD Crop BioScience), consisting of genistein and daidzein in a ratio of 8 : 2 w/w. Both liquid
25 formulations may be used together or separately for the treatment. For example improved results may be achieved, if a mixture of these liquid formulations is applied on corn seed and the use rate for the LCO and flavonoid products is about 97,9 and 12,0ml/100kg, respectively. For the application the products are each diluted with water appropriately. Then the prepared aqueous mixture is applied on seed at a slurry
30 rate of about 998ml/100kg. Preferably the treated seeds are planted after applying of nitrogen as urea in advance of planting. It is also advantageous to apply starter fertilizer at planting. For example a useful customary starter fertilizer is 7-21-7 starter fertilizer, which is made with two K sources, which are either sulfate of potash (SOP) or muriate of potash (MOP) and ammonium polyphosphate. After growth an increased

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grain yield can be found in a range of about 10 % in comparison to the growth yield of corn seeds, which were not treated with liquid formulations of LCO and flavonoid.

Improved yields are also achieved if the same liquid solutions of the LCO product and of the flavonoid product are prepared and applied separately on the seed and into the furrow or spray-applied to foliar. In a special embodiment the flavonoid product may be applied to the seed in the same use rate as described above (12,0ml/100 kg and slurry rate in water of 998 ml/100kg) and the LCO product may be applied to the seed furrow at planting at a rate of 1,17L/ha in 46,81L of water, whereby the concentration of LCO in the liquid formulation, which is used is about 1×10^{-8} M. Another preferred embodiment of the present invention is to apply the flavonoid product to the seed as described and to spray-apply the LCO product to foliar surfaces. The spray-application may be processed at a rate of 2,34 L/ha in 234L/ha of water at the V4 stage of corn development. As such the seed/furrow and seed/foliar applications may be processed at the same rates when applied alone or in combination.

In a more preferred embodiment of the present invention a combination of flavonoid and LCO is used for the seed and furrow application and in a most preferred embodiment such a combination is used for seed and foliar application. The latter type of application leads to the most increased growth yields.

In special experiments comparable results are achieved if soybeans are treated accordingly with the same liquid solutions of flavonoid and LCO. This result remains unchanged, if the application of flavonoid to the seed is varied to a slurry rate in water of 277ml/100kg.

As already mentioned above the same advantageous effects can be shown if seeds and plants of legumes, such as peas, chickpeas, dry beans, peanuts, clover, alfalfa, and of non-legumes such as corn, cotton, rice, tomatoes, canola, wheat, barley, sugar beet, and grass are treated according to the present invention.

The present description enables the person skilled in the art to apply the invention comprehensively. In the case of any lack of clarity, it goes without saying that the cited publications and patent literature should be employed. Accordingly, these documents are regarded as part of the disclosure content of the present description.

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For better understanding and in order to illustrate the invention, examples are given below which are within the scope of protection of the present invention. These examples also serve to illustrate possible variants. Owing to the general validity of the inventive principle described, however, the examples are not suitable for reducing the scope of protection of the present application to these alone.

It goes without saying to the person skilled in the art that, both in the examples given and also in the remainder of the description, the component amounts given in % present in the compositions always only add up to 100% based on the composition as a whole, and cannot go beyond this, even if higher values could arise from the percentage ranges indicated. In addition to this, % values are by weight based on the composition as a whole or as mol-%. Apart from that, concentrations are given as molar (M) concentrations, yields as kg per area like ha and applied quantities on seeds as ml or L per kg. Further scale units are self-explaining.

15

EXAMPLES

Example 1

A corn field trial was conducted evaluating the effect of liquid formulations of LCO and flavonoid on grain yield when applied alone or in combination on seed. The field trial was conducted at a site near Whitewater, WI in a Plano silt loam soil. The soil had a pH of 6.5 and soil test results showed an organic matter content of 4.4% by weight and phosphorus and potassium content of 42 and 146 ppm, respectively. The field was previously planted to soybeans. It was fall chisel plowed and field cultivated in the spring prior to planting.

25

The LCO product used in the trial contained approximately 1×10^{-8} M LCO from *Rhizobium leguminosarum* by *viceae*. The flavonoid product used (ReVV®, EMD Crop BioScience) had a 10 mM concentration of genistein and daidzein in a ratio of 8:2 w/w.

30

The corn seed used in the trial was Pioneer variety 38H52. The use rate for the LCO and flavonoid products were 97.9 and 12.0 ml/100 kg, respectively. The products

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were each diluted with water and applied on seed at a slurry rate of 998 ml/100 kg. The LCO/flavonoid combination was applied at the same concentration and slurry rate as when applied alone. The study was conducted in a randomized complete block design, with a plot size of 3.05 m by 15.24 m, with 76.2 cm row spacing, and four
 5 replications per treatment. Seeds were planted at a depth of 5.1 cm at a seeding rate of 81,500 seeds per ha. Planting was carried out using a four row precision vacuum planter. One hundred and fifty-seven kg/ha of nitrogen was applied as urea in advance of planting, and an additional 168 kg of 7-21-7 starter fertilizer was applied at planting.

10

Results of the study are shown in Table 1. The flavonoid treatment statistically increased grain yield by 332 kg/ha, while the LCO treatment numerically increased grain yield by 207 kg/ha. Application of the two products in combination resulted in a statistically significant increase in yield over each of the two products administered
 15 alone. The increase observed with the combination treatment of 1205 kg/ha unexpectedly exceeded the combined effect of the individual products alone (539 kg/ha) by more than two-fold.

Table 1

Treatment	Application	Grain yield (kg/ha)
Control	None	8945
LCO	Seed	9152
Flavonoid	Seed	9277
Flavonoid + LCO	Seed	10150
Probability %		<0.1
LSD 10%		263.6
CV%		4.4

20

Example 2

A second corn trial was conducted as described in Example 1 at a location near
 25 Fergus Falls, MN, in a nutrient rich loam soil previously planted to soybeans. The LCO

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and flavonoid products were applied alone or in combination on DynaGro variety 51K74 corn seed. The study was conducted in a randomized complete block design, with a plot size of 3.05 m by 6.10 m, with 76.2 cm row spacing, and four replications per treatment.

5

Results of the study are shown in Table 2. The LCO and flavonoid seed treatments numerically increased grain yield compared to the non-treated control by 461 and 965 kg/ha, respectively. Application of the two products in combination statistically increased yield compared to the control by 1506 kg/ha, and by 1045 kg/ha compared to the LCO treatment. As in Example 1, the increase in yield observed with the combined treatment exceeded the total increase in yield from the individual products alone.

15

Table 2

Treatment	Application	Grain yield (kg/ha)
Control	None	8860
LCO	Seed	9321
Flavonoid	Seed	9825
Flavonoid + LCO	Seed	10366
Probability %		<0.1
LSD 5%		871.4
CV%		6.3

Example 3

20

A corn field trial was conducted at the same site described above in Example 1 to evaluate the effect of flavonoid seed treatment on grain yield compared to application of LCO either in the seed furrow at planting or spray-applied as a foliar application. These individual product treatments were additionally compared to flavonoid seed treatment combined with in-furrow LCO application and flavonoid seed

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treatment combined with foliar LCO application. The LCO and flavonoid products were the same as those used in the prior examples.

5 The corn seed used in the trial was Dairyland variety DSR 4497. The flavonoid product was applied at the same use rate of 12.0 ml/100 kg and slurry rate in water of 998 ml/100 kg as in prior examples. The LCO product was applied at planting in the seed furrow at a rate of 1.17 L/ha in 46.81 L of water, or spray-applied to foliar surfaces at a rate of 2.34 L/ha in 234 L/ha of water at the V4 stage of corn development. The seed/furrow and seed/foliar applications were at the same rates for
10 the combination as when applied alone.

The study was conducted in a randomized complete block design, with a plot size of 3.05 m by 15.24 m, with 76.2 cm row spacing, and four replications per treatment. Seeds were planted at a depth of about 5.1 cm at a seeding rate of 81,500
15 seeds per ha. Planting was carried out using a four row precision vacuum planter. One hundred and fifty-seven kg/ ha of nitrogen was applied as urea in advance of planting, and an additional 168 kg of 7-21-7 starter fertilizer was applied at planting.

Results of the study are shown in Table 3. Application of flavonoid on seed and
20 LCO in the seed furrow numerically increased grain yield by 270 and 151 kg/ha, respectively, compared to the control treatment. In contrast, combined application of the two products on seed and in furrow statistically increased yield by 345 kg/ha.

Separate application of flavonoid on seed and LCO as a foliar application
25 resulted in a numerical increase in yield with flavonoid seed treatment of 270 kg/ha (as stated above) and a statistically significant increase of 464 kg/ha with LCO foliar application. Combined flavonoid seed treatment and LCO foliar application further increased yield by 577 kg/ha compared to the control treatment.

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Table 3

Treatment	Application	Grain yield (kg/ha)
Control	None	10897
Flavonoid	Seed	11167
LCO	Furrow	11048
LCO	Foliar	11361
Flavonoid / LCO	Seed, furrow	11242
Flavonoid / LCO	Seed, foliar	11474
Probability %		<0.1
LSD 10%		307.6
CV%		5.3

Example 4

A parallel corn field trial was conducted at the same location and with the same treatments and trial design as described in Example 3, but differing in the variety of corn used (Spangler 5775).

Results of the study are shown in Table 4. Application of flavonoid on seed statistically increased grain yield by 465 kg/ha compared to the non-treated control, while LCO application in the seed furrow numerically increased grain yield by 220 kg/ha. Combined flavonoid seed treatment and LCO furrow application further increased yield by 609 kg/ha compared to the control treatment.

Separate application of flavonoid on seed and LCO as a foliar application resulted in a statistically significant increase in yield with flavonoid seed treatment of 465 kg/ha (as stated above) and a numerical increase of 69 kg/ha with LCO foliar application. Application of the two products in combination resulted in a statistically significant increase in yield greater than that seen for each of the two products alone. Further, the increase observed with the combination treatment (1017 kg/ha) exceeded the combined effect of the individual products alone (534 kg/ha).

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Table 4

Treatment	Application	Grain yield (kg/ha)
Control	None	10087
Flavonoid	Seed	10552
LCO	Furrow	10307
LCO	Foliar	10156
Flavonoid / LCO	Seed, furrow	10696
Flavonoid / LCO	Seed, foliar	11104
Probability %		<0.1
LSD 10%		351.5
CV%		4.8

Example 5

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A soybean field trial was conducted to evaluate the effect of flavonoid seed treatment on grain yield compared to the effect of foliar application of LCO. The individual product treatments were additionally compared to flavonoid seed treatment combined with LCO foliar application. The LCO and flavonoid products were the same as those used in prior examples.

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The field trial was conducted at a site near Whitewater, WI in a Milford silty clay loam soil. The soil had a pH of 6.5 and soil test results showed an organic matter content of 4.7% and phosphorus and potassium content of 48 and 136 ppm, respectively. The field was no-till and was previously planted to corn.

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The soybean seed used in the trial was Dairyland variety DSR 1701. The flavonoid product was applied at a use rate of 12.0 ml/100 kg and slurry rate in water of 277 ml/100 kg. The LCO product was spray-applied to foliar surfaces at a rate of 2.34 L/ha in 234 L/ha of water at the V4 stage of soybean development. The combined seed/foliar application was at the same rate as when applied alone. The study was conducted in a randomized complete block design, with a plot size of 3.05 m by 15.24 m, with 76.2 cm row spacing, and four replications per treatment.

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Seeds were planted at a depth of 2.5 cm at a seeding rate of 395,400 seeds per ha. Planting was carried out using a John Deere 750 NT grain drill.

Results of the study are shown in Table 5. Application of flavonoid on seed statistically increased grain yield by 215 kg/ha compared to the non-treated control, while LCO foliar application numerically increased grain yield by 81 kg/ha. Application of the two products in combination resulted in a statistically significant increase above each of the two products alone, with the increase in yield (336 kg/ha) exceeding the combined effect of the individual products alone (296 kg/ha).

Table 5

Treatment	Application	Grain yield (kg/ha)
Control	None	3214
Flavonoid	Seed	3429
LCO	Foliar	3295
Flavonoid / LCO	Seed/foliar	3550
Probability %		<0.1
LSD 10%		87.4
CV%		5.2

Example 6

A parallel soybean field trial was conducted at the same location and with the same treatments and trial design as described in Example 5, but differing in the variety of soybean used (Dairyland variety DSR 2000).

Results of the study are shown in Table 6. Application of flavonoid on seed and LCO as a foliar application statistically increased grain yield by 175 and 303 kg/ha, respectively, compared to the non-treated control. Combined flavonoid seed treatment and LCO foliar application further increased yield by 478 kg/ha compared to the control treatment.

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Table 6

Treatment	Application	Grain yield (kg/ha)
Control	None	2750
Flavonoid	Seed	2925
LCO	Foliar	3053
Flavonoid / LCO	Seed/foliar	3228
Probability %		<0.1
LSD 10%		121.0
CV%		4.5

Although preferred embodiments of the invention have been shown and
described herein, it will be understood that such embodiments are provided by way of
example only. Numerous variations, changes and substitutions will occur to those
skilled in the art without departing from the spirit of the invention. Accordingly, it is
intended that the appended claims cover all such variations as fall within the spirit and
scope of the invention.

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What is Claimed:

1. A composition for enhancing plant growth and crop yield comprising at least one lipo-chitooligosaccharide and at least one flavonoid compound selected from the group consisting of flavones, flavanols, flavonols, flavanones, and isoflavones.
2. The composition of claim 1, wherein the lipo-chitooligosaccharide consists of a lipo-chitooligosaccharide produced by bacterial genera selected from the group consisting of *Bradyrhizobium*, *Rhizobium*, *Sinorhizobium*, and *Mesorhizobium*.
3. The composition of claim 1, wherein the lipo-chitooligosaccharide is produced at least in part by chemical synthesis.
4. The composition of claim 1, wherein the lipo-chitooligosaccharide is produced at least in part by a genetically modified cell or organism.
5. The composition of claim 1, wherein the flavonoid compound is selected from the group consisting of genistein, daidzein, formononetin, naringenin, hesperetin, luteolin, and apigenin.
6. The composition of claim 1, wherein the lipo-chitooligosaccharide is present at a concentration of between about 10^{-5} M to about 10^{-14} M.
7. The composition of claim 6, wherein the lipo-chitooligosaccharide is present at a concentration of between about 10^{-6} to 10^{-10} M.
8. The composition of claim 1 further comprising a bacterium that produces a lipo-chitooligosaccharide.
9. The composition of claim 1, wherein the flavonoid compound is present at a concentration of between about 20 μ M to about 800 μ M.
10. The composition of claim 9, wherein the flavonoid compound is present at a concentration of between about 100 μ M to about 500 μ M.
11. A method for enhancing plant growth or crop yield comprising administering to a plant or a seed the composition of claim 1 in an effective amount for enhancing plant growth or crop yield.
12. The method of claim 11, wherein the plant is a legume or a nonlegume.

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13. The method of claim 12, wherein the plant is selected from the group consisting of soybeans, peas, chickpeas, drybeans, peanuts, clover, alfalfa, corn, cotton, rice, tomatoes, canola, wheat, barley, sugar beet, and grass.

14. The method of claim 11, wherein the composition is administered by applying the composition to one or more of foliage, seeds, and soil that is in the immediate vicinity of the plant or seed.

15. A method for enhancing plant growth or crop yield comprising administering to a plant or a seed a composition comprising at least one lipo-chitooligosaccharide and at least one flavonoid compound selected from the group consisting of flavones, flavanols, flavonols, flavanones, and isoflavones, in an effective amount for enhancing plant growth or crop yield.

16. The method of claim 15, wherein the flavonoid compound is selected from the group consisting of genistein, daidzein, formononetin, naringenin, hesperetin, luteolin, and apigenin.

17. A method for enhancing plant growth or crop yield comprising sequentially administering to a plant or a seed, in either order, a first composition comprising at least one lipo-chitooligosaccharide in an effective amount for enhancing plant growth or crop yield, and a second composition comprising at least one flavonoid compound, selected from the group consisting of flavones, flavanols, flavonols, flavanones, and isoflavones, in an effective amount for enhancing plant growth or crop yield.

18. The method of claim 17, wherein the flavonoid compound is selected from the group consisting of genistein, daidzein, formononetin, naringenin, hesperetin, luteolin, and apigenin.