(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 July 2014 (10.07.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/106838 A2

(51) **International Patent Classification**:
C12N 15/82 (2006.01)

(21) **International Application Number**:
PCT/IL2013/051085

(22) **International Filing Date**:
30 December 2013 (30.12.2013)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:

61/748,095	1 January 2013 (01.01.2013)	US
61/748,094	1 January 2013 (01.01.2013)	US
61/748,101	1 January 2013 (01.01.2013)	US
61/748,099	1 January 2013 (01.01.2013)	US
61/814,888	23 April 2013 (23.04.2013)	US
61/814,892	23 April 2013 (23.04.2013)	US
61/814,899	23 April 2013 (23.04.2013)	US
61/814,890	23 April 2013 (23.04.2013)	US
61/908,965	26 November 2013 (26.11.2013)	US
61/908,855	26 November 2013 (26.11.2013)	US

(71) **Applicant**: **A.B. SEEDS LTD.** [IL/IL]; 1 HaGolan Street, P.O. Box 1061, 7111001 Lod (IL).

(72) **Inventors**: **AVNIEL, Amir**; 24 Remez Street, Apt. 7, 6219207 Tel-Aviv (IL). **LIDOR-NILI, Efrat**; 5 HaKatzir Street, 7405492 Nes Ziona (IL). **MAOR, Rudy**; 11 Kronnenberg Street, 7666206 Rechovot (IL). **MEIR, Ofir**; 74 Dolev Street, Moshav Gealya, 7688500 Doar-Na Emek Soreq (IL). **NOIVIRT-BRIK, Orly**; 9 HaTaAs Street, 5339711 Givataim (IL).

(74) **Agent**: **EHRLICH, Gal**; G.E. EHRLICH (1995) LTD., 11 Menachem Begin Road, 5268104 Ramat Gan (IL).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title**: METHODS OF INTRODUCING DSRNA TO PLANT SEEDS FOR MODULATING GENE EXPRESSION

IAP1-DS1

FIG. 10C

SCORE	EXPECT	IDENTITIES	GAPS	STRAND	FRAME
37.4 BITS(40)	0.91()	22/23(96%)	0/23(0%)	PLUS/PLUS	

FEATURES:

```
QUERY  501       TCTCCCTATAGTGAGTCGTATTA  523
                 || ||||||||||||||||||||
SBJCT  90683     TCGCCCTATAGTGAGTCGTATTA  90705
```

(57) **Abstract**: A method of introducing an exogenous non-transcribable polynucleotide trigger, for example dsRNA, molecule into a seed is provided. The method comprising contacting the seed with the exogenous non-transcribable polynucleotide trigger, for example dsRNA, molecule under conditions which allow penetration of the exogenous non-transcribable polynucleotide trigger, for example dsRNA, molecule into the seed, thereby introducing the exogenous non-transcribable polynucleotide trigger, for example dsRNA, molecule into the seed.

WO 2014/106838 A2

METHODS OF INTRODUCING dsRNA TO PLANT SEEDS FOR MODULATING GENE EXPRESSION

INCORPORATION OF SEQUENCE LISTING

The ASCII file, entitled 58066 P34098 12-22-2013_ST25.txt, created on 25 December 2013, comprising 109,372 bytes, submitted concurrently with the filing of this application.

FIELD OF THE DISCLOSURE

Methods and compositions for improving plant resistance to insect pests are provided. Methods and compositions for improving plant resistance to viral pathogens are also provided.

BACKGROUND

With a growing world population, increasing demand for food, fuel and fiber, and a changing climate, agriculture faces unprecedented challenges. Development of plants with improved traits is highly desirable, with some of the major traits that are of major interest to farmers and seed companies include improved abiotic stress tolerance, fertilizer use efficiency, disease resistance, yield and more.

Plant trait improvement is typically performed by either genetic engineering or classical breeding. New methods for trait improvement through specific gene alteration are highly desirable. These include methods for over-expression of genes or gene silencing. A powerful technique for sequence-specific gene silencing is through RNA interference (RNAi). First discovered in the nematode *C. elegans* (Fire *et al.* 1998, Nature, 391:806-811), RNAi is a mechanism in which expression of an individual gene can be specifically silenced by introducing a double-stranded RNA (dsRNA) that is homologous to the selected gene into cells. Inside the cell, dsRNA molecules are cut into shorter fragments of 21–27 nucleotides by an RNase III-related enzyme (Dicer). These fragments, called small interfering RNAs (siRNAs), get incorporated into the RNA-induced silencing complex (RISC). After additional processing, the siRNAs are transformed into single-stranded RNAs that act as guide sequences to eventually cleave target messenger RNAs. By using RNAi to specifically silence relevant target genes, one can alter basic traits of an organism. Specifically for plants, it holds incredible

potential for modifications that may lead to increased stress resistance and better crop yield.

In plants, RNAi is typically performed by producing transgenic plants that over-express a DNA fragment that is transcribed to produce a dsRNA. This dsRNA is then processed into siRNAs that mediate the cleavage and silencing of target genes.

The major technical limitation for this technology is that many important plant crop species are difficult or impossible to transform, precluding the constitutive expression of constructs directing production of dsRNA. Moreover, questions concerning the potential ecological impact of virus-resistant transgenic plants have so far significantly limited their use (Tepfer, 2002, Annu. Rev. Phytopathol. 40, 467–491). An additional hurdle for obtaining transgenic plants is attributed to the difficulty of having the transformation and regeneration events occur in the same cell types.

Therefore the development of a method for obtaining transformed seeds which is independent of the methods inherent to tissue culture procedures is at the cutting edge of plant molecular biology research.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a method of introducing naked dsRNA into a seed, the method comprising contacting the seed with the naked dsRNA under conditions which allow penetration of the dsRNA into the seed, thereby introducing the dsRNA into the seed.

According to an aspect of some embodiments of the present invention there is provided an isolated seed comprising an exogenous naked dsRNA, wherein the seed is devoid of a heterologous promoter for driving expression of the dsRNA in the plant.

According to an aspect of some embodiments of the present invention there is provided an isolated seed comprising an exogenous naked dsRNA3.

According to an aspect of some embodiments of the present invention there is provided a plant or plant part comprising an exogenous naked dsRNA and being devoid of a heterologous promoter for driving expression of the dsRNA in the plant.

According to an aspect of some embodiments of the present invention there is provided a seed containing device comprising a plurality of the seeds.

According to an aspect of some embodiments of the present invention there is provided a sown field comprising a plurality of the seeds.

According to an aspect of some embodiments of the present invention there is provided a method of producing a plant the method comprising:

(a) providing the seed; and

(b) germinating the seed so as to produce the plant.

According to an aspect of some embodiments of the present invention there is provided a method of modulating gene expression, the method comprising:

(a) contacting a seed of a plant with a naked dsRNA, under conditions which allow penetration of the dsRNA into the seed, thereby introducing the dsRNA into the seed; and optionally

(b) generating a plant of the seed.

According to some embodiments of the invention, the naked dsRNA is designed for down regulating expression of a gene of the plant.

According to some embodiments of the invention, the naked dsRNA is designed for down regulating expression of a gene of a phytopathogen.

According to some embodiments of the invention, the penetration is to an endosperm and alternatively or additionally an embryo of the seed.

According to some embodiments of the invention, the naked dsRNA does not integrate into the genome of the seeds.

According to some embodiments of the invention, the conditions result in presence of the dsRNA in the plant for at least 10 days following germination.

According to an aspect of some embodiments of the present invention there is provided a method of inhibiting expression of a target gene in a phytopathogenic organism, the method comprising providing to the phytopathogenic organism the plant or plant part, thereby inhibiting expression of a target gene in the phytopathogenic organism.

According to some embodiments of the invention, the phytopathogenic organism is selected from the group consisting of a fungus, a nematode and an insect.

According to some embodiments of the invention, the method further comprises observing death or growth inhibition of the phytopathogen following the providing.

According to an aspect of some embodiments of the present invention there is provided a kit for introducing naked dsRNA to seeds comprising:

(i) naked dsRNA; and

(ii) a priming solution.

According to some embodiments of the invention, the naked dsRNA and the priming solutions are comprised in separate containers.

According to some embodiments of the invention, the dsRNA comprises siRNA.

According to some embodiments of the invention, the dsRNA comprises siRNA and dsRNA.

According to some embodiments of the invention, the contacting is effected by inoculating the seed with the dsRNA.

According to some embodiments of the invention, the method further comprises priming the seed prior to the contacting.

According to some embodiments of the invention, the priming is effected by:

(i) washing the seed prior to the contacting; and

(ii) drying the seed following step (i).

According to some embodiments of the invention, the washing is effected in the presence of double deionized water.

According to some embodiments of the invention, the washing is effected for 2-6 hours.

According to some embodiments of the invention, the washing is effected at 4-28 ºC.

According to some embodiments of the invention, the drying is effected at 25-30 ºC for 10 - 16 hours.

According to some embodiments of the invention, the contacting is effected in a presence of the naked dsRNA at a final concentration of 0.1-100 µg/µl.

According to some embodiments of the invention, the contacting is effected in a presence of the naked dsRNA at a final concentration of 0.1-0.5 µg/µl.

According to some embodiments of the invention, the method further comprises treating the seed with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent following the contacting.

According to some embodiments of the invention, the treating comprises coating the seed with the agent.

According to some embodiments of the invention, the seed is free of an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent.

According to some embodiments of the invention, the dsRNA is for down regulating expression of a coding gene.

According to some embodiments of the invention, the dsRNA is for down regulating expression of a non-coding gene.

According to some embodiments of the invention, the seed is of the Viridiplantae super-family.

According to some embodiments of the invention, the conditions allow accumulation of the dsRNA in the endosperm and alternatively or additionally embryo of the seed.

According to some embodiments of the invention, a concentration of the naked dsRNA is adjusted according to a parameter selected from the group consisting of seed size, seed weight, seed volume, seed surface area, seed density and seed permeability.

According to some embodiments of the invention, the contacting is effected prior to breaking of seed dormancy and embryo emergence.

According to some embodiments of the invention, the seed is a primed seed.

According to some embodiments of the invention, the seed or the plant comprises RNA dependent RNA polymerase activity for amplifying expression of the dsRNA.

According to some embodiments of the invention, the seed is a hybrid seed.

According some embodiments, there is provided an isolated dsRNA comprising a nucleic acid sequence having:

(i) a homology level to a plant gene sufficient to induce amplification of secondary siRNA products of the dsRNA in a plant cell comprising same and wherein down-regulation of the plant gene by the dsRNA does not substantially affect any of biomass, vigor or yield of the plant; and

(ii) a homology level to a gene of a phytopathogenic organism sufficient to induce degradation of the gene of the phytopathogenic organism, wherein the phytopathogenic organism depends on the plant for growth and wherein the degradation induces a growth arrest or death of the phytopathogenic organism. According to some

embodiments, the nucleic acid sequence is at least 25 bp long. According to some embodiments, the nucleic acid sequence is 25-70 bp long. According to some embodiments, the dsRNA wherein the nucleic acid sequence is at least 80% identical to the plant gene. According to some embodiments, the nucleic acid sequence is more than 70 bp. According to some embodiments, the nucleic acid sequence comprises a nucleic acid segment at least 70 bp in length which is at least 65% identical to the plant gene, and/or a second nucleic acid segment at least 17 bp in length which is at least 85% identical to the plant gene. According to some embodiments, the first nucleic acid segment and the second nucleic acid segment overlap. According to some embodiments, the first nucleic acid segment and the second nucleic acid segment are in no overlap. According to some embodiments, the plant gene is expressed in most plant organs starting from germination. According to some embodiments of the invention, the isolated dsRNA is at least 80% homologous to the gene of the phytopathogen.

Several embodiments relate to a method of providing a plant having improved resistance to an insect pest, comprising: growing a plant from a seed, wherein the seed has been contacted with an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a gene of the insect pest or to the sequence of an RNA transcribed from said gene, wherein the plant exhibits improved resistance to the insect pest relative to a control plant, wherein the control plant is grown from a seed not contacted with the exogenous dsRNA molecule. In some embodiments, the plant is maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton or rapeseed. In some embodiments, the insect pest is *Spodoptera littoralis*, *Diabrotica virgifera virgifera* or *Leptinotarsa decemlineata*. In some embodiments, the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 21-26, 31, 34, 37, 38, 131-133, 144 or 145. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 146-190. In some embodiments, the exogenous dsRNA molecule comprises a nucleic acid sequence that is at least 80% identical to an endogenous plant gene over at

least 25 consecutive bp. In some embodiments, the seed is further treated with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent.

Several embodiments relate to a plant provided by a method comprising: growing a plant from a seed, wherein the seed has been contacted with an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a gene of the insect pest or to the sequence of an RNA transcribed from said gene, wherein the plant exhibits improved resistance to the insect pest relative to a control plant, wherein the control plant is grown from a seed not contacted with the exogenous dsRNA molecule. In some embodiments, the plant is maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton or rapeseed. In some embodiments, the insect pest is *Spodoptera littoralis*, *Diabrotica virgifera virgifera* or *Leptinotarsa decemlineata*. In some embodiments, the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 21-26, 31, 34, 37, 38, 131-133, 144 or 145. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 146-190. In some embodiments, the exogenous dsRNA molecule comprises a nucleic acid sequence that is at least 80% identical to an endogenous plant gene over at least 25 consecutive bp. In some embodiments, the seed is further treated with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent. In some embodiments, the plant does not comprise detectable levels of the exogenous dsRNA molecule.

Several embodiments relate to a method of providing a plant having improved resistance to an insect pest, comprising growing the plant from a seed, wherein the seed comprises an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a gene of the insect pest or to the sequence of an RNA transcribed from said gene, wherein the seed is devoid of a heterologous promoter for driving expression of the exogenous

dsRNA molecule, and wherein the plant exhibits improved resistance to the insect pest relative to a control plant, wherein the control plant is grown from a seed not comprising the exogenous dsRNA molecule. In some embodiments, the plant is maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton or rapeseed. In some embodiments, the insect pest is *Spodoptera littoralis*, *Diabrotica virgifera virgifera* or *Leptinotarsa decemlineata*. In some embodiments, the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 21-26, 31, 34, 37, 38, 131-133, 144 or 145. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 146-190. In some embodiments, the exogenous dsRNA molecule comprises a nucleic acid sequence that is at least 80 % identical to an endogenous plant gene over at least 25 consecutive bp. In some embodiments, the seed is further treated with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent.

Several embodiments relate to a plant provided by a method comprising growing the plant from a seed, wherein the seed comprises an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a gene of the insect pest or to the sequence of an RNA transcribed from said gene, wherein the seed is devoid of a heterologous promoter for driving expression of the exogenous dsRNA molecule, and wherein the plant exhibits improved resistance to the insect pest relative to a control plant, wherein the control plant is grown from a seed not comprising the exogenous dsRNA molecule. In some embodiments, the plant is maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton or rapeseed. In some embodiments, the insect pest is *Spodoptera littoralis*, *Diabrotica virgifera virgifera* or *Leptinotarsa decemlineata*. In some embodiments, the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or

essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 21-26, 31, 34, 37, 38, 131-133, 144 or 145. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 146-190. In some embodiments, the exogenous dsRNA molecule comprises a nucleic acid sequence that is at least 80% identical to an endogenous plant gene over at least 25 consecutive bp. In some embodiments, the seed is further treated with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent. In some embodiments, the plant does not comprise detectable levels of the exogenous dsRNA molecule.

Several embodiments relate to a method for generating a plant having insect resistance, the method comprising: a) introducing a non-transcribable trigger molecule comprising at least one polynucleotide strand comprising at least one segment of 18 or more contiguous nucleotides of an insect pest gene in either anti-sense or sense orientation into an ungerminated seed and b) germinating the seed to generate a plant exhibiting insect resistance after emerging from said seed. In some embodiments, the plant does not comprise detectable levels of the trigger molecule after emerging from the seed. In some embodiments, the non-transcribable trigger molecule is dsRNA. In some embodiments, the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin. In some embodiments, the plant is resistant to *Spodoptera littoralis*, *Diabrotica virgifera virgifera* or *Leptinotarsa decemlineata* infestation. In some embodiments, the non-transcribable trigger molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 21-26, 31, 34, 37, 38, 131-133, 144 or 145. In some embodiments, the non-transcribable trigger molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 146-190. In some embodiments, the non-transcribable trigger molecule is at least 80% identical to an endogenous plant gene over at least 25 consecutive bp. In some embodiments, the seed is primed prior to introducing the non-transcribable trigger molecule. In some embodiments, the priming is effected by: (i) washing the seed prior to said contacting; and (ii) drying the seed following step (i).

Several embodiments relate to a method of treating a seed to improve insect resistance of a plant grown from the seed, the method comprising: introducing an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of an insect pest gene or to the sequence of an RNA transcribed from the insect pest gene into the seed, wherein the plant grown from the seed exhibits improved insect resistance relative to a control plant. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 21-26, 31, 34, 37, 38, 131-133, 144 or 145. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 146-190. In some embodiments, the seed is primed prior to introducing the exogenous dsRNA molecule. In some embodiments, the priming is effected by: (i) washing the seed prior to said contacting; and (ii) drying the seed following step (i). In some embodiments, the seed is washed in double deionized water. In some embodiments, the seed is washed for 2-6 hours. In some embodiments, the seed is washed at 4-28 ºC. In some embodiments, the seed is dried at 25-30 ºC for 10 - 16 hours. In some embodiments, the dsRNA molecule is provided to the seed at a concentration of 20-150 µg/ml. In some embodiments, the dsRNA molecule is provided to the seed in a solution comprising 0.1 mM EDTA. In some embodiments, the dsRNA molecule is provided to the seed in the presence of a physical agent. In some embodiments, the physical agent is PEG-modified carbon nanotubes.

Several embodiments relate to a seed provided by a method comprising introducing an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of an insect pest gene or to the sequence of an RNA transcribed from the insect pest gene into the seed, wherein the plant grown from the seed exhibits improved insect resistance relative to a control plant. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 21-26, 31, 34, 37, 38, 131-133, 144 or 145. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides

of SEQ ID Nos.: 146-190. In some embodiments, the seed is primed prior to introducing the exogenous dsRNA molecule. In some embodiments, the priming is effected by: (i) washing the seed prior to said contacting; and (ii) drying the seed following step (i). In some embodiments, the seed is washed in double deionized water. In some embodiments, the seed is washed for 2-6 hours. In some embodiments, the seed is washed at 4-28 ºC. In some embodiments, the seed is dried at 25-30 ºC for 10-16 hours. In some embodiments, the dsRNA molecule is provided to the seed at a concentration of 20-150 μg/ml. In some embodiments, the dsRNA molecule is provided to the seed in a solution comprising 0.1 mM EDTA. In some embodiments, the dsRNA molecule is provided to the seed in the presence of a physical agent. In some embodiments, the physical agent is PEG-modified carbon nanotubes. Several embodiments relate to a seed containing device comprising one or more of the seeds. Several embodiments relate to a sown field comprising a plurality of the seeds.

Several embodiments relate to a seed comprising an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of an insect pest gene or to the sequence of an RNA transcribed from the insect pest gene, wherein the seed is devoid of a heterologous promoter for driving expression of said dsRNA molecule and wherein the exogenous dsRNA does not integrate into the genome of the seed. In some embodiments, the exogenous dsRNA molecule is present in an endosperm of the seed. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 21-26, 31, 34, 37, 38, 131-133, 144 or 145. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 146-190. In some embodiments, the exogenous dsRNA molecule is present in an embryo of the seed. In some embodiments, the exogenous dsRNA molecule is present at a similar concentration in an embryo and an endosperm of the seed. In some embodiments, the exogenous dsRNA molecule is present at a higher concentration in an endosperm than an embryo and of the seed. In some embodiments, the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin. In some embodiments, the insect pest is

Spodoptera littoralis, *Diabrotica virgifera virgifera* or *Leptinotarsa decemlineata*. In some embodiments, the exogenous dsRNA molecule comprises a nucleic acid sequence that is at least 80% identical over at least 25 consecutive bp to an endogenous gene of the seed. In some embodiments, the seed is treated with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent. In some embodiments, the seed is a primed seed. Several embodiments relate to a seed containing device comprising one or more of the seeds. Several embodiments relate to a sown field comprising a plurality of the seeds.

Several embodiments relate to a plant exhibiting insect resistance after emerging from a seed, wherein a non-transcribable trigger molecule comprising at least one polynucleotide strand comprising at least one segment of 18 or more contiguous nucleotides of an insect pest gene in either anti-sense or sense orientation is introduced into an ungerminated seed that gives rise to said plant. In some embodiments, the plant is selected from the group consisting of maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton and rapeseed. In some embodiments, the plant does not comprise a detectable level of the non-transcribable trigger molecule. In some embodiments, the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin. In some embodiments, the non-transcribable trigger molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 21-26, 31, 34, 37, 38, 131-133, 144 or 145. In some embodiments, the non-transcribable trigger molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 146-190. In some embodiments, the non-transcribable trigger molecule comprises a nucleic acid sequence that is at least 80% identical over at least 25 consecutive bp to an endogenous gene of the seed. In some embodiments, the non-transcribable trigger molecule comprises a nucleic acid sequence that is at least 17 bp in length and at least 85% identical to an endogenous gene of the seed. In some embodiments, the non-transcribable trigger molecule comprises a nucleic acid sequence that is at least 70 bp in length and at least 65% identical to an endogenous gene of the seed.

Several embodiments relate to a plant comprising a nucleic acid molecule for suppressing an insect pest gene, wherein the nucleic acid molecule is not integrated into

a chromosome of the plant, wherein the nucleic acid molecule is not transcribed from a heterologous transgene integrated into a chromosome of the plant, and wherein the insect pest gene is suppressed by introduction of a trigger molecule comprising at least one polynucleotide strand comprising at least one segment of 18 or more contiguous nucleotides of an insect pest gene in either anti-sense or sense orientation into an ungerminated seed giving rise to the plant. In some embodiments, the plant is selected from the group consisting of maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton and rapeseed. In some embodiments, the trigger molecule is dsRNA. In some embodiments, the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin. In some embodiments, the trigger molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 21-26, 31, 34, 37, 38, 131-133, 144 or 145. In some embodiments, the trigger molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 146-190. In some embodiments, the trigger molecule comprises a nucleic acid sequence that is at least 80% identical over at least 25 consecutive bp to an endogenous gene of the seed giving rise to the plant. In some embodiments, the trigger molecule comprises a nucleic acid sequence that is at least 17 bp in length and at least 85% identical to an endogenous gene of the seed giving rise to the plant. In some embodiments, the trigger molecule comprises a nucleic acid sequence that is at least 70 bp in length and at least 65% identical to an endogenous gene of the seed giving rise to the plant. In some embodiments, the plant does not comprise a detectable level of the trigger molecule.

Several embodiments relate to a method of reducing corn root worm pressure on a corn plant, the method comprising: a) introducing a trigger molecule comprising at least one polynucleotide strand comprising at least one segment of 18 or more contiguous nucleotides of a corn root worm gene in either anti-sense or sense orientation into an ungerminated corn seed and b) germinating the corn seed to generate a corn plant. In some embodiments, the trigger molecule is dsRNA. In some embodiments, the trigger molecule comprises at least one segment of 18 or more contiguous nucleotides of SEQ ID No. 144. In some embodiments, the trigger molecule comprises at least one segment of 18 or more contiguous nucleotides of SEQ ID Nos.:

146-190. In some embodiments, the ungerminated corn seed is primed prior to introducing the trigger molecule. In some embodiments, the seed is primed by: (i) washing the seed prior to said contacting; and (ii) drying the seed following step (i). In some embodiments, the seed is washed in double deionized water.

Several embodiments relate to a method of providing a plant having improved viral resistance, comprising: growing a plant from a seed, wherein the seed has been contacted with an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a viral gene or to the sequence of an RNA transcribed from said gene, wherein the plant exhibits improved viral resistance relative to a control plant, wherein the control plant is grown from a seed not contacted with the exogenous dsRNA molecule. In some embodiments, the plant is maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton or rapeseed. In some embodiments, the virus is Tomato golden mottle virus (ToGMoV), Cucumber Mosaic Virus (CMV) or Tomato Spotted Wilt Virus (TSWV). In some embodiments, the viral gene is selected from the group consisting of a ToGMoV gene, a CMV gene and a TSWV gene. In some embodiments, the viral gene is selected from the group consisting of Nucleocapsid (N) gene, a Replicase gene, a Coat gene and the AC1 gene. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 8, 11 or 185-190. In some embodiments, the exogenous dsRNA molecule comprises a nucleic acid sequence that is at least 80% identical to an endogenous plant gene over at least 25 consecutive bp. In some embodiments, the seed is further treated with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent.

Several embodiments relate to a plant provided by a method comprising: growing a plant from a seed, wherein the seed has been contacted with an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a viral gene or to the sequence of an RNA transcribed from said gene, wherein the plant exhibits improved resistance to the virus relative to a control plant, wherein the control plant is grown from a seed not contacted with the exogenous dsRNA molecule. In some embodiments, the plant is

maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton or rapeseed. In some embodiments, the virus is Tomato golden mottle virus (ToGMoV), Cucumber Mosaic Virus (CMV) or Tomato Spotted Wilt Virus (TSWV). In some embodiments, the viral gene is selected from the group consisting of a ToGMoV gene, a CMV gene and a TSWV gene. In some embodiments, the viral gene is selected from the group consisting of Nucleocapsid (N) gene, a Replicase gene, a Coat gene and the AC1 gene. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 8, 11 or 185-190. In some embodiments, the exogenous dsRNA molecule comprises a nucleic acid sequence that is at least 80% identical to an endogenous plant gene over at least 25 consecutive bp. In some embodiments, the seed is further treated with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent. In some embodiments, the plant does not comprise detectable levels of the exogenous dsRNA molecule.

Several embodiments relate to a method of providing a plant having improved viral resistance, comprising growing the plant from a seed, wherein the seed comprises an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a viral gene or to the sequence of an RNA transcribed from said gene, wherein the seed is devoid of a heterologous promoter for driving expression of the exogenous dsRNA molecule, and wherein the plant exhibits improved viral resistance relative to a control plant, wherein the control plant is grown from a seed not comprising the exogenous dsRNA molecule. In some embodiments, the virus is Tomato golden mottle virus (ToGMoV), Cucumber Mosaic Virus (CMV) or Tomato Spotted Wilt Virus (TSWV). In some embodiments, the viral gene is selected from the group consisting of a ToGMoV gene, a CMV gene and a TSWV gene. In some embodiments, the viral gene is selected from the group consisting of Nucleocapsid (N) gene, a Replicase gene, a Coat gene and the AC1 gene. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 8, 11 or 185-190. In some embodiments, the exogenous dsRNA molecule comprises a nucleic acid sequence that is at least 80% identical to an endogenous plant gene over at least 25 consecutive bp. In some embodiments, the seed

is further treated with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent.

Several embodiments relate to a plant provided by a method comprising growing the plant from a seed, wherein the seed comprises an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a viral gene or to the sequence of an RNA transcribed from said gene, wherein the seed is devoid of a heterologous promoter for driving expression of the exogenous dsRNA molecule, and wherein the plant exhibits improved viral resistance relative to a control plant, wherein the control plant is grown from a seed not comprising the exogenous dsRNA molecule. In some embodiments, the plant is maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton or rapeseed. In some embodiments, the virus is Tomato golden mottle virus (ToGMoV), Cucumber Mosaic Virus (CMV) or Tomato Spotted Wilt Virus (TSWV). In some embodiments, the viral gene is selected from the group consisting of a ToGMoV gene, a CMV gene and a TSWV gene. In some embodiments, the viral gene is selected from the group consisting of Nucleocapsid (N) gene, a Replicase gene, a Coat gene and the AC1 gene. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 8, 11 or 185-190. In some embodiments, the exogenous dsRNA molecule comprises a nucleic acid sequence that is at least 80% identical to an endogenous plant gene over at least 25 consecutive bp. In some embodiments, the seed is further treated with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent. In some embodiments, the plant does not comprise detectable levels of the exogenous dsRNA molecule.

Several embodiments relate to a method for generating a plant having viral resistance, the method comprising: a) introducing a non-transcribable trigger molecule comprising at least one polynucleotide strand comprising at least one segment of 18 or more contiguous nucleotides of an viral gene in either anti-sense or sense orientation into an ungerminated seed and b) germinating the seed to generate a plant exhibiting viral resistance after emerging from said seed. In some embodiments, the plant does not comprise detectable levels of the trigger molecule after emerging from the seed. In some embodiments, the non-transcribable trigger molecule is dsRNA. In some

embodiments, the virus is Tomato golden mottle virus (ToGMoV), Cucumber Mosaic Virus (CMV) or Tomato Spotted Wilt Virus (TSWV). In some embodiments, the viral gene is selected from the group consisting of a ToGMoV gene, a CMV gene and a TSWV gene. In some embodiments, the viral gene is selected from the group consisting of Nucleocapsid (N) gene, a Replicase gene, a Coat gene and the AC1 gene. In some embodiments, the non-transcribable trigger molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 8, 11 or 185-190. In some embodiments, the non-transcribable trigger molecule is at least 80% identical to an endogenous plant gene over at least 25 consecutive bp. In some embodiments, the seed is primed prior to introducing the non-transcribable trigger molecule. In some embodiments, the priming is effected by: (i) washing the seed prior to said contacting; and (ii) drying the seed following step (i).

Several embodiments relate to a method of treating a seed to improve viral resistance of a plant grown from the seed, the method comprising: introducing an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a viral gene or to the sequence of an RNA transcribed from the viral gene into the seed, wherein the plant grown from the seed exhibits improved viral resistance relative to a control plant. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 8, 11 or 185-190. In some embodiments, the seed is primed prior to introducing the exogenous dsRNA molecule. In some embodiments, the priming is effected by: (i) washing the seed prior to said contacting; and (ii) drying the seed following step (i). In some embodiments, the seed is washed in double deionized water. In some embodiments, the seed is washed for 2-6 hours. In some embodiments, the seed is washed at 4-28 ºC. In some embodiments, the seed is dried at 25-30 ºC for 10-16 hours. In some embodiments, the dsRNA molecule is provided to the seed at a concentration of 20-150 μg/ml. In some embodiments, the dsRNA molecule is provided to the seed in a solution comprising 0.1 mM EDTA. In some embodiments, the dsRNA molecule is provided to the seed in the presence of a physical agent. In some embodiments, the physical agent is PEG-modified carbon nanotubes.

Several embodiments relate to a seed provided by a method comprising introducing an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a viral gene or to the sequence of an RNA transcribed from the viral gene into the seed, wherein the plant grown from the seed exhibits improved viral resistance relative to a control plant. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 8, 11 or 185-190. In some embodiments, the seed is primed prior to introducing the exogenous dsRNA molecule. In some embodiments, the priming is effected by: (i) washing the seed prior to said contacting; and (ii) drying the seed following step (i). In some embodiments, the seed is washed in double deionized water. In some embodiments, the seed is washed for 2-6 hours. In some embodiments, the seed is washed at 4-28 ºC. In some embodiments, the seed is dried at 25-30 ºC for 10-16 hours. In some embodiments, the dsRNA molecule is provided to the seed at a concentration of 20-150 µg/ml. In some embodiments, the dsRNA molecule is provided to the seed in a solution comprising 0.1 mM EDTA. In some embodiments, the dsRNA molecule is provided to the seed in the presence of a physical agent. In some embodiments, the physical agent is PEG-modified carbon nanotubes. Several embodiments relate to a seed containing device comprising one or more of the seeds. Several embodiments relate to a sown field comprising a plurality of the seeds.

Several embodiments relate to a seed comprising an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a viral gene or to the sequence of an RNA transcribed from the viral gene, wherein the seed is devoid of a heterologous promoter for driving expression of said dsRNA molecule and wherein the exogenous dsRNA does not integrate into the genome of the seed. In some embodiments, the exogenous dsRNA molecule is present in an endosperm of the seed. In some embodiments, the exogenous dsRNA molecule is present in an embryo of the seed. In some embodiments, the exogenous dsRNA molecule is present at a similar concentration in an embryo and an endosperm of the seed. In some embodiments, the exogenous dsRNA molecule is present at a higher concentration in an endosperm than

an embryo and of the seed. In some embodiments, the virus is Tomato golden mottle virus (ToGMoV), Cucumber Mosaic Virus (CMV) or Tomato Spotted Wilt Virus (TSWV). In some embodiments, the viral gene is selected from the group consisting of a ToGMoV gene, a CMV gene and a TSWV gene. In some embodiments, the viral gene is selected from the group consisting of Nucleocapsid (N) gene, a Replicase gene, a Coat gene and the AC1 gene. In some embodiments, the exogenous dsRNA molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 8, 11 or 185-190. In some embodiments, the exogenous dsRNA molecule comprises a nucleic acid sequence that is at least 80% identical over at least 25 consecutive bp to an endogenous gene of the seed. In some embodiments, the seed is treated with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent. In some embodiments, the seed is a primed seed. Several embodiments relate to a seed containing device comprising one or more of the seeds. Several embodiments relate to a sown field comprising a plurality of the seeds.

Several embodiments relate to a plant exhibiting viral resistance after emerging from a seed, wherein a non-transcribable trigger molecule comprising at least one polynucleotide strand comprising at least one segment of 18 or more contiguous nucleotides of a viral gene in either anti-sense or sense orientation is introduced into an ungerminated seed that gives rise to said plant. In some embodiments, the plant is selected from the group consisting of maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton and rapeseed. In some embodiments, the plant does not comprise a detectable level of the non-transcribable trigger molecule. In some embodiments, the virus is Tomato golden mottle virus (ToGMoV), Cucumber Mosaic Virus (CMV) or Tomato Spotted Wilt Virus (TSWV). In some embodiments, the viral gene is selected from the group consisting of a ToGMoV gene, a CMV gene and a TSWV gene. In some embodiments, the viral gene is selected from the group consisting of Nucleocapsid (N) gene, a Replicase gene, a Coat gene and the AC1 gene. In some embodiments, the non-transcribable trigger molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 8, 11 or 185-190. In some embodiments, the non-transcribable trigger molecule comprises a nucleic acid sequence that is at least 80% identical over at least 25 consecutive bp to an

endogenous gene of the seed. In some embodiments, the non-transcribable trigger molecule comprises a nucleic acid sequence that is at least 17 bp in length and at least 85% identical to an endogenous gene of the seed. In some embodiments, the non-transcribable trigger molecule comprises a nucleic acid sequence that is at least 70 bp in length and at least 65% identical to an endogenous gene of the seed.

Several embodiments relate to a plant comprising a nucleic acid molecule for suppressing a viral gene, wherein the nucleic acid molecule is not integrated into a chromosome of the plant, wherein the nucleic acid molecule is not transcribed from a heterologous transgene integrated into a chromosome of the plant, and wherein the viral gene is suppressed by introduction of a trigger molecule comprising at least one polynucleotide strand comprising at least one segment of 18 or more contiguous nucleotides of a viral gene in either anti-sense or sense orientation into an ungerminated seed giving rise to the plant. In some embodiments, the plant is selected from the group consisting of maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton and rapeseed. In some embodiments, the trigger molecule is dsRNA. In some embodiments, the virus is Tomato golden mottle virus (ToGMoV), Cucumber Mosaic Virus (CMV) or Tomato Spotted Wilt Virus (TSWV). In some embodiments, the viral gene is selected from the group consisting of a ToGMoV gene, a CMV gene and a TSWV gene. In some embodiments, the viral gene is selected from the group consisting of Nucleocapsid (N) gene, a Replicase gene, a Coat gene and the AC1 gene. In some embodiments, the trigger molecule comprises a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of SEQ ID Nos.: 8, 11 or 185-190. In some embodiments, the trigger molecule comprises a nucleic acid sequence that is at least 80% identical over at least 25 consecutive bp to an endogenous gene of the seed giving rise to the plant. In some embodiments, the trigger molecule comprises a nucleic acid sequence that is at least 17 bp in length and at least 85% identical to an endogenous gene of the seed giving rise to the plant. In some embodiments, the trigger molecule comprises a nucleic acid sequence that is at least 70 bp in length and at least 65% identical to an endogenous gene of the seed giving rise to the plant. In some embodiments, the plant does not comprise a detectable level of the trigger molecule.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, examples of methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

FIG. 1 shows a time-course siGLO-treatment results on rice seeds. The effect of incubation time with siGLO dsRNA on fluorescence intensity, indicating quantity and quality of dsRNA penetration, was tested. Control seeds that were left untreated (1), were imaged along with seeds treated with siGLO dsRNA for four different incubation times; 10 min (2), 3.5 hours (3), 5.5 hours (4), and 24 hours (5).

FIGs. 2A-B show silencing the PDS-1 gene in rice by a dsRNA/siRNA mixture. FIG. 2A – A picture of germinated rice seeds 5 days after treatment, control on the left. FIG. 2B – A picture of germinated rice seeds 7 days after treatment, control on the bottom.

FIGs. 3A-C show PDS-1 expression levels as determined by Real-Time PCR. FIG. 3A is a picture of germinated rice seeds 7 days after treatment, control on the bottom. FIG. 3B – A picture of planted rice seeds 5 weeks after treatment, the control plant is on the left and has a darker green color compared to PDS-1 silenced plant. FIG. 3C - RNA was extracted from control and PDS-1 silenced plants and PDS-1 expression levels were checked by Real Time PCR. UBQ5 expression levels were served as normalizers and the PDS-1 expression levels in the control plants served as calibrators and got a value of 1.

FIGs. 4A-B show height distribution of control and NFY dsRNA-treated tomato plants 55 days post inoculation. FIG. 4A presents the height distribution of control plants (blue bars) and FIG. 4B shows the height distribution of treated plants (yellow bars).

FIGs. 5A-D show the specific distribution of height in control (blue bars) and ARF8 dsRNA-treated (maroon bars) tomato plants 55 (FIG. 5A), 62 (FIG. 5B) and 72 days (FIG. 5C) following treatment. FIG. 5D shows the average height of control plants compared with that of treated plants 62 days following treatment.

FIGs. 6A-B show the results of RT-PCR on RNA extracted from leaves of control and FW2.2 dsRNA treated tomato plants 9 weeks post germination. FIG. 6A shows the fold change of FW2.2 expression in control (shown in red bars) and dsRNA-treated (shown in blue bars) plants, which was plotted for each individual plant to demonstrate the variation in expression level of FW2.2 gene in the two plant groups. FIG. 6B shows the average expression of FW2.2 in control (red bar) compared to treated plants (blue bar). Down-regulation in expression level of FW2.2 gene is evident in treated plants compared to control plants.

FIGs. 7A-B show longer and more developed root system in rice seedlings grown from rice seeds treated against the Della gene (FIG. 7B) compared to control plants (FIG. 7A).

FIGs. 8A-B show longer and more developed root and shoot systems in rice seedlings grown from rice seeds treated against the NRR gene (FIG. 8B) compared to control plants (FIG. 8A) when the seedlings were grown on nitrogen free growth medium.

FIGs. 9A-C show the homology between the *Spodoptera littoralis* genes used for seed treatment and the corn genome. FIG. 9A – NADPH gene, sequence 1 (top panel, SEQ ID NO: 14 and 22) and sequence 2 (bottom panel, SEQ ID NO: 23 and 24) showing 82% identity over 71 nucleotides and 89% identity over 35 nucleotides respectively, FIG. 9B – ATPase (SEQ ID NOs: 25 and 26) showing 72% identity over 484 nucleotides, and FIG. 9C – IAP, sequence 1 (top panel, SEQ ID NO: 27 and 28) and sequence 2 (bottom panel, SEQ ID NO: 29 and 30) showing 81% identity over 36 nucleotides and 87% identity over 31 nucleotides respectively. "Query" stands for *S. littoralis* sequences and "Subject" stands for corn sequences.

FIGs. 10A-C show the homology between the *Spodoptera littoralis* genes used for seed treatment and the tomato genome. FIG. 10A – NADPH gene, sequence 1 (top panel, SEQ ID NO: 31 and 32) showing 93% identity over 30 nucleotides and 88% identity over 25 nucleotides respectively and sequence 2 (bottom panel, SEQ ID NO: 33 and 34) FIG. 10B – ATPase (SEQ ID NOs. 35 and 36) showing 73% identity over 359 nucleotides, and FIG. 10C – IAP (SEQ ID NOs. 37 and 38) showing 93 % identity over 28 nucleotides. "Query" stands for *S. littoralis* sequences and "Subject" stands for tomato sequences.

FIGs. 11A-D are bar graphs showing mortality and average weight of live *S. littoralis* larvae. FIG. 11A shows percentage of dead worms eight days after feeding on three 43-day-old ATPase dsRNA trigger-treated and control corn plants. FIG. 11B shows average weight of live *S. littoralis* larvae at the same time point. FIG. 11C is a bar graph showing percentage of dead *S. littoralis* larvae three days after feeding on 85-days old ATPase-treated and control corn plants. FIG. 11D is a bar graph showing percentage of dead *S. littoralis* larvae seven days after feeding on 91-day-old ATPase dsRNA trigger-treated and control corn plants.

FIG. 12 is a bar graph showing percentage of dead *S. littoralis* larvae seven days after feeding on 67-day-old dsRNA trigger treated (NADPH, IAP, and ATPase) and control (EDTA) corn plants.

FIGs. 13A-B: FIG. 13A is a bar graph showing average weight of live *S. littoralis* larvae eight days after feeding on 43-day-old EF1α dsRNA trigger-treated and control (EDTA) corn plants. FIG. 13B is a bar graph showing percentage of dead *S. littoralis* larvae five days after feeding on 87-day-old EF1α dsRNA trigger-treated and control (EDTA) corn plants.

FIG. 14 is a bar graph showing average weight of live *S. littoralis* larvae eight days after feeding on 43-day-old Beta-actin dsRNA trigger-treated and control (EDTA) corn plants.

FIGs. 15A-B: FIG. 15A is a bar graph showing average weight of live *S. littoralis* larvae eight days after feeding on 43-day-old NADPH dsRNA trigger-treated and control (EDTA) corn plants. FIG. 15B is a bar graph showing percentage of dead *S. littoralis* larvae seven days after feeding on 91-day-old NADPH dsRNA trigger-treated and control (EDTA) corn plants.

FIGs. 16A-B are bar graphs showing average weight of live *S. littoralis* larvae six days after feeding on 27-day-old dsRNA trigger-treated (IAP or MIX (IAP, NADPH and ATPase)) compared to control (EDTA) plants. FIG. 16A shows average weight per repeat and FIG. 16B shows average weight per treatment.

FIGs. 17A-B are bar graphs showing average weight of live *S. littoralis* larvae after feeding on EF1α dsRNA trigger-treated corn plants. FIG. 17A shows average weight nine days after feeding on 35-day-old plants. Error bars represent standard deviation for each treatment. FIG. 17B shows average weight five days after feeding on 36-day-old plants. Error bars represent standard deviation for each plant.

FIGs. 18A-B: FIG. 18A is a bar graph showing percentage of dead *S. littoralis* larvae 12 days after feeding on 56-day-old ATPase dsRNA trigger-treated and control (GUS) corn plants. FIG. 18B is a bar graph showing percentage of dead *S. littoralis* larvae four days after feeding on 57-day-old ATPase dsRNA trigger-treated and control (GUS) corn plants.

FIGs. 19A-B: FIG. 19A is a bar graph showing average weight of live *S. littoralis* larvae ten days after feeding on 24-day-old dsRNA trigger-treated and control (EDTA, EDTA/CNTP and GFP) corn plants. Error bars represent standard deviation for each plant. FIG. 19B is a bar graph showing average weight of live *S. littoralis* larvae ten days after feeding on 25-day-old dsRNA trigger-treated and control (EDTA, EDTA/CNTP and GFP/CNTP) corn plants. Error bars represent standard deviation for each plant.

FIGs. 20A-B are bar graphs showing average *S. littoralis* larvae weight 4 days after feeding on eight-day-old dsRNA trigger-treated (EF1α and EF1α/CNTP) and control (GUS and GUS/CNTP) corn plants. FIG. 20A shows average weight of *S. littoralis* larvae per plant and FIG. 20B shows average weight of *S. littoralis* larvae per treatment. Error bars represent standard deviation of the data.

FIG. 21 is a bar graph showing average weight of live *S. littoralis* larvae three and seven days after feeding on 48-day-old dsRNA trigger-treated (NADPH, IAP, and MIX (IAP, ATPase and NADPH))and control (EDTA) tomato plants.

FIG. 22 is a bar graph showing average weight of live *S. littoralis* larvae after feeding for four days on 42-day-old dsRNA trigger-treated (Beta-actin, ATPase and NADPH) and control (EDTA) tomato plants.

FIGs. 23A-B: FIG. 23A is a bar graph showing weight of *S. littoralis* larvae after feeding for six days on 85-day-old ATPase dsRNA trigger-treated and control (EDTA) tomato plants relative to their initial weight before feeding. FIG. 23B is a bar graph showing average weight of live *S. littoralis* larvae after feeding for five days on 88-day-old ATPase dsRNA trigger-treated and control (EDTA) tomato plants.

FIGs. 24A-B: FIG. 24A is a bar graph showing average weight of *S. littoralis* larvae after feeding for four days on 95-day-old NADPH dsRNA trigger-treated and control (EDTA) tomato plants. FIG. 24B is a bar graph showing average weight of *S. littoralis* larvae after feeding for seven days on 95-day-old NADPH dsRNA trigger-treated and control (ARF8) tomato plants.

FIGs. 25A-F: FIGs. 25A and C are bar graphs showing percentage of dead *S. littoralis* larvae per plant eight and ten days, respectively, after feeding on 31-day-old dsRNA trigger-treated (EF1α#1, EF1α#2, ATPase and NADPH) and control (EDTA and GFP) corn plants. FIGs. 25 B and D are bar graphs combining the data shown in FIGs. 25 A and C into treatments. FIG. 25E is a bar graph showing average weight of live *S. littoralis* larvae 11 days after feeding on treated and control corn plants. Error bars represent standard deviation of the data. FIG. 25F is a bar graph showing average weight of live *S. littoralis* larvae after feeding for eight and nine days on 32-days old treated and control corn plants. Weight scored after eight days is shown in dark colors and weight scored after nine days is shown in bright colors. Error bars represent standard deviation of the data.

FIGs. 26A-C are bar graphs showing larval recovery and weight of Western corn rootworm (WCR) fed on corn plants grown from seeds treated with 0ppm (Null control), 50ppm or 500ppm MON104454 or transgenic maize plants expressing an RNA suppression construct targeting WCR Snf7 (positive control). FIG. 26A is a bar graph showing the percentage of larval recovery after 4 weeks. FIG. 26B is a bar graph showing the total weight of WCR larvae recovered after 4 weeks. FIG. 26C is a bar graph showing the average weight of the WCR larvae recovered after 4 weeks.

FIGs. 27A-C are bar graphs showing the results of a Colorado potato beetle (CPB) infestation assay on tomato plants grown from seeds treated with T6593, buffer ("formulation") or a GFP dsRNA trigger. FIG. 27A shows the average defoliation of the T6593 treated and control (formulation and GFP) tomato plant by CPB. FIG. 27B

shows the percentage of CPB larvae recovered. FIG. 27C shows the average weight of WCR larvae recovered from the treated plants.

FIGs. 28A-B: FIG. 28 shows the results of the Quantigene analysis on plants treated with the Tomato golden mottle virus (ToGMoV) after seed imbibition with dsRNA polynucleotide sequences. FIG. 28A shows the results after treatment with the 5'AC1 dsRNA polynucleotide (5') compared to the GUS treated control (NTrC). FIG. 28B shows the results after treatment with the 3'AC1 dsRNA polynucleotide (3') compared to the GUS treated control (NTrC).

FIGs. 29A-B: FIG. 29 shows the results of the Quantigene analysis on plants treated with the Cucumber Mosaic Virus (CMV) after seed imbibitions with the dsRNA polynucleotide sequences. FIG. 29A shows the results after treatment with the 5' NC dsRNA polynucleotide (5') compared to the GUS treated control (NTrC). FIG. 29B shows the result after treatment with the 3'NC dsRNA polynucleotide (3') compared to the GUS control (NTrC).

FIG. 30 shows the results of the Quantigene analysis on plants treated with Tomato Spotted Wilt Virus (TSWV) 3' N dsRNA polynucleotide sequence (3') compared to the GUS treated control (NTrC).

FIGs. 31A-B show the homology between the *Spodoptera littoralis* EF1α gene used for seed treatment and the corn genome. FIG. 31A – EF1α gene, sequence 1 showing 75% identity over 400 nucleotides. FIG. 31B – EF1α gene, sequence 2 showing 75% identity over 446 nucleotides. "Query" stands for *S. littoralis* sequences and "Subject" stands for corn sequences.

FIGs. 32A-C are bar graphs showing real-time PCR analyses of corn EF1α mRNA expression in 20-day-old and 48-day-old corn plants germinated from seeds treated with 50 μg/ml dsRNA for 4 hours. FIG. 32A shows fold change in corn EF1α mRNA expression following treatment with *S. littoralis* EF1α dsRNA for which GFP dsRNA treatment was used as control baseline. Expression values per individual plants were normalized to the median expression of all plants treated with GFP dsRNA. The difference in expression relative to control group had a p-value of 0.016. FIG. 32B shows fold change in corn EF1α mRNA expression following treatment with a mixture of the same dsRNAs as in Figure 32A and PEG-modified carbon nanotubes (CNTP). Expression values per individual plants were normalized to the median expression of all

plants treated with GFP dsRNA\CNTP. The difference in expression relative to control group had a p-value of 0.003. FIG. 32C shows fold change in the same corn plants 48 days post seed treatment. Expression values per individual plants were normalized to the median expression of all plants treated with GFP dsRNA\CNTP. The difference in expression relative to control group had a p-value of 0.07.

FIG. 33 is a bar graph showing real-time PCR analysis of corn EF1α mRNA expression in nine-week-old corn plants germinated from seeds treated with 132 μg/ml dsRNA derived from *S. littoralis* sequence. Expression values per individual plants were normalized to the median expression of all control plants. The difference in expression relative to control group had a p-value of 0.12.

FIGs. 34A-B are bar graphs showing real-time PCR analyses of corn EF1α mRNA expression in six-day-old corn plants germinated from seeds treated with 160 μg/ml dsRNA for 7 hours. FIG. 34A shows fold change in corn EF1α mRNA expression with respect to the GUS dsRNA treatment. FIG. 34B shows the average fold change in corn EF1α mRNA expression for all plants treated with EF1α dsRNA (both dsRNA #1 and #2, with and without CNTP), GUS dsRNA (with and without CNTP) and EDTA (with and without CNTP). Error bars represent standard deviation of the data.

FIGs. 35A-C are bar graphs showing real-time PCR analyses of corn ATPase and NADPH mRNA expression in 27-days old corn plants germinated from seeds treated with 160 μg/ml dsRNA for 2 hours. FIG. 35A shows the average fold change in corn ATPase mRNA expression. FIGs. 35B and 35C shows the average fold change in corn NADPH mRNA expression. Expression values were normalized to the average expression of plants treated with GFP dsRNA (FIGs. 35A and 35B) or to the average expression of EDTA-treated control plants (FIG. 35C). Error bars represent standard deviation of the data.

DETAILED DESCRIPTION

Unless otherwise stated, nucleic acid sequences in the text of this specification are given, when read from left to right, in the 5' to 3' direction. Nucleic acid sequences may be provided as DNA or as RNA, as specified; disclosure of one necessarily defines the other, as is known to one of ordinary skill in the art. Further, disclosure of a nucleic acid sequence discloses the sequence of its reverse complement, as one necessarily

defines the other, as is known by one of ordinary skill in the art. Where a term is provided in the singular, the inventors also contemplate aspects of the invention described by the plural of that term.

Before explaining embodiments of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

With the extensive growth of the world-population and the limited habitats for plant growth and cultivation, there is an urgent need to improve plant yields under these changing conditions. RNAi has emerged as a powerful tool for modulating gene expression which can be used for generating plants with improved stress tolerance. In plants, RNAi is typically performed by producing transgenic plants that comprise a DNA fragment that is transcribed to produce a dsRNA. This dsRNA is then processed into siRNAs that mediate the silencing of target genes, typically by targeting cleavage of the target gene by an RNA Induced Silencing Complex (RISC) or by translational repression. The major technical limitation for this technology is that many important plant crop species are difficult or impossible to transform, precluding the constitutive expression of constructs directing production of dsRNA. Moreover, questions concerning the potential ecological impact of virus-resistant transgenic plants have so far significantly limited their use (Tepfer, 2002, Annu. Rev. Phytopathol. 40, 467–491).

The present embodiments include methods of introducing exogenous non-transcribable polynucleotide trigger, for example dsRNA, molecules into plant seeds for modulating gene expression in a plant grown from the seed and/or in a phytopathic organism that feeds on or infects a plant grown from the treated seed. Several embodiments relate to methods of introducing exogenous non-transcribable polynucleotide triggers into plant seeds for controlling insect pest infestation and/or viral infection of plants grown from the seeds. Ingestion of plant material produced from seeds treated with exogenous non-transcribable polynucleotide trigger, for example dsRNA, molecules according to the present embodiments results in the cessation of feeding, growth, development, reproduction, infectivity, and eventually may result in the death of the phytopathogen. In some embodiments, the exogenous non-transcribable polynucleotide triggers are designed to silence a target gene of an

insect pest or viral pathogen. The polynucleotide triggers can be single- or double-stranded RNA or single- or double-stranded DNA or double-stranded DNA/RNA hybrids or modified analogues thereof, and can be of oligonucleotide lengths or longer. Several embodiments relate to methods of introducing dsRNA to plant seeds for modulating gene expression.

The present inventors have now devised a novel technology for introducing exogenous non-transcribable polynucleotide triggers, for example dsRNA molecules, directly to the plant seed. These non-transcribable polynucleotide trigger, for example dsRNA, molecules enter seeds and start a silencing process, which is continued during the life cycle of the plant, resulting in a plant with an improved trait of interest. The introduced polynucleotide triggers are naked and as such no exogenous transcription regulatory elements are introduced into the plant thus lowering the environmental concerns associated with transgenic plants. In some embodiments, the introduced polynucleotide trigger is naked dsRNA and as such no exogenous transcription regulatory elements are introduced into the plant. In addition, the modified seed can be germinated to generate a plant without the need of going through the laborious and cumbersome steps of tissue culture regeneration.

The present embodiments provide, in part, a delivery system for the delivery of pest control agents to pests through their exposure to a diet containing plant material produced from seeds treated with exogenous non-transcribable polynucleotide trigger, for example dsRNA, molecules according to the present embodiments.

As is illustrated herein below and in the Examples section, which follows, the present embodiments include configuring the conditions necessary to introduce exogenous non-transcribable polynucleotide triggers, for example naked dsRNA into the seeds (see *e.g.*, Example 1). The exogenous non-transcribable polynucleotide trigger, for example naked dsRNA, doesn't integrate into the genome and is highly stable in the plant and in solution (see Examples 2-4). The exogenous non-transcribable polynucleotide trigger, for example naked dsRNA, penetrates through the seed coat (testa) of both monocot and dicot plants and distributes in the endosperm and seed embryo (Examples 5-6). In one aspect, the present embodiments include altering expression of endogenous genes (Examples 8-15). In some embodiments, the endogenous gene whose expression is altered is an ortholog of a targeted pest gene. In

another aspect, the present embodiments include introducing into seeds exogenous non-transcribable polynucleotide triggers, for example dsRNA, directed to exogenous genes (*e.g.*, insect pest genes or viral genes). These results are reproduced over a number of plants of both monocot and dicot groups. In a further aspect, the present embodiments include introducing into seeds exogenous non-transcribable polynucleotide triggers, for example dsRNA, directed to essential genes of insect pests or viral pathogens in a wide range of doses and kinetics which resulted in a significant alteration of gene expression. Interestingly, the dsRNA introduced according to the present embodiments is able to down-regulate essential genes in a phytopathogen which feeds on or infects a plant grown from a treated seed (*e.g.*, *Spodoptera littoralis*, Example 7). Thus, the present results are sufficient to show that the present teachings provide a cost-effective treatment of plant seeds to achieve a desired agricultural and horticultural phenotype, such as resistance to insect pests and viral pathogens.

Provided herein are compositions and methods for inducing systemic regulation (e.g., systemic suppression or silencing) of a target gene in a plant or phytopathogen by application to the plant seed of a polynucleotide trigger molecule with a segment in a nucleotide sequence essentially identical to, or essentially complementary to, a sequence of 18 or more contiguous nucleotides in either the target gene or RNA transcribed from the target gene, whereby the composition permeates the interior of the plant seed and induces systemic regulation of the target gene in the plant grown from the seed or in a phytopathogen of the plant grown from the seed. The polynucleotide trigger molecule can be one or more polynucleotide molecules with a single such segment, multiples of such a segment, multiple different such segments, or combination thereof.

Without being bound by a particular theory, it is suggested that the newly suggested transformation modality and modulation of gene expression is associated with:

(i) Introduction of an exogenous non-transcribable polynucleotide trigger molecule, for example naked dsRNA, into the interior of seeds (as opposed to mere seed coating). The introduction is effected by soaking the seeds in a solution which comprises the exogenous non-transcribable polynucleotide trigger, for example dsRNA, such that the exogenous non-transcribable polynucleotide trigger penetrates through the

seed coat or by dipping such that the exogenous non-transcribable polynucleotide trigger coats the seed and penetrates through the coat after sowing;

(ii) Amplification of the signal generated by the exogenous non-transcribable polynucleotide trigger, for example dsRNA; and

(iii) Spreading of the signal throughout the plant.

The first step occurs only once, during and shortly after the initial seed treatment, while the second and third steps occur in a repetitive loop for as long as the silencing signal remains active in the plant.

Without being bound by theory, a suggested unbinding mode of action for the described invention is based on each step:

Introduction of an exogenous non-transcribable polynucleotide trigger, for example dsRNA, into seeds.

A typical mature seed consists of an embryo encapsulated within a maternal seed coat (testa) and an abundant layer of endosperm tissue between the embryo and seed coat. The endosperm serves as a nutrient source for the embryo during seed development, germination and seedling establishment.

Seed germination typically begins with exposure of the seeds to water, which is absorbed by the embryo and endosperm. The endosperm then expands in volume, with the endosperm of some plant species being able to grow several-fold from their original volume. The embryo, which was dormant until this stage, is now released from dormancy and cell division, expansion and differentiation begin. The endosperm feeds the developing embryo until it is developed enough to begin photosynthesis and autotrophic growth.

Based on these known mechanisms of seed germination, two possible modes of action for the initial step of "Introduction of the exogenous non-transcribable polynucleotide trigger, for example dsRNA, into seeds" are suggested:

The exogenous non-transcribable polynucleotide trigger, for example dsRNA, molecules enter the embryo directly, carried by the water-based solution which is used for the seed treatment.

The exogenous non-transcribable polynucleotide trigger, for example dsRNA, molecules enter the endosperm as part of the endosperm's water-absorption process.

These molecules then feed the embryo as it develops as part of the nutrient flow from the endosperm during germination and seed development.

Based on the results described in Figures 7-13, it is estimated that a combination of the two options takes place. That is, some of the dsRNA enters the embryo directly and some is retained in the endosperm and feeds the developing embryo during seed germination.

Amplification of the signal

Once dsRNA molecules enter the embryo, they are recognized and processed by RNAse III-like enzymes such as Dicer or Dicer-like (DCL) enzymes. DCL enzymes process the long dsRNA molecules into short, double strand RNAs (known as siRNAs or shRNAs), which are typically 21-24 nucleotides (nt) long. One of the siRNA strands is typically rapidly degraded and the second one can be incorporated in RISC (RNA Induced Silencing Complex) protein complexes, which contain an Argonaute (AGO) protein. AGO proteins contain a PIWI domain to bind siRNAs and a PAZ domain with RNAse activity. Subsequently, the siRNA/AGO complex identifies an mRNA molecule, which is complementary to the siRNA and results in its silencing by cleavage or translational repression.

The siRNA is then released from the RISC complex and can now act as a primer for an RNA-Dependant RNA Polymerase (RDRP), this is an enzyme which is unique to the plant kingdom and can generate amplification of the silencing signal by generating new dsRNA molecules (secondary siRNA). These newly-synthesized dsRNAs can be processed again as described above, therefore maintaining and amplifying the silencing signal.

Spreading of the silencing signal

Silencing spreading is a known and well-understood phenomenon in plants. Not wishing to be bound by a particular theory, it is believed that short distance, cell-to-cell spreading occurs through plasmodesmata. This process is thought to be mediated by a 21nt-long siRNA, which is the product of a DCL enzyme. Additionally, systemic spreading is achieved through the phloem across the entire plant from source to sink.

Without being bound by particular theory, it is suggested that in the described methodology, spreading of the silencing signal occurs once the silencing signal begins

and is amplified as described above. This may include both short-distance and systematic spreading by various siRNA signal molecules.

According to one embodiment, there is provided a method of introducing an exogenous non-transcribable polynucleotide trigger, for example naked double-stranded RNA (dsRNA), into a seed, the method comprising contacting the seed with the exogenous non-transcribable polynucleotide trigger, for example naked dsRNA, under conditions which allow penetration of the exogenous non-transcribable polynucleotide trigger, for example naked dsRNA into the seed, thereby introducing the dsRNA into the seed.

Several embodiments described herein relate to a method of generating a plant having a desirable phenotype, comprising a) contacting an ungerminated seed with an exogenous non-transcribable polynucleotide trigger molecule under conditions which allow penetration of said trigger molecule into the seed and b) germinating said seed to generate a plant exhibiting the desired phenotype after emerging from said seed. In some embodiments, the desirable phenotype is insect resistance. In some embodiments, the desirable phenotype is viral resistance.

As used herein, the term "trigger" or "trigger polynucleotide" refers to a bioactive polynucleotide molecule that is substantially homologous or complementary to a polynucleotide sequence of a target gene or an RNA expressed from the target gene or a fragment thereof and functions to suppress the expression of the target gene or produce a knock-down phenotype. Trigger polynucleotides are generally described in relation to their "target sequence." Trigger polynucleotides may be single-stranded DNA (ssDNA), single-stranded RNA (ssRNA), double-stranded RNA (dsRNA), double-stranded DNA (dsDNA), or double-stranded DNA/RNA hybrids. Trigger polynucleotides may comprise naturally-occurring nucleotides, modified nucleotides, nucleotide analogues or any combination thereof. In some embodiments, a trigger polynucleotide may be incorporated within a larger polynucleotide, for example in a pri-miRNA molecule. In some embodiments, a trigger polynucleotide may be processed into a small interfering RNA (siRNA).

As used herein, the term "target sequence" refers to a nucleotide sequence that occurs in a gene or gene product against which a trigger polynucleotide is directed. In this context, the term "gene" means a locatable region of genomic sequence,

corresponding to a unit of inheritance, which includes regulatory regions, such as promoters, enhancers, 5' untranslated regions, intron regions, 3' untranslated regions, transcribed regions, and other functional sequence regions that may exist as native genes or transgenes in a plant genome. Depending upon the circumstances, the term target sequence can refer to the full-length nucleotide sequence of the gene or gene product targeted for suppression or the nucleotide sequence of a portion of the gene or gene product targeted for suppression.

As used herein, the term "derived from" refers to a specified nucleotide sequence that may be obtained from a particular specified source or species, albeit not necessarily directly from that specified source or species.

As used herein, the terms "sequence," "nucleotide sequence" or "polynucleotide sequence" refer to the nucleotide sequence of a DNA molecule, an RNA molecule or a portion thereof.

The term "polynucleotide" refers to any polymer of mononucleotides that are linked by internucleotide bonds. Polynucleotides may be composed of naturally-occurring ribonucleotides, naturally-occurring deoxyribonucleotides, analogs of naturally-occurring nucleotides (*e.g.*, enantiomeric forms of naturally-occurring nucleotides), or any combination thereof. Where a polynucleotide is single-stranded, its length can be described in terms of the number of nucleotides. Where a polynucleotide is double-stranded, its length can be described in terms of the number of base pairs.

As used herein, the term "non-transcribable polynucleotide" refers to a polynucleotide that does not comprise a complete polymerase II transcription unit.

The term "gene expression" refers to the process of converting genetic information encoded in genomic DNA into RNA (*e.g.*, mRNA, rRNA, tRNA, or snRNA) through transcription of the gene via the enzymatic action of an RNA polymerase, and into protein, through translation of mRNA. Gene expression can be regulated at many stages in the process.

As used herein, the phrases "inhibition of gene expression" or "gene suppression" or "silencing a target gene" and similar terms and phrases refer to the absence or observable reduction in the level of protein and/or mRNA product from the target gene. The consequences of inhibition, suppression, or silencing can be confirmed

by examination of the outward properties of a cell or organism or by biochemical techniques.

As used herein, the term "sequence identity," "sequence similarity" or "homology" is used to describe the degree of similarity between two or more nucleotide sequences. The percentage of "sequence identity" between two sequences is determined by comparing two optimally aligned sequences over a comparison window, such that the portion of the sequence in the comparison window may comprise additions or deletions (gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity. A sequence that is identical at every position in comparison to a reference sequence is said to be identical to the reference sequence and vice-versa. An alignment of two or more sequences may be performed using any suitable computer program. For example, a widely used and accepted computer program for performing sequence alignments is CLUSTALW v1.6 (Thompson, *et al.* Nucl. Acids Res., 22: 4673-4680, 1994).

By "essentially identical" or "essentially complementary" is meant that the bioactive polynucleotide trigger (or at least one strand of a double-stranded polynucleotide or portion thereof, or a portion of a single strand polynucleotide) hybridizes under physiological conditions to the endogenous gene, an RNA transcribed there from, or a fragment thereof, to effect regulation or suppression of the endogenous gene. For example, in some embodiments, a bioactive polynucleotide trigger has 100 percent sequence identity or at least about 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent sequence identity when compared to a sequence of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60 or more contiguous nucleotides in the target gene or RNA transcribed from the target gene. In some embodiments, a bioactive polynucleotide trigger has 100 percent sequence complementarity or at least about 83, 84, 85, 86, 87, 88, 89, 90, 91,

92, 93, 94, 95, 96, 97, 98, or 99 percent sequence complementarity when compared to a sequence of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60 or more contiguous nucleotides in the target gene or RNA transcribed from the target gene. In some embodiments, a bioactive polynucleotide trigger has 100 percent sequence identity with or complementarity to one allele or one family member of a given target gene (coding or non-coding sequence of a gene). In some embodiments, a bioactive polynucleotide trigger has at least about 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent sequence identity with or complementarity to multiple alleles or family members of a given target gene. In some embodiments, a bioactive polynucleotide trigger has 100 percent sequence identity with or complementarity to multiple alleles or family members of a given target gene.

As used herein, nucleic acid sequence molecules are said to exhibit "complete complementarity" when every nucleotide of one of the sequences read 5' to 3' is complementary to every nucleotide of the other sequence when read 3' to 5'. A nucleotide sequence that is completely complementary to a reference nucleotide sequence will exhibit a sequence identical to the reverse complement sequence of the reference nucleotide sequence.

Homologous sequences include both orthologous and paralogous sequences. The term "paralogous" relates to gene-duplications within the genome of a species leading to paralogous genes. The term "orthologous" relates to homologous genes in different organisms due to ancestral relationship.

As used herein, the terms "exogenous polynucleotide" and "exogenous nucleic acid molecule" relative to an organisms refer to a heterologous nucleic acid sequence which is not naturally expressed within that organism, for example a plant. An exogenous nucleic acid molecule may comprise a nucleic acid sequence which is identical or partially homologous to an endogenous nucleic acid sequence of the organism.

As used herein, the terms "endogenous polynucleotide" and "endogenous nucleic acid" refers to nucleic acid sequences that are found in an organism's cell. In certain aspects, an endogenous nucleic acid may be part of the nuclear genome or the

plastid genome. As used herein, endogenous nucleic acids do not include viral, parasite or pathogen nucleic acids, for example an endovirus sequence.

As used herein the phrase "naked dsRNA" refers to a dsRNA nucleic acid molecule which is non-transcribable in a plant cell. Thus, the naked dsRNA molecule is not comprised in a nucleic acid expression construct such as a viral vector. According to some embodiments of the invention, the naked dsRNA molecule is not derived from a viral vector. According to some embodiments, the dsRNA is not a product of a natural pathogenic or viral infection. According to some embodiments, the naked dsRNA may comprise regulatory elements for in-vitro transcription, such as the T7 promoter. According to some embodiments of the invention, the naked dsRNA may be modified *e.g.*, chemically modified, to confer higher bioavailability, penetration into the seeds and/or improved shelf-life.

As used herein the term "dsRNA" relates to two strands of anti-parallel polyribonucleic acids held together by base pairing. The dsRNA molecule may be formed by intramolecular hybridization or intermolecular hybridization. In some embodiments, the dsRNA may comprise a single strand of RNA that self-hybridizes to form a hairpin structure having an at least partially double-stranded structure including at least one segment that will hybridize to an RNA transcribed from the gene targeted for suppression. In some embodiments, the dsRNA may comprise two separate strands of RNA that hybridize through complementary base pairing. The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus. The two strands can be of identical length or of different lengths provided there is enough sequence homology between the two strands that a double stranded structure is formed with at least 80%, 90%, 95% or 100% complementarity over the entire length. According to an embodiment of the invention, there are no overhangs for the dsRNA molecule. According to another embodiment of the invention, the dsRNA molecule comprises overhangs. According to other embodiments, the strands are aligned such that there are at least 1, 2, or 3 bases at the end of the strands which do not align (*i.e.*, for which no complementary bases occur in the opposing strand) such that an overhang of 1, 2 or 3 residues occurs at one or both ends of the duplex when strands are annealed.

As will be appreciated by one of ordinary skill in the art, a dsRNA molecule of the present disclosure may refer to either strand of the anti-parallel nucleic acids. As will also be appreciated by one of ordinary skill in the art, a dsRNA molecule of the present disclosure includes both a 'sense' and 'antisense' strand and that the sense and antisense strands are reverse complements of each other in a region of base pairing. As used herein the sequence of a dsRNA molecule for regulating a target gene of interest is provided as the 'sense' orientation with respect to the target gene of interest. As used herein, "the reverse complement of a dsRNA molecule for regulating a target gene of interest" refers to a nucleic acid sequence in the 'antisense' orientation.

As mentioned, any dsRNA molecule can be used in accordance with the present teachings. In some embodiments, dsRNA used in the present application is subject to amplification by RNA-Dependant RNA Polymerase (RDRP). Without being limited, dsRNA can be siRNA, shRNA, pre-miRNA, or pri-miRNA.

The polynucleotides, DNA, RNA, dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA or miRNA of the present embodiments may be produced chemically or enzymatically by one skilled in the art through manual or automated reactions or in vivo in another organism. RNA may also be produced by partial or total organic synthesis; any modified ribonucleotide can be introduced by in vitro enzymatic or organic synthesis. The RNA may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (*e.g.*, T3, T7, SP6). The use and production of an expression construct are known in the art (see, for example, WO 97/32016; U.S. Pat. Nos. 5,593,874, 5,698,425, 5,712,135, 5,789,214, and 5,804,693). If synthesized chemically or by in vitro enzymatic synthesis, the RNA may be purified prior to introduction into the seed. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the RNA may be used with no or a minimum of purification to avoid losses due to sample processing. The RNA may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

The present teachings relate to various lengths of dsRNA, whereby the shorter version *i.e.*, x is shorter or equals 50 bp (*e.g.*, 17-50), is referred to as siRNA or miRNA.

Longer dsRNA molecules of 51-600 or more than 600 bp are referred to herein as dsRNA, which can be further processed for siRNA molecules.

In one embodiment, the dsRNA in the present application is between 20 and 100 bp, between 25 and 90 bp, between 30 and 80 bp, between 30 and 70 bp, between 30 and 60 bp, or between 30 and 50 bp. In another embodiment, the dsRNA in the present application is about 50 bp. In a further embodiment, the dsRNA comprises 1-base, 2-base or 3-base 5'-overhangs on one or both termini. In another embodiment, the dsRNA does not comprise 1-base, 2-base or 3-base 5'-overhangs on one or both termini. In a further embodiment, the dsRNA comprises 1-base, 2-base or 3-base 3'-overhangs on one or both termini. In another embodiment, the dsRNA does not comprise 1-base, 2-base or 3-base 3'-overhangs on one or both termini.

In another embodiment, the dsRNA in the present application is between 100 and 1,000 bp, between 200 and 900 bp, between 300 and 800 bp, between 400 and 700 bp, between 400 and 600 bp, or between 400 and 500 bp. In another embodiment, the dsRNA in the present application is about 450 bp. In another embodiment, the dsRNA in the present application is about 550 bp. In another embodiment, the dsRNA in the present application is about 650 bp. In another embodiment, the dsRNA in the present application is about 750 bp. In another embodiment, the dsRNA in the present application is about 850 bp. In a further embodiment, the dsRNA comprises 1-base, 2-base or 3-base 5'-overhangs on one or both termini. In another embodiment, the dsRNA does not comprise 1-base, 2-base or 3-base 5'-overhangs on one or both termini. In a further embodiment, the dsRNA comprises 1-base, 2-base or 3-base 3'-overhangs on one or both termini. In another embodiment, the dsRNA does not comprise 1-base, 2-base or 3-base 3'-overhangs on one or both termini.

In one embodiment, the dsRNA in the present application is between 15 and 500 bp, between 15 and 450 bp, between 15 and 400 bp, between 15 and 350 bp, between 15 and 300 bp, between 15 and 250 bp, between 15 and 200 bp, between 15 and 150 bp, between 15 and 100 bp, between 15 and 90 bp, between 15 and 80 bp, between 15 and 70 bp, between 15 and 60 bp, between 15 and 50 bp, between 15 and 40 bp, between 15 and 35 bp, between 15 and 30 bp, or between 15 and 25 bp. In another embodiment, the dsRNA in the present application is at least about 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 500, 600, 800, 900, 1000 bp long. In a further

embodiment, the dsRNA in the present application is between 100 and 1000 bp, between 200 and 1000 bp, between 300 and 1000 bp, between 400 and 1000 bp, between 500 and 1000 bp, between 600 and 1000 bp, between 700 and 1000 bp, between 800 and 1000 bp, or between 900 and 1000 bp.

The term "siRNA" refers to small inhibitory RNA duplexes (generally between 17-30 basepairs, but also longer *e.g.*, 31-50 bp) that induce the RNA interference (RNAi) pathway. Typically, siRNAs are chemically synthesized as 21mers with a central 19 bp duplex region and symmetric 2-base 3'-overhangs on the termini, although it has been recently described that chemically synthesized RNA duplexes of 25-30 base length can have as much as a 100-fold increase in potency compared with 21mers at the same location. The observed increased potency obtained using longer RNAs in triggering RNAi is theorized to result from providing Dicer with a substrate (27mer) instead of a product (21mer) and that this improves the rate or efficiency of entry of the siRNA duplex into RISC.

It has been found that position of the 3'-overhang influences potency of a siRNA and asymmetric duplexes having a 3'-overhang on the antisense strand are generally more potent than those with the 3'-overhang on the sense strand (Rose *et al.*, 2005). This can be attributed to asymmetrical strand loading into RISC, as the opposite efficacy patterns are observed when targeting the antisense transcript.

The strands of a double-stranded interfering RNA (*e.g.*, a siRNA) may be connected to form a hairpin or stem-loop structure (*e.g.*, a shRNA). Thus, as mentioned the RNA silencing agent of some embodiments of the invention may also be a short hairpin RNA (shRNA).

The term "shRNA," as used herein, refers to an RNA agent having a stem-loop structure, comprising a first and second region of complementary sequence, the degree of complementarity and orientation of the regions being sufficient such that base pairing occurs between the regions, the first and second regions being joined by a loop region, the loop resulting from a lack of base pairing between nucleotides (or nucleotide analogs) within the loop region. The number of nucleotides in the loop is a number between and including 3 to 23, or 5 to 15, or 7 to 13, or 4 to 9, or 9 to 11. Some of the nucleotides in the loop can be involved in base-pair interactions with other nucleotides in the loop. Examples of oligonucleotide sequences that can be used to form the loop

include 5'-UUCAAGAGA-3' (Brummelkamp, T. R. *et al.* (2002) Science 296: 550) and 5'-UUUGUGUAG-3' (Castanotto, D. *et al.* (2002) RNA 8:1454). It will be recognized by one of skill in the art that the resulting single chain oligonucleotide forms a stem-loop or hairpin structure comprising a double-stranded region capable of interacting with the RNAi machinery.

As used herein, the phrase "microRNA (also referred to herein interchangeably as "miRNA" or "miR") or a precursor thereof" refers to a microRNA (miRNA) molecule acting as a post-transcriptional regulator. Typically, the miRNA molecules are RNA molecules of about 20 to 22 nucleotides in length which can be loaded into a RISC complex and which direct the cleavage of another RNA molecule, wherein the other RNA molecule comprises a nucleotide sequence essentially complementary to the nucleotide sequence of the miRNA molecule.

Typically, a miRNA molecule is processed from a "pre-miRNA" or as used herein a precursor of a pre-miRNA molecule by proteins, such as DCL proteins, present in any plant cell and loaded onto a RISC complex where it can guide the cleavage of the target RNA molecules.

Pre-microRNA molecules are typically processed from pri-microRNA molecules (primary transcripts). The single stranded RNA segments flanking the pre-microRNA are important for processing of the pri-miRNA into the pre-miRNA. The cleavage site appears to be determined by the distance from the stem-ssRNA junction (Han *et al.* 2006, Cell 125, 887-901, 887-901).

As used herein, a "pre-miRNA" molecule is an RNA molecule of about 100 to about 200 nucleotides, preferably about 100 to about 130 nucleotides which can adopt a secondary structure comprising an imperfect double stranded RNA stem and a single stranded RNA loop (also referred to as "hairpin") and further comprising the nucleotide sequence of the miRNA (and its complement sequence) in the double stranded RNA stem. According to a specific embodiment, the miRNA and its complement are located about 10 to about 20 nucleotides from the free ends of the miRNA double stranded RNA stem. The length and sequence of the single stranded loop region are not critical and may vary considerably, *e.g.,* between 30 and 50 nt in length. The complementarity between the miRNA and its complement need not be perfect and about 1 to 3 bulges of unpaired nucleotides can be tolerated. The secondary structure adopted by an RNA

molecule can be predicted by computer algorithms conventional in the art such as mFOLD. The particular strand of the double stranded RNA stem from the pre-miRNA which is released by DCL activity and loaded onto the RISC complex is determined by the degree of complementarity at the 5′ end, whereby the strand which at its 5′ end is the least involved in hydrogen bounding between the nucleotides of the different strands of the cleaved dsRNA stem is loaded onto the RISC complex and will determine the sequence specificity of the target RNA molecule degradation. However, if empirically the miRNA molecule from a particular synthetic pre-miRNA molecule is not functional (because the "wrong" strand is loaded on the RISC complex); it will be immediately evident that this problem can be solved by exchanging the position of the miRNA molecule and its complement on the respective strands of the dsRNA stem of the pre-miRNA molecule. As is known in the art, binding between A and U involving two hydrogen bounds, or G and U involving two hydrogen bounds is less strong than between G and C involving three hydrogen bounds. Examples of hairpin sequences are provided in Tables 3, 4, 6, 7, 13, 18, 26, 27, 28, 34, 35, 36, and 37 below.

Naturally occurring miRNA molecules may be comprised within their naturally occurring pre-miRNA molecules but they can also be introduced into existing pre-miRNA molecule scaffolds by exchanging the nucleotide sequence of the miRNA molecule normally processed from such existing pre-miRNA molecule for the nucleotide sequence of another miRNA of interest. The scaffold of the pre-miRNA can also be completely synthetic. Likewise, synthetic miRNA molecules may be comprised within, and processed from, existing pre-miRNA molecule scaffolds or synthetic pre-miRNA scaffolds. Some pre-miRNA scaffolds may be preferred over others for their efficiency to be correctly processed into the designed microRNAs, particularly when expressed as a chimeric gene wherein other DNA regions, such as untranslated leader sequences or transcription termination and polyadenylation regions are incorporated in the primary transcript in addition to the pre-microRNA.

According to the present teachings, the dsRNA molecules may be naturally occurring or synthetic.

The dsRNA can be a mixture of long and short dsRNA molecules such as, dsRNA, siRNA, siRNA+dsRNA, siRNA+miRNA, or any combination of same. According to a specific embodiment, the dsRNA is a siRNA (100 %). According to a

specific embodiment the dsRNA is a siRNA+dsRNA combination in various ratios. Any dsRNA to siRNA ratio can be used for the siRNA+dsRNA combination. For example, a ratio of 1 to 1: one dsRNA mixed with the same sequence after RNAse III treatment. According to another embodiment, the dsRNA to siRNA ratio is 2:1, 1.5:1, 1.3:1, 1:0.01, 1:0.05 or 1:0.1. According to a further embodiment, the dsRNA to siRNA ratio is 2:1 to 1:0.1. According to a specific embodiment, the dsRNA is purified dsRNA (100 %). According to another embodiment, the dsRNA to siRNA ratio is 1:2, 1:5, 1:10, 1:20, or 1:50. According to a further embodiment, the dsRNA is purified siRNA (100%).

The dsRNA molecule can be designed for specifically targeting a target gene of interest. In some embodiments, the target gene is an essential gene of an insect pest. In some embodiments, the target gene is a viral gene. It will be appreciated that the dsRNA can be used to down-regulate one or more target genes. If a number of target genes are targeted, a heterogenic composition which comprises a plurality of dsRNA molecules for targeting a number of target genes is used. Alternatively said plurality of dsRNA molecules are separately applied to the seeds (but not as a single composition). According to a specific embodiment, a number of distinct dsRNA molecules for a single target are used, which may be separately or simultaneously (*e.g.*, co-formulation) applied.

According to one embodiment, the target gene is endogenous to the plant. Down regulating such a gene is typically important for conferring the plant with an improved, agricultural, horticultural, nutritional trait ("improvement" or an "increase" is further defined herein below). It will be appreciated that the treatment with the dsRNA may result in an up-regulation of the target gene (which follows a suggested mechanism that is provided herein below) however such an up-regulation may be transient.

According to another embodiment, the target gene is exogenous to the plant. In some embodiments, the target gene is an insect pest gene. In some embodiments, the target gene is a viral gene. It will further be appreciated that the treatment with the dsRNA may result in an up-regulation of a plant ortholog of the target gene.

Several embodiments described herein relate to guidelines for the design and selection of non-transcribable polynucleotide trigger, for example dsRNA, molecules for efficient RNA silencing in phytopathogens, which nourish or depend on a plant for

growth/replication and/or survival. Not wishing to be bound by a particular theory, non-transcribable polynucleotide trigger, for example dsRNA, molecules having a sufficient level of homology to an endogenous plant gene allows for degradation and amplification of the primary siRNAs (those which are triggered by Dicer processing) to generate secondary siRNAs formed by DICER-LIKE 4 (DCL4). Such non-transcribable polynucleotide trigger, for example dsRNA, molecules can be selected for having minimal effect on the plant growth and viability. In some embodiments, the secondary siRNAs are of sufficient homology to a gene of a phytopathogen so as allow the degradation of the targeted phytopathogen gene via an RNA interference mode. In some embodiments, a phytopathogen provided with a plant material grown from a seed treated with a non-transcribable polynucleotide trigger, for example dsRNA, molecule as described herein will lose viability either by the induction of growth arrest or death. Such non-transcribable polynucleotide trigger, for example dsRNA molecules are considered as valuable pesticides and can have wide applications in agriculture and horticulture.

Without being bound by particular theory, it is suggested that one mode of modulation of gene expression is associated with: (i) introduction of non-transcribable polynucleotide trigger, for example dsRNA, molecules into the interior of seeds (as opposed to mere seed coating); (ii) amplification of the signal produced from introduction of the non-transcribable polynucleotide trigger, for example dsRNA, molecule; and spreading of the signal throughout the plant. The first step occurs only once, during and shortly after the initial seed treatment, while the second and third steps occur in a repetitive loop for as long as the silencing signal remains active in the plant. As mentioned, introduction of the compositions of the present invention can also be performed to other organs/cells of the plant (as opposed to seeds) using conventional delivery methods such as particle bombardment, grafting, soaking, topical application with a transfer agent and the like. Thus steps (i) and (ii), defined above, are shared also by this mode of administration.

A phytopathogen feeding-on or infecting a plant which comprises any of the dsRNA, primary siRNA or secondary siRNAs which target an essential gene of the phytopathogen will exhibit a growth arrest or death, thereby reducing its injurious effect on the plant or plant product.

In some embodiments, there is provided a method of introducing naked double-stranded RNA (dsRNA) into a seed, the method comprising contacting the seed with the naked dsRNA under conditions which allow penetration of a nucleic acid sequence having: a homology level to a gene of a phytopathogenic organism sufficient to induce degradation of said gene of said phytopathogenic organism, wherein said phytopathogenic organism depends on said plant for growth and wherein said degradation induces a growth arrest or death of said phytopathogenic organism. In some embodiments, the dsRNA targets a gene that contains regions that are poorly conserved between individual phytopathogenic organisms, or between the phytopathogenic organism and the host plant. In certain embodiments it may be desirable to target a gene in a phytopathogenic organism that has no known homologs in other organisms, such as the host plant.

In some embodiments, a non-transcribable polynucleotide trigger, for example dsRNA, molecule is selected of sufficient homology to a plant gene to mediate its degradation in an RNA interference mediated function.

According to one embodiment, there is provided a method of introducing naked double-stranded RNA (dsRNA) into a seed, the method comprising contacting the seed with the naked dsRNA under conditions which allow penetration of a nucleic acid sequence having:

(i) a homology level to a plant gene sufficient to induce amplification of secondary siRNA products of said dsRNA in a plant cell comprising the same and wherein modification of the expression of the plant gene by said dsRNA does not substantially affect any of biomass, vigor or yield of said plant; and

(ii) a homology level to a gene of a phytopathogenic organism sufficient to induce degradation of said gene of said phytopathogenic organism, wherein said phytopathogenic organism depends on said plant for growth and wherein said degradation induces a growth arrest or death of said phytopathogenic organism.

In some embodiments, the dsRNA has a homology level to a plant gene sufficient to induce amplification of secondary siRNA products of said dsRNA in a plant cell comprising the dsRNA and wherein altering expression of the plant gene by said dsRNA does not substantially affect any of biomass, vigor or yield of said plant.

The plant gene can be naturally expressed in the plant (endogenous) or a result of genetic transformation (transgenic plant).

In some embodiments, the dsRNA has a homology level to a plant gene that:

(i) is expressed in all or most plant organs, starting from germination;

(ii) is a non-vital gene, such that its down regulation or up regulation doesn't affect the plant's any of plant's biomass, yield, vigor; and/or

(iii) is not associated with endurance of abiotic or biotic stress.

The plant gene can be selected having at least one of the above characteristics *i.e.*, (i), (ii) or (iii). Alternatively, the plant gene fulfils two criteria such as (i) and (ii), (i) and (iii) or (ii) and (iii). Alternatively all the three criteria prevail *i.e.*, (i), (ii) and (iii). In some embodiments, the dsRNA has a homology level to a plant gene that doesn't affect the biomass, yield, and/or vigor of the plant when measures are taken to grow the plant under optima/normal conditions or conditions which do not require function of the gene for optimal growth, vigor, biomass, and/or yield. As used herein the phrase "doesn't substantially affect" refers to no effect as compared to the same characteristic in an isogenic plant of the same developmental stage and growth conditions. Alternatively, said characteristic is only slightly affected by no more than 10 %, 8 %, 7 %, 6 %, 5 %, 4 %, 3 %, 2 % or 1 %.

According to some embodiments, the nucleic acid sequence of the non-transcribable polynucleotide trigger, for example dsRNA, molecule is selected so as to exhibit sufficient homology to recruit the RDR6 system and generate secondary siRNA transcripts. Such a homology level is typically at least 80 % identity to an endogenous plant gene over at least 25 consecutive bp. According to an alternative embodiment, the homology level of the non-transcribable polynucleotide trigger, for example dsRNA, molecule is at least 85% identity to a plant gene over at least 25 consecutive bp. According to an alternative embodiment, the homology level of the non-transcribable polynucleotide trigger, for example dsRNA, molecule is at least 88% identity to the plant gene over at least 25 consecutive bp. According to an alternative embodiment, the homology level of the non-transcribable polynucleotide trigger, for example dsRNA, molecule is at least 90 % identity to the plant gene over at least 25 consecutive bp of the target gene. According to an alternative embodiment, the homology level of the non-transcribable polynucleotide trigger, for example dsRNA, molecule is at least 92%

identity to the plant gene over at least 25 consecutive bp. According to an alternative embodiment, the homology level of the non-transcribable polynucleotide trigger, for example dsRNA, molecule is at least 95% identity to the plant gene over at least 25 consecutive bp. According to an alternative embodiment, the homology level of the non-transcribable polynucleotide trigger, for example dsRNA, molecule is at least 25 consecutive bp.

According to some embodiments, the non-transcribable polynucleotide trigger, for example dsRNA, molecule is at least is 70 bp or longer say 70-700, 70-600, 70-500, 70-400, 70-300, 70-200, 70-100 bp.

According to some embodiments, the non-transcribable polynucleotide trigger, for example dsRNA, molecule comprises a nucleic acid segment at least 70 bp in length which is at least 65 % identical to the plant gene. According to a specific embodiment, the nucleic acid sequence comprises a nucleic acid segment at least 70 bp in length which is at least 70 % identical (over the entire sequence) to the plant gene. According to a specific embodiment, the nucleic acid sequence comprises a nucleic acid segment at least 70 bp in length which is at least 75 % identical (over the entire sequence) to the plant gene. According to a specific embodiment, the nucleic acid sequence comprises a nucleic acid segment at least 70 bp in length which is at least 80% identical (over the entire sequence) to the plant gene. According to a specific embodiment, the nucleic acid sequence comprises a nucleic acid segment at least 70 bp in length which is at least 85 % identical (over the entire sequence) to the plant gene. According to a specific embodiment, the nucleic acid sequence comprises a nucleic acid segment at least 70 bp in length which is at least 90 % identical (over the entire sequence) to the plant gene. According to a specific embodiment, the nucleic acid sequence comprises a nucleic acid segment at least 70 bp in length which is at least 95 % identical (over the entire sequence) to the plant gene. According to a specific embodiment, the nucleic acid sequence comprises a nucleic acid segment at least 70 bp in length which is 100 % identical (over the entire sequence) to the plant gene.

In some embodiments, the nucleic acid sequence of the non-transcribable polynucleotide trigger, for example dsRNA, molecule comprises a second nucleic acid segment at least 17 bp in length (over at least 17 consecutive bp) which is at least 85% identical to a plant gene. According to a specific embodiment, the nucleic acid

sequence of the non-transcribable polynucleotide trigger, for example dsRNA, molecule comprises a second nucleic acid segment at least 17 bp in length (over at least 17 consecutive bp) which is at least 90 % identical to a plant gene. According to a specific embodiment, the nucleic acid sequence of the non-transcribable polynucleotide trigger, for example dsRNA, molecule comprises a second nucleic acid segment at least 17 bp in length (over at least 17 consecutive bp) which is at least 95 % identical to a plant gene. According to a specific embodiment, the nucleic acid sequence of the non-transcribable polynucleotide trigger, for example dsRNA, molecule comprises a second nucleic acid segment at least 17 bp in length (over at least 17 consecutive bp) which is 100 % identical to a plant gene.

According to a specific embodiment, the first nucleic acid segment and the second nucleic acid segment overlap (by at least 5 %, 10 %, 20 %, 40 %, 50 % or more). According to a specific embodiment, the overlap is by 5-99 %, 5-95 %, 5-90 %, 5-80 %, 5-70 %, 5-60 %. According to a specific embodiment, the first nucleic acid segment and the second nucleic acid segment are in no overlap.

In some embodiments, the nucleic acid sequence of the non-transcribable polynucleotide trigger, for example dsRNA, molecule is selected having a homology level to a gene of a phytopathogenic organism sufficient to induce degradation of the gene of the phytopathogenic organism, wherein the phytopathogenic organism depends on the plant for growth and wherein the degradation induces a growth arrest or death of the phytopathogenic organism.

Thus, the non-transcribable polynucleotide trigger, for example dsRNA, molecule exhibits at least 80 %, 85 %, 88 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or even 100 % identity to the gene of the phytopathogen.

In some embodiments, the non-transcribable polynucleotide trigger, for example dsRNA, molecule can be designed for specifically targeting a target gene of interest. It will be appreciated that the non-transcribable polynucleotide trigger, for example dsRNA, molecule can be used to down-regulate one or more target genes of the phytopathogen or plant (in the latter case to increase the amplification). If a number of target genes are targeted, a heterogenic composition which comprises a plurality of non-transcribable polynucleotide trigger, for example dsRNA, molecules for targeting a number of target genes is used. Alternatively said plurality of non-transcribable

polynucleotide trigger, for example dsRNA molecules are separately applied to the seeds (but not as a single composition).

Down regulation of the target gene may be important for conferring improved tolerance to biotic stress induced by phytopathogen. The biotic stress can affect any of the plant's biomass, vigor or yield, as well as tolerance to abiotic stress and nitrogen use efficiency. The target gene (plant of phytopathogen) may comprise a nucleic acid sequence which is transcribed to an mRNA which codes for a polypeptide.

As used herein, the term "endogenous" refers to a gene whose expression (mRNA or protein) takes place in the plant. Typically, the endogenous gene is naturally expressed in the plant or originates from the plant. Thus, the plant may be a wild-type plant. However, the plant may also be a genetically modified plant (transgenic).

As used herein the term "isolated" refers to the isolation from the physiological, natural environment. In the case of dsRNA, isolation from cellular organelles, such as the cytosol or nucleus. In the case of a seed, isolation from other plant parts such as the fruit. According to a specific embodiment, an isolated dsRNA molecule is in a form of naked RNA.

Down regulation of the target gene may be important for conferring improved one of-, or at least one of (*e.g.*, two of- or more), biomass, vigor, yield, abiotic stress tolerance, biotic stress tolerance or improved nitrogen use efficiency.

Examples of target genes include, but are not limited to, an enzyme, a structural protein, a plant regulatory protein, a miRNA target gene, or a non-coding RNA such as a miRNA of the plant. WO2011067745, WO 2009125401 and WO 2012056401 provide examples of miRNA sequences or targets of miRNAs (*e.g.*, mRNA167, miRNA 156, miR164 and targets thereof NFY, SPL17 and NAC, respectively) which expression can be silenced to improve a plant trait. Other examples of target genes which may be subject to modulation according to the present teachings are described in the Examples section which follows.

The target gene may comprise a nucleic acid sequence which is transcribed to an mRNA which codes for a polypeptide. Alternatively, the target gene can be a non-coding gene such as a miRNA or a siRNA.

For example, in order to silence the expression of an mRNA of interest, synthesis of the dsRNA suitable for use with some embodiments of the invention can be

selected as follows. First, the mRNA sequence is scanned including the 3' UTR and the 5' UTR.

Second, the mRNA sequence is compared to an appropriate genomic database using any sequence alignment software, such as the BLAST software available from the NCBI server (wwwdotncbidotnlmdotnihdotgov/BLAST/). Putative regions in the mRNA sequence which exhibit significant homology to other coding sequences are filtered out.

Qualifying target sequences are selected as template for dsRNA synthesis. Preferred sequences are those that have as little homology to other genes in the genome to reduce an "off-target" effect.

In one embodiment, the dsRNA may comprise a target sequence in an intron, exon, 3' UTR, 5' UTR, or a regulatory element of a target gene, or combinations thereof. In one embodiment, the dsRNA of the present application may comprise a target site residing in a promoter.

It will be appreciated that the RNA silencing agent of some embodiments of the invention need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides.

The dsRNA may be synthesized using any method known in the art, including either enzymatic syntheses or solid-phase syntheses. These are especially useful in the case of short polynucleotide sequences with or without modifications as explained above. Equipment and reagents for executing solid-phase synthesis are commercially available from, for example, Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the capabilities of one skilled in the art and can be accomplished via established methodologies as detailed in, for example: Sambrook, J. and Russell, D. W. (2001), "Molecular Cloning: A Laboratory Manual"; Ausubel, R. M. *et al.*, eds. (1994, 1989), "Current Protocols in Molecular Biology," Volumes I-III, John Wiley & Sons, Baltimore, Maryland; Perbal, B. (1988), "A Practical Guide to Molecular Cloning," John Wiley & Sons, New York; and Gait, M. J., ed. (1984), "Oligonucleotide Synthesis"; utilizing solid-phase chemistry, *e.g.,* cyanoethyl phosphoramidite followed by deprotection, desalting, and purification by, for example, an automated trityl-on method or HPLC.

As mentioned, the naked dsRNA molecule is directly contacted with the seed.

The seed may be of any plant, such as of the Viridiplantae super family including monocotyledon and dicotyledon plants. Other plants are listed herein below. According to an embodiment of the invention, the cells of the plant comprise RNA dependent RNA polymerase activity and the target RNA molecule of the dsRNA to ensure amplification of the dsRNA.

The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and isolated plant cells, tissues and organs. The plant may be in any form including suspension cultures, embryos, meristematic regions, callus tissue, leaves, gametophytes, sporophytes, pollen, and microspores. It will be appreciated, that the plant or seed thereof may be transgenic plants.

As used herein the phrase "plant cell" refers to plant cells which are derived and isolated from disintegrated plant cell tissue or plant cell cultures. Plant cells may be reproductive cells (*i.e.*, cells from a tissue contributing directly to the sexual reproduction of a plant) or non-reproductive cells (*i.e.*, cells from a tissue not involved in the sexual reproduction of a plant). Plant cells may be cells that are capable of regenerating into a whole plant or cells that cannot regenerate into a whole plant, for example, enucleated mature sieve tube cells.

As used herein the phrase "plant cell culture" refers to any type of native (naturally occurring) plant cells, plant cell lines and genetically modified plant cells, which are not assembled to form a complete plant, such that at least one biological structure of a plant is not present. Optionally, the plant cell culture of this aspect of the present invention may comprise a particular type of a plant cell or a plurality of different types of plant cells. It should be noted that optionally plant cultures featuring a particular type of plant cell may be originally derived from a plurality of different types of such plant cells.

Any commercially or scientifically valuable plant is envisaged in accordance with some embodiments of the invention. Plants that are particularly useful in the methods of the invention include all plants which belong to the super family Viridiplantae, in particular monocotyledonous and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the

list comprising *Acacia spp.*, *Acer spp.*, *Actinidia spp.*, *Aesculus spp.*, *Agathis australis*, *Albizia amara*, *Alsophila tricolor*, *Andropogon spp.*, *Arachis spp*, *Areca catechu*, *Astelia fragrans*, *Astragalus cicer*, *Baikiaea plurijuga*, *Betula spp.*, *Brassica spp.*, *Bruguiera gymnorrhiza*, *Burkea africana*, *Butea frondosa*, *Cadaba farinosa*, *Calliandra spp*, *Camellia sinensis*, *Canna indica*, *Capsicum spp.*, *Cassia spp.*, *Centroema pubescens*, *Chacoomeles spp.*, *Cinnamomum cassia*, *Coffea arabica*, *Colophospermum mopane*, *Coronillia varia*, *Cotoneaster serotina*, *Crataegus spp.*, *Cucumis spp.*, *Cupressus spp.*, *Cyathea dealbata*, *Cydonia oblonga*, *Cryptomeria japonica*, *Cymbopogon spp.*, *Cynthea dealbata*, *Cydonia oblonga*, *Dalbergia monetaria*, *Davallia divaricata*, *Desmodium spp.*, *Dicksonia squarosa*, *Dibeteropogon amplectens*, *Dioclea spp*, *Dolichos spp.*, *Dorycnium rectum*, *Echinochloa pyramidalis*, *Ehraffia spp.*, *Eleusine coracana*, *Eragrestis spp.*, *Erythrina spp.*, *Eucalyptus spp.*, *Euclea schimperi*, *Eulalia vi/losa*, *Pagopyrum spp.*, *Feijoa sellowlana*, *Fragaria spp.*, *Flemingia spp*, *Freycinetia banksli*, *Geranium thunbergii*, *GinAgo biloba*, *Glycine javanica*, *Gliricidia spp*, *Gossypium hirsutum*, *Grevillea spp.*, *Guibourtia coleosperma*, *Hedysarum spp.*, *Hemaffhia altissima*, *Heteropogon contoffus*, *Hordeum vulgare*, *Hyparrhenia rufa*, *Hypericum erectum*, *Hypeffhelia dissolute*, *Indigo incamata*, *Iris spp.*, *Leptarrhena pyrolifolia*, *Lespediza spp.*, *Lettuca spp.*, *Leucaena leucocephala*, *Loudetia simplex*, *Lotonus bainesli*, *Lotus spp.*, *Macrotyloma axillare*, *Malus spp.*, *Manihot esculenta*, *Medicago saliva*, *Metasequoia glyptostroboides*, *Musa sapientum*, *Nicotianum spp.*, *Onobrychis spp.*, *Ornithopus spp.*, *Oryza spp.*, *Peltophorum africanum*, *Pennisetum spp.*, *Persea gratissima*, *Petunia spp.*, *Phaseolus spp.*, *Phoenix canariensis*, *Phormium cookianum*, *Photinia spp.*, *Picea glauca*, *Pinus spp.*, *Pisum sativam*, *Podocarpus totara*, *Pogonarthria fleckii*, *Pogonaffhria squarrosa*, *Populus spp.*, *Prosopis cineraria*, *Pseudotsuga menziesii*, *Pterolobium stellatum*, *Pyrus communis*, *Quercus spp.*, *Rhaphiolepsis umbellata*, *Rhopalostylis sapida*, *Rhus natalensis*, *Ribes grossularia*, *Ribes spp.*, *Robinia pseudoacacia*, *Rosa spp.*, *Rubus spp.*, *Salix spp.*, *Schyzachyrium sanguineum*, *Sciadopitys vefficillata*, *Sequoia sempervirens*, *Sequoiadendron giganteum*, *Sorghum bicolor*, *Spinacia spp.*, *Sporobolus fimbriatus*, *Stiburus alopecuroides*, *Stylosanthos humilis*, *Tadehagi spp*, *Taxodium distichum*, *Themeda triandra*, *Trifolium spp.*, *Triticum spp.*, *Tsuga heterophylla*, *Vaccinium spp.*, *Vicia spp.*, *Vitis vinifera*, *Watsonia pyramidata*, *Zantedeschia aethiopica*, *Zea mays*, amaranth,

artichoke, asparagus, broccoli, Brussels sprouts, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugar beet, sugar cane, sunflower, tomato, squash tea, maize, wheat, barley, rye, oat, peanut, pea, lentil and alfalfa, cotton, rapeseed, canola, pepper, sunflower, tobacco, eggplant, eucalyptus, a tree, an ornamental plant, a perennial grass and a forage crop. Alternatively algae and other non-Viridiplantae can be used for the methods of the present invention.

According to some embodiments of the invention, the plant used by the method of the invention is a crop plant including, but not limited to, cotton, Brassica vegetables, oilseed rape, sesame, olive tree, palm oil, banana, wheat, corn or maize, barley, alfalfa, peanuts, sunflowers, rice, oats, sugarcane, soybean, turf grasses, barley, rye, sorghum, sugar cane, chicory, lettuce, tomato, zucchini, bell pepper, eggplant, cucumber, melon, watermelon, beans, hibiscus, okra, apple, rose, strawberry, chili, garlic, pea, lentil, canola, mums, *Arabidopsis*, broccoli, cabbage, beet, quinoa, spinach, squash, onion, leek, tobacco, potato, sugarbeet, papaya, pineapple, mango, *Arabidopsis* thaliana, and also plants used in horticulture, floriculture or forestry, such as, but not limited to, poplar, fir, eucalyptus, pine, an ornamental plant, a perennial grass and a forage crop, coniferous plants, moss, algae, as well as other plants listed in World Wide Web (dot) nationmaster (dot) com/encyclopedia/Plantae.

According to a specific embodiment, the plant is selected from the group consisting of corn, rice, wheat, tomato, cotton and sorghum.

According to a specific embodiment, the seed is an uncoated or fresh seed that hasn't been subjected to chemical/physical treatments.

In some embodiments, washing of the seeds is effected for 30 minutes to 4 hours. Other examples of wash ranges are 1 minute to 10 minutes, 10 minutes to 30 minutes. According to some embodiments, washing of the seeds can be as short as 5, 10, 20, 30, 45, or 60 seconds. The wash solution may include a weak detergent such as Tween-20. The concentration of the detergent may be 0.01-0.2% or 0.2-1%. According to another embodiment, the detergent concentration can be about 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1% or higher.

The seed may be subjected to priming or washing prior to contacting with the dsRNA.

As used herein the term "priming" refers to controlling the hydration level within seeds so that the metabolic activity necessary for germination can occur but radicle emergence is prevented. Different physiological activities within the seed occur at different moisture levels (Leopold and Vertucci, 1989; Taylor, 1997). The last physiological activity in the germination process is radicle emergence. The initiation of radicle emergence requires a high seed water content. By limiting seed water content, all the metabolic steps necessary for germination can occur without the irreversible act of radicle emergence. Prior to radicle emergence, the seed is considered desiccation tolerant, thus the primed seed moisture content can be decreased by drying. After drying, primed seeds can be stored until time of sowing.

Several different priming methods are used commercially. Among them, liquid or osmotic priming and solid matrix priming appear to have the greatest following (Khan *et al.*, 1991).

According to an embodiment of the invention, priming is effected in the presence of salt, a chelating agent, polyethylene glycol or a combination of same (*e.g.*, chelating agent and salt).

Alternatively, priming is effected in the presence of water such as deionized water or double deionized water. According to a specific embodiment, the priming is effected in the presence of 100% ddW.

Several types of seed priming are commonly used:

Osmopriming (osmoconditioning) - is the standard priming technique. Seeds are incubated in well aerated solutions with a low water potential, and afterwards washes and dried. The low water potential of the solutions can be achieved by adding osmotica like mannitol, polyethyleneglycol (PEG) or salts like KCl.

Hydropriming (drum priming) - is achieved by continuous or successive addition of a limited amount of water to the seeds. A drum is used for this purpose and the water can also be applied by humid air. 'On-farm steeping' is a cheap and useful technique that is practiced by incubating seeds (cereals, legumes) for a limited time in warm water.

Matrixpriming (matriconditioning) - is the incubation of seeds in a solid, insoluble matrix (vermiculite, diatomaceous earth, cross-linked highly water-absorbent polymers) with a limited amount of water. This method confers a slow imbibition.

Pregerminated seeds - is only possible with a few species. In contrast to normal priming, seeds are allowed to perform radicle protrusion. This is followed by sorting for specific stages, a treatment that reinduces desiccation tolerance, and drying. The use of pregerminated seeds causes rapid and uniform seedling development.

Thus, according to one embodiment, the seeds are primed seeds.

Of note, it may be possible that the seeds are treated with water (double-distilled water, ddW), prior to contacting with the dsRNA without effecting any priming on the seeds. For instance, treatment for a short while with water (*e.g.*, 30 seconds to 1 hour, 30 seconds to 0.5 hour, 30 seconds to 10 minutes, 30 seconds to 5 minutes or 45 seconds to 5 minutes). According to some embodiments, treatment with water can be as short as 5, 10, 20, or 30 seconds.

It will be appreciated that the non-transcribable polynucleotide trigger, for example dsRNA, molecule can be comprised in water (*e.g.*, tap water, distilled water or double distilled water) *i.e.*, free of any of the above mentioned priming effective concentration of salts, a chelating agents, polyethylene glycol or combinations of same (*e.g.*, chelating agent and salt). In some embodiments, the non-transcribable polynucleotide trigger, for example dsRNA, molecule is provided to the seed in a buffer solution, such as EDTA.

In some embodiments, the seeds are non-primed seeds.

A non-limiting method of introducing the dsRNA into the seed is provided in Example 1, which is considered as an integral part of the specification.

The temperature at the washing/priming and drying steps may be the same or differ.

According to one embodiment, the washing/priming is effected at 4-28 ºC.

According to one embodiment, the priming/washing solution or the dsRNA containing solution is devoid of a solid carrier.

According to one embodiment, the priming/washing solution or the dsRNA containing solution is devoid of a transferring agent such as a surfactant or a salt.

According to a further embodiment of the invention, the seeds subject to contacting with the dsRNA molecule are washed in order to remove agents, to which the seeds have been subjected, such as a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent.

Thus, according to one embodiment, the seeds (prior to treatment with dsRNA) are substantially free (*i.e.*, do not comprise effective amounts) of pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent.

The seeds are then subjected to drying. In some embodiments, drying is optional.

According to one embodiment, the drying is effected at 20-37 ºC, 20-30 ºC, 22-37 ºC, 15-22 ºC or 20-25 ºC for 10-20 hours, 10-16 hours or even 2-5 hours.

Various considerations are to be taken when calculating the concentration of the dsRNA in the contacting solution.

These are dependent on at least one of seed size, seed weight, seed volume, seed surface area, seed density and seed permeability.

For example, related to seed size, weight, volume and surface area, it is estimated that corn seeds will require longer treatment than *Arabidopsis* and tomato seeds. Regarding permeability and density, it is estimated that wheat seeds will require longer treatments at higher concentrations than tomato seeds.

Examples of concentrations of dsRNA in the treating solution include, but are not limited to, 0.01-0.3 µg/µl, 0.01-0.15 µg/µl, 0.04-0.15 µg/µl, 0.1-100 µg/µl; 0.1-50 µg/µl, 0.1-10, µg/µl, 0.1-5 µg/µl, 0.1-1 µg/µl, 0.1-0.5 µg/µl, 0.15-0.5 µg/µl , 0.1-0.3 µg/µl, 0.01-0.1 µg/µl, 0.01-0.05 µg/µl, 0.02-0.04 µg/µl , 0.001-0.02 µg/µl. According to a specific embodiment, the concentration of the dsRNA in the treating solution is 0.01-0.15 or 0.04-0.15 µg/µl.

In one embodiment, the dsRNA concentration in the treating solution is 0.01-0.3 µg/ml, 0.01-0.15 µg/ml, 0.04-0.15 µg/ml, 0.1-100 µg/ml; 0.1-50 µg/ml, 0.1-10 µg/ml, 0.1-5 µg/ml, 0.1-1 µg/ml, 0.1-0.5 µg/ml, 0.15-0.5 µg/ml , 0.1-0.3 µg/ml, 0.01-0.1 µg/ml, 0.01-0.05 µg/ml, 0.02-0.04 µg/ml , or 0.001-0.02 µg/ml.

In another embodiment, the dsRNA concentration in the treating solution is about 5-10 µg/ml, 10-15 µg/ml, 15-20 µg/ml, 20-25 µg/ml; 25-30 µg/ml, 30-35 µg/ml, 35-40 µg/ml, 40-45 µg/ml, 45-50 µg/ml, 50-55 µg/ml, 55-60 µg/ml, 60-65 µg/ml, 65-70 µg/ml, 70-75 µg/ml, 75-80 µg/ml, 80-85 µg/ml, 85-90 µg/ml, 90-95 µg/ml, 95-100 µg/ml, 100-105 µg/ml, 105-110 µg/ml, 110-115 µg/ml, 115-120 µg/ml, 120-125 µg/ml; 125-130 µg/ml, 130-135 µg/ml, 135-140 µg/ml, 140-145 µg/ml, 145-150 µg/ml, 150-155 µg/ml, 155-160 µg/ml, 160-165 µg/ml, 165-170 µg/ml, 170-175 µg/ml, 175-180

μg/ml, 180-185 μg/ml, 185-190 μg/ml, 190-195 μg/ml, 195-200 μg/ml, 200-210 μg/ml, 210-220 μg/ml, 220-230 μg/ml, 230-240 μg/ml, 240-250 μg/ml, 250-260 μg/ml, 260-270 μg/ml, 270-280 μg/ml, 280-290 μg/ml, 290-300 μg/ml, 300-310 μg/ml, 310-320 μg/ml, 320-330 μg/ml, 330-340 μg/ml, 340-350 μg/ml, 350-360 μg/ml, 360-370 μg/ml, 370-380 μg/ml, 380-390 μg/ml, 390-400 μg/ml, 400-410 μg/ml, 410-420 μg/ml, 420-430 μg/ml, 430-440 μg/ml, 440-450 μg/ml, 450-460 μg/ml, 460-470 μg/ml, 470-480 μg/ml, 480-490 μg/ml, or about 490-500 μg/ml.

In another embodiment, the dsRNA concentration in the treating solution is 0.0001-3 μg/μl, 0.0001-2.5 μg/μl, 0.0001-2 μg/μl, 0.0001-1.5 μg/μl, 0.0001-1 μg/μl, 0.0001-0.9 μg/μl, 0.0001-0.8 μg/μl, 0.0001-0.7 μg/μl, 0.0001-0.6 μg/μl, 0.0001-0.5 μg/μl, 0.0001-0.4 μg/μl, 0.0001-0.3 μg/μl, 0.0001-0.2 μg/μl, 0.0001-0.1 μg/μl, 0.0001-0.05 μg/μl, 0.0001-0.02 μg/μl, 0.0001-0.01 μg/μl, 0.0001-0.005 μg/μl, 0.0001-0.001 μg/μl, or 0.0001-0.0005 μg/μl.

In another embodiment, the dsRNA concentration in the treating solution is 0.0001-3 μg/μl, 0.0005-3 μg/μl, 0.001-3 μg/μl, 0.005-3 μg/μl, 0.01-3 μg/μl, 0.05-3 μg/μl, 0.1-3 μg/μl, 0.2-3 μg/μl, 0.3-3 μg/μl, 0.4-3 μg/μl, 0.5-3 μg/μl, 0.6-3 μg/μl, 0.7-3 μg/μl, 0.8-3 μg/μl, 0.9-3 μg/μl, 1-3 μg/μl, or 2-3 μg/μl.

In another embodiment, the dsRNA concentration in the treating solution is 0.0001-3 μg/μl, 0.0005-2.5 μg/μl, 0.001-2 μg/μl, 0.005-1.5 μg/μl, 0.01-1 μg/μl, 0.05-0.5 μg/μl, 0.1-0.4 μg/μl, or 0.2-0.3 μg/μl.

According to a specific embodiment, the contacting with the dsRNA is effected in the presence of a chelating agent such as EDTA or another chelating agent such as DTPA (0.01-0.1 mM).

In some embodiments, the treating solution may comprise a transferring agent such as a surfactant or a salt. Examples of such transferring agents include but are not limited salts such as sodium or lithium salts of fatty acids (such as tallow or tallowamines or phospholipids lipofectamine or lipofectin (1-20 nM, or 0.1-1 nM)) and organosilicone surfactants. Other useful surfactants include organosilicone surfactants including nonionic organosilicone surfactants, *e.g.*, trisiloxane ethoxylate surfactants or a silicone polyether copolymer such as a copolymer of polyalkylene oxide modified heptamethyl trisiloxane and allyloxypolypropylene glycol methylether (commercially available as SilwetTM L-77 surfactant having CAS Number 27306-78-1 and EPA

Number: CAL.REG.NO. 5905-50073-AA, currently available from Momentive Performance Materials, Albany, N.Y.).

In some embodiments, the treating solution may comprise a physical agent. Examples of physical agents include: (a) abrasives such as carborundum, corundum, sand, calcite, pumice, garnet, and the like, (b) nanoparticles such as carbon nanotubes and (c) a physical force. Carbon nanotubes are disclosed by Kam *et al.* (2004) J. Am. Chem. Soc., 126 (22):6850-6851, Liu *et al.* (2009) Nano Lett., 9(3):1007-1010, and Khodakovskaya *et al.* (2009) ACS Nano, 3(10):3221-3227. Physical force agents can include heating, chilling, the application of positive pressure, or ultrasound treatment. Agents for laboratory conditioning of a plant to permeation by polynucleotides include, *e.g.*, application of a chemical agent, enzymatic treatment, heating or chilling, treatment with positive or negative pressure, or ultrasound treatment. Agents for conditioning plants in a field include chemical agents such as surfactants and salts.

Contacting of the seeds with the dsRNA can be effected using any method known in the art as long as an effective amount of the dsRNA enters the seeds. These examples include, but are not limited to, soaking, spraying or coating with powder, emulsion, suspension, or solution; similarly, the polynucleotide molecules are applied to the plant by any convenient method, *e.g.*, spraying or wiping a solution, emulsion, or suspension.

As used herein "an effective amount" refers to an amount of dsRNA which is sufficient to down regulate the target gene by at least 20%, 30%, 40%, 50%, or more, say 60%, 70%, 80%, 90% or more even 100%. The effective amount can be a result of the formation of amplification in the plant or the phytopathogen.

According to a specific embodiment contacting may be effected by soaking (*i.e.*, inoculation) so that shaking the seeds with the treating solution may improve penetration and soaking and therefore reduce treatment time. Shaking is typically performed at 50-150 RPM and depends on the volume of the treating solution. Shaking may be effected for 4-24 hours (1-4 hours, 10 minutes to 1 hour or 30 seconds to 10 minutes). The present teachings further envisage short incubation time such as up to 10 minutes. Examples include but are not limited to 30 seconds to 7 minutes, to 30 seconds to 5 minutes, to 30 seconds to 3 minutes, to 30 seconds to 2 minutes, to 30 seconds to 1 minute, 1 minute to 10 minutes or to 1 minute to 5 minutes.

In one embodiment, the incubation time may be between 1 and 60, between 2 and 60, between 5 and 60, between 10 and 60, between 20 and 60, between 30 and 60, between 40 and 60, between 50 and 60, between 1 and 50, between 1 and 40, between 1 and 30, between 1 and 20, between 1 and 10, between 1 and 5, between 5 and 50, between 10 and 40, and between 20 and 30 seconds.

In another embodiment, the incubation time may be between 1 and 60, between 2 and 60, between 5 and 60, between 10 and 60, between 20 and 60, between 30 and 60, between 40 and 60, between 50 and 60, between 1 and 50, between 1 and 40, between 1 and 30, between 1 and 20, between 1 and 10, between 1 and 5, between 5 and 50, between 10 and 40, and between 20 and 30 minutes.

Dipping is also considered under the scope of the present embodiments. Thus, the seeds are dipped into the dsRNA solution for seconds *e.g.*, 1-10 seconds, 1-5 seconds, 1-3 seconds or 1-2 seconds. During this period, the dsRNA may adsorb on the seed surface. The adsorbed dsRNA, which coats the seed, may penetrate the seed or the seedling during germination. The incubation takes place in the dark at 4-28 °C or 15-22 °C (*e.g.*, 8-15 °C, 4-8 °C, 22-28 °C).

In one embodiment, the dipping time may be between 1 and 60, between 2 and 60, between 5 and 60, between 10 and 60, between 20 and 60, between 30 and 60, between 40 and 60, between 50 and 60, between 1 and 50, between 1 and 40, between 1 and 30, between 1 and 20, between 1 and 10, between 1 and 5, between 5 and 50, between 10 and 40, and between 20 and 30 minutes.

In one embodiment, the dipping time may be between 1 and 60, between 2 and 60, between 5 and 60, between 10 and 60, between 20 and 60, between 30 and 60, between 40 and 60, between 50 and 60, between 1 and 50, between 1 and 40, between 1 and 30, between 1 and 20, between 1 and 10, between 1 and 5, between 5 and 50, between 10 and 40, and between 20 and 30 seconds.

According to a specific embodiment, contacting occurs prior to breaking of seed dormancy and embryo emergence.

Following contacting, preferably prior to breaking of seed dormancy and embryo emergence, the seeds may be subjected to treatments (*e.g.*, coating) with the above agents (*e.g.*, pesticide, fungicide etc.).

Contacting is effected such that the dsRNA enters the embryo, endosperm, the coat, or a combination of the three.

After contacting with the treatment solution, the seeds may be subjected to drying for up to 30 hours at 25-37 °C. For example, the seeds may be dried for 16 hours at 30 °C.

According to a specific embodiment, the seed (*e.g.*, isolated seed) comprises the exogenous naked dsRNA and wherein at least 10 or 20 molecules of the dsRNA are in the endosperm of the isolated seed.

As used herein the term "isolated" refers to separation from the natural physiological environment. In the case of seed, the isolated seed is separated from other parts of the plant. In the case of a nucleic acid molecule (*e.g.*, dsRNA) separated from the cytoplasm.

According to a specific embodiment, the dsRNA is not expressed from the plant genome, thereby not being an integral part of the genome.

According to a specific embodiment there is provided an isolated seed comprising an exogenous dsRNA being present at a similar concentration (*e.g.*, about 1:1, 2:1 or 1:2) in an embryo and an endosperm of the seed. It is suggested that the direct introduction of the naked dsRNA to the seed results in higher concentration of the dsRNA in the endosperm than that observed when the dsRNA is expressed from a nucleic acid expression construct.

According to a specific embodiment there is provided an isolated seed comprising an exogenous dsRNA being spatially distributed in an embryo and an endosperm of the plant seed in a spatial distribution that differs from a spatial distribution of the exogenous dsRNA in a seed derived from a transgenic plant that recombinantly expresses said exogenous dsRNA.

Methods of measuring the localization of RNA molecules in the seed are well known in the art. The use of siGlo as described in the Examples section is an example for such.

According to an alternative or an additional embodiment, there is provided an isolated seed comprising an exogenous dsRNA, wherein a concentration ratio of said exogenous dsRNA to siRNA maturing there from is higher in the seed as compared to a transgenic seed recombinantly expressing said exogenous dsRNA.

As used herein the term "higher" refers to at least about 3%, 5%, 7%, 10%, 15%, 20%, 25%, 30%, 50%, 60%, 70%, 80%, 90% or even a few folds higher.

According to an alternative or an additional embodiment, there is provided an isolated seed comprising an exogenous dsRNA, wherein the plant seed is devoid of a heterologous promoter for driving expression of said exogenous dsRNA, wherein a spatial distribution of said exogenous dsRNA and/or siRNA maturing there from is altered in the seed as compared to same in a transgenic seed recombinantly expressing said exogenous dsRNA.

The term "recombinantly expressing" refers to an expression from a nucleic acid construct.

According to a further embodiment there is provided a plant seed obtainable (or obtained) by any of the methods described herein.

Methods of qualifying successful introduction of the dsRNA include but are not limited to, RT-PCR (*e.g.*, quantifying the level of the target gene or the naked dsRNA), phenotypic analysis such as biomass, vigor, yield and stress tolerance, root architecture, leaf dimensions, grain size and weight, oil content, cellulose, as well as cell biology techniques.

According to some embodiments, an alteration of the expression level of the plant ortholog of the insect pest gene targeted by the seed treatment, as described herein, is observed. See for instance Examples 45 and 46 of the Examples section which follows.

Seeds may be stored for 1 day to several months prior to planting (*e.g.*, at 4-10 °C).

The resultant seed can be germinated in the dark so as to produce a plant.

Thus there is provided a plant or plant part comprising an exogenous naked dsRNA and devoid of a heterologous promoter for driving expression of the dsRNA in the plant.

As used herein "devoid of a heterologous promoter for driving expression of the dsRNA" means that the plant or plant cell doesn't include a cis-acting regulatory sequence (*e.g.*, heterologous) transcribing the dsRNA in the plant. As used herein the term "heterologous" refers to exogenous, not-naturally occurring within the native plant cell (such as by position of integration, or being non-naturally found within the plant

cell). Thus the isolated seed in the absence of a heterologous promoter sequence for driving expression of the dsRNA in the plant, comprises a homogenic (prior to amplification) or heterogenic (secondary siRNAs, following amplification) population of plant non-transcribable dsRNA.

The present methodology can be used for modulating gene expression such as in a plant, the method comprising:

(a) contacting a seed of the plant with a naked dsRNA, under conditions which allow penetration of the dsRNA into the seed, thereby introducing the dsRNA into the seed; and optionally

(b) generating a plant of the seed.

When used for down-regulating a plant gene, the naked dsRNA is designed of the desired specificity using bioinformatic tools which are well known in the art (*e.g.*, BLAST).

This methodology can be used in various applications starting from basic research such as in order to assess gene function and lasting in generating plants with altered traits which have valuable commercial use.

Such plants can exhibit agricultural beneficial traits including altered morphology, altered flowering, altered tolerance to stress (*i.e.*, biotic and/or abiotic), altered biomass vigor and/or yield and the like.

The phrase "abiotic stress" as used herein refers to any adverse effect on metabolism, growth, viability and/or reproduction of a plant. Abiotic stress can be induced by any of suboptimal environmental growth conditions such as, for example, water deficit or drought, flooding, freezing, low or high temperature, strong winds, heavy metal toxicity, anaerobiosis, high or low nutrient levels (*e.g.* nutrient deficiency), high or low salt levels (*e.g.* salinity), atmospheric pollution, high or low light intensities (*e.g.* insufficient light) or UV irradiation. Abiotic stress may be a short term effect (*e.g.* acute effect, *e.g.* lasting for about a week) or alternatively may be persistent (*e.g.* chronic effect, *e.g.* lasting for example 10 days or more). The present invention contemplates situations in which there is a single abiotic stress condition or alternatively situations in which two or more abiotic stresses occur.

According to one embodiment, the abiotic stress refers to salinity.

According to another embodiment, the abiotic stress refers to drought.

According to another embodiment, the abiotic stress refers to a temperature stress.

As used herein the phrase "abiotic stress tolerance" refers to the ability of a plant to endure an abiotic stress without exhibiting substantial physiological or physical damage (*e.g.* alteration in metabolism, growth, viability and/or reproducibility of the plant).

As used herein the phrase "nitrogen use efficiency (NUE)" refers to a measure of crop production per unit of nitrogen fertilizer input. Fertilizer use efficiency (FUE) is a measure of NUE. Crop production can be measured by biomass, vigor or yield. The plant's nitrogen use efficiency is typically a result of an alteration in at least one of the uptake, spread, absorbance, accumulation, relocation (within the plant) and use of nitrogen absorbed by the plant. Improved NUE is with respect to that of a non-transgenic plant (*i.e.*, lacking the transgene of the transgenic plant) of the same species and of the same developmental stage and grown under the same conditions.

As used herein the phrase "nitrogen-limiting conditions" refers to growth conditions which include a level (*e.g.*, concentration) of nitrogen (*e.g.*, ammonium or nitrate) applied which is below the level needed for optimal plant metabolism, growth, reproduction and/or viability.

As used herein the term/phrase "biomass", "biomass of a plant" or "plant biomass" refers to the amount (*e.g.*, measured in grams of air-dry tissue) of a tissue produced from the plant in a growing season. An increase in plant biomass can be in the whole plant or in parts thereof such as aboveground (*e.g.* harvestable) parts, vegetative biomass, roots and/or seeds or contents thereof (*e.g.*, oil, starch etc.).

As used herein the term/phrase "vigor", "vigor of a plant" or "plant vigor" refers to the amount (*e.g.*, measured by weight) of tissue produced by the plant in a given time. Increased vigor could determine or affect the plant yield or the yield per growing time or growing area. In addition, early vigor (*e.g.* seed and/or seedling) results in improved field stand.

As used herein the term/phrase "yield", "yield of a plant" or "plant yield" refers to the amount (*e.g.*, as determined by weight or size) or quantity (*e.g.*, numbers) of tissues or organs produced per plant or per growing season. Increased yield of a plant

can affect the economic benefit one can obtain from the plant in a certain growing area and/or growing time.

According to one embodiment, the yield is measured by cellulose content, oil content, starch content and the like.

According to another embodiment, the yield is measured by oil content.

According to another embodiment, the yield is measured by protein content.

According to another embodiment, the yield is measured by seed number, seed weight, fruit number or fruit weight per plant or part thereof (*e.g.*, kernel, bean).

A plant yield can be affected by various parameters including, but not limited to, plant biomass; plant vigor; plant growth rate; seed yield; seed or grain quantity; seed or grain quality; oil yield; content of oil, starch and/or protein in harvested organs (*e.g.*, seeds or vegetative parts of the plant); number of flowers (*e.g.* florets) per panicle (*e.g.* expressed as a ratio of number of filled seeds over number of primary panicles); harvest index; number of plants grown per area; number and size of harvested organs per plant and per area; number of plants per growing area (*e.g.* density); number of harvested organs in field; total leaf area; carbon assimilation and carbon partitioning (*e.g.* the distribution/allocation of carbon within the plant); resistance to shade; number of harvestable organs (*e.g.* seeds), seeds per pod, weight per seed; and modified architecture [such as increase stalk diameter, thickness or improvement of physical properties (*e.g.* elasticity)].

Improved plant NUE is translated in the field into either harvesting similar quantities of yield, while implementing less fertilizers, or increased yields gained by implementing the same levels of fertilizers. Thus, improved NUE or FUE has a direct effect on plant yield in the field.

As used herein "biotic stress" refers stress that occurs as a result of damage done to plants by other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants. Examples 7, and 20-38 of the Examples section which follows, describes implementation the present teachings towards conferring resistance to *Spodoptera littoralis*. Examples 38 and 39 of the Examples section which follows, describes implementation the present teachings towards conferring resistance to *Coleopteran* pests. Examples 40-52 of the Examples

section which follows, describes implementation the present teachings towards conferring resistance to viral infection.

As used herein the term "improving" or "increasing" refers to at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or greater increase in NUE, in tolerance to stress, in yield, in biomass or in vigor of a plant, as compared to a native or wild-type plants [*i.e.*, isogenic plants (not grown from seeds treated with dsRNA) of the present embodiments].

In some embodiments, the target gene of the dsRNA may not be an endogenous plant gene but rather a gene exogenous to the plant, such as a gene of a phytopathogenic organism which feeds on the plant or depends thereon for growth/replication (*e.g.*, bacteria or viruses) and/or survival. In some embodiments, the target gene is an essential gene of an insect pest. In some embodiments, the target gene is a viral gene.

As used herein, the term "phytopathogen" refers to an organism that benefits from an interaction with a plant, and has a negative effect on that plant. The term "phytopathogen" includes insects, arachnids, crustaceans, fungi, bacteria, viruses, nematodes, flatworms, roundworms, pinworms, hookworms, tapeworms, trypanosomes, schistosomes, botflies, fleas, ticks, mites, and lice and the like that may ingest or contact one or more cells, tissues, or fluids produced by a plant.

The methods described herein can be used to generate a plant that is resistant to one or more phytopathogens. In some embodiments, the phytopathogen is an insect pest. When an insect is the target pest for the present invention, such pests include but are not limited to: from the order *Lepidoptera*, for example, *Acleris spp.*, *Adoxophyes spp.*, *Aegeria spp.*, *Agrotis spp.*, *Alabama argillaceae*, *Amylois spp.*, *Anticarsia gemmatalis*, *Archips spp*, *Argyrotaenia spp.*, *Autographa spp.*, *Busseola fusca*, *Cadra cautella*, *Carposina nipponensis*, *Chilo spp.*, *Choristoneura spp.*, *Clysia ambiguella*, *Cnaphalocrocis spp.*, *Cnephasia spp.*, *Cochylis spp.*, *Coleophora spp.*, *Crocidolomia binotalis*, *Cryptophlebia leucotreta*, *Cydia spp.*, *Diatraea spp.*, *Diparopsis castanea*, *Earias spp.*, *Ephestia spp.*, *Eucosma spp.*, *Eupoecilia ambiguella*, *Euproctis spp.*, *Euxoa spp.*, *Grapholita spp.*, *Hedya nubiferana*, *Heliothis spp.*, *Hellula undalis*, *Hyphantiria*

cunea, *Keiferia lycopersicella*, *Leucoptera scitella*, *Lithocollethis spp.*, *Lobesia botrana*, *Lymantria spp.*, *Lyonetia spp.*, *Malacosoma spp.*, *Mamestra brassicae*, *Manduca sexta*, *Operophtera spp.*, *Ostrinia Nubilalis*, *Pammene spp.*, *Pandemis spp.*, *Panolis flammea*, *Pectinophora gossypiella*, *Phthorimaea operculella*, *Pieris rapae*, *Pieris spp.*, *Plutella xylostella*, *Prays spp.*, *Scirpophaga spp.*, *Sesamia spp.*, *Sparganothis spp.*, *Spodoptera spp.*, *Synanthedon spp.*, *Thaumetopoea spp.*, *Tortrix spp.*, *Trichoplusia ni* and *Yponomeuta spp.;* from the order *Coleoptera*, for example, *Agriotes spp.*, *Anthonomus spp.*, *Atomaria linearis*, *Chaetocnema tibialis*, *Cosmopolites spp.*, *Curculio spp.*, *Denrmestes spp.*, *Diabrotica spp.*, *Epilachna spp.*, *Eremnus spp.*, *Leptinotarsa decemlineata*, *Lissorhoptrus spp.*, *Melolontha spp.*, *Orycaephilus spp.*, *Otiorhynchus spp.*, *Phlyctinus spp.*, *Popillia spp.*, *Psylliodes spp.*, *Rhizopertha spp.*, *Scarabeidae*, *Sitophilus spp.*, *Sitotroga spp.*, *Tenebrio spp.*, *Tribolium spp.* and *Trogoderma spp.;* from the order *Orthoptera*, for example, *Blatta spp.*, *Blattella spp.*, *Gryllotalpa spp.*, *Leucophaea maderae*, *Locusta spp.*, *Periplaneta ssp.*, *and Schistocerca spp.;* from the order *Isoptera*, for example, *Reticulitemes ssp;* from the order *Psocoptera*, for example, *Liposcelis spp.;* from the order *Anoplura*, for example, *Haematopinus spp.*, *Linognathus spp.*, *Pediculus spp.*, *Pemphigus spp.* and *Phylloxera spp.;* from the order *Mallophaga*, for example, *Damalinea spp.* and *Trichodectes spp.;* from the order *Thysanoptera*, for example, *Franklinella spp.*, *Hercinothrips spp.*, *Taeniothrips spp.*, *Thrips palmi*, *Thrips tabaci* and *Scirtothrips aurantii;* from the order *Heteroptera*, for example, *Cimex spp.*, *Distantiella theobroma*, *Dysdercus spp.*, *Euchistus spp.*, *Eurygaster spp.*, *Leptocorisa spp.*, *Nezara spp.*, *Piesma spp.*, *Rhodnius spp.*, *Sahlbergella singularis*, *Scotinophara spp.*, *Triatoma spp.*, *Miridae family spp.* such as *Lygus hesperus* and *Lygus lineoloris*, *Lygaeidae family spp.* such as *Blissus leucopterus*, and *Pentatomidae family spp.;* from the order *Homoptera*, for example, *Aleurothrixus floccosus*, *Aleyrodes brassicae*, *Aonidiella spp.*, *Aphididae*, *Aphis spp.*, *Aspidiotus spp.*, *Bemisia tabaci*, *Ceroplaster spp.*, *Chrysomphalus aonidium*, *Chrysomphalus dictyospermi*, *Coccus hesperidum*, *Empoasca spp.*, *Eriosoma larigerum*, *Erythroneura spp.*, *Gascardia spp.*, *Laodelphax spp.*, *Lacanium corni*, *Lepidosaphes spp.*, *Macrosiphus spp.*, *Myzus spp.*, *Nehotettix spp.*, *Nilaparvata spp.*, *Paratoria spp.*, *Pemphigus spp.*, *Planococcus spp.*, *Pseudaulacaspis spp.*, *Pseudococcus spp.*, *Psylla ssp.*, *Pulvinaria aethiopica*, *Quadraspidiotus spp.*,

Rhopalosiphum spp., *Saissetia spp.*, *Scaphoideus spp.*, *Schizaphis spp.*, *Sitobion spp.*, *Trialeurodes vaporariorum*, *Trioza erytreae* and *Unaspis citri;* from the order *Hymenoptera*, for example, *Acromyrmex*, *Atta spp.*, *Cephus spp.*, *Diprion spp.*, *Diprionidae*, *Gilpinia polytoma*, *Hoplocampa spp.*, *Lasius spp.*, *Monoimorium pharaonis*, *Neodiprion spp*, *Solenopis spp.* and *Vespa ssp.;* from the order *Diptera*, for example, *Aedes spp.*, *Antherigona soccata*, *Bibio hortulanus*, *Calliphora erythrocephala*, *Ceratitis spp.*, *Chrysomyia spp.*, *Culex spp.*, *Cuterebra spp.*, *Dacus spp.*, *Drosophila melanogaster*, *Fannia spp.*, *Gastrophilus spp.*, *Glossina spp.*, *Hypoderma spp.*, *Hyppobosca spp.*, *Liriomysa spp.*, *Lucilia spp.*, *Melanagromyza spp.*, *Musca ssp.*, *Oestrus spp.*, *Orseolia spp.*, *Oscinella frit*, *Pegomyia hyoscyami*, *Phorbia spp.*, *Rhagoletis pomonella*, *Sciara spp.*, *Stomoxys spp.*, *Tabanus spp.*, *Tannia spp.* and *Tipula spp.*, from the order *Siphonaptera*, for example, *Ceratophyllus spp. und Xenopsylla cheopis* and from the order *Thysanura*, for example, *Lepisma saccharina*.

Thus, according to one embodiment, there is provided a method of inhibiting expression of a target gene in a phytopathogenic organism, the method comprising providing (*e.g.*, feeding or contacting under infecting conditions) to the phytopathogenic organism the plant as described herein (at least part thereof includes the naked dsRNA), thereby inhibiting expression of a target gene in the phytopathogenic organism. In some embodiments, the target gene is an "essential gene." As used herein, the term "essential gene" refers to a gene of an organism that is essential for its survival or reproduction. In some embodiments, the target gene is expressed in the insect gut, for example, V-ATPase. In some embodiments, the target gene is involved in the growth, development, and reproduction of an insect. Examples of such genes include, but are not limited to, CHD3 gene and a beta-tubulin gene.

The phytopathogenic organism refers to a multicellular organism *e.g.*, insects, fungi, animals or a microorganism that can cause plant disease, including viruses, bacteria, fungi as well as oomycetes, chytrids, algae, and nematodes.

Reference herein to a "nematode" refers to a member of the phylum Nematoda. Members of the family *Heteroderidae* are sedentary parasites that form elaborate permanent associations with the target host organism. They deprive nutrients from cells of an infected organism through a specialized stylet. The cyst nematodes (genera *Heterodera* and *Globodera*) and root-knot nematodes (genus *Meliodogyne*), in

particular, cause significant economic loss in plants, especially crop plants. Examples of cyst nematodes include, inter alia, *H. avenae* (cereal cyst nematodes), *H. glycines* (beet cyst nematode) and *G. pallida* (potato cyst nematode). Root-knot nematodes include, for example, *M. javanica*, *M. incognita* and *M. arenaria*. These pathogens establish "feeding sites" in the plant, by causing the morphological transformation of root cells into giant cells. Hence, nematode "infestation" or "infection" refers to invasion of and feeding upon the tissues of the host plant. Other nematodes that cause significant damage include the lesion nematodes such as *Pratylenchus*, particularly *P. penetrans*, which infects maize, rice and vegetables, *P. brachyurus* which infects pineapple and *P. thornei* which infects inter alia, wheat.

Several embodiments relate to a method of inhibiting expression of a target gene in an insect pest, the method comprising providing (*e.g.*, feeding) to the insect pest a plant grown from a seed treated with an exogenous dsRNA as described herein, thereby inhibiting expression of the target gene in the insect pest. Insects that may cause damage and disease in plants belong to three categories, according to their method of feeding: chewing, sucking and boring. Major damage is caused by chewing insects that eat plant tissue, such as leaves, flowers, buds and twigs. Examples from this large insect category include beetles and their larvae (grubs), web-worms, bagworms and larvae of moths and sawflies (caterpillars). By comparison, sucking insects insert their mouth parts into the tissues of leaves, twigs, branches, flowers or fruit and suck out the plant's juices. Typical examples of sucking insects include but are not limited to aphids, mealy bugs, thrips and leaf-hoppers. Damage caused by these pests is often indicated by discoloration, drooping, wilting and general lack of vigor in the affected plant.

Several embodiments relate to a method of providing resistance to an insect pest, the method comprising growing a plant from a seed treated with an exogenous dsRNA as described herein. In some embodiments, the insect pest is selected from the orders *Coleoptera*, *Lepidoptera*, *Diptera*, *Orthoptera*, *Heteroptera*, *Ctenophalides*, *Arachnidiae*, and *Hymenoptera*. In some embodiments, the insect pest is a beetle or larvae. According to a specific embodiment, the phytopathogen is prodentia of the family *Noctuidae e.g.*, *Spodoptera littoralis*.

Examples of significant bacterial plant pathogens include, but are not limited to, *Burkholderia*, Proteobacteria (*Xanthomonas spp.* and *Pseudomonas spp.*, *Pseudomonas syringae pv. tomato*).

A number of virus genera are transmitted, both persistently and non-persistently, by soil borne zoosporic protozoa. These protozoa are not phytopathogenic themselves, but parasitic. Transmission of the virus takes place when they become associated with the plant roots. Examples include *Polymyxa graminis*, which has been shown to transmit plant viral diseases in cereal crops and *Polymyxa betae* which transmits Beet necrotic yellow vein virus. Plasmodiophorids also create wounds in the plant's root through which other viruses can enter.

Specific examples of viruses which can be targeted according to the present teachings include, but are not limited to:

(1) Tobacco mosaic virus (TMV, RNA virus) which infects plants, especially tobacco and other members of the family *Solanaceae*.

(2) Tomato spotted wilt virus (TSWV, RNA virus) which causes serious diseases of many economically important plants representing 35 plant families, including dicots and monocots. This wide host range of ornamentals, vegetables, and field crops is unique among plant-infecting viruses. Belongs to tospoviruses in the Mediterranean area, affect vegetable crops, especially tomato, pepper and lettuce (Turina *et al.*, 2012, Adv Virus Res 84;403-437).

(3) Tomato yellow leaf curl virus (TYLCV) which is transmitted by whitefly, mostly affects tomato plants. Geminiviruses (DNA viruses) in the genus Begomovirus (including sweepoviruses and legumoviruses) - most devastating pathogens affecting a variety of cultivated crops, including cassava, sweet potato, beans, tomato, cotton and grain legumes (Rey *et al.* 2012, Viruses 4;1753-1791). Members include TYLCV above and tomato leaf curl virus (ToLCV).

(4) Cucumber mosaic virus (CMV) - CMV has a wide range of hosts and attacks a great variety of vegetables, ornamentals, and other plants (as many as 191 host species in 40 families). Among the most important vegetables affected by cucumber mosaic are peppers (*Capsicum annuum L.*), cucurbits, tomatoes (*Lycopersicon esculentum Mill.*), and bananas (*Musa L. spp.*).

Other vegetable hosts include: cucumber, muskmelon, squash, tomato, spinach, celery, peppers, water cress, beet, sweet potato, turnip, chayote, gherkin, watermelon, pumpkin, citron, gourd, lima bean, broad bean, onion, ground-cherry, eggplant, potato, rhubarb, carrot, dill, fennel, parsnip, parsley, loofah, and artichoke (Chabbouh and Cherif, 1990, FAO Plant Prot. Bull. 38:52-53.).

Ornamental hosts include: China aster, chrysanthemum, delphinium, salvia, geranium, gilia, gladiolus, heliotrope, hyacinth, larkspur, lily, marigold, morning glory, nasturtium, periwinkle, petunia, phlox, snapdragon, tulip, and zinnia (Chupp and Sherf, 1960; Agrios, 1978).

(5) Potato virus Y (PVY) - one of the most important plant viruses affecting potato production.

(6) Cauliflower mosaic virus (CaMV, DNA virus (Rothnie *et al.*, 1994)).

(7) African cassava mosaic virus (ACMV).

(8) Plum pox virus (PPV) is the most devastating viral disease of stone fruit from the genus *Prunus*.

(9) Brome mosaic virus (BMV) - commonly infects *Bromus inermis* and other grasses, can be found almost anywhere wheat is grown.

(10) Potato virus X (PVX) There are no insect or fungal vectors for this virus. This virus causes mild or no symptoms in most potato varieties, but when Potato virus Y is present, synergy between these two viruses causes severe symptoms in potatoes.

Additional viruses:

Citrus tristeza virus (CTV) - causes the most economically damaging disease to Citrus, including sour orange (*Citrus aurantium*), and any Citrus species grafted onto sour orange root stock, sweet orange (*C. sinensis*), grapefruit (*C. paradisi*), lime and Seville orange (*C. aurantifolia*), and mandarin (*C. reticulata*). CTV is also known to infect *Aeglopsis chevalieri*, *Afraegle paniculata*, *Pamburus missionis*, and *Passiflora gracilis*. CTV is distributed worldwide and can be found wherever citrus trees grow.

Barley yellow dwarf virus (BYDV) - most widely distributed viral disease of cereals. It affects the economically important crop species barley, oats, wheat, maize, triticale and rice.

Potato leafroll virus (PLRV) infects potatoes and other members of the family *Solanaceae*.

Tomato bushy stunt virus (TBSV), RNA virus, a member of the genus Tombusvirus and mostly affects tomatoes and eggplant.

Additional reviews:

Hamilton *et al.*, 1981, J Gen Virol 54;223-241 – mentions TMV, PVX, PVY, CMV, CaMV.

Additional scientific papers:

Makkouk *et al.*, 2012, Adv Virus Res 84;367-402 -Viruses affecting peas and beans with narrow (Faba bean necrotic yellow virus (FBNYN)) and wide (alfalfa mosaic virus (AMV) and CMV) host range.

Insect pests causing plant disease include those from the families of, for example, *Apidae*, *Curculionidae*, *Scarabaeidae*, *Tephritidae*, *Tortricidae*, amongst others.

The target gene of the phytopathogenic organism encodes a product essential to the viability and/or infectivity of the pathogen, therefore its down-regulation (by the naked dsRNA) results in a reduced capability of the pathogen to survive and infect host cells. Hence, such down-regulation results in a "deleterious effect" on the maintenance viability and/or infectivity of the phytopathogen, in that it prevents or reduces the pathogen's ability to feed off and survive on nutrients derived from host cells. By virtue of this reduction in the phytopathogen's viability and/or infectivity, resistance and/or enhanced tolerance to infection by a pathogen is facilitated in the cells of the plant. Genes in the pathogen may be targeted at the mature (adult), immature (juvenile) or embryo stages.

Examples of genes essential to the viability and/or infectivity of the pathogen are provided herein. Such genes may include genes involved in development and reproduction, *e.g.* transcription factors (see, *e.g.* Xue *et al.*, 1993; Finney *et al.*, 1988), cell cycle regulators such as wee-1 and ncc-1 proteins (see, *e.g.* Wilson *et al.*, 1999; Boxem *et al.*, 1999) and embryo-lethal mutants (see, *e.g.* Schnabel *et al.*, 1991); proteins required for modeling such as collagen, ChR3 and LRP-1 (see, *e.g.* Yochem *et al.*, 1999; Kostrouchova *et al.*, 1998; Ray *et al.*, 1989); genes encoding proteins involved in the motility/nervous system, *e.g.* acetycholinesterase (see, *e.g.* Piotee *et al.*, 1999; Talesa *et al.*, 1995; Arpagaus *et al.*, 1998), ryanodine receptor such as *unc-68* (see, *e.g.* Maryon *et al.*, 1998; Maryon *et al.*, 1996) and glutamate-gated chloride

channels or the avermeetin receptor (see, *e.g.*, Cully *et al.*, 1994; Vassilatis *et al.*, 1997; Dent *et al.*, 1997); hydrolytic enzymes required for deriving nutrition from the host, *e.g.* serine proteinases such as HGSP-1 and HGSP-III (see, *e.g.* Lilley *et al.*, 1997); parasitic genes encoding proteins required for invasion and establishment of the feeding site, *e.g.* cellulases (see, *e.g.* de Boer *et al.*, 1999; Rosso *et al.*, 1999) and genes encoding proteins that direct production of stylar or amphidial secretions such as sec-1 protein (see, *e.g.* Ray *et al.*, 1994; Ding *et al.*, 1998); genes encoding proteins required for sex or female determination, *e.g.* tra-1, tra-2 and egl-1, a suppressor of ced9 (see, *e.g.* Hodgkin, 1980; Hodgkin, 1977; Hodgkin, 1999; Gumienny *et al.*, 1999; Zarkower *et al.*, 1992); and genes encoding proteins required for maintenance of normal metabolic function and homeostasis, *e.g.* sterol metabolism, embryo lethal mutants (see, *e.g.* Schnabel *et al.*, 1991) and trans-spliced leader sequences (see, *e.g.* Ferguson et al, 1996), pos-1, cytoplasmic Zn finger protein; pie-1, cytoplasmic Zn finger protein; mei-1, ATPase; dif-1, mitochondrial energy transfer protein; rba-2, chromatin assembly factor; skn-1, transcription factor; plk-1, kinase; gpb-1, G-protein B subunit; par-1, kinase; bir-1, inhibitor of apoptosis; mex-3, RNA-binding protein, unc-37, G-protein B subunit; hlh-2, transcription factor; par-2, dnc-1, dynactin; par-6, dhc-1, dynein heavy chain; and pal-1, homeobox. Such genes have been cloned from parasitic nematodes such as Meliodogyne and Heterodera species or can be identified by one of skill in the art using sequence information from cloned *C. elegans* orthologs (the genome of *C. elegans* has been sequenced and is available, see The *C. elegans* Sequencing Consortium (1998)).

Several embodiments relate to a method of conferring pathogen resistance on a plant, the method comprising contacting a seed with an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a gene of a phytopathogenic organism, and growing a plant from the seed. As used herein, a "pathogen resistance" trait is a characteristic of a plant that causes the plant host to be resistant to attack from a pathogen that typically is capable of inflicting damage or loss to the plant. Not wishing to be bound by a particular theory, once the phytopathogen is provided with the plant material produced from a seed comprising the naked dsRNA, expression of the gene within the target

pathogen is suppressed, and the suppression of expression of the gene in the target pathogen results in the plant being resistant to the pathogen.

In the embodiments described herein, the target gene can encode an essential protein or transcribe an non-coding RNA which, the predicted function is for example selected from the group consisting of ion regulation and transport, enzyme synthesis, maintenance of cell membrane potential, amino acid biosynthesis, amino acid degradation, development and differentiation, infection, penetration, development of appressoria or haustoria, mycelial growth, melanin synthesis, toxin synthesis, siderophore synthesis, sporulation, fruiting body synthesis, cell division, energy metabolism, respiration, and apoptosis, among others.

According to a specific embodiment, the phytopathogenic organism is selected from the group consisting of a fungus, a nematode, a virus, a bacteria and an insect.

To substantiate the anti-pest activity, the present teachings also contemplate observing death or growth inhibition and the degree of host symptomatology following said providing.

To improve the anti-phytopathogen activity, embodiments of the present invention further provide a composition that contains two or more different agents each toxic to the same plant pathogenic microorganism, at least one of which comprises a dsRNA described herein. In certain embodiments, the second agent can be an agent selected from the group consisting of inhibitors of metabolic enzymes involved in amino acid or carbohydrate synthesis; inhibitors of cell division; cell wall synthesis inhibitors; inhibitors of DNA or RNA synthesis, gyrase inhibitors, tubulin assembly inhibitors, inhibitors of ATP synthesis; oxidative phosphorylation uncouplers; inhibitors of protein synthesis; MAP kinase inhibitors; lipid synthesis or oxidation inhibitors; sterol synthesis inhibitors; and melanin synthesis inhibitors.

In some embodiments, a seed comprising an exogenous dsRNA as described herein is treated with a non-polynucleotide pesticide. It is believed that the combination of a plant exhibiting bioactivity against a target pest as a result of treating the seed from which the plant is grown with an exogenous dsRNA coupled with treatment of the seed with certain chemical or protein pesticides provides unexpected synergistic advantages to seeds having such treatment, including unexpectedly superior efficacy for protection against damage to the resulting plant by the target pest. The seeds of the present

embodiments are believed to have the property of decreasing the cost of pesticide use, because less of the pesticide can be used to obtain a required amount of protection than if the innovative composition and method is not used. Moreover, because less pesticide is used it is believed that the subject method is therefore safer to the operator and to the environment, and is potentially less expensive than conventional methods.

When it is said that some effects are "synergistic," it is meant to include the synergistic effects of the combination on the pesticidal activity (or efficacy) of the combination of the bioactivity of a plant grown from a dsRNA treated seed and the pesticide. However, it is not intended that such synergistic effects be limited to the pesticidal activity, but that they should also include such unexpected advantages as increased scope of activity, advantageous activity profile as related to type and amount of damage reduction, decreased cost of pesticide and application, decreased pesticide distribution in the environment, decreased pesticide exposure of personnel who produce, handle and plant seeds, and other advantages known to those skilled in the art.

In addition, plants generated according to the teachings of the present embodiments or parts thereof can exhibit altered nutritional or therapeutic efficacy and as such can be employed in the food or feed and drug industries. Likewise, the plants generated according to the teachings of the present embodiments or parts thereof can exhibit altered oil or cellulose content and as such can be implemented in the construction or oil industry.

The seeds of the present invention can be packed in a seed containing device which comprises a plurality of seeds at least some of which (*e.g.*, 5%, 10% or more) containing an exogenous naked dsRNA, wherein the seed is devoid of a heterologous promoter for driving expression of the dsRNA.

The seed containing device can be a bag, a plastic bag, a paper bag, a soft shell container or a hard shell container.

Several embodiments described herein relate to a solution for treating seeds comprising a non-transcribable polynucleotide trigger, for example dsRNA, molecule comprising a sequence that is essentially complementary or essentially identical to at least 18 contiguous nucleotides of a target gene. In some embodiments, the solution may further comprise buffer, for example, EDTA. As used herein "solution" refers to homogeneous mixtures and non-homogeneous mixtures such as suspensions, colloids,

micelles, and emulsions. In some embodiments, the solution may be provided in a kit. In some embodiments, the kit may further comprise one or more of seeds, containers, priming solution, and seed growth medium.

Reagents of the present invention can be packed in a kit including the non-transcribable polynucleotide trigger, for example dsRNA, molecule, instructions for introducing the non-transcribable polynucleotide trigger, for example dsRNA, molecule into the seeds and optionally a priming solution.

Compositions of some embodiments of the invention may, if desired, be presented in a pack or dispenser device, which may contain one or more dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for introduction to the seed.

According to one embodiment, the non-transcribable polynucleotide trigger, for example dsRNA, molecule and priming solution are comprised in separate containers.

As used herein the term "about" refers to ± 10%.

The terms "comprises," "comprising," "includes," "including," "having" and their conjugates mean "including but not limited to."

The term "consisting of" means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a," "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such

as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals there between.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the agronomic, chemical, pharmacological, biological, biochemical and medical arts.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following Examples. The following Examples are presented for the purposes of illustration and should not be construed as limitations.

EXAMPLES

Reference is now made to the following Examples, which together with the above descriptions illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and

recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook *et al.*, (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel *et al.*, "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson *et al.*, "Recombinant DNA", Scientific American Books, New York; Birren *et al.* (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites *et al.* (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak *et al.*, "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1: PROTOCOLS FOR dsRNA PRODUCTION AND SEED TREATMENT

Generating the dsRNA/siRNA Sequences

The dsRNA sequences were custom-created for each gene using *in vitro* transcription of PCR products. Part of the mRNA, including either the ORF, 3' UTR or 5' UTR for which dsRNA to be produced was PCR-amplified using gene-specific primers, which contain the sequence of the T7 promoter on either side. This product was used as a template for dsRNA production using commercial kits such as the MaxiScript dsRNA kit (Life Technologies) or T7 High Yield RNA Synthesis kit (NEB). Next, the sample is treated with DNase Turbo at 37 °C for 15-30 min followed by phenol treatment and nucleic acid precipitation. Next, one of two different reactions is carried out: (1) dsRNA is ready to use, or (2) processing of the dsRNA with Dicer (Shortcut RNase III (NEB)) to create small interfering RNAs (siRNA).

Either dsRNA or a combination of dsRNA and siRNA were used for seed treatments as described below.

General Seed Treatment Protocol for Gene Silencing using a dsRNA/siRNA Mixture.

Uncoated organic corn seeds were from variety "popcorn," uncoated organic whole grain rice seeds, organic soybean and wheat seeds were purchased from Nitsat Haduvdevan (Israel). Fresh tomato seeds were retrieved from M82 tomato fruits, which are propagated in-house. Uncoated or fresh plant seeds were washed with double distilled water (DDW) prior to treatment for four hours. Next, seeds were dried at 30 °C for 10-16 hours. Following the drying step, seeds were treated with a solution containing the dsRNA formulation, which is made of dsRNA at a final concentration of 40-150 µg/ml in 0.1mM EDTA. Treatment was performed by gently shaking the seeds in the solution for 24 hours in a dark growth chamber at 15 °C. Finally, seeds were washed twice briefly and planted on soil or dried for 0-30 hours and germinated at 25 °C in a dark growth chamber and planted in soil or planted directly in soil. Control seeds were treated in a similar way, with a formulation that lacked the dsRNA or with non-specific dsRNA.

EXAMPLE 2: STABILITY OF THE DSRNA IN SEEDLINGS OF RICE, TOMATO AND SORGHUM

As an example for an exogenous gene that is not present/expressed in plants, the ORFs encoding the replicase and coat protein of CGMMV (accession number AF417242) were used to as targets for dsRNA treatment of plant seeds using the protocol described in Example 1. Rice, tomato and sorghum seeds were washed for 4 hours at 20 °C, tomato and sorghum were dried at 30 °C and rice at 20 °C for overnight. Seeds were immediately treated at 15 ºC with 132.7 µg/ml dsRNA (final concentration) for 39 hours for rice, 93.8 µg/ml dsRNA (final concentration) for 48 hours for tomato, and 75 µg/ml dsRNA (final concentration) for 40 hours for sorghum.

Briefly, the virus-derived ORFs were amplified by PCR with specifically designed forward and reverse primers that contain the T7 sequence (5'-TAATACGACTCACTATAGGG-3', SEQ ID NO: 1) at their 5' (see Table 1, below). PCR products were purified from agarose gel and since they carry T7 promoters at both ends they were used as templates for T7-dependent in-vitro transcription, resulting in dsRNA product of the CGMMV genes. PCR on a housekeeping gene, tubulin, was used as a positive control (forward primer 5'-GGTGCTCTGAACGTGGATG-3' (SEQ ID NO: 2), and reverse primer 5'-CATCATCGCCATCCTCATTCTC-3'(SEQ ID NO: 3)).

Table 1: PCR primers served as Templates for in vitro Transcription and detection of CGMMV and CGMMV dsRNA products.

Virus Name	Product Name	Product Sequence/SEQ ID NO:	Forward primer/SEQ ID NO:	Reverse primer/SEQ ID NO:
1) CGMMV (NCBI Accession number AF417242)	CGMVV dsRNA product 1	TAATACGACTCACTATAGGGGGTAAGCG GCATTCTAAACCTCCAAATCGGAGGTTG GACTCTGCTTCTGAAGAGTCCAGTTCTGT TTCTTTTGAAGATGGCTTACAATCCGATC ACACCTAGCAAACTTATTGCGTTTAGTG CTTCTTATGTTCCCGTCAGGACTTTACTT AATTTTCTAGTTGCTTCACAAGGTACCGC TTTCCAGACTCAAGCGGGAAGAGATTCT TTCCGCGAGTCCCTGTCTGCGTTACCCTC GTCTGTCGTAGATATTAATTCTAGATTCC CAGATGCGGGTTTTTACGCTTTCCTCAAC GGTCCTGTGTTGAGGCCTATCTTCGTTTC GCTTCTCAGCTCCACGGATACGCGTAAT AGGGTCATTGAGGTTGTAGATCCTAGCA ATCCTACGACTGCTGAGTCGCTTAACGC CGTAAAGCGTACTGATGACGCGTCTACG GCCGCTAGGGCTGAGATAGATAATTTAA TAGAGTCTATTTCTAAGGGTTTTGATGTT TACGATAGGGCTTCATTTGAAGCCGCGT	TAATACGACT CACTATAGGG GGTAAGCGGC ATTCTAAACC/ (SEQ ID NO:5)	Set 1: TAATACGACTCA CTATAGGGGAAG ACCCTCGAAACT AAGC/(SEQ ID NO:4)
			CTTCTTATGTT CCCGTCAGG/ (SEQ ID NO:7)	Set 2: ACTCAGCAGTCG TAGGATTG/(SEQ ID NO:6)

Virus Name	Product Name	Product Sequence/SEQ ID NO:	Forward primer/SEQ ID NO:	Reverse primer/SEQ ID NO:
		TTTCGGTAGTCTGGTCAGAGGCTACCAC CTCGAAAGCTTAGTTTCGAGGGTCTTCC CCTATAGTGAGTCGTATTA/(SEQ ID NO:8)		
	CGMVV dsRNA product 2	TAATACGACTCACTATAGGGGCTTTACC GCCACTAAGAACTCTGTACACTCCCTTG CGGGTGGTCTGAGGCTTCTTGAATTGGA ATATATGATGATGCAAGTGCCCTACGGC TCACCTTGTTATGACATCGGCGGTAACT ATACGCAGCACTTGTTCAAAGGTAGATC ATATGTGCATTGCTGCAATCCGTGCCTA GATCTTAAAGATGTTGCGAGGAATGTGA TGTACAACGATATGATCACGCAACATGT ACAGAGGCACAAGGGATCTGGCGGGTG CAGACCTCTTCCAACTTTCCAGATAGAT GCATTCAGGAGGTACGATAGTTCTCCCT GTGCGGTCACCTGTTCAGACGTTTTCCA AGAGTGTTCCTATGATTTTGGGAGTGGT AGGGATAATCATGCAGTCTCGTTGCATT CAATCTACGATATCCCTTATTCTTCGATC GGACCTGCTCTTCATAGGAAAAATGTGC GAGTTTGTTATGCAGCCTTTCATTTCTCG GAGGCATTGCTTTTAGGTTCGCCTGTAG GTAATTTAAATAGTATTGGGGCTCAGTT TAGGGTCGATGGTGATGCCCTATAGTGA GTCGTATTA/(SEQ ID NO:11)	TAATACGACT CACTATAGGG GCTTTACCGC CACTAAGAAC /(SEQ ID NO:10)	Set 3: TAATACGACTCA CTATAGGGCATC ACCATCGACCCT AAAC /(SEQ ID NO:9)

dsRNA homologous to green mottle mosaic virus is stable in rice seedlings. Rice seeds were treated at 15 ºC with 132.7 µg/ml dsRNA (final concentration) for 39 hours and dsRNA was detected. At one week post germination, dsRNA was detectable in 9 out of 10 seedlings. Detection of tubulin cDNA served as a positive control for the cDNA quality. At two weeks post germination, dsRNA is detectable in 10 out of 10 seedlings. At 3 weeks post germination, dsRNA homologous to green mottle mosaic virus is detected in 5 out of 5 samples in rice seedlings

Tomato seeds were treated at 15 ºC with 93.8 µg/ml dsRNA (final concentration) for 48 hours and sorghum seeds treated at 5 µg/ml dsRNA (final concentration) for 40 hours. CGMMV dsRNA was detected by RT-PCR in 5 out of 13 tomato seedlings tested at 10 day post-germination and 3 out of four sorghum seedlings 4 weeks after germination.

The exogenous dsRNA was found to be stable for at least three weeks in rice seedlings and at least 10 days in tomato seedlings and four weeks in Sorghum plants.

EXAMPLE 3: THE dsRNA IS NOT INTEGRATED INTO THE GENOME OF RICE

Rice seeds were treated with an exogenous dsRNA as in Example 2. Plants were germinated and grown for five weeks, DNA was extracted and PCR reactions were performed to demonstrate that the dsRNA did not integrate into the Rice's genome. Two sets of primers that gave a positive reaction when checked on the RNA level were used, set 1 (see Table 2) of primers were the set of primers used to amplify the template (all the dsRNA sequence). Set 2 (see Table 3) are the primers that were used in the PCR above. A Rice endogenous housekeeping gene (tubulin) was used as a positive control for the PCR reaction (see Table 2).

Three different DNA PCR reactions were carried out on dsRNA treated and untreated plants. No amplified DNA corresponding to CGMMV was detected in any treated or untreated plant.

Table 2: Tubulin Primers Used for PCR Amplification.

Primer Name and Direction	Primer Sequence/(SEQ ID NO:)	Primer Length
osa_TubA1_736F	GGTGCTCTGAACGTGGATG (SEQ ID NO: 12)	19
osa_TubA1_1342R	CATCATCGCCATCCTCATTCTC (SEQ ID NO: 13)	22

EXAMPLE 4: EXOGENOUS dsRNA MOLECULES ARE HIGHLY STABLE IN SOLUTION AND DO NOT GET INCORPORATED INTO THE GENOME OF TREATED PLANTS

Corn seeds were treated using the protocol described in Example 1, seeds were washed for 4 h at 20 °C, dried at 30 °C overnight and immediately treated with 40 µg/ml dsRNA (final concentration) directed against the β-glucuronidase (GUS) reporter gene for 60 hours at 15 °C, dried and were germinated. Leaves and roots were harvested from control and dsGUS-treated plants 7 and 15 days following germination. RNA was extracted from the harvested tissues and RT-PCR with specific GUS primers was run (Table 3). In addition, a corn endogenous housekeeping gene (ubiquitin) was used as a positive control for the PCR reaction. The GUS dsRNA molecules were found to be extremely stable in the treated seeds, and can be detected in corn plants 7 and 15 days post germination of the seeds.

GUS dsRNA can is detected in corn seedlings by RT-PCR at 7 and 15 days after germination according to an aspect of the present disclosure. At one week, GUS dsRNA is detected in shoots of nine of eleven corn seedlings tested. GUS dsRNA is not detected in untreated plants. At 1 week post-germination, GUS dsRNA is detected in five of five treated corn seedlings' roots 1 week post germination. At 15 days post germination, GUS dsRNA is detected in corn seedlings' roots.

GUS dsRNA molecules do not get incorporated in the genome of treated corn plants one week after germination as determined by agarose gel electrophoresis of DNA PCR reactions on GUS sequence.

Table 3: Primers for PCR Amplification of GUS and Ubiquitin Genes and GUS dsRNA product.

Primer Length	Primer Sequence/SEQ ID NO:	Primer Name
GUS_T7_For	TAATACGACTCACTATAGGGAGATCGACGGCCTGTGGGCATTC /(SEQ ID NO:15)	
GUS_T7_Rev	TAATACGACTCACTATAGGGAGCATTCCCGGCGGGATAGTCTG /(SEQ ID NO:16)	43
GUS208For	CAGCGCGAAGTCTTTATACC/(SEQ ID NO:17)	43
GUS289Rev	CTTTGCCGTAATGAGTGACC/(SEQ ID NO:18)	20
zmaUBQ-947F	CCATAACCCTGGAGGTTGAG/(SEQ ID NO:19)	20
zmaUBQ1043R	ATCAGACGCTGCTGGTCTGG/(SEQ ID NO:20)	20
GUS dsRNA product	TAATACGACTCACTATAGGGAGATCGACGGCCTGTGGGCATTC AGTCTGGATCGCGAAAACTGTGGAATTGATCAGCGTTGGTGG GAAAGCGCGTTACAAGAAAGCCGGGCTATTGCTGTGCCAGGC AGTTTTAACGATCAGTTCGCCGATGCAGATATTCGTAATTATG CGGGCAACGTCTGGTATCAGCGCGAAGTCTTTATACCGAAAG GTTGGGCAGGCCAGCGTATCGTGCTGCGTTTCGATGCGGTCAC TCATTACGGCAAAGTGTGGGTCAATAATCAGGAAGTGATGGA GCATCAGGGCGGCTATACGCCATTTGAAGCCGATGTCACGCC GTATGTTATTGCCGGGAAAAGTGTACGTATCACCGTTTGTGTG AACAACGAACTGAACTGGCAGACTATCCCGCCGGGAATGCTC CCTATAGTGAGTCGTATTA/(SEQ ID NO:21)	

EXAMPLE 5: FLUORESCENCE MICROSCOPY OF siRNA SEQUENCES IN VARIOUS PLANT SEEDS

Plant seeds as per the protocol described in Example 1. Seeds were washed for 4 h at 20 °C, dried at 25 °C and were immediately treated with a fluorescent siRNA (siGLO, 2μM final concentration, Thermo Scientific) at 15 ºC for 24 h. The quality of the siGLO before application to a plant seed was verified by gel electrophoresis analysis Bands c corresponding to the expected size of 20-24 bp of the fluorescent siRNA molecules was detected.

Fluorescent pictures of the seeds were taken 24-48 hours post treatment using an Olympus microscope at the lowest objective magnification (5X for bigger seeds such as rice and tomato seeds, and 10X for smaller seeds such as *Arabidopsis* seeds). To eliminate the possibility of non-specific auto-fluorescence, dsRNA-treated seeds are compared to control untreated seeds. Penetration of fluorescent siRNA molecules into plant seeds was observed at 24 hours after seed treatment with siRNA at 2 μM final concentration in *Arabidopsis* seeds, rice seeds, and tomato seeds.

Penetration of fluorescent siRNA molecules into rice seeds was observed at 24 hours following treatment with siGLO dsRNA.

In order to evaluate the distribution efficiency of the fluorescent siRNA inside the seeds, different plant seeds were cut into slices and imaged with a fluorescent microscope 48 hours after treatment. Each treated seed was imaged alongside a control untreated seed. Light and fluorescent images were taken where applicable for rice, tomato, cucumber, bean, sorghum and wheat seed samples.

Penetration of fluorescent siRNA molecules into rice seeds was observed at 48 hours following treatment with siGLO dsRNA. siGLO-treated and control rice seeds were sliced to view the interior distribution of the fluorescent dsRNA using a fluorescent microscope and fluorescent siRNA molecules detected in the treated seed. Fluorescent siGLO RNA is detected in the endosperm and the embryo.

Penetration of fluorescent siRNA molecules into tomato seeds was observed at 48 hours following treatment with siGLO dsRNA. siGLO-treated and control tomato seeds were sliced to view the interior distribution of the fluorescent dsRNA using a fluorescent microscope. Fluorescent siGLO RNA is detected in the endosperm and the embryo.

Penetration of fluorescent siRNA molecules into cucumber seeds was observed at 48 hours following treatment with siGLO dsRNA. siGLO-treated and control cucumber seeds were sliced to view the interior distribution of the florescent dsRNA using a fluorescent microscope. Fluorescent siGLO RNA is detected in the endosperm and the embryo.

Penetration of fluorescent siRNA molecules is detected in sliced seeds of various plant species, including bean, tomato, sorghum and wheat, 48 hours following treatment with siGLO dsRNA. siGLO-treated and control seeds were sliced to view the

interior distribution of the fluorescent dsRNA using a fluorescent microscope. Light images were also taken for each seed and are shown alongside the fluorescent image of the seed for reference.

Figure 1 presents fluorescent images of siGLO-treatment of rice seeds over a 24 hour period. The effect of incubation time with siGLO dsRNA on fluorescence intensity, indicating quantity and quality of dsRNA penetration, was tested. Control seeds that were left untreated (1), were imaged along with seeds treated with siGLO dsRNA for four different incubation times; 10 min (2), 3.5 hours (3), 5.5 hours (4), and 24 hours (5).

It is clear that the siRNA is distributed at various levels between the embryo and the endosperm. Accordingly, dsRNA molecules enter the embryo directly. Though not to be limited by any particular theory, the dsRNA molecules are carried by the water-based solution used for the seed treatment. The dsRNA molecules enter the endosperm as part of the endosperm's water-absorption process. These molecules then are transferred to the embryo as it develops as part of the endosperm to embryo nutrient flow during germination and seed development.

These present findings suggest the RNA molecules used to treat the seeds both penetrate the embryo and function in the embryo as it develops and also penetrate the endosperm and feed the embryo following germination.

EXAMPLE 6: TIME COURSE EXPERIMENT WITH siGLO TREATMENT

A time course experiment was performed on rice seeds to monitor the kinetics of siGLO penetration into the seeds following the seed treatment (Figure 1). The results indicate that the siRNA efficiently penetrates the plant seeds using the protocol described in Example 1.

EXAMPLE 7: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* GENES

Spodoptera littoralis (or *Prodenia littoralis*), also known as the African Cotton Leafworm or Egyptian Cotton Leafworm, is a moth found widely in Africa and Mediterranean Europe. It is a common pest on vegetables, fruits, flowers and other crops.

RNA was extracted for dsRNA production from *Spodoptera littoralis* larvae, and a cDNA library was prepared from 0.5 μg total RNA. Several genes (ATPase,

NADPH Cytochrome P450 oxidoreductase (herein referred to as NADPH), inhibitor of apoptosis (IAP) and Chitin Synthase) were selected to test the effect of feeding S. litoralis with plants grown from seeds treated with dsRNA directed against these genes (see Table 4). Corn seeds were washed for 4 h, dried at 30ºC and immediately were treated with dsRNA molecules at a final concentration of 40µg/ml (for IAP and ATPase), 80µg/ml (for NADPH, 40µg/ml for each dsRNA sequence, see Table 4), or a mix solution (80µg/ml final) containing all three genes (20µg/ml for each of the four dsRNA sequences), for 24 hours. Fresh tomato seeds were not washed and immediately treated with dsRNA molecules at a final concentration of 66µg/ml (for IAP), 133µg/ml (for NADPH), or a mix solution (80µg/ml final) containing dsRNA targeting these two genes, for 48 hours. Treated seeds were germinated and grown into plants. Control seeds which were not treated with dsRNA directed against *S. littoralis* genes but were incubated with a similar solution, either not containing dsRNA or containing dsRNA directed against an unrelated gene, such as GUS, were germinated and grown alongside the treated plants. The leaves of treated and control plants were placed in petri dishes and used as sole food source for *S. littoralis* (typically, about 5 caterpillars per plate). Total body weight of the caterpillars was recorded at the beginning of each experiment, and was tracked throughout. New leaves were supplemented as needed and their weight was recorded as well. Body weight gain of the caterpillars was calculated and used as an indicator to their well-being and survivability.

Table 4 Sequences of *Spodoptera littoralis* Genes for Down regulation and Primers used for dsRNA Molecules Generation.

Gene Name	Organism	SEQ ID NO
NADPH	*Spodoptera littoralis* NADPH cytochrome P450 oxidoreductase mRNA, complete cds (JX310073.1)	21
ATPase	*Spodoptera littoralis* H(+)-ATPase B subunit mRNA, partial cds (AY169409.1)	22
IAP	*Spodoptera littoralis* mRNA for inhibitor of apoptosis (iap gene) (AM709785.1)	23
Chitin synthase	*Spodoptera exigua* chitin synthase A mRNA, complete cds (DQ062153)	24
NADPH dsRNA#1	*Spodoptera littoralis*	25
NADPH dsRNA#2	*Spodoptera littoralis*	26
NADPH dsRNA#1 frwd	*Spodoptera littoralis*	27
NADPH dsRNA#1 rev	*Spodoptera littoralis*	28

NADPH dsRNA#2 frwd	*Spodoptera littoralis*	29
NADPH dsRNA#2 rev	*Spodoptera littoralis*	30
ATPase dsRNA#1	*Spodoptera littoralis*	31
ATPase dsRNA#1 frwd	*Spodoptera littoralis*	32
ATPase dsRNA#1 rev	*Spodoptera littoralis*	33
IAP dsRNA#1	*Spodoptera littoralis*	34
IAP dsRNA#1 frwd	*Spodoptera littoralis*	35
IAP dsRNA#1 rev	*Spodoptera littoralis*	36
Chitin synthase dsRNA#1	*Spodoptera exigua*	37
Chitin synthase dsRNA#2	*Spodoptera exigua*	38
Chitin synthase dsRNA#1 frwd	*Spodoptera exigua*	39
Chitin synthase dsRNA#2 frwd	*Spodoptera exigua*	40
Chitin synthase dsRNA#2 rev	*Spodoptera exigua*	41
Chitin synthase dsRNA#1 rev	*Spodoptera exigua*	42

Experiment 1

Spodoptera littoralis leafworms were placed in petri dishes with corn leaves from germinated control or dsRNA-treated seeds and were monitored daily for consumption of leaves and for body weight gain. Data for *S. littoralis* body weight gain after 24 hours, 48 hours and 5 days are shown in Table 5 respectively. A negative effect on body weight gain of the worms feeding on any dsRNA-treated leaves compared to worms feeding on control untreated leaves is noted. Body weight gain of *S. littoralis* fed on the control leaves was normalized to a value of '1'.

Experiment 2

In this experiment, dsRNA molecules for silencing of the *S. littoralis* NADPH or IAP genes were used to treat corn seeds. Leaves from seedlings grown from these seeds, as well as control leaves, were used as a food source for 5 *Spodoptera littoralis* leafworms in a single petri dish (two plates for each treatment). Control leaves were treated with dsRNA directed against the GUS gene. Body weight gain was recorded for control and treated groups 48 hours from beginning of the experiment (Table 5). The strongest effect on body weight gain was seen in worms feeding on NADPH-dsRNA treated leaves. Body weight gain of *S. littoralis* fed on the control leaves was normalized to a value of '1'.

Experiment 3

In this experiment, dsRNA molecules for silencing of the *S. littoralis* NADPH or IAP genes were used to treat tomato seeds. An additional treatment was also included, where seeds were treated with a mix solution containing the dsRNA molecules targeted against both genes. Leaves from seedlings grown from these seeds, as well as control leaves, were used as a food source for 5 *Spodoptera littoralis* leafworms in a single petri

dish. Body weight gain was recorded for control and treated groups 72 hours after treatment is presented in Table 5. Body weight gain of *S. littoralis* fed on the control leaves was normalized to a value of '1'.

Experiment 4

In this experiment, dsRNA molecules for silencing of the *S. littoralis* NADPH, IAP or ATPase genes were used to treat corn seeds. An additional treatment was also included, where seeds were treated with a mix solution containing the dsRNA molecules targeted against all three genes. Leaves from seedlings grown from these seeds, as well as control leaves, were used as a food source for 5 *Spodoptera littoralis* leafworms in a single petri dish. On day 4, the treated corn leaves were replaced with untreated lettuce leaves as the only food source. Body weight gain was recorded for control and treated groups for up to 8 days. The body weight of all worms at 24 hours was used as a reference point and body weight gain of *S. littoralis* fed on the control leaves was normalized to a value of '1'. Data of relative body weight gain of worms feeding on control or treated corn leaves is presented in Table 5.

Table 5: *Spodoptera littoralis* body weight gain after twenty four hours on dsRNA treated leaves

Expt.	Time	control	NADPH	IAP	Mix	ATPase	gus
	24 hours	1.0	0.64	0.38	n/a	0.8	n/a
1	48 hours	1.0	0.69	0.57	n/a	0.7	n/a
1	5 days	1.0	0.36	0.84	na/	0.94	n/a
2	48 hours	1.0	0.55	0.9			1.0
3	48 hours	1	0.55	0.9			
3	72 hours	1	0.95	0.91	0.90		
4	5 days[1]	1.0	0.76	0.73	0.99	1.11	
4	7 days[2]	1.0	0.88	0.87	0.89	0.91	
4	8 days[3]	1.0	0.9	0.78	0.97	1.12	

[1] four days of treated corn and 1 day of lettuce;
[2] four days of treated corn and 3 days of lettuce;
[3] four days of treated corn and 4 days of lettuce

EXAMPLE 8: SILENCING THE PDS-1 GENE IN RICE BY A dsRNA/siRNA MIXTURE

Rice seeds were washed in wash solution for 4 h at 20 ºC, dried at 25 ºC and immediately treated with a mixture of dsRNA/siRNA at a total concentration of 5 µg/ml at 15 ºC. Seeds were germinated at room temperature for several days and seed development was monitored. Seeds treated with the PDS and dsRNA/siRNA mixture exhibited stunted and delayed development, as seen by smaller seedlings and reduced

rooting. For efficiency considerations and in order to increase the likelihood of an observed effect, two products of the PDS-1 gene are combined (see Table 6).

Table 6: Two PDS-1 Gene Products to be Silenced by dsRNA/siRNA Mixture.

Sequence name	Organism	NCBI Accession Number	SEQ ID NO
Phytoene Desaturase PDS1 dsRNA1	*Zea mays*	BT084155.1	43
Phytoene desaturase PDS1 dsRNA2	*Zea mays*	BT084155.1	44

The experiment was performed in three biological repeats and the results are presented in Figures 2A-B.

EXAMPLE 9: CHLOROPHYLL BLEACHING AND GROWTH INHIBITION FOLLOWING PDS SILENCING

Rice seeds were treated as described in Example 8 and their subsequent development and seedling growth were monitored. Thirty days post PDS-1 silencing treatment the overall phenotype of the two plant groups, control and PDS-silenced, was recorded. PDS silencing has been reported to cause chlorophyll bleaching and growth inhibition (Peretz *et al.*, 2007, Plant Physiol 145: 1251-1263), which correlates with the phenotype of the PDS-silenced plants of the invention. Treated rice plants after thirty days appeared smaller in size and paler in color, respectively, compared to control plants.

EXAMPLE 10: DETECTION OF THE TWO PDS-1 GENE PRODUCTS BY REAL-TIME PCR

Following treatment with the dsRNA/siRNA mixture (ratio 1:1) as described in Example 8, expression levels of PDS-1 gene products are determined by real-time PCR using specifically designed primers:

Forward: GATTGCTGGAGCAGGATTAG SEQ ID NO: 45;

Reverse: CCCTTGCCTCAAGCAATATG, SEQ ID NO: 46.

For normalization purposes, UBQ5 expression was also determined using primers:

forward – ACCACTTCGACCGCCACTACT, SEQ ID NO: 47;

reverse - ACGCCTAAGCCTGCTGGTT, SEQ ID NO: 48.

The results are shown in Figures 3A-C.

EXAMPLE 11: HAP2E TARGET GENE SILENCING

Rice seeds were treated using the protocol described in Example 1. Seeds were washed for 4 h at room temperature, dried overnight at 25 °c and immediately treated with a Hap2e dsRNA concentration of 152µg/ml, for 41 hours at 15 °C (for Hap2e dsRNA sequences see Table 7). Control and Hap2e dsRNA-treated rice seeds that were germinated 5 days post treatment did not exhibit any differences in their root development. RNA was extracted from shoots of germinated seeds, 5 and 7 days post germination, and RT-PCR was run. After testing 3 different sets of primers (see Table 7), located in various regions of the dsRNA molecules (Table 8, showing the fold change relative to the control), the best primer set (primer set 3) was used to evaluate the endogenous Hap2e expression levels in dsRNA-treated plants versus control (untreated) plants. Down-regulation of Hap2e mRNA expression in the treated plants, at a level of over 50% silencing compared to control plants, was achieved with an efficiency of 31.25% (Table 9).

Other rice seeds were treated in same conditions with a Hap2e dsRNA concentration of 145.7 µg/ml, for 42 hours. RT-PCR using random primers+Oligo dT on RNA extracted from seedlings 18 days post germination also exhibited down-regulation of Hap2e mRNA in dsRNA-treated plants (Table 10), with 50% efficiency of reaching down-regulation of over 25 % compared to control.

Table 7: Primers used for RT-PCR of Hap2e dsRNA Molecules.

Primer Set	Primer Set Location	Primer Name and Direction	Primer Sequence	SEQ ID No.:
1	In dsRNA	osaHAP2E501F3	ACCGGCATCAGCTCAGTCTC	49
		osaHAP2E589R3	TGCTGTTCTCTGGGCACAGG	50
2	Junction	osaHAP2E11F5	TCCCCTCAGATATTAACAAC	51
		osaHAP2E108R5	AGGAGGAAAGGCAGCTTCTGTG	52
3	Out of dsRNA	osaHAP2E122F7	GTGACTCGTCACCAACAAAG	53
		osaHAP2E202R7	TGTGTTGTCCGTTGAGACTG	54

Table 8: Treatment of Rice seeds with Hap2e dsRNA (target of mir 169) Primer evaluation.

	control	EM47766	EM47767	EM47769	EM47772	EM47773
Primer set 1	1.0	0.87	0.7	-	0.81	0.62
Primer set 2	1.0	0.99	0.82	-	0.89	0.44
Primer set 3	1.0	0.76	0.73	-	0.78	0.4

Fold change relative to untreated control (control = 1.0)

Table 9: Treatment of Rice seeds with Hap2e dsRNA (target of mir 169) at 7 days.

	control	EM47796	EM47798	EM47799	EM47803	EM47804	EM47769
Relative Fold change	1.0	0.41	0.77	0.52	0.47	0.83	0.0

Fold change relative to untreated control (control = 1.0)

Table 10: Treatment of Rice seeds with Hap2e dsRNA (target of mir 169) at 18 days.

control	EM 49050	EM 49051	EM 49052	EM 49053	EM 49054	EM 49056	EM 49047	EM 49060	EM 49061	EM 49063	EM 49064
1.0	0.33	0.41	0.93	0.54	0.65	0.54	0.73	0.73	0.90	0.64	0.96

Fold change relative to untreated control (control = 1.0)

EXAMPLE 12: NFY TARGET GENE SILENCING IN CORN SEEDS

Corn seeds were treated using the protocol described in Example 1, Seeds were washed for 4 h at room temperature, dried overnight at 30 °C and immediately treated with a NFY dsRNA concentration of 56µg/ml, for 40 hours at 15 °C (for NFY dsRNA sequence see Table 11). RT-PCR on RNA extracted from control and NFY dsRNA-treated corn seeds 10 days after germination was performed to determine the expression level of NFY target gene (see Table 11). Down-regulation of the gene was successfully achieved as exhibited in Table 12.

Table 11: Primers used for RT-PCR of NFYA dsRNA Molecules in Corn Seeds 3 10 Days after Germination.

Primer Name and Direction	Primer Sequence	SEQ ID No.:
zma-NFYA3_345 F3	TCGGAAGCCGTACCTTCGTG	55
zma-NFYA3 _442R3	CCTGGAGCTGCTGCTTTGTG	56
zma-NFYA3_457F4	TACCAGGCGTCGAGTGGTTC	57
zma-NFY-A3_542R4	GAAGAGGGCGTGCAAATGGG	58

Table 12: Treatment of corn seeds with NFY dsRNA (target of mir169).

control	EM 48006	EM 48007	EM 48009	EM 48010	EM 48011	EM 48012	EM 48013	EM 48014
1.0	0.51	0.62	0.67	0.33	0.50	0.76	0.85	0.11

Fold change relative to untreated control (control = 1.0)

EXAMPLE 10: NFY TARGET GENE SILENCING IN TOMATO SEEDS

Tomato seeds were treated using the protocol described in Example 1. Un washed seeds were treated with a NFY dsRNA concentration of 200µg/ml, for 24 hours at 15ºC, seeds were washed twice briefly and immediately planted in soil without drying. RT-PCR on RNA extracted from control and NFY dsRNA-treated tomato seeds 3 weeks after germination was performed to determine the expression level of NFY

target gene (see Table 13). Down-regulation of the gene was successfully achieved as exhibited in Table 14.

Tomato plants 55 days post treatment with NFY dsRNA molecules were compared to same age control plants. Major phenotypic differences were evident upon comparison, most notably was a shift in height, where treated plants appeared significantly shorter than untreated control plants (Figure 4).

Table 13: Primers used for RT-PCR of NFYA dsRNA Molecules in Tomato and NFY dsRNA product.

Sequence Name	Sequence	SEQ ID No.:
slyNFYA125F3	CTATTGCGTGTGCTCCAAAC	59
slyNFYA212R3	ACATGAGGAGGAACCAAAGG	60
NFY dsRNA product 1	CTAATACGACTCACTATAGGGAGAGGCTCAAGAACCAG TTTATGTTAATGCTAAGCAGTATCGAAGGATCCTGCAGC GAAGACAGTCACGTGCTAAAGCAGAACTTGAAAAGAAG CAAATAAAGGGTAGAAAGCCATATCTTCACGAGTCTCG ACATCAGCATGCACTGAGGAGGGTAAGGGCCTCGGGTG GACGTTTTGCCAAAAAGACAGATGCTTCTAAGGGTACT GGTTCTGTGAGTTCATCGGGTTCTGAACCTTTGCAGTTC AATGCTGCTGATATTCAAAAGAGGAATGAAAATGGAAG GTTGGCCGAGCTTCAGCAGTCTTATTCAAATGGTAGCAG TTATGGCAATCAAAGTAGCTTTCAAGAATCCAAGGATG AGTACCAGTTTGCTAAAAGCAGGGAAGGAGGTTTTTTT GTCAAGTAATTGGAGATACGTTCATGTGTAAACTAGCTC TTGCCCTCTCCCTATAGTGAGTCGTATTAG	61
NFY dsRNA product 2	CTAATACGACTCACTATAGGGAGAGCAGTTATGGCAAT CAAAGTAGCTTTCAAGAATCCAAGGATGAGTACCAGTT TGCTAAAAGCAGGGAAGGAGGTTTTTTTGTCAAGTAATT GGAGATACGTTCATGTGTAAACTAGCTCTTGCCCTGCAA CGAGGGTAGAGTATGAGCAAGAGGAGTTTACAGGGATT GTTTCATTTCTTGGCTTTTCAAGATAGGCGGCAATTCAT TCTTGGCTTTTTACTTTAGTGTTAAAGGGAGCAACAGAG GTGACGAGGGTATCAGTGTTGCAGCATTTGCTTGGAGAT TACATCTTCCCTTATGTACAGAGATGGATGAACTTAGAA CTAGGATTAGAAAGTTTTTCAGTAAGTTTATGTTTGGCC AGTTACTGTAGTTTTAGTTTAGGAGACCATGTAAAAAGG TTGTTAGTTTTGCAAAAGGATCTTTTTTCTTTCCCTAATT GGTGCATTCTCCCTATAGTGAGTCGTATTAG	62

Table 14: Treatment of Tomato seeds with NFY dsRNA (target of mir169) at 3 weeks.

Plant	EM 49778	EM 49812	EM 49816	EM 49818	EM 49819	EM 49826	EM 49827	EM 49829
Relative fold change	1.0	0.8	0.9	0.5	0.6	0.9	0.8	0.6
Plant	EM 49832	EM 49833	EM 49834	EM 49835	EM 49836	EM 49837	EM 49838	EM 49839
Relative fold change	0.7	0.8	0.9	0.5	0.9	0.5	0.5	0.8

Fold change relative to untreated control (control = 1.0)

EXAMPLE 11: NAC TARGET GENE SILENCING IN CORN SEEDS

Corn seeds were treated using the protocol described in Example 1, seeds were washed for 4 h at room temperature, dried overnight at 30 °C and immediately treated with a NAC dsRNA concentration of 90µg/ml, for 40 hours at 15 °C and immediately germinated (for NAC dsRNA sequence see Table 15). RT-PCR on RNA extracted from control and NAC dsRNA-treated corn seeds 10 days after germination was performed to determine the expression level of NAC target gene (see Table 15). Down-regulation of the gene was successfully achieved as exhibited in Table 16.

Table 15: Primers used for RT-PCR of NAC dsRNA Molecules in Corn.

Primer Name and Direction	Primer Sequence	SEQ ID No.:
zmaNAC5_267F3	CGAGTCGGGATACTGGAAGG	63
zmaNAC5_342R3	CTTCTTCATGCCGACGAGGG	64
zmaNAC5_187F4	ACGATGGGCGAGAAGGAGTG	65
zmaNAC5_250R4	TCAGTCCCGTCGGGTACTTG	66

Table 16: Treatment of Corn Seeds with NAC dsRNA (target of mir164) at 10 days post germination.

Plant	Control	1	2	3	4	5	6	7
Relative fold change	1.0	0.22	0.14	0.22	0.20	0.43	0.16	0.55
Plant	8	9	10	11	12	13	14	
Relative fold change	0.00	0.09	0.13	0.21	0.26	0.26	0.18	

Fold change relative to untreated control (control = 1.0)

EXAMPLE 12: ARF-8 TARGET GENE SILENCING IN RICE SEEDS

Rice seeds were treated using the protocol described in Example 1, seeds were washed for 4 h, dried overnight at 20 °C and immediately treated with a ARF-8 dsRNA concentration of 66.2µg/ml, for 42 hours at 15 °C. RT-PCR on RNA extracted from control and ARF-8 dsRNA-treated rice seeds 18 days after germination was performed to determine the expression level of ARF-8 target gene (see Table 17). Down-regulation of the gene was successfully achieved as exhibited in Table 18 and Table 19.

Table 17: Primers used for RT-PCR of ARF-8 dsRNA Molecules in Corn and ARF-8 dsRNA product.

Sequence Name	Sequence	SEQ ID No.:
osaARF8_140F3	AGGGTCACATCCCGAACTAC	67
osaARF8_233R3	ACCTCGTCAGTCTCCACATC	68
osaARF8_1674F4	GTTGGATTCGAGCTTCCTTC	69
osaARF8_1757R4	TGCTGCTGCTCACTAGCTAC	70
ARF8 dsRNA product	CTAATACGACTCACTATAGGGAGACAGTCCGTTGGCCTAGT TCCTATTGGAGATCTGTGAAGGTTGGTTGGGATGAATCAAC TGCAGGGGAAAGACCACCAAGAGTTTCTTTATGGGAAATT	71

	GAACCATTGACAACCTTTCCAATGTATCCATCTCTGTTCCC ACTGAGAGTTAAGCATCCTTGGTATTCAGGAGTTGCTTCCC TGCATGATGACAGCAATGCTTTAATGTGGCTGAGAGGAGT TGCTGGTGAGGGAGGTTTTCAGTCTCTGAACTTTCAGTCAC CTGGTATTGGCTCCTGGGGACAACAGAGGCTCCATCCATCC TTACTGAGCAGCGATCACGATCAGTACCAAGCAGTAGTTG CTGCTGCTGCTGCTTCCCAATCTGGTGGTTACTTAAAACAG CAATTCTTGCACCTTCAGCAACCTATGCAGTCCCCTCAAGA ACACTGCAACCTCAACCCTCTCCCTATAGTGAGTCGTATTA G	

Table 18: Treatment of Rice Seeds with ARF-8 dsRNA (target of mir167) at 18 days post germination.

Plant	Control	EM 48977	EM 48983	EM 48984	EM 48986	EM 48987	EM 48989
Fold change	1.0	0.67	0.28	0.86	0.74	0.59	0.47

Fold change relative to untreated control (control = 1.0)

Table 19: Treatment of Rice Seeds with ARF-8 dsRNA (target of mir167) at 18 days post germination.

Plant	Control	EM 49194	EM 49196	EM 49198	EM 49200	EM 49201	EM 493203	EM 49204	EM 49206	EM 49209
Fold change	1.0	0.44	0.88	0.45	0.22	0.26	0.12	0.06	0.31	0.92

Fold change relative to untreated control (control = 1.0)

EXAMPLE 13: SPL17 TARGET GENE SILENCING IN RICE SEEDS

Rice seeds were treated using the protocol described in Example 1, seeds were washed for 4 h, dried overnight at 20 °C and immediately treated with a SPL17 dsRNA concentration of 200µg/ml, for 41 hours at 15 °C (for SPL17 dsRNA sequence see Table 20). Control and SPL17 dsRNA-treated rice seeds that were germinated 5 days post treatment did not exhibit any visual differences. RNA was extracted from 5 days old shoots of these germinated seeds and RT-PCR was run to determine SPL17 expression levels in control and treated plant groups. Two different sets of primers (see Table 20), located in various regions of the dsRNA molecules, were tested (Table 21). When RT-PCR was run on RNA extracted from 14-week old plants, down-regulation of SPL17 mRNA expression in the treated plants was achieved with high efficiency compared to control plants, (Table 22).

Table 20: Primers used for RT-PCR of SPL17 dsRNA Molecules in Rice Seeds 5 Days after Germination.

Primer Set and Location	Sequence Name	Primer Sequence	SEQ ID No.:
1 – in dsRNA	osaSPL17_119F3	CTCAGCCATGGGATACTACC	72
	osaSPL17_189R3	GCTGGCCGTTGACGACATTG	73
2 – out of dsRNA	osaSPL17_55F4	ACCTCAGGTGGATGTCTC	74
	osaSPL17_151R4	TGCTGGTGCTTTGGGTAG	75

Table 21: Treatment of Rice Seeds with SPL17 dsRNA (target of mir156) at 5 days post germination.

Plant	Control	EM 47708	EM 47709	EM 47710	EM 47711	EM 47712
Primer set 1	1	1.42	3.14	11.97	2.33	9.01
Primer set 2	1	.76	.92	1	.69	.84

Fold change relative to untreated control (control = 1.0)

Table 22: Treatment of Rice Seeds with SPL17 dsRNA (target of mir156) at 14 week post germination.

Plant	Control	EM 49502	EM 49503	EM 49511	EM 49513	EM 49515	EM 49517	EM 49519
Fold change	1.0	0.085	0.141	0.27	0.337	0.275	0.129	0.321

Fold change relative to untreated control (control = 1.0)

EXAMPLE 15: Silencing of MicroRNA Target Genes with Complementary dsRNA/siRNA

The high specificity and efficiency of posttranscriptional gene silencing by target gene-specific dsRNA has become a preferred method to generate preferred phenotype eukaryotic organisms, wherein expression of one or more genes is reduced or inactivated. Specific dsRNA sequences designed to silence corn (*Zea mays*) and rice (*Oryza sative*) microRNA target genes. Specifically, microRNAs shown to associate with improved abiotic stress tolerance will be used. Table 23 below provides several examples for target gene sequences that are produced using PCR amplification to test the gene silencing capabilities of their respective dsRNA/siRNA mixture. These dsRNA molecules will then be used to knock down the endogenous level of the selected target genes.

Table 23: Target Gene Sequences and Primers for PCR.

Sequence name	Organism	SEQ ID NO
miR169/NFY-A3	*Zea mays*	76
miR169/NFY-A3 frwd	artificial Sequence	77
miR169/NFY-A3 rev	artificial Sequence	78

miR169/NFY-A3 frwd	artificial Sequence	79
miR169/NFY-A3 rev	artificial Sequence	80
HAP2	*Oryza sativa*	81
HAP2 frwd	artificial Sequence	82
HAP2 rev	artificial Sequence	83
HAP2 frwd	artificial Sequence	84
HAP2 rev	artificial Sequence	85
miR156/SPL17	*Oryza sativa*	86
miR156/SPL17 frwd	artificial Sequence	87
miR156/SPL17 rev	artificial Sequence	88
miR156/SPL17 frwd	artificial Sequence	89
miR156/SPL17 rev	artificial Sequence	90
miR156/SBP-A3 HQ858696.1	*Zea mays*	91
miR156/SBP-A3 frwd	artificial Sequence	92
miR156/SBP-A3 rev	artificial Sequence	93
miR156/SBP-A3 frwd	artificial Sequence	94
miR156/SBP-A3 rev	artificial Sequence	95
miR164/NAC NM_001064881.1	*Oryza sativa*	96
miR164/NAC frwd	artificial Sequence	97
mir164/NAC rev	artificial Sequence	98
miR164/NAC frwd	artificial Sequence	99
mir164/NAC rev	artificial Sequence	100
NAC5 NM_001154298.1	*Zea mays*	101
NAC5 frwd	artificial Sequence	102
NAC5 rev	artificial Sequence	103
NAC5 frwd	artificial Sequence	104
NAC5 rev	artificial Sequence	105

EXAMPLE 16: ARF-8 GENE SILENCING IN TOMATO SEEDS

Tomato seeds were treated using the protocol described in Example 1, unwashed seeds were treated with a ARF-8 dsRNA concentration of 200µg/ml, for 24 hours at 15 °C and immediately planted in soil. Expression levels of the gene were examined using RT-PCR, 3 and 8 weeks after treatment (see Table 25). Changes in expression were achieved in dsRNA-treated plants 3 weeks after treatment (Table 24).

Table 24: Treatment of Tomato Seeds with ARF-8 dsRNA (target of mir167) at 3 weeks and 8 weeks post germination.

Plant	Control	EM 49933	EM 49950	EM 49951	EM 49952
3 weeks	1.0	0.6	0.6	0.5	0.8
Plant	EM 49953	EM 49954	EM 49955	EM 49957	
3 weeks	0.8	0.5	0.6	0.9	
Plant	Control	EM 50374	EM 50377	EM 50378	EM 50379

8 weeks	1.0	0.97	0.68	0.98	0.68
Plant	EM 50381	EM 50383	EM 50398	EM 50399	EM 50402
8 weeks	0.60	0.69	0.47	0.99	0.47

Fold change relative to untreated control (control = 1.0)

Plants that were treated with dsRNA molecules specific for the ARF8 gene showed a phenotypic difference compared to control plants. This phenotypic difference was observed at different time points (55, 62 and 72 days) and was demonstrated by a decrease in height (Figures 5A-C). While the average height of control plants was ~36cm, the dsRNA treated plants were ~30cm tall on average (Figure 5D). In addition to their decreased height (delayed vertical development), dsRNA-treated plants appeared more branched (increased horizontal development) compared to control plants. Thus, plants treated with dsRNA specific for ARF8 appeared shorter and more branched relative to their control counterparts 55 and 72 days after treatment.

Table 25: Primers used for RT-PCR of ARF-8 dsRNA Molecules in Tomato and ARF-8 dsRNA product.

Sequence Name	Sequence	SEQ ID No.:
slyARF_8_1816F4	CCTCAACAGTCCTGGATGTC	106
sly ARF_8_1896R4	CCCGTAAGTTGGAAGTGATG	107
ARF 8 dsRNA product 1	CTAATACGACTCACTATAGGGAGAGCTTCTCCTCCCTA CAACTGTGTCTAACGTCGCTACTACATCAATTGATGCT GATATATCCTCTATGCCACTAGGGACTTCTGGATTTCC GAATCCCTTGTATAGTTATGTGCAAGATTCTACTGACT TGTTGCATAATGTAGGGCAAGCTGATGCACAAACTGT GCCCCGTACATTTGTCAAGGTTTACAAATCAGCGTCCC TTGGGAGGTCATTGGACATCACTCGGTTCAACAGCTAT CATGAGCTGCGACAGGAATTAGGGCAGATGTTCGGTA TCGAAGGGTTGCTTGAAGACCCTCAAAGATCAGGCTG GCAGCTTGTATTTGTTGACAGGGAGAATGATGTCCTTC TCCTTGGAGACGATCCGTGGGAGGAATTTGTCAATAA TGTTTGGTACATCAAAATTCTTTCACCCGAGGATGTGC AGAAACTGGGGAAAGAGGAGGTTGGATCCCTCTCCCT ATAGTGAGTCGTATTAG	108
ARF 8 dsRNA product 2	CTAATACGACTCACTATAGGGAGATGGGAGATTGAGC CTTTGACTACTTTTCCGATGTATCCATCTCTTTTTCCTC TAAGGCTAAAGAGGCCTTTCTATCAAGGAACCTCATCT TATCAGGATAGTAACAATGAAGCTATTAATCGAATGT CATGGTTAAGAGGGAATGCTGGTGAGCTAGGACATCA TTCAATGAATCTTCAGTCTTTTGGCATGCTTCCTTGGAT GCAACAGAGAGTCGATTCAACAATTCTCCCAAATGAT ATTAATCAGCACTATCAAGCTATGCTGGCTACTGGC TTGCAAAGTTTTGGGAGTGGAGATTTACTGAAACAGC AATTAATGCAGTTTCAGCAGCCTGTCCAATATCTGCAA CATGCAAGTACTGAGAATTCAATTTTGCATCAGCAGC AGCAGCAGCAGCAGCAAATAATGCAGCAAGCAGTTCA TCAGCATATGCTGCCTGCTCAAACCCAAATGCTGTCAG AGAACCTTCAAAGGCAATCCCAGCATCAATCCATCTC CCTATAGTGAGTCGTATTAG	109

EXAMPLE 17: FW2.2 GENE SILENCING IN TOMATO SEEDS

Tomato seeds were treated using the protocol described in Example 1, unwashed seeds were treated with a FW2.2 dsRNA concentration of 100µg/ml, for 24 hours at 15 °C and immediately planted in soil Expression levels of the gene were examined using RT-PCR, 9 weeks following germination (primers are listed in Table 26). An approximate 2-fold reduction in the expression level of FW2.2 in dsRNA treated plants compared to control plants was detected (Figure 6).

Even so, plants that were treated with dsRNA molecules specific for the FW2.2 gene showed no phenotypic differences compared to control plants, ruling out a toxic effect as an alternative explanation for the phenotypic effects seen in Example 15. The plants presented similar height and appearance 72 days after treatment.

Table 26: Primers used for RT-PCR of FW2.2 dsRNA Molecules in Tomato and FW2.2 dsRNA product.

Sequence Name	Sequence	SEQ ID No.:
slyFW2_316F2	GAGGCACCTTGTGTTGATTG	110
slyFW2_406R2	CAAAGCCACGGTTCTTAAGC	111
FW2.2 dsRNA product	CTAATACGACTCACTATAGGGAGATCCAGGTCCAATGAAA CAACCTTATGTTCCTCCTCACTATGTATCTGCCCCCGGCAC CACCACGGCGCGGTGGTCGACTGGTCTTTGTCATTGTTTTG ATGACCCTGCTAACTGTTTAGTTACTAGTGTTTGCCCTTGTA TCACCTTTGGACAGATTTCTGAAATACTAAACAAAGGAAC AACTTCATGTGGGAGTAGAGGTGCATTATATTGTTTGCTGG GATTGACAGGATTGCCTAGCCTATATTCCTGCTTCTACAGG TCTAAAATGAGGGGGCAATATGATCTGGAAGAGGCACCTT GTGTTGATTGTCTTGTACATGTATTCTGTGAACCTTGTGCTC TTTGCCAAGAATACAGAGAGCTTAAGAACCGTGGCTTTGA TATGGGAATAGGGTGGCAAGCTAATATGGATAGACAAAGC CGAGGAGTTACCATGCCCCCTTATCATGCAGGCATGACCTC TCCCTATAGTGAGTCGTATTAG	112

EXAMPLE 17: DELLA GENE DOWN-REGULATION IN RICE RESULTS IN MORE DEVELOPED ROOTS OF GERMINATED SEEDS

Rice seeds were treated using the protocol described in Example 1, seeds were washed for 4 h, dried for 24 h at room temperature and immediately treated with a DELLA dsRNA concentration of 66µg/ml, for 36 hours at 15 °C. Rice seeds were treated with dsRNA directed against the Della gene (see Table 28), which is a known plant growth repressor. *Arabidopsis* seedlings with mutant Della gene are larger with a longer root system (Josse, E.M., Gan, Y., Bou-Torrent, J., Stewart, K.L., Gilday, A.D., Jeffree, C.E., Vaistij, F.E., Martínez-García, J.F., Nagy, F., Graham, I.A., and Halliday, K.J. (2011). A DELLA in disguise: SPATULA restrains the growth of the developing

Arabidopsis seedling. Plant Cell 23: 1337–1351.). Figure 7 shows mimicking of the *Arabidopsis* phenotypes using dsRNA seed treatment, with treated seedlings being larger with longer roots than control seedlings.

EXAMPLE 18: NRR GENE DOWN-REGULATION IN RICE RESULTS IN MORE DEVELOPED ROOTS AND SHOOTS OF GERMINATED SEEDS

Rice seeds were treated using the protocol described in Example 1, seeds were washed for 4 h, dried for 24 h at room temperature and immediately treated with a NRR dsRNA concentration of approximately 4µg/ml, for 36 hours at 15 °C. Rice seeds were treated with dsRNA directed against the NRR gene, which was found to regulate root growth in response to macronutrients in rice (Zhang *et al.*, 2012, Mol Plant 5(1):63-72). Transgenic rice seedlings, with reduced NRR levels using RNAi were shown to have longer roots when grown under nitrogen limiting conditions. Figure 8 shows mimicking of this phenotype using dsRNA seed treatment, with resulting treated seedlings being larger and with longer roots than control seedlings.

Table 27: Products of NRR dsRNA Molecules in rice.

Sequence Name	Sequence	SEQ ID No.:
NRR dsRNA product 1	CTAATACGACTCACTATAGGGAGAAGCTCCTGAACCCAT CATTGAAGAACCAGTGCTTAGCCTTGATCCAGTTGCAGCA GCCATTTCGATGATGTCTGGCAGTGAGAACGTAATGGAT GAAACTATAGAGGTTGCAGATATCAGCGACATTCAGAAT GACTCTCTTTTAAGCGAAGTATTATACGAGTGCGAGAAG GAACTCATGGAGAAGTCCGCAATCGAAGAGACTATTTCT GAACTGCTGGACGTCAAGATTCCTATGCTGCAAGTGGAA GAGTTCCCTAGGGAAACCCAAGTACAACTACCGGCCATG GAGAAGGAGAAGCCATCAGTTCCTGAATGTTGTTCACTC CAGAAAAGTGTCAGTTCTGGGTGCCTCAACTCAGCTGATT GGATCAATGGACCAGCCAGGCCAAACTTCCTGGACTTCC AAGGATTGGACTTTGAGACAGCGTTTGGGTTGAGGAGGG CATACAGCGAAGGAGACATTCTCCCTATAGTGAGTCGTA TTAG	113
NRR dsRNA product 2	CTAATACGACTCACTATAGGGAGACATGGAGAAGTCCGC AATCGAAGAGACTATTTCTGAACTGCTGGACGTCAAGAT TCCTATGCTGCAAGTGGAAGAGTTCCCTAGGGAAACCCA AGTACAACTACCGGCCATGGAGAAGGAGAAGCCATCAGT TCCTGAATGTTGTTCACTCCAGAAAAGTGTCAGTTCTGGG TGCCTCAACTCAGCTGATTGGATCAATGGACCAGCCAGG CCAAACTTCCTGGACTTCCAAGGATTGGACTTTGAGACAG CGTTTGGGTTGAGGAGGGCATACAGCGAAGGAGACATTC AGAATCTTGGAGCTAGCACCCCTCGACCCGGGAACTCAG GAAACGCTCAATTAGCATCTTGCGAGAGGCTTGTAACCA TCAGTGACCTGAAATCTGAAGAAAGGAAGCAGAAGCTAT CTAGGTACAGAAAGAAGAAGGTGAAGAGAAACTTTGGC AGAAAGATCAAGTATGCTTGCAGGAAGGCTCTCTCCCTA TAGTGAGTCGTATTAG	114

EXAMPLE 19: SIMULTANEOUS SILENCING OF THREE ENDOGENOUS GENES

In the present Example, the effect of silencing three genes simultaneously is tested. Rice seeds were treated using the protocol described in Example 1, seeds were washed for 4 hours, dried overnight at room temperature and immediately treated with a solution containing a mixture (152.7µg/ml final concentration) of dsRNA against three genes: Hap2e (59.9µg/ml, see Table 28), Della (44µg/ml see Table 28 below) and SQS (48.4µg/ml see Table 28 below) for 42 h at 15 °C. RNA was extracted from shoots of germinated seeds, 18 days post germination, and RT-PCR for each of the three genes was run (see Table 28 below). As can be seen in Table 29, down-regulation of all three genes was highly effective, with treated plants exhibiting decrease in expression of each individual gene at various amounts, ranging from a minimum of 10% decrease to total silencing of the gene (equals 100% down-regulation).

Table 28: Primers Used for RT-PCR Analysis for Expression Level of Hap2e, Della and SQS Genes and dsRNA products.

Sequence Name	Sequence	SEQ ID No.:
osaHAP2E122F7	GTGACTCGTCACCAACAAAG	115
osaHAP2E202R7	TGTGTTGTCCGTTGAGACTG	116
osaDella1410F5	CAGTTCGCGCACACCATTCG	117
osaDella1494R5	GCAGCATGAACGGCTCCAAG	118
osaSQS465F3	TCCGCAATGCCGTGTGCATC	119
osaSQS543R3	GCGGCAGGAATGCTAGTGTC	120
Della dsRNA product	CTAATACGACTCACTATAGGGAGAGCCCACTTCTACGA GTCCTGCCCCTACCTCAAGTTCGCCCACTTCACCGCAAA TCAAGCCATCCTCGAGGCTTTCGCCGGCTGCCACCGCGT CCACGTCGTCGACTTCGGCATCAAGCAGGGGATGCAAT GGCCAGCTCTCCTCCAGGCCCTCGCCCTTCGTCCCGGCG GCCCCCCATCGTTCCGCCTCACCGGCGTCGGCCCCCCGC AGCCGGACGAGACCGACGCCTTGCAGCAGGTGGGTTGG AAGCTTGCCCAGTTCGCGCACACCATTCGCGTCGACTTC CAGTACCGGGGACTCGTCGCCGCCACTCTCGCGGACTT GGAGCCGTTCATGCTGCAGCCGGAGGGCGAGGCGGACG CGAACGAGGAGCCTGAGGTGATCGCCGTCAACTCGGTG TTCGAGCTGCACCGGCTGCTCGCGCAGCCCGGCGCGCT GGAGAAGGTCCTGGGCACGGTGCACGCGGTGCGGCCAA GGATCGTCACCGTGGTAGAGTCTCCCTATAGTGAGTCGT ATTAG	121
SQS dsRNA product 1	CTAATACGACTCACTATAGGGAGAATATCTACAACCGC GACTGGCATTATTCATGTGGAACAAAAGACTACAAATT ACTGATGGATAAGTTTCGCCTTGTCTCCACGGCTTTCTT GGAGCTTGGTCAAGGTTATCAAGAGGCAATTGAAGAAA TCACTAGGCTAATGGGAGCAGGAATGGCAAAATTTATC TGCAAGGAGGTTGAAACTGTTGATGACTACAATGAGTA CTGTCACTATG TAGCAGGGCTAGTGGGGTATGGGCTTTCCAGGCTCTTTC	122

	ATGCTGGTGGGACGGAAGATCTGGCTTCAGATTCACTTT CAAATTCAATGGGCTTGTTTCTGCAGAAAATCAATATAA TTAGGGATTATTTGGAGGACATAAACGAGATACCAAAG TCACGTATGTTCTGGCCTCGAGAAATATGGAGTAAATAT GTCAATAAACTCGAGGATTTGAAATACGAGGAAAATTC AGAAAAGGCAGTTCAGTGTTTGAATGATATGGTGACTA ACGCTCTGTCTCATCTCCCTATAGTGAGTCGTATTAG	
SQS dsRNA product 2	CTAATACGACTCACTATAGGGAGACGCTCTGTCTCATGC TGAAGACTGCCTCCAATACATGTCAGCATTGAAGGATC ATGCCATTTTCCGTTTTTGTGCAATACCTCAGATAATGG CAATTGGGACATGTGCTATTTGCTACAATAATGTGAATG TCTTTAGAGGAGTTGTTAAGATGAGGCGTGGGCTCACT GCACGAGTAATTGATGAGACAAACACAATGTCAGATGT CTATACTGCTTTCTATGAGTTCTCTTCGCTGATAGAATC GAAGATTGATAATAATGATCCAAATGCTTCCCTAACGC GGAAACGTGTTGATGCGATAAAGAGAACCTGCAAGTCA TCTTGCTCACTAAAGAGAAGGGGATACGATTTGGAGAA GTCAAAGTACAACTCCATGCTGATAATGGTTGTACTTCT GTTGGTGGCTCTCCCTATAGTGAGTCGTATTA	123

Table 29: Simultaneous Knockdown of Expression in Rice seeds at 18 days post germination.

RNA	Control	EM 49174	EM 49175	EM 49177	EM 49178	EM 49179	EM 49180
Hap2e	1.0	0.19	0.20	0.53	0.88	0.28	0.14
Della	1.0	0.14	0.10	0.47	1.00	0.42	0.10
SQS	1.0	0.15	0.01	0.23	0.71	0.42	0.27
RNA	EM 49181	EM 49183	EM 49184	EM 49185	EM 49186	EM 49187	EM 49188
Hap2e	0.06	0.27	0.92	0.24	0.27	0.29	0.37
Della	0.01	0.14	0.60	0.27	0.29	0.37	0.16
SQS	0.56	0.08	0.87	0.49	0.09	0.13	0.10

Fold change relative to untreated control (control = 1.0)

EXAMPLE 20: GENERATION OF dsRNA MOLECULES FOR SILENCING A TARGET GENE OF A PHYTOPATHOGEN

dsRNAs encoding *S. littoralis* genes were analyzed against the corn and tomato genomes (Figures 9 and 10 respectively) using BLAST searches with the following parameters: Expect threshold – 10; Word size – 11; Match/Mismatch score 2,-3; Gap costs: Existence:5 Extension:2; Max matches in a query range: 0. BLAST searches were performed against the databases of corn (*Zea mays* - taxid:4577) and tomato (*Solanum lycopersicum* taxid:4081) sequences that meet the teachings of the present invention are presented.

EXAMPLE 21: SEED TREATMENT AGAINST SPODOPTERA LITTORALIS NADPH GENE

Corn seeds (var. 01DKD2) were treated with dsRNA molecules (SEQ ID No. 26) having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* NADPH gene according to the protocol described in Example 1. A final concentration of 80 µg/ml dsRNA diluted with 0.1mM EDTA was used. Treatment was performed by gently shaking the seeds in the solution for 3.5 hours in a dark growth chamber at 15 °C. After treatment, seeds were planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with GFP dsRNA (SEQ ID No. 124), or with a similar solution not containing dsRNA (EDTA control), were germinated and grown alongside the treated plants as a control.

28 days after seed treatment, the leaves of treated and control plants were placed in petri dishes and used as sole food source for *S. littoralis*. For each plant, 15 larvae were used (5 larvae per plate, three plates per plant). Five plants from each seed treatment (NADPH, GFP and EDTA) were tested. New leaves were supplemented as needed. Body weight of each larva was recorded 12 days after the beginning of feeding and was used as an indicator of their well-being and survivability. A significant (one-way ANOVA, p-value=$8.36x10^{-5}$) negative effect on the body weight of the larvae fed on NADPH dsRNA-treated plants compared to larvae fed on control plants was observed. See Table 30. The average weight of larvae fed on NADPH-treated plants was 23% and 20% lower than the average weight of larvae fed on GFP and EDTA-treated plants, respectively.

Table 30: *Spodoptera littoralis* average weight (mg) after 12 days of feeding on treated plants.

Sample	1	2	3	4	5	Average	std. dev.
EDTA	40.8	46.7	45.3	38.9	47.1	43.8	3.7
GFP	42.9	41	47.3	48.9	49.2	45.9	3.7
sI-NADPH #1	38.5	26.8	32.5	35.2	43.2	35.2	6.2

73 days after seed treatment, the leaves of treated and control plants were used again as sole food source for *S. littoralis*. Five plants from each group were included in

the feeding experiment. The leaves of each plant were placed in five petri dishes containing five larvae each, summing to 25 larvae per plant and 125 larvae per group. Seven days into the experiment, an unusual large number of larvae were found dead in the EDTA control group. Due to the large number of deaths in the control group, the effect of feeding plant tissue collected 73 days after dsRNA seed treatment on *S. littoralis* well-being and survivability was not analyzed further.

The expression levels of NADPH in subsets of larvae fed on plants grown from seeds treated with dsRNA molecules targeting NADPH or GFP (28 days after seed treatment) were determined.

Table 31: Larvae from which RNA was extracted.

Leaf source			Weight (mg)
Treatment	Plant number	Repeat	
NADPH	2	2	22
			29
			15
			35
			18
	3	3	36
			28
			32
			30
			39
		2	29
			28
GFP	1	2	37
			49
			33
			27
			37
	2	3	28
			40
			26
			39
			31
		2	34
			39

Total RNA was extracted from the larvae and cDNA was prepared using oligo-dT primers and the expression level of *S. littoralis* NADPH mRNA was determined in treated and control larvae by real-time PCR (RT-PCR) with SYBR Green (Quanta BioSciences), using the house-keeping genes Actin and EF1α as normalizers. The sequences of the primers used in the RT-PCR analysis are shown in Table 32.

Table 32: Primers Used for RT-PCR Analysis for Expression Level of NADPH.

Primer Name and Direction	Primer Sequence	SEQ ID No.
NADPH_F	ATGGCTGTTGACGTAAGG	125
NADPH_R	TGCAGCTTCAGCTTCTGTG	126
EF1α_F	ACCGTCGTACTGGTAAATCC	127
EF1α_R	TGGCGGCATCTCCAGATTTG	128
Actin_F	CTGGTCGTACCACCGGTAT	129
Actin_R	GCAGAGCGTAACCTTCGTAG	130

No significant change in NADPH expression levels (Wilcoxon rank-sum test, p-value>0.05) was observed by RT-PCR analysis in larvae fed on plants grown from seeds treated with dsRNA molecules targeting NADPH or GFP (28 days after seed treatment).

EXAMPLE 22: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* ATPase GENE

Corn seeds were treated according to the protocol described in Example 1 with dsRNA molecules having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* ATPase gene (SEQ ID No. 31). Briefly, seeds were washed with double distilled water (DDW) prior to treatment for four hours. Next, seeds were dried at 30 °C over-night. Following the drying step, a final concentration of 53 µg/ml dsRNA diluted with 0.1mM EDTA was used. Treatment was performed by gently shaking the seeds in the solution for 26 hours in a dark growth chamber at 15 °C. After treatment, seeds were germinated on wet paper for seven days and then planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with a similar solution not containing dsRNA were germinated and grown alongside the treated plants as a control (EDTA control).

43 days after seed treatment, the leaves of treated and control plants were used as sole food source for *S. littoralis*. Plant number 1 served as a food source for 20 larvae placed in 130x170mm box. Plant number 2 served as a food source for 15 larvae placed in 124x95mm box. Plant number 3 served as a food source for 8 larvae placed in petri dish. The surface of all boxes and plates was covered with vermiculite, and new leaves were supplemented as needed. Mortality and body weight of the larvae were tracked throughout the experiment. Figure 11A shows mortality and Figure 11B shows the average weight of live *S. littoralis* larvae eight days after the beginning of feeding. While the larvae fed on plants 1 and 3 grown from ATPase dsRNA treated seeds gained

comparable weight and showed similar mortality to that of the control group, the larvae fed on plant number 2 grown from an ATPase dsRNA treated seed were almost 3-fold smaller compared to the control group, which had a higher death rate.

To test the persistence of the effects of dsRNA seed treatment, the leaves of plant number 2 were collected 85 days after seed treatment and used as sole food source for *S. littoralis*. A total of 15 larvae, in three petri dishes containing five larvae each, were used. Figure 11C shows the percentage of dead larvae three days after the beginning of experiment. In the ATPase dsRNA-treated group 12 out of 15 larvae were dead, while no dead larvae were found in the control group.

The persistence of the effects of dsRNA seed treatment were further tested by collecting the leaves of plants number 1 and 2 at 91 days after seed treatment, and using the leaves as the sole food source for *S. littoralis*. A total of 15 larvae, in three petri dishes containing five larvae each, were fed on each plant. Four days into the experiment, both groups were fed also on plant number 3. Figure 11D shows the percentage of dead larvae seven days after the beginning of feeding, compared to the control group.

EXAMPLE 23: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* ATPase, IAP AND NADPH GENES

The plants described in this Example were treated with dsRNA molecules having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* ATPase, IAP or NADPH gene, and are the same plants described in Example 7.

67 days after seed treatment, leaves of the treated and control plants described in Example 7 were used as sole food source for *S. littoralis*. One plant from each treatment served as a food source for 10 larvae placed in a petri dish. The surface of the plates was covered with vermiculite. Figure 12 shows the percentage of dead larvae after seven days of feeding.

EXAMPLE 24: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* EF1α GENE

Corn seeds were treated with dsRNA molecules having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* EF1α gene (Table 33) according to the protocol described

in Example 1. Briefly, corn seeds were washed with double distilled water (DDW) prior to treatment for four hours. Next, the seeds were dried at 30 °C over-night. Following the drying step, a final concentration of 132 µg/ml dsRNA diluted with 0.1mM EDTA was used. Treatment was performed by gently shaking the seeds in the solution for 26 hours in a dark growth chamber at 15 °C. After treatment, seeds were germinated on wet paper for seven days and then planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with a similar solution not containing dsRNA were germinated and grown alongside the treated plants as a control (EDTA control).

Table 33: dsRNAs derived from the *S. littoralis* EF1α gene.

Sequence Name	Sequence	SEQ ID No.:
EF1α dsRNA #1	CTAATACGACTCACTATAGGGAGAATGCCCTGGTTCAAGGGATGG AACGTTGAGCGCAAGGAAGGCAAGGCTGAAGGTAAATGCCTCATT GAGGCCCTCGACGCCATCCTGCCCCCTGCTCGCCCCACAGACAAG CCCCTGCGTCTTCCCCTCCAGGACGTATACAAAATCGGTGGTATTG GTACGGTGCCCGTAGGCAGAGTTGAAACTGGTATCCTCAAGCCTG GTACCATCGTCGTCTTCGCCCCCGCCAACATCACCACTGAAGTCAA GTCTGTGGAGATGCACCACGAAGCTCTCCAAGAGGCCGTACCCGG TGACAACGTTGGTTTCAACGTAAAGAACGTTTCCGTCAAGGAGTT GCGTCGTGGTTACGTCGCTGGTGACTCCAAGAACAACCCACCCAA GGGCGCCGCCGATTTCACAGCACAGGTCATCGTGCTCAACCACCC TGGTCAAATCTCAAACGGATACACACCTGTGCTGGATTGCCACAC AGCCCACATTGCCTGCAAGTTCGCTGTCTCCCTATAGTGAGTCGTA TTAG	131
EF1α dsRNA #2	CTAATACGACTCACTATAGGGAGAGGCCCAGGAAATGGGTAAGG GTTCCTTCAAATACGCCTGGGTATTGGACAAACTGAAGGCTGAGC GTGAACGTGGTATCACCATTGATATTGCTCTGTGGAAGTTCGAAA CCGCTAAATACTATGTCACCATTATTGACGCTCCCGGACACAGAG ATTTCATCAAGAACATGATCACTGGAACCTCCCAGGCCGATTGCG CCGTACTCATTGTCGCCGCTGGTACCGGTGAATTCGAGGCTGGTAT CTCGAAGAACGGACAGACCCGTGAGCACGCTCTGCTCGCTTTCAC ACTCGGTGTCAAGCAGCTGATTGTGGGCGTCAACAAAATGGACTC CACTGAGCCCCCATACAGCGAATCCCGTTTCGAGGAAATCAAGAA GGAAGTGTCCTCCTACATCAAGAAGATCGGTTACAACCCAGCTGC TGTCGCTTTCGTACCCATTTCTGGCTGGCACGGAGTCTCCCTATAG TGAGTCGTATTAG	132

43 days after seed treatment, the leaves of treated and control plants were used as sole food source for *S. littoralis*. Plant number 1 served as a food source for 20 larvae placed in 130x170mm box. Plant number 2 served as a food source for 15 larvae placed in 124x95mm box. Plant number 3 served as a food source for 8 larvae placed in petri dish. The surface of all boxes and plates was covered with vermiculite, and new leaves were supplemented as needed. Mortality and body weight of the larvae were tracked throughout the experiment. Eight days after the beginning of the feeding

experiment, eight larvae out of 43 were found dead in the EF1α treated group, and three out of 43 larvae were dead in the control group. Figure 13A shows the average weight of live *S. littoralis* larvae eight days after the beginning of feeding.

87 days after seed treatment, the leaves of plants number 2 and 3 were used for a second time as sole food source for *S. littoralis*. A total of 15 larvae, in three petri dishes containing five larvae each, were fed on each EF1α-treated plant, and on two control plants (plants number 3 and 6). Figure 13B shows the percentage of dead larvae five days after the beginning of experiment.

EXAMPLE 25: SEED TREATMENT AGAINST SPODOPTERA LITTORALIS BETA ACTIN GENE

Corn seeds were treated with dsRNA molecules having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* Beta actin gene (Table 34) according to the protocol described in Example 1. Seeds were washed with double distilled water (DDW) prior to treatment for four hours. Next, seeds were dried at 30 °C overnight. Following the drying step, a final concentration of 76 µg/ml dsRNA diluted with 0.1mM EDTA was used. Treatment was performed by gently shaking the seeds in the solution for 26 hours in a dark growth chamber at 15 °C. After treatment, seeds were germinated on wet paper for seven days and then planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with a similar solution not containing dsRNA were germinated and grown alongside the treated plants as a control (EDTA control).

Table 34: dsRNA derived from the *S. littoralis* Beta actin gene.

Sequence Name	Sequence	SEQ ID No.:
Beta actin dsRNA #1	CTAATACGACTCACTATAGGGAGAATGGCTCCG GCATGTGCAAGGCCGGTTTCGCCGGCGACGACG CGCCCCGCGCCGTCTTCCCATCCATCGTAGGTCG CCCTCGTCACCAGGGTGTGATGGTTGGTATGGGT CAGAAGGACTCCTACGTAGGCGATGAGGCCCAG AGCAAGAGAGGTATCCTCACCCTGAAGTACCCC ATCGAGCACGGTATCATCACCAACTGGGACGAC ATGGAGAAGATCTGGCACCACACCTTCTACAAC GAGCTGCGCGTCGCCCCTGAGGAACACCCAGTC CTCCTGACTGAGGCTCCCCTCAACCCTAAGGCCA ACAGGGAGAAGATGACCCAGATCATGTTTGAGA CCTTCAACTCCCCCGCCATGTACGTCGCCATCCA GGCTGTGCTCTCTCTGTACGCCTCTGGTCGTACC ACCGGTATCGTCCTGGACTCCGGTGATGGTGTCT CCCACACCGTTCTCCCTATAGTGAGTCGTATTAG	133

43 days after seed treatment, the leaves of treated and control plants were used as sole food source for *S. littoralis*. Plant number 1 served as a food source for 20 larvae placed in 130x170mm box. Plant number 2 served as a food source for 15 larvae placed in 124x95mm box. Plant number 3 served as a food source for 8 larvae placed in petri dish. The surface of all boxes and plates was covered with vermiculite, and new leaves were supplemented as needed. Mortality and body weight of the caterpillars were tracked throughout the experiment. Eight days after the beginning of the feeding experiment, three larvae out of 43 were found dead in both the Beta-actin treated group and the control group. Figure 14 shows average weight of live *S. littoralis* larvae eight days after the beginning of feeding.

EXAMPLE 26: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* NADPH GENE

Corn seeds were treated with dsRNA molecules (SEQ ID No. 26) having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* NADPH gene according to the protocol described in Example 1. Briefly, seeds were washed with double distilled water (DDW) prior to treatment for four hours. Next, seeds were dried at 30 °C overnight. Following the drying step, a final concentration of 154 µg/ml dsRNA diluted with 0.1mM EDTA was used. Treatment was performed by gently shaking the seeds in the solution for 26 hours in a dark growth chamber at 15 °C. After treatment, seeds were germinated on wet paper for seven days and then planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with a similar solution not containing dsRNA were germinated and grown alongside the treated plants as a control (EDTA control).

43 days after seed treatment, the leaves of treated and control plants were used as sole food source for *S. littoralis*. Plant number 1 served as a food source for 20 larvae placed in 130x170mm box. Plant number 2 served as a food source for 15 larvae placed in 124x95mm box. Plant number 3 served as a food source for 8 larvae placed in petri dish. The surface of all boxes and plates was covered with vermiculite, and new leaves were supplemented as needed. Mortality and body weight of the larvae were tracked throughout the experiment. Eight days after the beginning of the feeding experiment, three larvae out of 43 were found dead in both the NADPH treated group

and the control group. Figure 15A shows average weight of live *S. littoralis* larvae eight days after the beginning of feeding.

91 days after seed treatment, the leaves of plant number 2 were used for a second time as sole food source for *S. littoralis*. A total of 15 larvae, in three petri dishes containing five larvae each, were fed on the NADPH-treated plant. Additional 15 larvae, in three petri dishes containing five larvae each, were fed on control plant. Figure 15B shows the percentage of dead larvae seven days after the beginning of experiment. In the NADPH-treated group 9 out of 15 larvae were dead, while in the control group 2 out of 15 larvae were dead.

EXAMPLE 27: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* IAP, ATPase AND NADPH GENES

Corn seeds were treated according to the protocol described in Example 1 with dsRNA molecules (SEQ ID No. 34) having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* IAP gene or with a solution containing a mix of dsRNAs (SEQ ID Nos. 34, 25, 26, and 31) having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* IAP, NADPH and ATPase genes. These two solutions were first used for the seed treatment described in Example 7, and then re-used in the experiment described here. Seeds were washed with double distilled water (DDW) prior to treatment for four hours. Next, seeds were dried at 30 °C overnight. Treatment was performed by gently shaking the seeds in the solution for 24 hours in a dark growth chamber at 15 °C. After treatment, the seeds were dried overnight at 30 °C, planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with a similar solution (EDTA) not containing dsRNA were germinated and grown alongside the treated plants as a control.

27 days after seed treatment, the leaves of the treated and control plants were used as sole food source for *S. littoralis*. A total of 24 larvae, in three petri dishes containing eight larvae each, were used for each treatment. One repeat from the IAP treatment contained nine larvae. Each repeat was fed from one plant, and three days into the experiment a second plant from the same treatment was added to the plate. Mortality and body weight of the caterpillars were tracked throughout the experiment.

Figures 16A-B shows average weight of live *S. littoralis* larvae six days after the beginning of the feeding experiment.

EXAMPLE 28: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* EF1α GENE

Corn seeds were treated according to the protocol described in Example 1 with two dsRNA molecules having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* EF1α gene (Table 33). Briefly, seeds were washed with double distilled water (DDW) prior to treatment for four hours. Next, seeds were dried at 30°C overnight. Two dsRNA sequences (SEQ ID No. 131 and SEQ ID No. 132) were used separately in two different seed treatments; each at a final concentration of 67 µg/ml dsRNA diluted with 0.1mM EDTA. Treatment was performed by gently shaking the seeds in the solution for 24 hours in a dark growth chamber at 15 °C. After treatment, seeds were dried at 30 °C overnight and then planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with dsRNA molecules having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the corn DWF1 gene (Table 35) (44µg/ml for DWF1#1 (SEQ ID No. 134) and 51µg/ml for DWF1#2 (SEQ ID No. 135)) were germinated and grown alongside the treated plants as two separated controls.

Table 35: Control dsRNAs derived from the corn DWF gene.

Sequence Name	Sequence	SEQ ID No.:
DWF1 dsRNA #1	CTAATACGACTCACTATAGGGAGTGTCAACATGGGTCAGAT AACCAGAGCTACCTGCCCAATGAACCTTGCCCTTGCGGTCG TCGCCGAGCTCGACGACCTCACTGTTGGTGGGCTGATCAAC GGTTACGGCATCGAGGGGAGCTCTCACCTCTATGGCCTTTTC TCCGACACGGTTGTCGCGATGGAGGTTGTTCTCGCAGATGG CCGGGTCGTCAGAGCCACCAAGGACAACGAGTACTCTGACC TTTTCTATGGAATTCCCTGGTCCCAGGGAACACTGGGGTTCC TTGTCTCTGCAGAGATCAAGCTGATCCCCATCAAGGAGTAC ATGAAGCTCACCTACACTCCAGTCAAGGGGGGTCTAAAGGA GATCGCGCAGGCCTACGCGGATTCTTTCGCTCCGAGGGACG GTGACCCGGCAAAGGTCCCTGACTTTGTTGAAGGGATGGTG TACACAGAGAGCGAGGGTGTCATGATGACGGGCGTGTACGC TTCGAAAGAAGAGGCGAAGAAGAAGGGCAACAAGATCAAC TGCGTGGGGTGGTGGTTTAAGCCCTGGTTCTACCTCTCCCTA TAGTGAGTCGTATTAG	134
DWF1 dsRNA #2	CTAATACGACTCACTATAGGGAGAGCGAGTTTGTGGAGTAC ATCCCGACGAGGGAGTACTACCACCGGCACACCCGGTGCCT	135

	GTACTGGGAGGGGAAGCTGATCCTGCCCTTCGGCGACCAGT TCTGGTTCAGGTTCCTGCTGGGCTGGCTGATGCCACCGAAG GTGTCCCTGCTGAAGGCGACCCAGGGCGAGGCTATCAGGAA CTACTACCACGACAACCATGTGATCCAGGACATGCTGGTGC CGCTGTACAAGGTTGGGGATGCGCTGGAGTTCGTGCACCGC GAGATGGAGGTGTATCCTCTGTGGCTGTGCCCTCACCGGCT GTACAAGCTGCCGGTGAAGACGATGGTGTACCCGGAGCCTG GGTTCGAGCACCAGCACAGGCAGGGCGACGCGAGCTACGC ACAGATGTTCACGGACGTGGGCGTGTACTACGCCCCCGGGG CGGTGCTGAGGGGGGAGGAGTTCAACGGCGCGGAGGCTGT GCACAGGCTGGAGCAGTGGCTGATCGAGAACCACAGCTAC CAGCCGCAGTACGCGGTGTCGGAGCTGAACGAGAAGGACT CCTGTCTCCCTATAGTGAGTCGTATTAG	

35 days after seed treatment, the leaves of the treated and control plants were used as sole food source for *S. littoralis*. Two plants from each treatment and from the DWF1#1 control were included in the feeding experiment. The leaves of each plant were placed in two petri dishes containing 10 larvae each, summing to 40 larvae fed on each seed treatment. Mortality and body weight of the larvae were tracked throughout the experiment. Nine days after the beginning of the feeding experiment, four larvae out of 40 were found dead in the EF1α #2 treated group and in the control group. Six larvae out of 40 were found dead in the EF1α #1 treated group. Figure 17A shows average weight of live *S. littoralis* larvae nine days after the beginning of the feeding experiment.

36 days after seed treatment, other plants from the same EF1α#1 and DWF1#1 seed treatment were used as sole food source for *S. littoralis*. Fifteen plants from the treatment were included in the feeding experiment. The leaves of each plant were placed in three petri dishes containing 5 larvae each, summing to 15 larvae per plant and 225 larvae total. Two days into the experiment, plant number 15 was replaced by plant number 8. Two plants from the control group were included in the feeding experiment. The leaves of each control plant were placed in three petri dishes containing 5 larvae each, summing to 15 larvae per plant and 30 larvae total. Body weight of the larvae was tracked throughout the experiment. Figure 17B shows average weight of *S. littoralis* larvae after five days of feeding.

71 days after seed treatment, the leaves of the EF1α #2 treated and DWF1#2 control plants were used as sole food source for *S. littoralis*. Ten plants from the treatment were included in the feeding experiment, from which two plants were tested for the second time (see Figure 17A) and eight plants were tested for the first time. The leaves of each plant were placed in three petri dishes containing five larvae each,

summing to 15 larvae per plant and 150 larvae total. Two plants from the control group, that were not tested previously, were included in the feeding experiment. The leaves of each control plant were placed in three petri dishes containing five larvae each, summing to 15 larvae per plant and 30 larvae total. Eight days into the experiment, an unusually large number of larvae were found dead in both treatment and control groups. Therefore, this time point was not analyzed further.

EXAMPLE 29: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* ATPase GENE

Corn seeds were treated with dsRNA molecules (SEQ ID No. 31) having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* ATPase gene according to the protocol described in Example 1. Briefly, seeds were washed with double distilled water (DDW) prior to treatment for four hours. Next, seeds were dried at 30 °C overnight. Following the drying step, a final concentration of 145 µg/ml dsRNA diluted with 0.1mM EDTA was used. The dsRNA solution contained a mixture of un-treated dsRNA molecules and phenol-treated dsRNA molecules as described in Example 1. Treatment was performed by gently shaking the seeds in the solution for 24 hours in a dark growth chamber at 15 °C. After treatment, seeds were dried at 30 °C overnight and then planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with 67 µg/ml dsRNA (SEQ ID No. 20) derived from GUS sequence were germinated and grown alongside the treated plants as a control.

56 days after seed treatment, the leaves of treated and control plants were used as sole food source for *S. littoralis*. Ten plants from the treatment were included in the feeding experiment. The leaves of each plant were placed in three petri dishes containing five larvae each, except for plants number 3 and 14 that were placed together in the same plates. A total of 15 larvae per plant and 135 larvae total were tested. Two plants from the control group were included in the feeding experiment. The leaves of each control plant were placed in three petri dishes containing five larvae each, summing to 15 larvae per plant and 30 larvae total. After twelve days of feeding, 12 out of 135 larvae were found dead in the ATPase treated group and 21 out of 30 larvae were

found dead in the control group. Figure 18A shows the percentage of dead larvae 12 days after the beginning of experiment.

57 days after seed treatment, other plants from the same treated and control groups were used as sole food source for *S. littoralis*. Fourteen plants from the treatment were included in the feeding experiment. The leaves of each plant were placed in three petri dishes containing five larvae each, except for plants number 13 and 4, which were placed together in the same plates, and plants number 10 and 19, which were placed together in the same plates (plants 4 and 10 were analyzed for the second time, see Figure 18A). A total of 15 larvae per plant and 180 larvae overall were tested. Two plants from the control group were included in the feeding experiment. The leaves of each control plant were placed in three petri dishes containing five larvae each, summing to 15 larvae per plant and 30 larvae total. Four days after feeding begun, 29 larvae out of 180 were found dead in the ATPase treated group and 29 larvae out of 30 were found dead in the control group. Figure 18B shows the percentage of dead larvae four days after the beginning of experiment.

EXAMPLE 30: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* EF1α GENE

Corn seeds (var. Vivani) were treated with dsRNA molecules (SEQ ID Nos. 131 and 132) having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* EF1α gene according to the protocol described in Example 1. A mixture of 25 µg/ml from each of the two dsRNAs was used. The dsRNA was diluted either with 0.1mM EDTA alone, or additionally mixed with 40 µg/ml of PEG-modified carbon nanotubes (CNTP). Treatment was performed by gently shaking the seeds in the solution for 4 hours in a dark growth chamber at 15 °C. After treatment, seeds were planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with 50 µg/ml dsRNA derived from GFP sequence (SEQ ID No. 124), or with a similar solution not containing dsRNA, with or without 40 µg/ml of PEG-modified carbon nanotubes, were germinated and grown alongside the treated plants as a control.

24 days after seed treatment, leaves of treated and control plants were used as sole food source for *S. littoralis*. Ten plants from the EF1α treatment group, two plants

from the GFP control, one plant from the EDTA control and one plant from the EDTA/CNTP control were included in the feeding experiment. The leaves of each plant were placed in three petri dishes containing five larvae each, summing to 15 larvae per plant, 150 larvae for EF1α treatment, 30 larvae for GFP control and 15 for both of the EDTA controls. Figure 19A shows average weight of *S. littoralis* larvae after ten days of feeding.

25 days after seed treatment, leaves of treated and control plants were used as sole food source for *S. littoralis*. Thirteen plants from the EF1α/CNTP treatment group, two plants from the GFP/CNTP control, one plant from the EDTA/CNTP control and one plant from the EDTA control were included in the feeding experiment. The leaves of each plant were placed in three petri dishes containing five larvae each, summing to 15 larvae per plant, except for plant 9 in the EF1α/CNTP group, where only two plates were analyzed. A total of 190 larvae for EF1α/CNTP treatment, 30 larvae for GFP/CNTP control and 15 larvae for both of the EDTA controls were tested. Seven days into the feeding experiment, plant 1 from the EF1α/CNTP group was replaced by plant 6 from the same group. Figure 19B shows average weight of *S. littoralis* larvae after ten days of feeding. To determine the expression levels of EF1α in the larvae ten days after feeding on treated plants, each repeat (plate) of five larvae was pooled together, and total RNA was extracted. cDNA was prepared using oligo-dT primers (SEQ ID Nos. 136-143) and the expression level of *S. littoralis* EF1α mRNA was determined in treated and control larvae by real-time PCR with SYBR Green (Quanta BioSciences), using Actin and ATPase as normalizers. No significant change in EF1α expression levels (Wilcoxon rank-sum test, p-value>0.05) was observed.

61 days after seed treatment, leaves of treated and control plants were used again as sole food source for *S. littoralis*. Thirteen plants from the EF1α/CNTP treatment group and three plants from the GFP/CNTP control were included in the feeding experiment. Some of the plants from the EF1α/CNTP group were tested for the first time and some were tested for the second time (see Figure 19B). The three plants from the GFP/CNTP control were tested for the first time. The leaves of each plant were placed in three petri dishes containing five larvae each, summing to 15 larvae per plant, except for plant 8 in the EF1α/CNTP group, where only two plates were analyzed. A total of 190 larvae for EF1α/CNTP treatment and 45 larvae for GFP/CNTP control were

tested. Twelve days into the experiment, an unusually large number of larvae were found dead in both treatment and control groups. Therefore, this time point was not further analyzed.

EXAMPLE 31: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* EF1α GENE

Corn seeds (var. 01DKD2) were treated with dsRNA molecules (SEQ ID Nos. 131 and 132) having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* EF1α gene according to the protocol described in Example 1. A mixture of 25 µg/ml from each of the two dsRNAs was used. The dsRNA was diluted either with 0.1mM EDTA alone, or additionally mixed with 40 µg/ml of PEG-modified carbon nanotubes (CNTP). Treatment was performed by gently shaking the seeds in the solution for 4 hours in a dark growth chamber at 15 °C. After treatment, seeds were planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with 50 µg/ml dsRNA (SEQ ID No. 20) derived from GUS sequence, with or without 40 µg/ml of PEG-modified carbon nanotubes, were germinated and grown alongside the treated plants as a control.

Eight days after seed treatment, the leaves of treated and control plants were used as sole food source for *S. littoralis*. Thirteen plants from the EF1α treatment, thirteen plants from the EF1α/CNTP treatment, ten plants from the GUS control and four plants from the GUS/CNTP control were included in the feeding experiment. The leaves of each plant were placed in two petri dishes covered with 1% agar. Each plate contained three larvae, summing to six larvae per plant, 78 larvae for both the EF1α and EF1α/CNTP treatments, 60 larvae for the GFP control and 24 for the GUS/CNTP control. Body weight of the larvae was recorded four days after feeding. Figures 20A-B shows average weight of *S. littoralis* larvae in control and treatment groups.

EXAMPLE 32: SEED TREATMENT AGAINST SPODOPTERA LITTORALIS IAP, ATPase AND NADPH GENES

Tomato plants grown from the tomato seeds described in Example 7, which were treated with dsRNA molecules (SEQ ID Nos. 34, 35, 25 and 26) having a nucleotide sequence that is essentially identical or essentially complementary to at least

18 contiguous nucleotides of the *S. littoralis* IAP gene, ATPase gene or NADPH gene were examined further for control of *S. littoralis*.

48 days after seed treatment, the leaves of treated and control plants were used as sole food source for *S. littoralis*. One plant from each treatment served as a food source for seven larvae placed in a petri dish. The surface of all plates was covered with vermiculite. Mortality and body weight of the larvae were tracked throughout the experiment. Three days into the experiment, one larva was found dead in the IAP treated group, and two larvae were found dead in the MIX treated group. No further death occurred in the following days up to day 7. Figure 21 shows the average weight of live *S. littoralis* larvae after three and seven days of feeding.

EXAMPLE 33: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* BETA ACTIN, ATPase AND NADPH GENES

Tomato seeds were treated with dsRNA molecules (SEQ ID Nos. 133, 31, 25, and 26) having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* Beta actin gene (see Table 34), ATPase gene or NADPH gene according to the protocol described in Example 1. A final concentration of 96 µg/ml dsRNA for Beta actin, 73 µg/ml dsRNA for ATPase and 164 µg/ml dsRNA for NADPH, diluted with 0.1mM EDTA was used. Treatment was performed by gently shaking the seeds in the solution for 26 hours in a dark growth chamber at 15 °C. After treatment, seeds were germinated in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with a similar solution (EDTA) not containing dsRNA were germinated and grown alongside the treated plants as a control.

42 days after seed treatment, the leaves of treated and control plants were used as sole food source for *S. littoralis*. Plants number 1 and 2 from the Beta actin and ATPase treatments and plants number 21 and 23 from the NADPH treatment were used. The plants from each treatment served as a food source for five larvae placed in a petri dish. The surface of all plates was covered with vermiculite. Body weight of the larvae was tracked throughout the experiment. Figure 22 shows average weight of *S. littoralis* larvae after four days of feeding.

EXAMPLE 34: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* ATPase GENE

The tomato plants described in this Example originate from the seed treatment with ATPase dsRNA described in Example 33 above.

85 days after seed treatment, the leaves of treated and control plants described in Example 33 were used again as sole food source for *S. littoralis*. One plant from the ATPase treatment and one plant from the control were used. Leaves from these plants were placed in three petri dishes contain five larvae each. Three days into the experiment, another plant from the treatment and another plant from the control were added to their respective plates. Body weight of the larvae was tracked throughout the experiment. Since at the onset of the feeding experiment the larvae fed from the control group were 30 % smaller when the larvae fed from the treatment, the weight of the larvae relative to their initial weight was recorded. Figure 23A shows relative weight of *S. littoralis* larvae after six days of feeding.

88 days after seed treatment, other plants from the same seed treatment were used as sole food source for *S. littoralis*. Three plants from the ATPase treatment and two plants from the control were used. Leaves from these plants were placed in three petri dishes contain five larvae each. Mortality and body weight of the larvae were tracked throughout the experiment. After feeding for five days, 4 out of 15 and 1 out of 15 worms were found dead in the ATPase and control group, respectively. Figure 23B shows average weight of live *S. littoralis* larvae after five days of feeding.

EXAMPLE 35: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* NADPH GENE

The tomato plants described in this Example originate from the seed treatment with NADPH dsRNA described in Example 33 above.

95 days after seed treatment, leaves of treated and control plants were used as sole food source for *S. littoralis*. Two plants from the NADPH treatment (not tested previously) and a pool of plants from the control were used. Leaves from these plants were placed in three petri dishes contain five larvae each. Body weight of the caterpillars was tracked throughout the experiment. Figure 24A shows average weight of *S. littoralis* larvae after four days of feeding. On the fourth day, the control plants were replaced by plants that were germinated from seeds treated against the tomato

gene AFR8. These seeds were treated with a mixture of two dsRNA sequences (SEQ ID Nos. 25 and 26) at a final concentration of 200 µg/ml (100 µg/ml from each dsRNA) for 24 hours. On the sixth day, another plant was added to each of the two NADPH treated plants. Figure 24B shows average weight of *S. littoralis* larvae seven days after the feeding experiment begun.

EXAMPLE 36: SEED TREATMENT AGAINST NON-*SPODOPTERA LITTORALIS* GENES

The corn plants described in this Example originate from the seed treatments described in Example 28 (DWF1 dsRNA#2, SEQ ID NO 135) and in Example 29 (GUS, SEQ ID NO 20).

69 days after seed treatment, the leaves of the germinated plants were used as sole food source for *S. littoralis*. Two plants from the DWF1 dsRNA#2 treatment and five plants from the GUS treatment were included in the feeding experiment. The leaves of each plant were placed in three petri dishes containing five larvae each, summing to 15 larvae per plant, 30 larvae for the DWF1 dsRNA#2 treatment and 75 larvae for the GUS treatment. Ten days into the experiment, an unusually large number of larvae were found dead in both treatments. Due to the large number of death in both treatment groups, this experimental time point was not further analyzed.

70 days after seed treatment, other plants from the same treatments were used as sole food source for *S. littoralis*. Two plants from the DWF1 dsRNA#2 treatment and 16 plants from the GUS treatment were included in the feeding experiment. The leaves of each plant were placed in three petri dishes containing five larvae each, summing to 15 larvae per plant, 30 larvae for the DWF1 dsRNA #2 treatment and 240 larvae for the GUS treatment. Nine days into the experiment, an unusually large number of larvae were found dead in both treatments. Due to the large number of death in both treatment groups, this experimental time point was not further analyzed.

EXAMPLE 37: SEED TREATMENT AGAINST *SPODOPTERA LITTORALIS* ATPase, EF1α AND NADPH GENES

Corn seeds (var. Vivani) were treated with dsRNA molecules (SEQ ID Nos. 131, 132, 31, 25 and 26) having a nucleotide sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of the *S. littoralis* EF1α gene, ATPase gene or NADPH gene according to the protocol described in Example 1,

without pre-treatment wash. The two EFIα dsRNAs were used separately. A final concentration of 160 µg/ml dsRNA, diluted with 0.1mM EDTA, was used. Treatment was performed by gently shaking the seeds in the solution for 2 hours in a dark growth chamber at 15 °C. After treatment, seeds were washed briefly with DDW, planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with 160 µg/ml dsRNA (SEQ ID No. 124) derived from GFP sequence, or with a similar solution not containing dsRNA (EDTA) were germinated and grown alongside the treated plants as a control.

31 days after seed treatment, the leaves of germinated plants were used as sole food source for *S. littoralis*. The larvae taken for this experiment were up to five hours old (*i.e.* up to five hours after hatching). Six plants from each treatment were included in the feeding experiment. The leaves of each plant were placed in 16 wells of 24-well plate containing one larva each, summing to 16 larvae per plant and 96 larvae per treatment. The surface of the wells were covered with 1 % agarose. Eight days after feeding had begun, 57 larvae were found dead in the ATPase treated group and 42 larvae were found dead in the NADPH treated group. The number of dead larvae in other groups ranged between 13 and 23. The average number of dead larvae in the six ATPase treated plants was significantly higher than the average number of dead larvae in the six GFP control plants, with a p-value of 0.03 (t-test). Similarly, the average number of dead larvae in the NADPH treated plants was higher compared to the average number of dead larvae in the GFP control plants (t-test, p-value=0.07). Figure 25A and B shows the percentage of dead larvae eight days after the beginning of feeding. Figure 25C and D shows the percentage of dead larvae ten days after the beginning of feeding. Figure 25E shows average weight of live *S. littoralis* larvae 11 days after the feeding experiment had begun.

32 days after seed treatment, other plants from the same seed treatment were used as sole food source for *S. littoralis*. The larvae taken for this experiment were up to 24 hours old. Five to seven plants from each treatment were included in the feeding experiment. The leaves of each plant were placed in 16 wells of 24-well plate containing one larva each, summing to 16 larvae per plant, 80 larvae for EDTA, 96 larvae for GFP and ATPase and 112 larvae for NADPH and for the two EF1α treatments. The surface of the wells were covered with 1 % agarose. Body weight of

the larvae was recorded eight and nine days after the start of feeding; some of the larvae, feeding on a subset of the plants, were recorded in the eighth day and the remaining larvae were recorded in the ninth day. Figure 25F shows the average weight of live *S. littoralis* larvae per plant.

EXAMPLE 38: SEED TREATMENTS TARGETING COLEOPTERAN PESTS

This Example illustrates non-limiting embodiments of a method of providing a plant having improved resistance to an coleopteran pest, including the step of growing a plant from a seed that has been contacted with a exogenous non-transcribable dsRNA, wherein said plant exhibits improved resistance to said coleopteran pest, relative to a plant grown from a seed not contacted with said dsRNA. More specifically this Example illustrates a method of providing a maize plant having improved resistance to a corn rootworm (*Diabrotica sp.*), including the step of growing a maize plant from a maize seed that has been contacted with at least one dsRNA designed to silence a target gene endogenous to a corn rootworm, wherein the maize plant germinated from the maize seed exhibits improved resistance to the corn rootworm, relative to a maize plant grown from a maize seed not contacted with the dsRNA.

A 228 bp dsRNA trigger with the sense strand sequence of GGCTGATAGCACTTAAGGAGCTTCCTAATCACGAAAGAATTCTGCAGGATT TAGTTATGGACATACTGAGAGTACTCTCTGCTCCTGACTTAGAAGTCCGCAA GAAGACTTTAAGTCTAGCCCTTGAATTAGTCTCTTCACGGAACATAGAAGA AATGGTATTAGTATTAACAAAGGAAGTGAGTAAAACGGTAGACAGTGAACA TGAGGATACAGGAAAGTACAGGC (MON104454, SEQ ID No.:144) was tested in a corn rootworm infestation assay in maize plants grown from maize seeds contacted prior to germination with the dsRNA trigger. Maize seeds (70 seeds, variety LH244) were placed in a 50-milliliter Falcon tube with 35 milliliters of a solution of the dsRNA trigger in buffer (0.1 millimolar EDTA, diluted from a 0.5 molar pH 8 stock) or 35 milliliters of buffer alone as a null control, and incubated in the dark at 15 °C with gentle agitation for 8 hours. Seeds of a transgenic maize plant that expresses an RNA suppression construct targeting DvSnf7 and that has resistance to corn rootworm were used in a transgenic positive control and were similarly incubated prior to germination in 35 milliliters of buffer alone. DvSnf7 is the Snf7 ortholog from *Diabrotica virgifera virgifera* (Western corn rootworm, WCR) and is a component of the ESCRT-III

complex (endosomal sorting complex required for transport); see Bolognesi *et al.* (2012) PLoS ONE 7(10): e47534, doi:10.1371/journal.pone.0047534. The following day, the seeds were washed 3 times (1 minute/wash with gentle agitation) in enough water to fill the Falcon tube. The washed seeds were planted at a depth of 0.5 inch in 6" closed-bottom polyethylene pots filled with Metromix 200 soil. Greater than 85 % of the seeds germinated in all treatments. At the V2/V3 stage (approximately 2 weeks after planting), 50 neonate *Diabrotica virgifera virgifera* larvae were added to each pot (12–15 replicates performed). As a transgenic positive control, maize plants expressing a recombinant Snf7 transgene and similarly challenged with *Diabrotica virgifera virgifera* larvae were used. After ~4 weeks, the larvae were isolated using a Berlese funnel, counted, and weighed. Larval recovery and weight were calculated. The results are shown in Figure 26. Larval recovery per plant did not differ significantly between larvae fed on maize plants grown from seed treated with the dsRNA trigger at 50 ppm (micrograms/milliliter) and larvae fed on control plants (Figure 26A), but total larval weight (Figure 26B) and average larval weight (Figure 26C) were significantly reduced in the larvae fed on maize plants grown from seed treated with the dsRNA trigger at 50 ppm (micrograms/milliliter), compared to larvae fed on control plants. The plants grown from seed treated with the dsRNA trigger at 500 ppm exhibited stunted plant growth and root growth, which may have affected the observed results; nonetheless, larval recovery per plant was significantly decreased (Figure 26A), and total larval weight (Figure 26B) and average larval weight (Figure 26C) were significantly reduced in the larvae fed on maize plants grown from seed treated with the dsRNA trigger at 500 ppm (micrograms/milliliter), compared to larvae fed on control plants. Quantigene assays did not detect a significant amount of MON104454 RNA in either leaf or root tissue of the maize plants grown from seed treated with the dsRNA trigger at 500 ppm.

A similar experiment was carried out in tomato plants grown from seeds treated prior to germination by incubating overnight in 100 ppm (micrograms/milliliter) of a 279 bp blunt-ended dsRNA trigger with the sense strand sequence of

TACCTGTGGCTCTCACAGGCAGCGAAGATGGTACCGTTAGAGTTTGGCATA
CGAATACACACAGATTAGAGAATTGTTTGAATTATGGGTTCGAGAGAGTGT
GGACCATTTGTTGCTTGAAGGGTTCGAATAATGTTTCTCTGGGGTATGACGA
GGGCAGTATATTAGTGAAAGTTGGAAGAGAAGAACCGGCAGTTAGTATGGA

TGCCAGTGGCGGTAAAATAATTTGGGCAAGGCACTCGGATTACAACAAGCT AATTTGAAGGCGCTGCCAGAAGG (T6593, SEQ ID No.:145) and subjected to a *Leptinotarsa decemlineata* (Colorado potato beetle, CPB) infestation assay. Control plants were treated with either buffer ("formulation") or a dsRNA trigger for green fluorescent protein (GFP). Germination rate was >90 % and no obvious effects on plant growth were observed for the treated plants, compared to the control plants. No significant effect on either the tomato plant defoliation rate (Figure 27A) or on recovery of viable larvae (Figure 27B) or average larval weight (Figure 27C) were observed for the plants treated with either T6593 dsRNA or GFP dsRNA, compared to the control plants. Quantigene assays did not detect a significant amount of T6593 RNA in analyzed tissues (young leaf, old leaf, cotyledon, root) of the tomato plants grown from seed treated with the T6593 dsRNA trigger at 500 ppm.

EXAMPLE 39: SEED TREATMENTS TARGETING ESSENTIAL COLEOPTERAN GENES

This Example illustrates non-limiting embodiments of a method of providing a plant having improved resistance to an coleopteran pest, including the step of growing a plant from a seed that has been contacted with a exogenous non-transcribable dsRNA, wherein said plant exhibits improved resistance to said coleopteran pest, relative to a plant grown from a seed not contacted with said dsRNA. More specifically this Example illustrates a method of providing a maize plant having improved resistance to a corn rootworm (*Diabrotica sp.*), including the step of growing a maize plant from a maize seed that has been contacted with at least one polynucleotide trigger designed to silence a target gene endogenous to a corn rootworm, wherein the maize plant germinated from the maize seed exhibits improved resistance to the corn rootworm, relative to a maize plant grown from a maize seed not contacted with the polynucleotide trigger.

Double-stranded RNA (dsRNA) triggers for the target genes identified in Table 36 are produced. Suitable triggers are of 21-1,000 base pairs in length, in some embodiments, 21-50, 50-100, 100-200, 200-500, 500-700, or 700-1,000 base pairs in length. The triggers provided in Table 36 are between 173–504 base pairs in length, but both shorter or longer triggers are useful in the methods disclosed herein. All of the dsRNA triggers provided in Table 36 were determined to cause significant larval

stunting and mortality at 10 ppm and at 0.1 ppm in a diet bioassay with *Diabrotica virgifera virgifera* (Western corn rootworm, WCR) as described in the working examples in US Patent Application Publication 2009/0307803, which are incorporated by reference herein, where the dsRNA trigger is delivered as an overlayer on the surface of a solid insect diet in a 96-well plate.

Table 36: dsRNA triggers.

Trigger ID	Trigger Length (bp)	Target Gene	SEQ ID NO. OF TARGET GENE
T33514	501	Croquemort	146
T33515	502	predicted: similar to ENSANGP00000020392	147
T33516	500	Cathepsin L-like proteinase	148
T33519	501	Uncharacterized conserved protein	149
T30147	502	Eukaryotic translation initiation factor 3 subunit, putative	150
T30502	501	Cleavage and polyadenylation specificity factor subunit 6	151
T32275	502	Cleavage and polyadenylation specificity factor subunit 6	152
T32328	504	Cleavage and polyadenylation specificity factor subunit 6	153
T30501	501	Lissencephaly-1 homolog	154
T30145	502	Wd-repeat protein	155
T33520	501	Sodium-dependent phosphate transporter	156
T30139	502	T-complex protein 1 subunit delta	157
T30137	502	Putative uncharacterized protein	158
T32250	502	Solute carrier family 2, facilitated glucose transporter member 6	159
T30133	501	26S proteasome non-ATPase regulatory subunit, putative	160
T30471	501	WD repeat-containing protein 75	161
T30132	501	THO complex subunit 5-like protein	162
T30469	502	Another transcription unit protein	163
T30467	502	CG8315	164
T30466	374	Putative uncharacterized protein	165
T33522	500	E3 ubiquitin-protein ligase UBR2	166
T30463	502	TMEM9 domain family member B	167
T30462	501	Eukaryotic translation initiation factor 2 subunit 1	168
T32319	500	Eukaryotic translation initiation factor 2 subunit 1	169
T30126	502	Pre-mRNA-processing factor 6	170
T32320	496	Delta-aminolevulinic acid dehydratase	171
T30456	502	StAR-related lipid transfer protein 7	172
T32316	496	26S proteasome non-ATPase regulatory subunit, putative	173
T30117	502	Putative uncharacterized protein	174
T30112	502	General transcription factor IIF subunit 2	175
T30423	501	Proliferating cell nuclear antigen	176
T32201	502	Proliferating cell nuclear antigen	177
T30420	501	Cactin	178
T30417	501	Vesicle-trafficking protein SEC22b	179

T30106	482	Putative uncharacterized protein	180
T33528	501	Anon-15Ab	181
T30411	502	ATP-dependent RNA helicase SUV3, mitochondrial	182
T33531	501	ATP-binding cassette transporter	183
T30371	490	Nuclear pore complex protein Nup107	184

** (+) significant stunting or mortality compared with water-treated control; (-) no significant stunting or mortality compared with water-treated control; NT = either (1) trigger was not tested, or (2) both of the following occurred: the sample did not provide significant stunting/mortality and the positive control did not provide significant stunting/mortality in that test. Positive control used in this assay was dsRNA with the sequence previously disclosed as SEQ ID NO. 880 in U. S. Patent No. 7,943,819.

Blunt-ended double-stranded RNA (dsRNA) triggers for each of the trigger sequences provided in Table 36 are synthesized and tested in a corn rootworm infestation assay in maize plants grown from maize seeds contacted prior to germination with the individual dsRNA trigger as described above in Example 38 using *Diabrotica virgifera virgifera* larvae, wherein mortality or stunting of the larvae due to contact with or ingestion of the polynucleotide triggers is assayed. Triggers that are found to be effective in causing larval stunting or mortality or both are further tested.

It is anticipated that methods using a combination of certain polynucleotide triggers according to the present embodiments (*e.g.*, the dsRNA triggers described herein) with one or more non-polynucleotide pesticidal agents will result in a synergetic improvement in prevention or control of insect infestations, when compared to the effect obtained with the polynucleotide triggers alone or the non-polynucleotide pesticidal agent alone. In one embodiment, maize plants having improved resistance to corn rootworm infestation are grown from seed having in their genome a recombinant DNA sequence encoding a non-polynucleotide pesticidal agent, wherein the seed are contacted prior to germination with an effective amount of a polynucleotide trigger. Bioassays such as the corn rootworm infestation assay described herein are useful for defining dose-responses for larval mortality or growth inhibition using combinations of the polynucleotide triggers of the present embodiments and one or more non-polynucleotide pesticidal agents (*e.g.*, a patatin, a plant lectin, a phytoecdysteroid, a *Bacillus thuringiensis* insecticidal protein, a *Xenorhabdus* insecticidal protein, a Photorhabdus insecticidal protein, a *Bacillus laterosporous* insecticidal protein, and a *Bacillus sphearicus* insecticidal protein). One of skill in the art can test combinations of

polynucleotide triggers and non-polynucleotide pesticidal agents in routine bioassays to identify combinations of bioactives that are synergistic and desirable for use in protecting plants from insect infestations.

EXAMPLE 40: Seed Treatment with dsRNA polynucleotides targeting Tomato Golden Mottle Virus (ToGMoV)

The following Example illustrates a method of providing a plant with improved resistance to a viral pathogen, including the step of growing a plant from a seed imbibed with an exogenous non-transcribable dsRNA polynucleotide comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a viral pathogen gene. In this experiment, each treatment was applied to forty seeds.

dsRNA polynucleotide triggers comprising a sequence homologous to either a 5' or 3' sequence of the AC1 (replicase-associated protein) gene of Tomato golden mottle virus (ToGMoV) as described in Table 37 were diluted to 100µg/ml in 0.1mM EDTA pH 8.0, in a final volume of 0.6 ml. For each dsRNA polynucleotide trigger, forty tomato seeds (Solanum lycopersicum var. HP375) were placed in 2ml Eppendorf tubes and allowed to incubate in the dsRNA polynucleotide solution. An additional set of forty tomato seeds was incubated in a solution containing a dsRNA polynucleotide targeting the E.coli β-glucuronidase (GUS) gene sequence as a negative control. Incubation was performed in the dark at 15 °C with gentle agitation for 24 hours.

Table 37: Sequences of ToGMoV used in seed treatment experiments.

SEQ ID NO	Target Gene	Species
185	ToGMoV AC1/Rep1 (5')	Tomato Golden Mottle Virus
186	ToGMoV AC1/Rep1 (3')	Tomato Golden Mottle Virus

The following day the seeds were washed three times (1 minute/wash with gentle agitation) in enough water to fill the Eppendorf tube. The washed seeds were planted at a depth of 0.5 inch in 6" polyethylene pots filled with Metromix 200 soil and incubated under standard growth chamber conditions: 25 °C day, 22 °C night; 12 hour day; light intensity ~15000 lux. Approximately 2 weeks after planting, the emerging cotyledons were inoculated with ToGMoV via agroinoculation.

Agroinfection of tomato plants was performed essentially as described in Grimsley N, Hohn T, Davies JW, Hohn B (1987) *Agrobacterium*-mediated delivery of

infectious Maize streak virus into maize plants. Nature 325:177-179. Briefly, agroinfectious clones of the DNA-A and DNA-B components of ToGMoV were grown in Luria-Broth media supplemented with the selective antibiotic spectinomycin (resistance to which was conferred by the binary vector). Cultures were grown to an OD600 of 0.4 - 0.5 at 28°C in a shaking incubator. Agroinfection was conducted 2 times on each plant. Agroinfected plants were maintained under growth chamber conditions and monitored for signs of viral infection alongside uninoculated plants growing in the same growth chamber.

Fourteen days after virus inoculation, plant leaves, approximately 226 mm^2, equivalent to 2 standard leaf punches, were harvested and prepared for Quantigene analysis. As can be seen in Figure 28A, plants treated with the Tomato golden mottle virus (ToGMoV) 5' AC1 (SEQ ID NO. 185) dsRNA triggers accumulated virus (as measured by RNA levels of the AC1 gene) at a level not significantly different from the control group. Conversely, plants treated with the Tomato golden mottle virus (ToGMoV) 3' AC1 (SEQ ID NO 186) dsRNA triggers showed a statistically significant decrease in accumulation of ToGMoV virus levels relative to the GUS-treated control group, significant at an alpha of 0.05. See Figure 28B.

EXAMPLE 41: SEED TREATMENT WITH DSRNA POLYNUCLEOTIDES TARGETING CUCUMBER MOSAIC VIRUS (CMV)

The following Example illustrates a method of providing a plant with improved resistance to a viral pathogen, including the step of growing a plant from a seed imbibed with an exogenous non-transcribable dsRNA polynucleotide comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a Cucumber Mosaic Virus (CMV) gene. In this experiment, each treatment was applied to forty seeds.

dsRNA polynucleotide triggers comprising a sequence homologous to either a 5' or 3' sequence of the 3b Nucleocapsid (NC) gene of CMV as described in Table 38 were diluted to 100µg/ml in 0.1mM EDTA pH 8.0, in a final volume of 0.6 ml. For each dsRNA polynucleotide trigger, forty tomato seeds (Solanum lycopersicum var. HP375) were placed in 2ml Eppendorf tubes and allowed to incubate in the dsRNA polynucleotide solution. An additional set of forty tomato seeds were incubated in the presence of a dsRNA polynucleotide targeting the E.coli β-glucuronidase (GUS) gene

sequence as a negative control. Incubation was performed in the dark at 15 °C with gentle agitation for 24 hours.

Table 38: Sequences of CMV used in seed treatment experiments.

SEQ ID NO	Target Gene	Species
187	3b-Nucleocapsid (NC)	Cucumber Mosaic Virus
188	3b-Nucleocapsid (NC)	Cucumber Mosaic Virus

The following day, the seeds were washed three times (1 minute/wash with gentle agitation) in enough water to fill the Eppendorf tube. The washed seeds were then planted at a depth of 0.5 inch in 6" polyethylene pots filled with Metromix 200 soil and incubated under standard greenhouse conditions: 28 °C day, 21 °C night; 16 hour day cycle. Approximately 2 weeks after planting, the emerging cotyledons were inoculated with CMV via rub infection using a standard protocol known in the literature (Roger Hull: Mechanical Inoculation of Plant Viruses; Current Protocols in Microbiology, 2005, 13:16B6.1-16B6.4). Briefly, one gram of symptomatic leaf tissue from known CMV-infected plants was ground in a sterile mortar and pestle in 25 ml of ice-cold 0.1M phosphate buffer (pH 7.8). This inoculation buffer was gently rubbed onto cotyledons of plants dusted with carborundum powder. After inoculation, plants remained under greenhouse conditions and were monitored for signs of infection.

Fourteen days after virus inoculation, plant leaves, approximately 226 mm^2, equivalent to 2 standard leaf punches, were harvested and prepared for Quantigene analysis. As can be seen in Figure 29A, plants treated with the Cucumber Mosaic Virus (CMV) 5' 3b dsRNA polynucleotide trigger (SEQ ID NO. 187) accumulated virus (as measured by RNA levels of the 3b-NC gene) at a level not significantly different from the control group, albeit with a slight downward trend. A similar result was observed in plants treated with the Cucumber Mosaic Virus (CMV) 5' 3b dsRNA polynucleotide trigger (SEQ ID NO. 188). See Figure 29B.

EXAMPLE 42: Seed Treatment with dsRNA polynucleotides targeting Tomato Spotted Wilt Virus (TSWV)

The following Example illustrates a method of providing a plant with improved resistance to a viral pathogen, including the step of growing a plant from a seed imbibed with an exogenous non-transcribable dsRNA polynucleotide comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a Tomato Spotted Wilt Virus (TSWV) gene.

A dsRNA polynucleotide trigger comprising a sequence homologous to a 3' sequence of the Nucleocapsid (N) gene of TSWV (SEQ ID NO. 190) was diluted to 100µg/ml in 0.1mM EDTA pH 8.0, in a final volume of 0.6 ml. Forty tomato seeds (Solanum lycopersicum var. HP375) were placed in a 2mL Eppendorf tube and allowed to incubate in the dsRNA polynucleotide solution. An additional set of forty tomato seeds was incubated in the presence of a dsRNA polynucleotide targeting the *E. coli* β-glucuronidase (GUS) gene sequence as a negative control. Incubation was performed in the dark at 15 °C with gentle agitation for 24 hours. The following day, the seeds were washed three times (1 minute/wash with gentle agitation) in enough water to fill the Eppendorf tube. The washed seeds were planted at a depth of 0.5 inch in 6" polyethylene pots filled with Metromix 200 soil and incubated under standard greenhouse conditions: 28 °C day, 21 °C night; 16 hour day cycle.

Approximately 2 weeks after planting, the emerging cotyledons were inoculated with TSWV via rub infection using a standard protocol described in the literature (Roger Hull: Mechanical Inoculation of Plant Viruses; Current Protocols in Microbiology, 2005, 13:16B6.1-16B6.4). Briefly, one gram of symptomatic leaf tissue from known TSWV-infected plants was ground in a sterile mortar and pestle in 25 ml of ice-cold 0.1M phosphate buffer (pH 7.8). This inoculation buffer was gently rubbed onto cotyledons of plants dusted with carborundum powder. After inoculation, the plants remained under greenhouse conditions and were monitored for signs of infection.

Fourteen days after virus inoculation, plant leaves, approximately 226 mm^2, equivalent to 2 standard leaf punches, were harvested and prepared for Quantigene analysis. As can be seen in Figure 30, plants treated with the Tomato Spotted Wilt Virus (TSWV) 3' N dsRNA polynucleotide (SEQ ID NO. 190) accumulated virus (as measured by RNA levels of the 3b-NC gene) at a level not significantly different from the control group.

EXAMPLE 43: Seed Treatment with dsRNA polynucleotides targeting Tomato Spotted Wilt Virus (TSWV)

The following Example illustrates a method of providing a plant with improved resistance to a viral pathogen, including the step of growing a plant from a seed imbibed with an exogenous non-transcribable dsRNA polynucleotide comprising a sequence that

is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a Tomato Spotted Wilt Virus (TSWV) gene.

A dsRNA polynucleotide trigger comprising a sequence homologous to a 5' sequence of the Nucleocapsid (N) gene of TSWV (SEQ ID NO. 189) is diluted to 100µg/ml in 0.1mM EDTA pH 8.0, in a final volume of 0.6 ml. Forty tomato seeds (Solanum lycopersicum var. HP375) are placed in a 2mL Eppendorf tube containing the dsRNA polynucleotide solution. An additional set of forty tomato seeds is incubated in the presence of a dsRNA polynucleotide targeting the *E. coli* β-glucuronidase (GUS, SEQ ID No. 20) gene sequence as a negative control. Incubation is performed in the dark at 15 °C with gentle agitation for 24 hours. The next day, the seeds are washed three times (1 minute/wash with gentle agitation) in enough water to fill the Eppendorf tube. The washed seeds are planted at a depth of 0.5 inch in 6" polyethylene pots filled with Metromix 200 soil and incubated under standard greenhouse conditions: 28 °C day, 21 °C night; 16 hour day cycle.

Approximately 2 weeks after planting, the emerging cotyledons are inoculated with TSWV via rub infection using a standard protocol described in the literature (Roger Hull: Mechanical Inoculation of Plant Viruses; Current Protocols in Microbiology, 2005, 13:16B6.1-16B6.4). Briefly, one gram of symptomatic leaf tissue from known TSWV-infected plants is ground in a sterile mortar and pestle in 25 ml of ice-cold 0.1M phosphate buffer (pH 7.8). This inoculation buffer is gently rubbed onto cotyledons of plants dusted with carborundum powder. After inoculation, the plants remain under greenhouse conditions and are monitored for signs of infection.

Fourteen days after virus inoculation, plant leaves, approximately 226 mm^2, equivalent to 2 standard leaf punches, are harvested and prepared for Quantigene analysis. Plants treated with TSWV 5' N dsRNA polynucleotide (SEQ ID NO. 189) are expected to accumulate virus (as measured by RNA levels of the 3b-NC gene) at a level lower than that of the control group.

EXAMPLE 44: GENERATION OF dsRNA MOLECULES FOR SILENCING EF1α GENE OF *S. LITTORALIS*

dsRNA polynucleotide triggers derived from the *S. littoralis* EF1α gene were analyzed against the corn genome (*Zea mays* - taxid:4577, Figure 31) using the same

BLAST parameters as described in Example 7. dsRNAs targeting *S. littoralis* EF1α and having homology to a corn gene are selected.

EXAMPLE 45: ALTERED CORN EF1α EXPRESSION FOLLOWING SEED TREATMENT WITH *S. LITTORALIS* dsRNA

Corn seeds (var. Vivani) were treated according to the protocol described in Example 1 with exogenous non-transcribable dsRNA trigger molecules (SEQ ID Nos. 131 and 132) derived from the *S. littoralis* EF1α gene sequence, with no pre- and post-treatment washes. A mixture of 25 µg/ml from each dsRNA was used. The dsRNA was diluted either with 0.1mM EDTA, or mixed with 40 µg/ml of PEG-modified carbon nanotubes. Treatment was performed by gently shaking the seeds in the solution for 4 hours in a dark growth chamber at 15 °C. After treatment, seeds were planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds treated with 50 µg/ml dsRNA derived from GFP sequence were germinated and grown alongside the EF1α dsRNA treated plants as a control.

Total RNA was extracted from leaves of germinated seeds, 20 days post treatment. cDNA was prepared using oligo-dT primers and the expression level of corn EF1α mRNA was determined in treated and control plants by real-time PCR with SYBR Green (Quanta BioSciences). The house-keeping genes GPM120 and NFE101 were used as endogenous control genes to normalize for input amounts. Primers were designed so as to not amplify the dsRNA trigger and thus detect only corn-derived EF1α mRNA.

Table 39: Primers Used for RT-PCR Analysis for Expression Level of EF1α.

Target Gene	Forward / Reverse	Primer Sequence	SEQ ID No.
EF1α	Forward	GCAACCACTCCCAAATACTC	191
EF1α	Reverse	CAGGGTTGTACCCAACTTTC	192
GPM120	Forward	AGGCTTTCGCTGCGTGTT	193
GPM120	Reverse	TGGCCCATCCAAACTCAGA	194
NFE101	Forward	GCTCAAGTTCTTCGGATGAC	195
NFE101	Reverse	ACTTCTTCCAGCAGACTAGC	196

This analysis showed a significant (Wilcoxon rank-sum test, p-value<0.05) up-regulation of corn EF1α mRNA. The median expression level of EF1α in plants treated with *S. littoralis* dsRNA was 2.12 and 1.68-fold higher than in control plants treated

with GFP dsRNA, with or without PEG-modified carbon nanotubes, respectively. See Figure 32A and B.

The plants treated with dsRNA/CNTP were analyzed again for EF1α expression level 48 days post treatment. This analysis showed an up-regulation of corn EF1α mRNA. The median expression level of EF1α in plants treated with *S. littoralis* dsRNA was 1.66-fold higher than in control plants treated with GFP dsRNA. See Figure 32C.

EXAMPLE 46: CORN EF1α, BETA ACTIN, ATPase AND NADPH EXPRESSION FOLLOWING SEED TREATMENT WITH *S. LITTORALIS* dsRNAs

Corn seeds were treated with exogenous non-transcribable dsRNA trigger molecules derived from *S. littoralis* genes according to the protocol described in Example 1. Seeds were washed with double distilled water (DDW) prior to treatment for four hours. Next, seeds were dried at 30°C overnight. Following the drying step, a final concentration of 132 µg/ml dsRNA for EF1α (a mixture of dsRNA#1 (SEQ ID No. 131) and dsRNA#2 (SEQ ID No. 132) at about equal concentrations), 53 µg/ml dsRNA for ATPase (SEQ ID No. 31), 76 µg/ml dsRNA for Beta actin (SEQ ID No. 133) and 154 µg/ml dsRNA for NADPH (SEQ ID Nos. 25 and 26), all diluted with 0.1mM EDTA, was used. Treatment was performed by gently shaking the seeds in the solution for 26 hours in a dark growth chamber at 15 °C. After treatment seeds were washed briefly with DDW and placed on wet paper for germination without a drying step. Seven days after germination the seedlings were planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with a similar solution (EDTA) not containing dsRNA were germinated and grown alongside the treated plants as a control.

Nine weeks after treatment, total RNA was extracted from leaves of germinated seeds. cDNA was prepared using oligo-dT and random primers and the expression level of corn EF1α, Beta actin, ATPase and NADPH was determined in treated and control plants. The numbers of plants analyzed were 3, 4, 3, 3 and 7 for EF1α, Beta actin, ATPase, NADPH and control respectively. The house-keeping gene FKBP was used as endogenous control gene to normalize for input amounts. Primers were designed so as to not amplify the dsRNA triggers and thus detect only corn-derived mRNAs.

Table 40: Primers Used for RT-PCR Analysis for Expression Level of EF1α, BETA ACTIN, ATPase AND NADPH.

Target Gene	Forward / Reverse	Primer Sequence	SEQ ID No.
EF1α	Forward	GCAACCACTCCCAAATACTC	197
EF1α	Reverse	CAGGGTTGTACCCAACTTTC	198
Beta actin	Forward	TCTGGCATCACACCTTCTAC	199
Beta actin	Reverse	TTCTCACGGTTAGCCTTTGG	200
ATPase	Forward 1	TGTCCTGCCATCTCTATCTC	201
ATPase	Reverse 1	ACATCCGAATGGTCTCTACG	202
ATPase	Forward 2	CACAACCGTGCAGTTTACAG	203
ATPase	Reverse 2	AAATGCGCCCAAGCATATCG	204
NADPH	Forward	CAGAGGACGAGGAATATGAG	205
NADPH	Reverse	CTAGCAGCATTGTCAGTAGG	206
FKBP	Forward	CGGTGTTCGACAGCAGCTAC	207
FKBP	Reverse	CTTCGCCGCCAACAATACCC	208

Due to the small group size, this analysis showed no significant difference in the expression of these genes (Wilcoxon rank-sum test, p-value>0.05), but the expression of EF1α showed an up-regulation trend. The median expression level of EF1α in plants treated with *S. littoralis* EF1α dsRNA was 2.28-fold higher than in control plants (Figure 33).

EXAMPLE 47: CORN EF1α AND ATPase EXPRESSION FOLLOWING SEED TREATMENT WITH *S. LITTORALIS* dsRNAs

Corn seeds were treated with exogenous non-transcribable dsRNA trigger molecules derived from the *S. littoralis* EF1α and ATPase genes according to the protocol described in Example 1. Seeds were washed with double distilled water (DDW) prior to treatment for four hours and dried at 30 °C overnight. Two EF1α dsRNA sequences (dsRNA#1 (SEQ ID No. 131) and #2 (SEQ ID No. 132)) were used separately in two different seed treatments; each at a final concentration of 67 µg/ml dsRNA. ATPase dsRNA (SEQ ID No. 31) was used at a final concentration of 145 µg/ml. All dsRNAs were diluted with 0.1mM EDTA. Treatment was performed by gently shaking the seeds in the solution for 24 hours in a dark growth chamber at 15 °C. After treatment, seeds were dried at 30 °C overnight and then planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with 67 µg/ml dsRNA (SEQ ID No. 20) derived from GUS sequence were germinated and grown alongside the treated plants as a control.

Seven days after treatment, total RNA was extracted from leaves of germinated seeds and the expression level of corn EF1α and ATPase was determined in treated and control plants as described in Example 2 above. The house-keeping gene GPM120 was

used as endogenous control gene to normalize for input amounts. Primers were designed so as to not amplify the dsRNA triggers and thus detect only corn-derived mRNA.

Table 41: Primers Used for RT-PCR Analysis for Expression Level of EF1α and ATPase.

Target Gene	Forward / Reverse	Primer Sequence	SEQ ID No.
EF1α	Forward	GCAACCACTCCCAAATACTC	191
EF1α	Reverse	CAGGGTTGTACCCAACTTTC	192
ATPase	Forward	GCGCAAGTTTTTCGTAGATGAC	209
ATPase	Reverse	ACCATAGTCCACAGATGACAC	210
GMP120	Forward	GCTGCGTGTTGTGCGTTCTG	211
GMP120	Reverse	TCGTCGCGTGCTGTCTGTTC	212

No significant difference in the expression of these genes was observed.

EXAMPLE 48: CORN NADPH EXPRESSION FOLLOWING SEED TREATMENT WITH *S. LITTORALIS* dsRNA

Corn seeds (var. 01DKD2) were treated according to the protocol described in Example 45 with exogenous non-transcribable dsRNA trigger molecules (SEQ ID No. 26) derived from the *S. littoralis* NADPH gene. A final concentration of 80 µg/ml dsRNA diluted with 0.1mM EDTA was used. Treatment was performed by gently shaking the seeds in the solution for 3.5 hours in a dark growth chamber at 15 °C. After treatment, seeds were planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with GFP dsRNA, or with a similar solution not containing dsRNA, were germinated and grown alongside the treated plants as a control.

20 days after treatment, total RNA was extracted from leaves of germinated seeds and the expression level of corn NADPH was determined in treated and control plants as described in Example 45 above. The house-keeping genes GPM120 and NFE101 were used as endogenous control genes to normalize for input amounts. Primers were designed so as to not amplify the dsRNA trigger and thus detect only corn-derived mRNA.

Table 42: Primers Used for RT-PCR Analysis for Expression Level of NADPH.

Target Gene	Forward / Reverse	Primer Sequence	SEQ ID No.
NADPH	Forward	CAGAGGACGAGGAATATGAG	205
NADPH	Reverse	CTAGCAGCATTGTCAGTAGG	206
GPM120	Forward	AGGCTTTCGCTGCGTGTT	213
GMP120	Reverse	TGGCCCATCCAAACTCAGA	214
NFE101	Forward	GCTCAAGTTCTTCGGATGAC	215
NFE101	Reverse	ACTTCTTCCAGCAGACTAGC	216

No significant difference in the expression of NADPH was observed.

EXAMPLE 49: CORN EF1α EXPRESSION FOLLOWING SEED TREATMENT WITH *S. LITTORALIS* dsRNAs

Corn seeds (var. 01DKD2) were treated according to the protocol described in Example 45 with exogenous non-transcribable dsRNA trigger molecules (SEQ ID No. 131) derived from the *S. littoralis* EF1α gene. A mixture of 25 µg/ml from each of the two dsRNAs was used. The dsRNA was diluted either with 0.1mM EDTA alone, or additionally mixed with 40 µg/ml of PEG-modified carbon nanotubes (CNTP). Treatment was performed by gently shaking the seeds in the solution for 4 hours in a dark growth chamber at 15 °C. After treatment, seeds were planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with 50 µg/ml dsRNA derived from GUS sequence, with or without 40 µg/ml of PEG-modified carbon nanotubes, were germinated and grown alongside the treated plants as a control.

20 days after treatment, total RNA was extracted from leaves of germinated seeds and the expression level of corn EF1α was determined in treated and control plants as described in Example 45. The house-keeping genes GPM120, NFE101 and Expressed were used as endogenous control genes to normalize for input amounts. Primers were designed so as to not amplify the dsRNA trigger and thus detect only corn-derived mRNA.

Table 43: Primers Used for RT-PCR Analysis for Expression Level of EF1α.

Target Gene	Forward / Reverse	Primer Sequence	SEQ ID No.
EF1α	Forward	GCAACCACTCCCAAATACTC	198
EF1α	Reverse	CAGGGTTGTACCCAACTTTC	199
GPM120	Forward	AGGCTTTCGCTGCGTGTT	193

GMP120	Reverse	TGGCCCATCCAAACTCAGA	194
NFE101	Forward	GCTCAAGTTCTTCGGATGAC	215
Expressed	Forward	GGATGCTACTCGCCAGACA	217
Expressed	Reverse	GTGGTCAGCCTGCTTCAAC	218

No significant difference in the expression of EF1α was observed.

EXAMPLE 50: CORN EF1α EXPRESSION FOLLOWING SEED TREATMENT WITH *S. LITTORALIS* dsRNAs

Corn seeds (var. Vivani) were treated according to the protocol described in Example 1, with exogenous non-transcribable dsRNA trigger molecules (SEQ ID Nos. 131 and 132) derived from the *S. littoralis* EF1α gene, without pre-treatment wash. A mixture of 25 µg/ml from each of the two dsRNAs was used. The dsRNA was diluted either with 0.1mM EDTA alone, or additionally mixed with 40 µg/ml of PEG-modified carbon nanotubes (CNTP). Treatment was performed by gently shaking the seeds in the solution for 4 hours in a dark growth chamber at 15 °C. After treatment seeds were washed briefly with DDW and directly germinated in soil without a drying step. Plants were grown at about 25 °C with 16 hours photoperiod and watered with tap water as necessary. Seeds that were treated with a similar solution not containing dsRNA, or with 50 µg/ml dsRNA derived from GFP sequence, with or without 40 µg/ml of PEG-modified carbon nanotubes, were germinated and grown alongside the treated plants as a control.

14 days after treatment, total RNA was extracted from leaves of germinated seeds and the expression level of corn EF1α was determined in treated and control plants as described in Example 45 above. The house-keeping genes NFE101 and Expressed were used as endogenous control genes to normalize for input amounts. Primers were designed so as to not amplify the dsRNA trigger and thus detect only corn-derived mRNA.

Table 44: Primers Used for RT-PCR Analysis for Expression Level of EF1α.

Target Gene	Forward / Reverse	Primer Sequence	SEQ ID No.
EF1α	Forward	GCAACCACTCCCAAATACTC	197
EF1α	Reverse	CAGGGTTGTACCCAACTTTC	198
NFE101	Forward	GCTCAAGTTCTTCGGATGAC	215
Expressed	Forward	GGATGCTACTCGCCAGACA	217
Expressed	Reverse	GTGGTCAGCCTGCTTCAAC	218

No significant difference in the expression of EF1α was observed.

EXAMPLE 51: CORN EF1α EXPRESSION FOLLOWING SEED TREATMENT WITH *S. LITTORALIS* dsRNAs

Corn seeds (var. Vivani) were treated according to the protocol described in Example 1, with exogenous non-transcribable dsRNA trigger molecules (SEQ ID Nos. 131 and 132) derived from the *S. littoralis* EF1α gene, without pre-treatment wash. The two dsRNAs were used separately, each at a final concentration of 160 µg/ml. The dsRNAs were diluted either with IDT buffer alone (30 mM HEPES, pH 7.5, 100 mM Potassium Acetate), or additionally mixed with 40 µg/ml of PEG-modified carbon nanotubes (CNTP). Treatment was performed by gently shaking the seeds in the solution for 7 hours in a dark growth chamber at 25 °C. After treatment, seeds were washed briefly with DDW and directly germinated in soil without a drying step. Plants were grown at about 25 °C with 16 hours photoperiod and watered with tap water as necessary. Seeds that were treated with a similar solution not containing dsRNA, or with 160 µg/ml dsRNA derived from GFP sequence, with or without 40 µg/ml of PEG-modified carbon nanotubes, were germinated and grown alongside the treated plants as a control.

Six days after treatment, total RNA was extracted from leaves of germinated seeds and the expression level of corn EF1α was determined in treated and control plants as described in Example 45 above. The house-keeping genes GPM120 and Expressed were used as endogenous control genes to normalize for input amounts. Primers were designed so as to not amplify the dsRNA trigger and thus detect only corn-derived mRNA.

Table 45: Primers Used for RT-PCR Analysis for Expression Level of EF1α.

Target Gene	Forward / Reverse	Primer Sequence	SEQ ID No.
EF1α	Forward 1	GCAACCACTCCCAAATACTC	197
EF1α	Reverse 1	CAGGGTTGTACCCAACTTTC	198
EF1α	Forward 2	CCCAGGTCATCATCATGAAC	191
EF1α	Reverse 2	GAGCTCAGCAAACTTGACAG	192
GPM120	Forward	GCTGCGTGTTGTGCGTTCTG	211
Expressed	Forward	GGATGCTACTCGCCAGACA	217
Expressed	Reverse	GTGGTCAGCCTGCTTCAAC	218

The results of this analysis are shown in Figure 34. A significant up-regulation of corn EF1α mRNA was observed in plants following treatment with EF1α dsRNA #1 (t-test, p-value=0.004). The average expression level of EF1α in plants treated with this dsRNA was 1.8 higher than in control plants treated with GUS dsRNA. When grouping all the plants treated with EF1α dsRNAs (both dsRNA #1 and #2, with and without CNTP) and comparing to all plants treated with GUS dsRNA (with and without CNTP) a significant up-regulation of corn EF1α mRNA was observed. See Figure 34B. The average expression level of EF1α in plants treated with EF1α dsRNAs was 1.73 higher than in control plants treated with GUS dsRNA (t-test, p-value=0.005).

EXAMPLE 52: CORN ATPase AND NADPH EXPRESSION FOLLOWING SEED TREATMENT WITH *S. LITTORALIS* dsRNAs

Corn seeds (var. Vivani) were treated according to the protocol described in Example 1 with exogenous non-transcribable dsRNA trigger molecules (SEQ ID Nos. 31 and 26) derived from the *S. littoralis* ATPase and NADPH genes, without pre-treatment wash. A final concentration of 160 µg/ml dsRNA, diluted with 0.1mM EDTA, was used. Treatment was performed by gently shaking the seeds in the solution for 2 hours in a dark growth chamber at 15°C. After treatment, seeds were washed briefly with DDW, planted in soil and grown at about 25 °C with 16 hours photoperiod. The plants were watered with tap water as necessary. Seeds that were treated with 160 µg/ml dsRNA (SEQ ID No. 124) derived from GFP sequence, or with a similar solution not containing dsRNA (EDTA) were germinated and grown alongside the treated plants as a control.

27 days after treatment, total RNA was extracted from leaves of germinated seeds and the expression levels of corn ATPase and NADPH were determined in treated and control plants as described in Example 45 above. The house-keeping gene, Expressed, was used as endogenous control genes to normalize for input amounts. Primers were designed so as to not amplify the dsRNA trigger and thus detect only corn-derived mRNA.

Table 46: Primers Used for RT-PCR Analysis for Expression Level of ATPase and NADPH.

Target Gene	Forward / Reverse	Primer Sequence	SEQ ID No.
ATPase	Forward	GCGCAAGTTTTTCGTAGATGAC	219
ATPase	Reverse	ACCATAGTCCACAGATGACAC	220
NADPH	Forward	CAGAGGACGAGGAATATGAG	205
NADPH	Reverse	CTAGCAGCATTGTCAGTAGG	206
Expressed	Forward	GGATGCTACTCGCCAGACA	217
Expressed	Reverse	GTGGTCAGCCTGCTTCAAC	218

The results of this analysis are shown in Figure 35A (ATPase expression) and 35B and C (NADPH expression). No difference in corn ATPase expression levels was detected following treatment with *S. littoralis* ATPase dsRNA. However, a trend of down-regulation of corn NADPH mRNA was observed in plants following treatment with NADPH dsRNA triggers. The average expression level of NADPH in plants treated with this dsRNA trigger was 1.37 fold lower than in control plants treated with GFP dsRNA trigger (t-test, p-value=0.11). When grouping all control plants (those treated with GFP dsRNA and those treated with EDTA) and comparing to plants treated with NADPH dsRNA trigger, a significant down-regulation of corn NADPH mRNA was observed, with an average decrease of 1.67 fold in NADPH expression levels following treatment with NADPH dsRNAs (t-test, p-value=0.02).

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. A method of providing a plant having improved resistance to an insect pest, comprising: growing a plant from a seed, wherein the seed has been contacted with an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a gene of the insect pest or to the sequence of an RNA transcribed from said gene, wherein the plant exhibits improved resistance to the insect pest relative to a control plant, wherein the control plant is grown from a seed not contacted with the exogenous dsRNA molecule.
2. A method of providing a plant having improved resistance to an insect pest, comprising growing the plant from a seed, wherein the seed comprises an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a gene of the insect pest or to the sequence of an RNA transcribed from said gene, wherein the seed is devoid of a heterologous promoter for driving expression of the exogenous dsRNA molecule, and wherein the plant exhibits improved resistance to the insect pest relative to a control plant, wherein the control plant is grown from a seed not comprising the exogenous dsRNA molecule.
3. The method of Claims 1 or 2, wherein the plant is maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton or rapeseed.
4. The method of Claims 1, 2 or 3, wherein the insect pest is *Spodoptera littoralis*, *Diabrotica virgifera virgifera* or *Leptinotarsa decemlineata*.
5. The method of any one of Claims 1-4, wherein the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin.
6. The method of any one of Claims 1-5, wherein the exogenous dsRNA molecule comprises a nucleic acid sequence that is at least 80% identical to an endogenous plant gene over at least 25 consecutive bp.
7. The method of any one of Claims 1-6, wherein the seed is further treated with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent.
8. A plant provided by the method of any one of Claims 1-7.

9. The plant of Claim 8, wherein the plant does not comprise detectable levels of the exogenous dsRNA molecule.
10. A method for generating a plant having insect resistance, the method comprising: a) introducing a non-transcribable trigger molecule comprising at least one polynucleotide strand comprising at least one segment of 18 or more contiguous nucleotides of an insect pest gene in either anti-sense or sense orientation into an ungerminated seed and b) germinating the seed to generate a plant exhibiting insect resistance after emerging from the seed.
11. The method of Claim 10, wherein the plant does not comprise detectable levels of the trigger molecule after emerging from the seed.
12. The method of Claim 10 or 11, wherein the non-transcribable trigger molecule is dsRNA.
13. The method of any one of Claims 10-12, wherein the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin.
14. The method of any one of Claims 10-13, wherein the plant is resistant to *Spodoptera littoralis*, *Diabrotica virgifera virgifera* or *Leptinotarsa decemlineata* infestation.
15. The method of any one of Claims 10-14, wherein the non-transcribable trigger molecule is at least 80% identical to an endogenous plant gene over at least 25 consecutive bp.
16. The method of any one of Claims 10-15, wherein the method further comprises priming the seed prior to introducing the non-transcribable trigger molecule.
17. The method of Claim 16, wherein the priming is effected by:
    (i) washing the seed prior to introducing the non-transcribable trigger molecule; and
    (ii) drying the seed following step (i).
18. A method of treating a seed to improve insect resistance of a plant grown from the seed, the method comprising: introducing an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of an insect pest gene or to the sequence of an RNA transcribed from the insect pest gene into the seed, wherein the plant

grown from the seed exhibits improved insect resistance relative to a control plant.

19. The method of Claim 18, wherein the method further comprises priming the seed prior to introducing the exogenous dsRNA molecule.
20. The method of Claim 19, wherein the priming is effected by:
    (i) washing the seed prior to introducing the exogenous dsRNA molecule; and
    (ii) drying the seed following step (i).
21. The method of Claim 20, wherein the washing is effected in the presence of double deionized water.
22. The method of Claim 20, wherein the washing is effected for 2-6 hours.
23. The method of Claim 20, wherein the washing is effected at 4-28 ºC.
24. The method of Claim 20, wherein the drying is effected at 25-30 ºC for 10-16 hours.
25. The method of Claim 18, wherein the introducing is effected in a presence of the exogenous dsRNA molecule at a concentration of 20-150 µg/ml.
26. The method of Claim 18, wherein the exogenous dsRNA molecule is introduced to the seed in a solution comprising 0.1 mM EDTA.
27. The method of Claim 18, wherein the introducing of the exogenous dsRNA molecule is effected in a presence of a physical agent.
28. The method of Claim 27, wherein the physical agent is PEG-modified carbon nanotubes.
29. A seed provided by the method of any one of Claims 18-28.
30. A seed containing device comprising a plurality of the seeds of Claim 29.
31. A sown field comprising a plurality of the seeds of Claim 29.
32. A seed comprising an exogenous dsRNA molecule comprising a sequence that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of an insect pest gene or to the sequence of an RNA transcribed from the insect pest gene, wherein the seed is devoid of a heterologous promoter for driving expression of the dsRNA molecule and wherein the exogenous dsRNA molecule does not integrate into the genome of the seed.
33. The seed of Claim 32, wherein the exogenous dsRNA molecule is present in an endosperm of the seed.

34. The seed of Claim 32, wherein the exogenous dsRNA molecule is present in an embryo of the seed.
35. The seed of Claim 32, wherein the exogenous dsRNA molecule is present at a similar concentration in an embryo and an endosperm of the seed.
36. The seed of Claim 32, wherein the exogenous dsRNA molecule is present at a higher concentration in an endosperm than an embryo of the seed.
37. The seed of Claim 32, wherein the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin.
38. The seed of Claim 37, wherein the insect pest is *Spodoptera littoralis*, *Diabrotica virgifera virgifera* or *Leptinotarsa decemlineata*.
39. The seed of Claim 32, wherein the exogenous dsRNA molecule comprises a nucleic acid sequence that is at least 80% identical over at least 25 consecutive bp to an endogenous gene of the seed.
40. The seed of Claim 32, wherein the seed is treated with an agent selected from the group consisting of a pesticide, a fungicide, an insecticide, a fertilizer, a coating agent and a coloring agent.
41. The seed of Claim 32, wherein the seed is a primed seed.
42. A seed containing device comprising a plurality of the seeds of any of Claim 32-41.
43. A sown field comprising a plurality of the seeds of any of Claim 32-41.
44. A plant exhibiting insect resistance after emerging from a seed, wherein a non-transcribable trigger molecule comprising at least one polynucleotide strand comprising at least one segment of 18 or more contiguous nucleotides of an insect pest gene in either anti-sense or sense orientation is introduced into an ungerminated seed that gives rise to the plant.
45. The plant of Claim 44, wherein the plant is selected from the group consisting of maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton and rapeseed.
46. The plant of Claim 44 or 45, wherein the plant does not comprise a detectable level of the non-transcribable trigger molecule.

47. The plant of any one of Claims 44-46, wherein the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin.
48. The plant of any one of Claims 44-47, wherein the non-transcribable trigger molecule comprises a nucleic acid sequence that is at least 80% identical over at least 25 consecutive bp to an endogenous gene of the plant.
49. The plant of any one of Claims 44-47, wherein the non-transcribable trigger molecule comprises a nucleic acid sequence that is at least 17 bp in length and at least
    85% identical to an endogenous gene of the plant.
50. The plant of any one of Claims 44-47, wherein the non-transcribable trigger molecule comprises a nucleic acid sequence that is at least 70 bp in length and at least
    65% identical to an endogenous gene of the plant.
51. A plant comprising a nucleic acid molecule for suppressing an insect pest gene, wherein the nucleic acid molecule is not integrated into a chromosome of the plant, wherein the nucleic acid molecule is not transcribed from a heterologous transgene integrated into a chromosome of the plant, and wherein the insect pest gene is suppressed by introduction of a trigger molecule comprising at least one polynucleotide strand comprising at least one segment of 18 or more contiguous nucleotides of an insect pest gene in either anti-sense or sense orientation into an ungerminated seed giving rise to the plant.
52. The plant of Claim 51, wherein the plant is selected from the group consisting of maize, soybean, rice, wheat, tomato, cucumber, lettuce, cotton and rapeseed.
53. The plant of Claim 51, wherein the trigger molecule is dsRNA.
54. The plant of Claim 51, wherein the insect pest gene is selected from the group consisting of ATPase, NADPH Cytochrome P450 Oxidoreductase, IAP, Chitin Synthase, EF1α, and β-actin.
55. The plant of any of Claims 51-54, wherein the trigger molecule comprises a nucleic acid sequence that is at least 80% identical over at least 25 consecutive bp to an endogenous gene of the seed giving rise to the plant.

56. The plant of any one of Claims 51-54, wherein the trigger molecule comprises a nucleic acid sequence that is at least 17 bp in length and at least 85% identical to an endogenous gene of the seed giving rise to the plant.
57. The plant of any one of Claims 51-54, wherein the trigger molecule comprises a nucleic acid sequence that is at least 70 bp in length and at least 65% identical to an endogenous gene of the seed giving rise to the plant.
58. The plant of Claim 51, wherein the plant does not comprise a detectable level of the trigger molecule.
59. A method of reducing corn root worm pressure on a corn plant, the method comprising:
    a) introducing a trigger molecule comprising at least one polynucleotide strand comprising at least one segment of 18 or more contiguous nucleotides of a corn root worm gene in either anti-sense or sense orientation into an ungerminated corn seed and
    b) germinating the corn seed to generate the corn plant.
60. The method of Claim 59, wherein the trigger molecule is dsRNA.
61. The method of Claim 59, wherein the ungerminated corn seed is primed prior to introducing the trigger molecule.
62. The method of Claim 61, wherein the priming is effected by:
    (i) washing the seed prior to introducing the trigger molecule; and
    (ii) drying the seed following step (i).
63. The method of Claim 62, wherein the washing is effected in the presence of double deionized water.

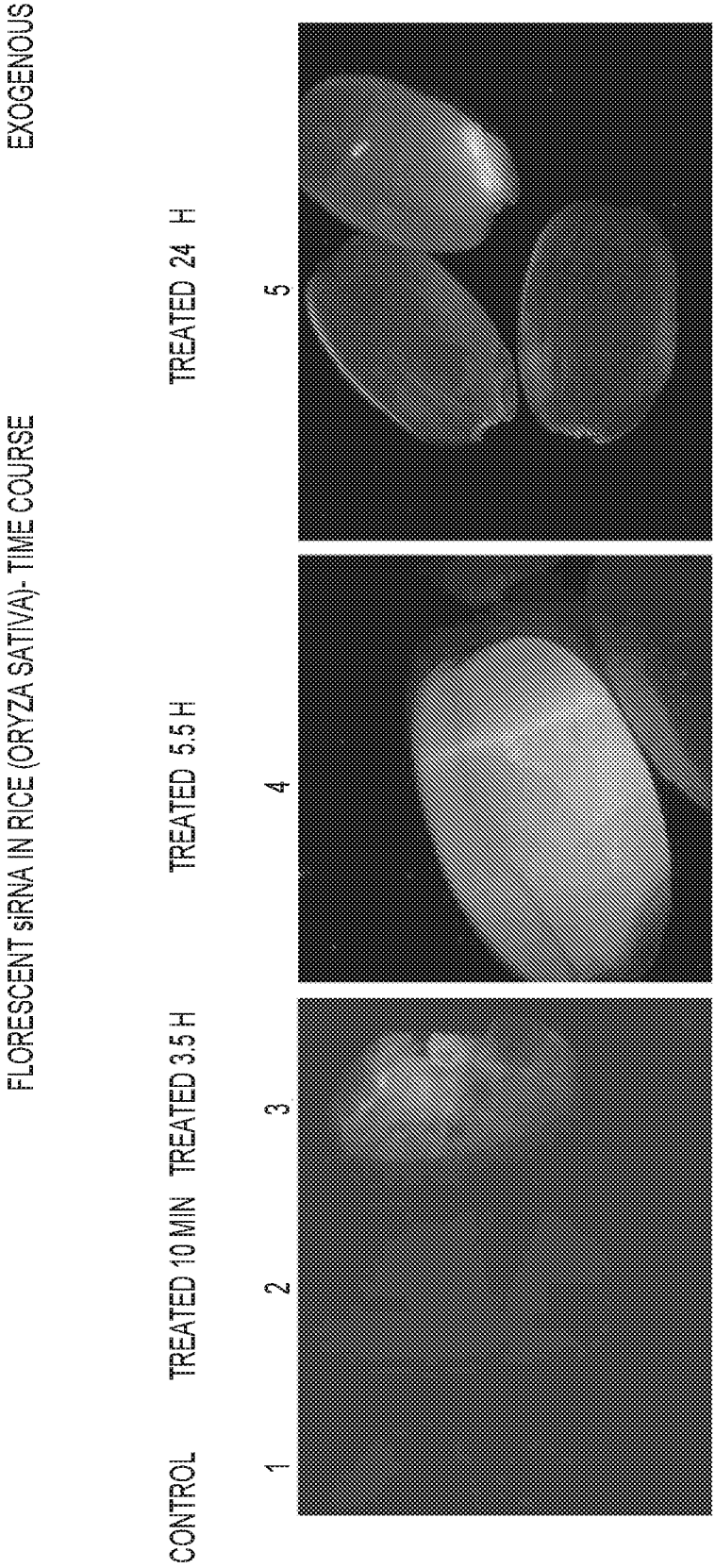

FLORESCENT siRNA IN RICE (ORYZA SATIVA)- TIME COURSE
EXOGENOUS
CONTROL
TREATED 10 MIN
TREATED 3.5 H
TREATED 5.5 H
TREATED 24 H
1
2
3
4
5

FIG. 1

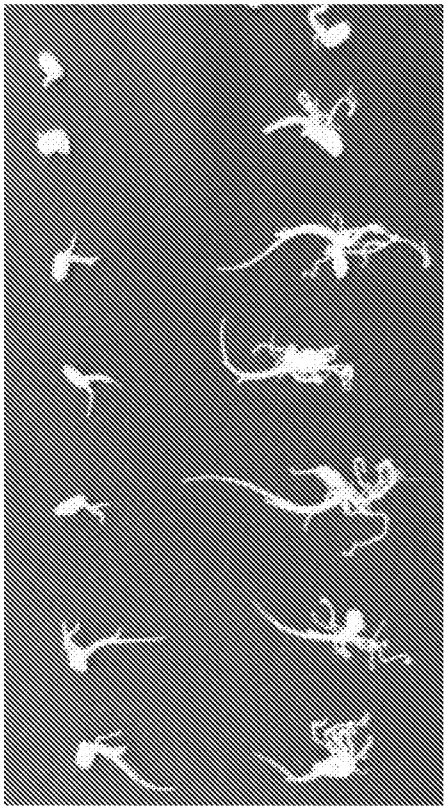


FIG. 2B

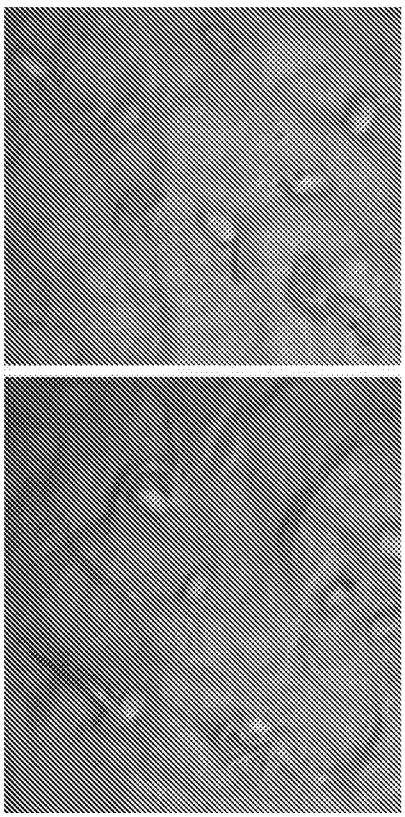


FIG. 2A

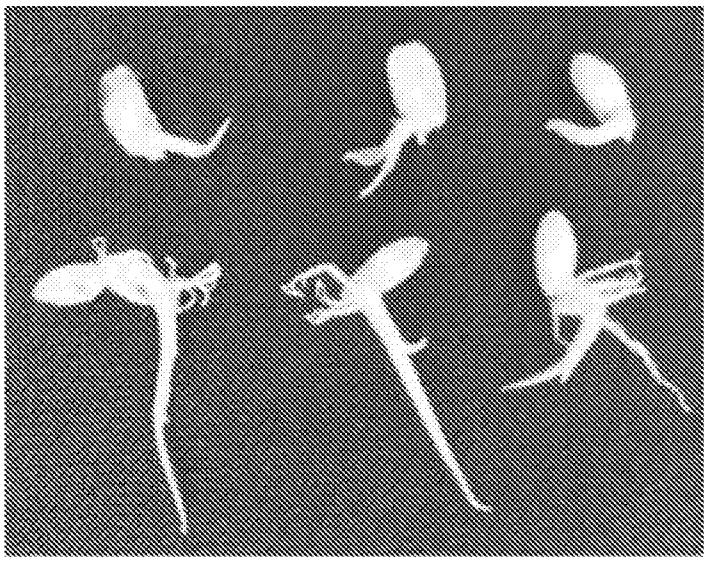


FIG. 3A

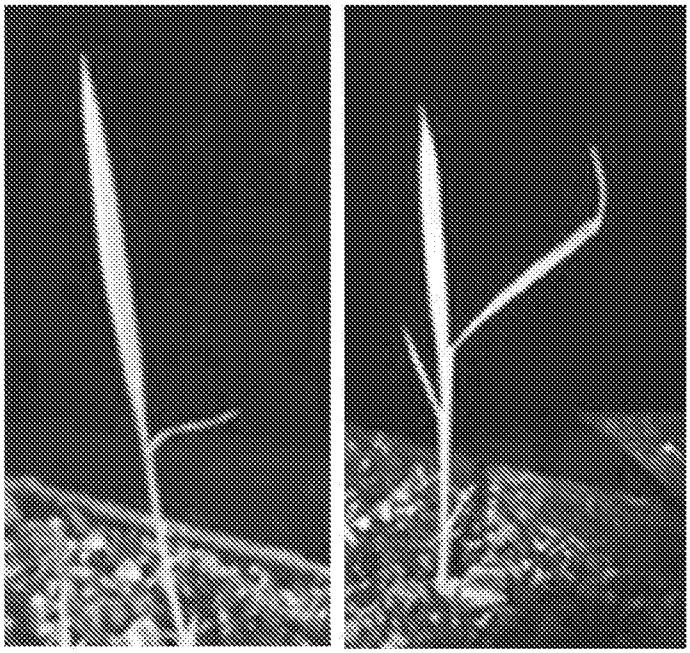


FIG. 3B

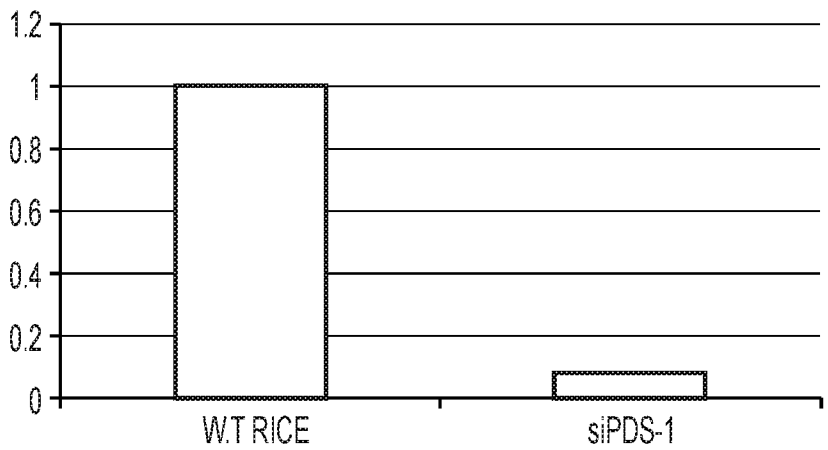


FIG. 3C

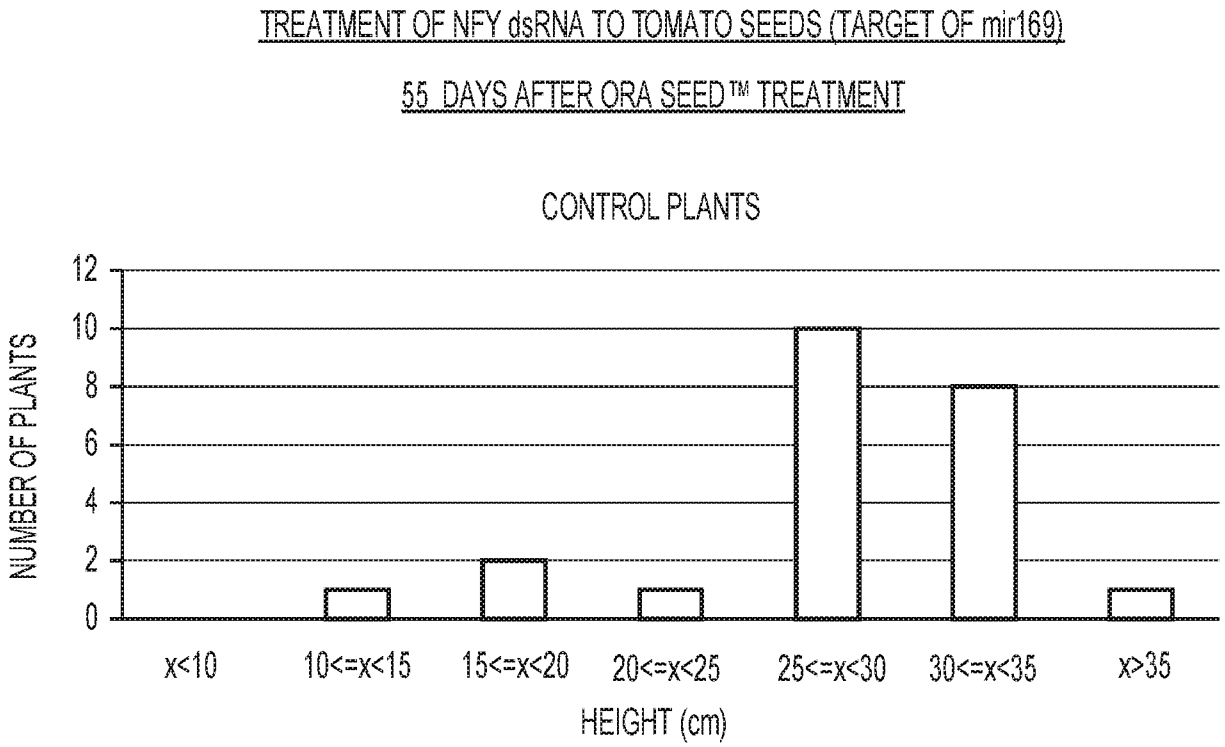

TREATMENT OF NFY dsRNA TO TOMATO SEEDS (TARGET OF mir169)
55 DAYS AFTER ORA SEED™ TREATMENT
CONTROL PLANTS
NUMBER OF PLANTS
12
10
8
6
4
2
0
x<10
10<=x<15
15<=x<20
20<=x<25
25<=x<30
30<=x<35
x>35
HEIGHT (cm)

FIG. 4A

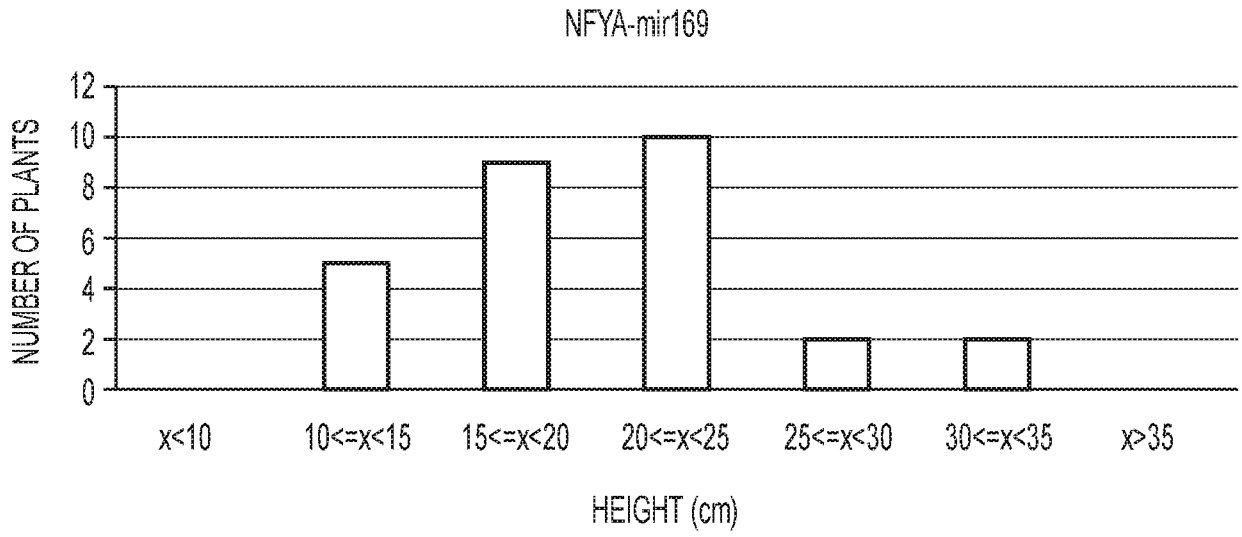

NFYA-mir169
NUMBER OF PLANTS
12
10
8
6
4
2
0
x<10
10<=x<15
15<=x<20
20<=x<25
25<=x<30
30<=x<35
x>35
HEIGHT (cm)

FIG. 4B

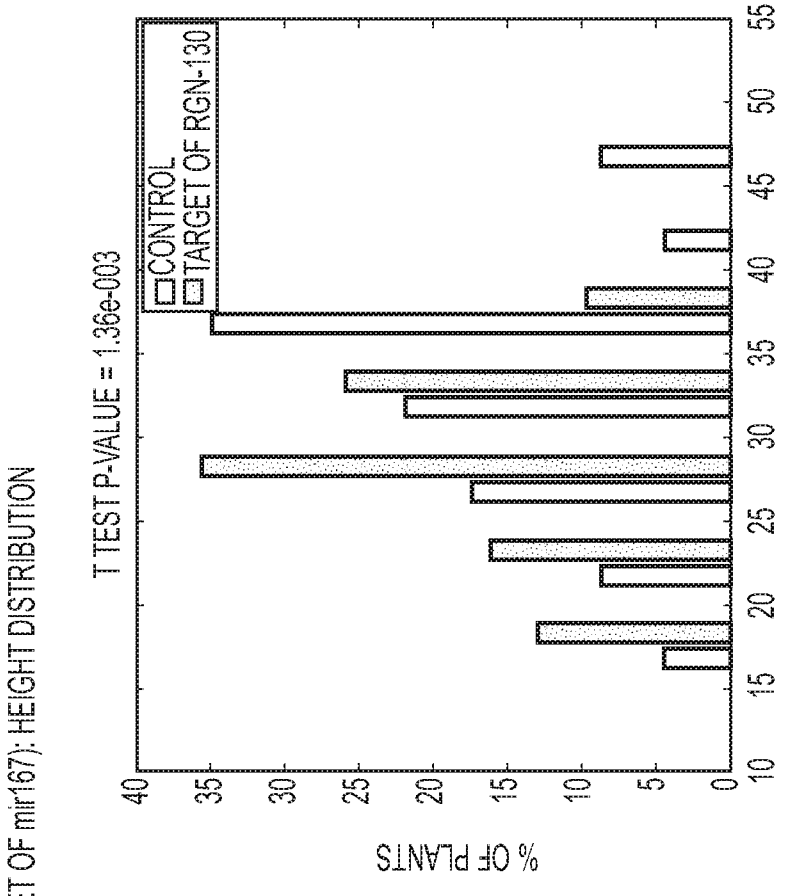

TREATMENT OF ARF8 dsRNA TO TOMATO SEEDS (TARGET OF mir167); HEIGHT DISTRIBUTION
T TEST P-VALUE = 1.22e-003
CONTROL
TARGET OF RGN-130
% OF PLANTS
PLANTS HEIGHT (CM)
T TEST P-VALUE = 1.36e-003
CONTROL
TARGET OF RGN-130
% OF PLANTS
PLANTS HEIGHT (CM)

FIG. 5A

FIG. 5B

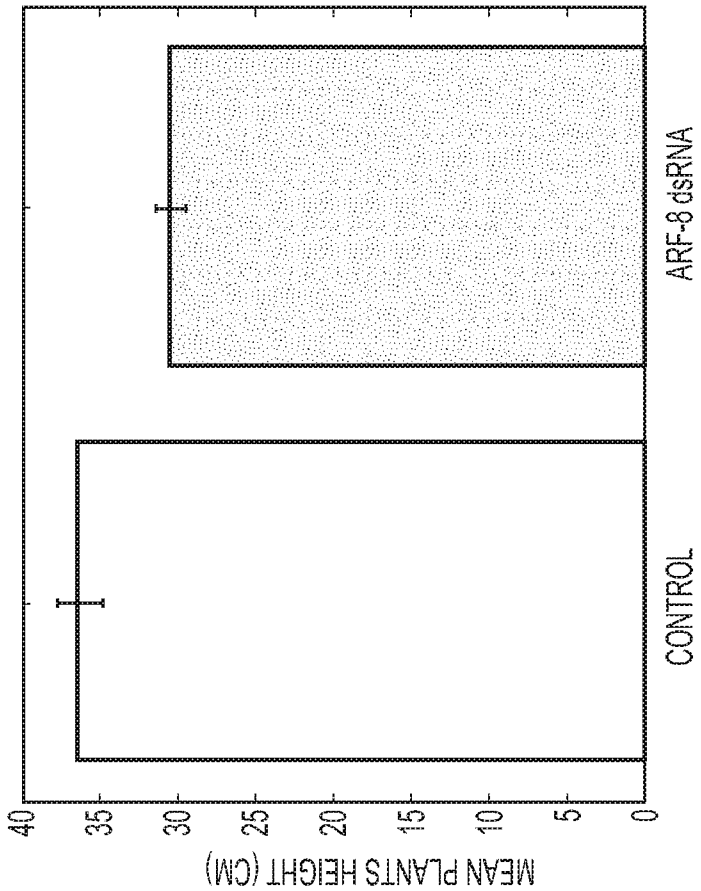

MEAN PLANTS HEIGHT (CM)
0
5
10
15
20
25
30
35
40
CONTROL
ARF-8 dsRNA

FIG. 5D

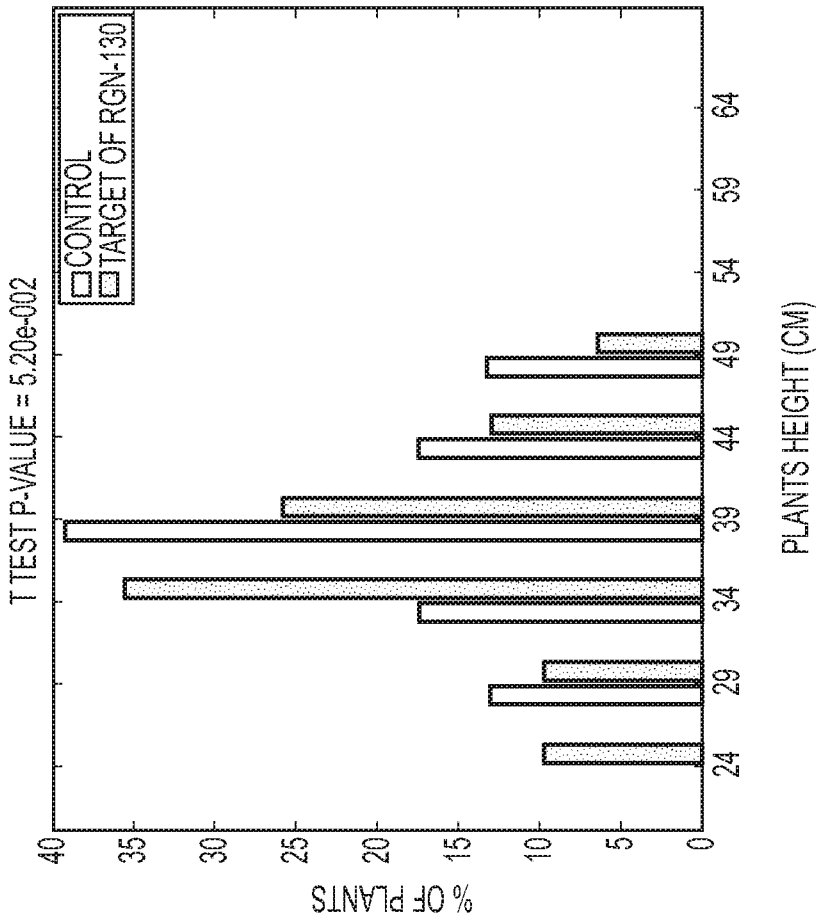

% OF PLANTS
0
5
10
15
20
25
30
35
40
PLANTS HEIGHT (CM)
24
29
34
39
44
49
54
59
64
T TEST P-VALUE = 5.20e-002
CONTROL
TARGET OF RGN-130

FIG. 5C

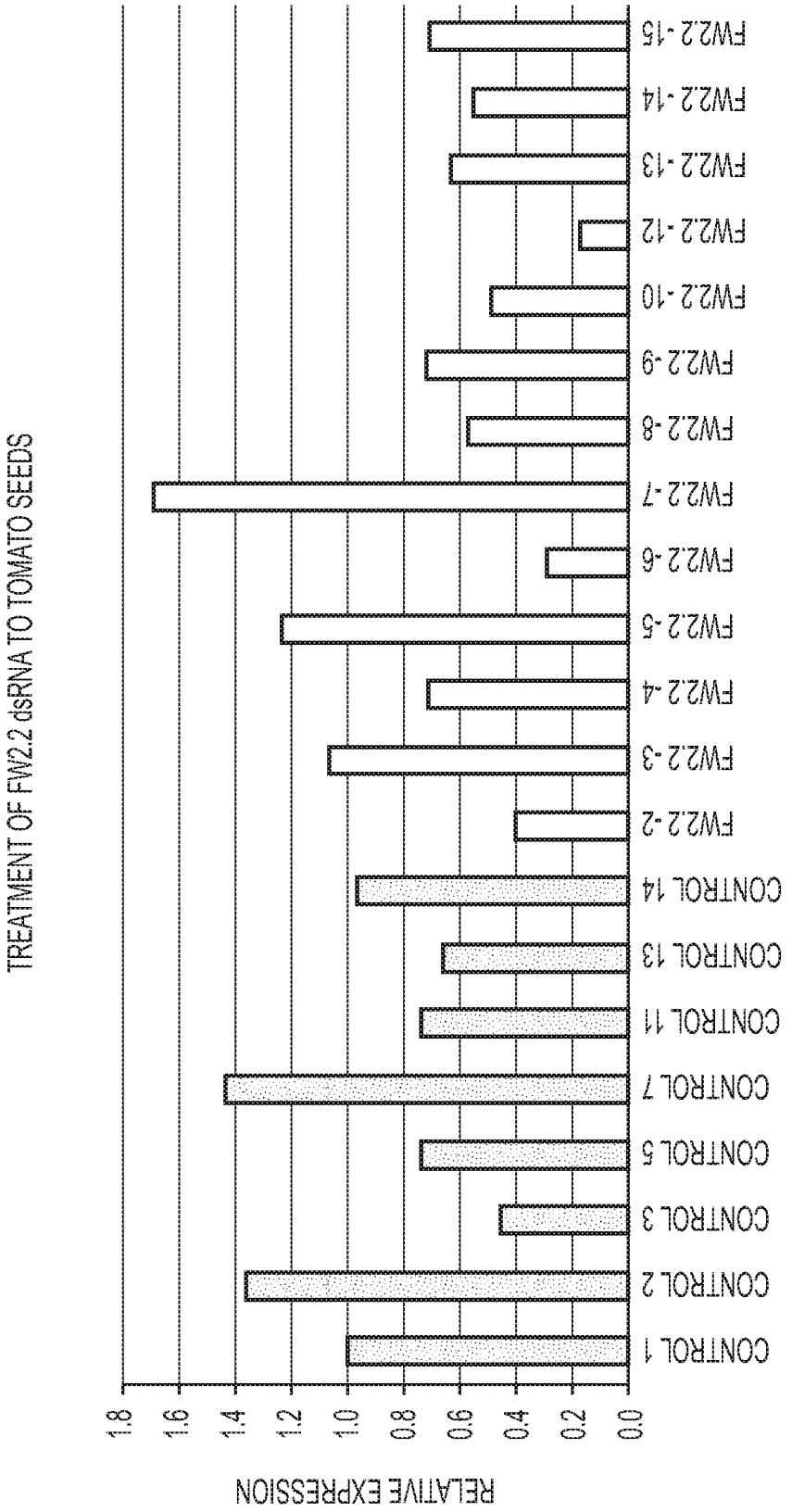

TREATMENT OF FW2.2 dsRNA TO TOMATO SEEDS
RELATIVE EXPRESSION
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CONTROL 1
CONTROL 2
CONTROL 3
CONTROL 5
CONTROL 7
CONTROL 11
CONTROL 13
CONTROL 14
FW2.2 -2
FW2.2 -3
FW2.2 -4
FW2.2 -5
FW2.2 -6
FW2.2 -7
FW2.2 -8
FW2.2 -9
FW2.2 -10
FW2.2 -12
FW2.2 -13
FW2.2 -14
FW2.2 -15
PLANTS

FIG. 6A

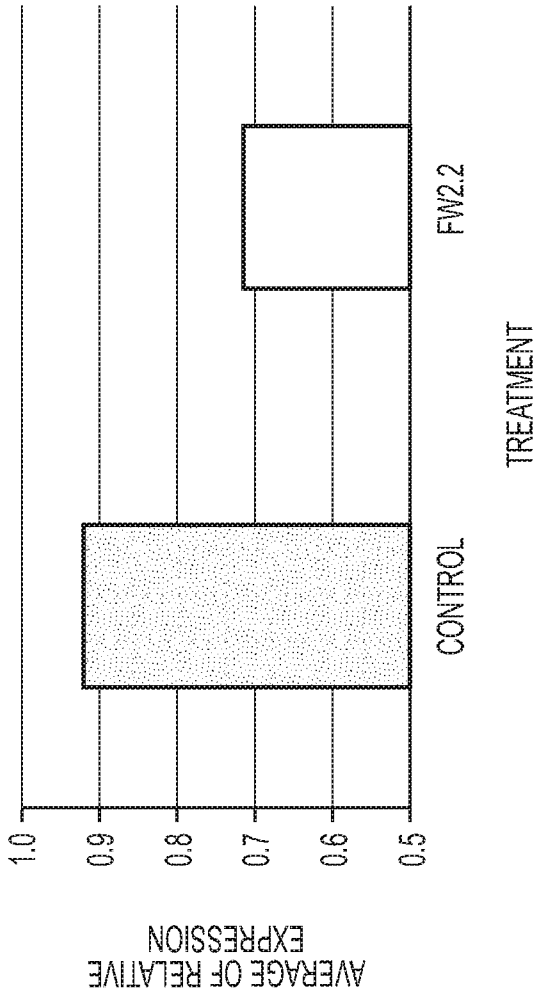

AVERAGE OF RELATIVE EXPRESSION
1.0
0.9
0.8
0.7
0.6
0.5
CONTROL
FW2.2
TREATMENT

FIG. 6B

TREATMENT OF DELLA dsRNA TO RICE SEEDS

CONTROL SEEDS

FIG. 7A

DELLA TREATED SEEDS

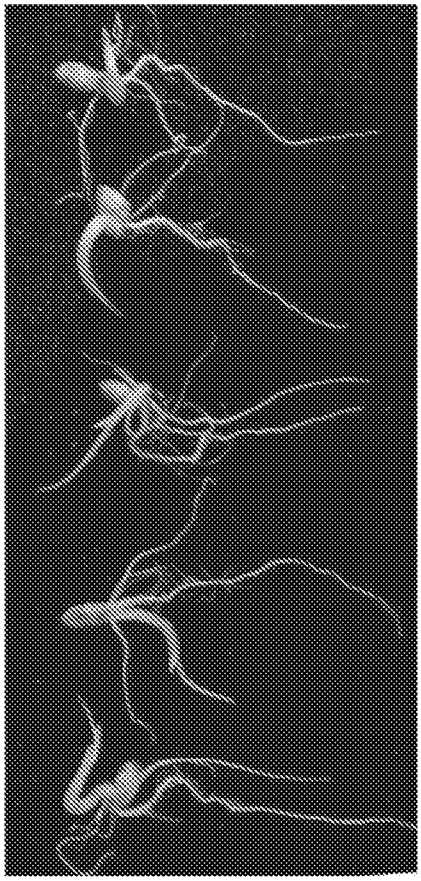


FIG. 7B

TREATMENT OF NRR dsRNA TO RICE SEEDS

CONTROL

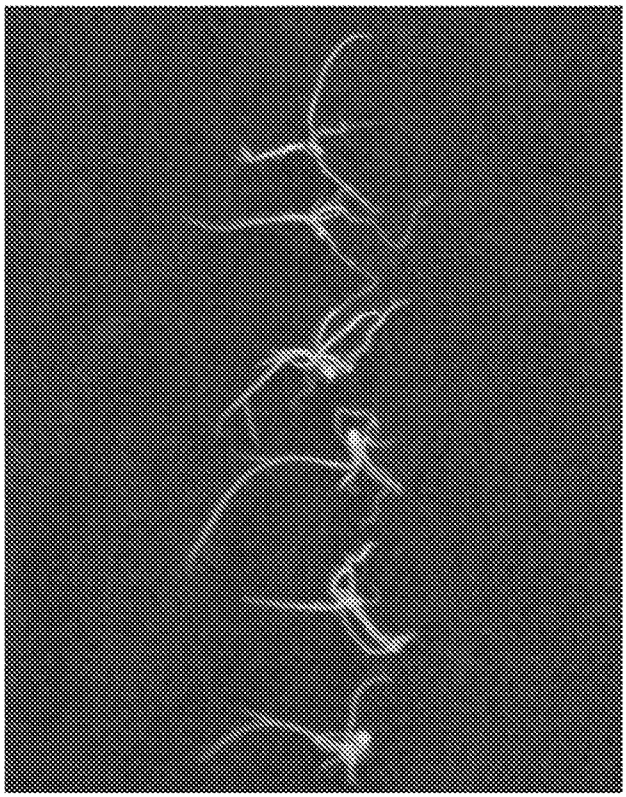


FIG. 8A

TREATED

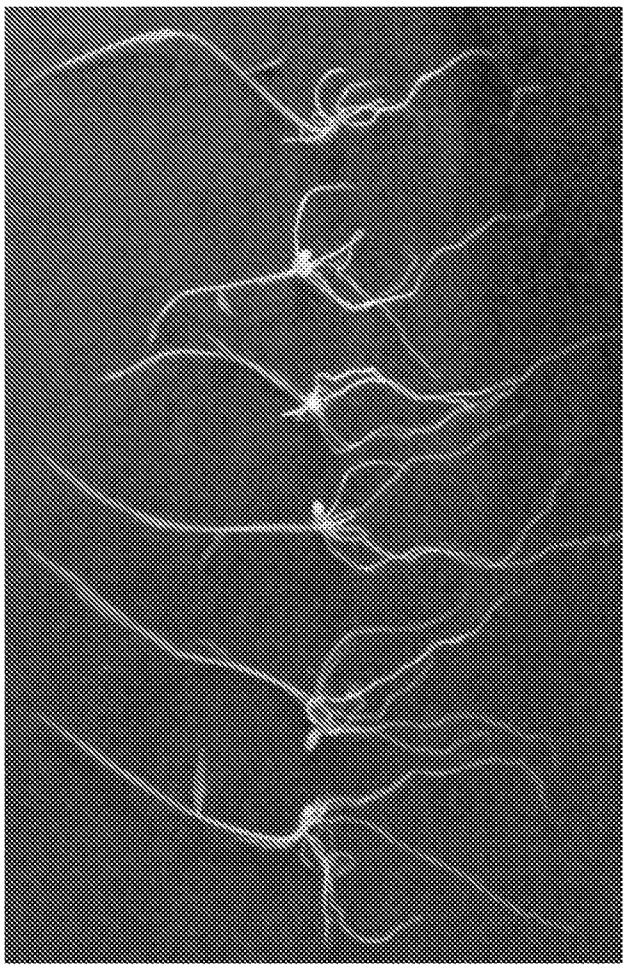


FIG. 8B

NADPH-DS#1

SCORE	EXPECT	IDENTITIES	GAPS	STRAND	FRAME
64.4 BITS(70)	3E-09()	58/71(82%)	4/71(5%)	PLUS/PLUS	

FEATURES:

```
QUERY  27     TTGGGCTTGGAGATGATGACGCTAAT--ATTGAAGATGACTTTATCACCTGGAAAGAAAA
              |||| ||||||||||||||||  | |||  ||||| |||||||| | ||| ||||||||||
SBJCT  864    TTGGTCTTGGAGATGATGA--CCAATGCATTGAGGATGACTTCAACACATGGAAAGAAAC
921
```

```
QUERY  85     GTTCTGGCCAG  95
                 |||||||||
SBJCT  922    TCTCTGGCCAG  932
```

NADPH-DS2

SCORE	EXPECT	IDENTITIES	GAPS	STRAND	FRAME
46.4 BITS(50)	8e-04()	31/35(89%)	0/35(0%)	PLUS/PLUS	

FEATURES:

```
QUERY  351     CCTCGCCTACAACCAAGATACTACTCCATCTCATC  385
               |||||||| ||||||||||||||| || || |||||
SBJCT  1604    CCTCGCCTTCAACCAAGATACTATTCTATTTCATC  1638
```

FIG. 9A

ATPASE-DS1

SCORE	EXPECT	IDENTITIES	GAPS	STRAND	FRAME
251 BITS(278)	LE-65()	348/484(72%)	10/484(2%)	PLUS/PLUS	

FEATURES:

```
QUERY   27      GGAAAGCCCATTGACAAGGGTCCCCCAATCCTCGCCGAGGACTTCTTGGACATCCAGGGA  86
                |||||  ||  ||||||||  ||  ||||||  ||   |   |  ||  |  ||  ||||||  |||    |||
SBJCT   462     GGAAAACCTATTGACAATGGCCCCCCGATATTACCTGAAGCCTACTTGGATATCTCTGGA  521


QUERY   87      CAGCCCATCAACCCATGGTCCCGTATCTACCCCGAGGAGATGATCCAGACTGGTATCTCC  146
                    |  ||  ||  ||   |      |  |  |||  ||  ||||||||||||||  |||||  ||  |||
SBJCT   522     AGTTCTATTAATCCCAGTGAGAGAACCTATCCAGAGGAGATGATCCAAACTGGGATATCC  581


QUERY   147     GCTATCGACGTGATGAACTCCATTGCTCGTGGTCAGAAGATCCCCATCTTCTCTGCCGCT  206
                 |  ||  ||  ||||||||||||||||||||||||  ||  ||  ||||||  |  ||  |||||  |||
SBJCT   582     ACCATTGATGTGATGAACTCCATTGCTCGTGGGCAAAAAATCCCCCTATTTTCTGCTGCT  641


QUERY   207     GGTCTGCCCCACAATGAAATTGCCGCCCAGATCTGTAGACAGGCCGGTCTTGTTAAGATC  266
                ||  ||  ||  ||||||||||||||  ||  |||||||||  |  |||||  ||  |||||  ||||
SBJCT   642     GGACTCCCTCACAATGAAATTGCTGCTCAGATCTGTCGTCAGGCTGGCCTTGTGAAGACA  701
```

FIG. 9B

```
QUERY  267  CCCG----GCAAATCA-GTGTTGGATGACCACGAGGACAACTTCGCCATCGTATTCGCA  320
SBJCT  702  TTGGAGAAAGGAAAGCATGCAGAGGGTGGTGAAGATGACAACTTTGCTATTGTGTTTGCT  761

QUERY  321  GCTATGGGTGTGAACATGGAAACCGCCCGGTTCTTCAAACAGGACTTCGAAGAGAACGGT  380
SBJCT  762  GCTATGGGAGTGAACATGGAAACAGCTCAATTCTTCAAACGTGATTTTGAAGAGAACGGT  821

QUERY  381  TCTATGGAGAACGTGT--GCCTGTTCTTGAACTTGGCCAATGACCCCACTATTGAGAGAA  438
SBJCT  822  TCCATG--GAACGGGTCACCCTTTTTCTGAATCTGGCTAATGATCCCACCATTGAACGTA  879

QUERY  439  TTATCACACCCCGTCTTGCTTTGACTGCTGCCGAGTTCTTGGCCTACCAGTGCGAGAAAC  498
SBJCT  880  TTATCACCCCTCGGATTGCTCTAACAACAGCAGAATATTTGGCATATGAATGTGGGAAGC  939

QUERY  499  ACGT  502
SBJCT  940  ATGT  943
```

FIG. 9B
CONTINUED

IAP-DS1

SCORE	EXPECT	IDENTITIES	GAPS	STRAND	FRAME
37.4 BITS(40)	0.43()	22/23(96%)	0/23(0%)	PLUS/PLUS	

FEATURES:

```
QUERY  2    TAATACGACTCACTATAGGGAGA  24
            ||||||||||||||||||||||| ||
SBJCT  441  TAATACGACTCACTATAGGGCGA  463
```

IAP-DS2

SCORE	EXPECT	IDENTITIES	GAPS	STRAND	FRAME
35.6 BITS(38)	1.5()	19/19(100%)	0/19(0%)	PLUS/PLUS	

FEATURES:

```
QUERY  444  CCGGTACCTCTCCGCCACG  462
            ||||||||||||||||||||
SBJCT  607  CCGGTACCTCTCCGCCACG  625
```

FIG. 9C

NADPH-DS1

SCORE	EXPECT	IDENTITIES	GAPS	STRAND	FRAME
42.8 BITS(46)	0.021()	28/30(93%)	1/30(3%)	PLUS/PLUS	

FEATURES:

```
QUERY  2        TAATACGACTCACTATAGGGAGAACTTGGG  31
                |||||||||||||||||||| ||| |||||
SBJCT  283      TAATACGACTCACTATAGGGCGAA-TTGGG  311
```

ANOTHER SEGMENT:

SCORE	EXPECT	IDENTITIES	GAPS	STRAND	FRAME
37.4 BITS(40)	0.91()	20/20(100%)	0/20(0%)	PLUS/PLUS	

FEATURES:

```
QUERY  523      CCCTATAGTGAGTCGTATTA  542
                ||||||||||||||||||||
SBJCT  755      CCCTATAGTGAGTCGTATTA  774
```

NADPH-DS2

SCORE	EXPECT	IDENTITIES	GAPS	STRAND	FRAME
39.2 BITS(42)	0.26()	21/21(100%)	0/21(0%)	PLUS/PLUS	

FEATURES:

```
QUERY  189      GATGAAGAAGACAAAAAGAAA  209
                |||||||||||||||||||||
SBJCT  42818    GATGAAGAAGACAAAAAGAAA  42838
```

FIG. 10A

ATPASE-DS1

SCORE	EXPECT	IDENTITIES	GAPS	STRAND	FRAME
214 BITS(236)	6e-54()	263/359(73%)	9/359(2%)	PLUS/PLUS	

FEATURES:

```
QUERY  114   TACCCCGAGGAGATGATCCAGACTGGTATCTCCGCTATCGACGTGATGAACTCCATTGCT  173
             || || || || ||||| ||||| || || ||| |  | ||||| ||||| || ||||||
SBJCT  487   TATCCTGAAGAAATGATACAGACAGGAATTTCCACAGTAGACGTCATGAATTCAATTGCT  546

QUERY  174   CGTGGTCAGAAGATCCCCATCTTCTCTGCCGCTGGTCTGCCCCACAATGAAATTGCCGCC  233
              | || |||||||| ||  | |||||||| |||||||| || || ||||||||||| |||
SBJCT  547   AGAGGGCAGAAGATTCCTCTTTTCTCTGCTGCTGGTCTTCCTCATAATGAAATTGCAGCC  606

QUERY  234   CAGATCTGTAGACAGGCCGGTCTTGTTAAGATCCCCGGCAAATCAG-------TGTTG--  284
             ||||||||| | ||||| || || || ||||     |  ||||| |       | |||
SBJCT  607   CAGATCTGTCGTCAGGCTGGACTGGTGAAGAGGTTGGAAAAATCTGACAACCTTCTTGAG  666

QUERY  285   GATGACCACGAGGACAACTTCGCCATCGTATTCGCAGCTATGGGTGTGAACATGGAAACC  344
             | ||   | |||||||| || ||||| || || || |||||||| || ||||||||||
SBJCT  667   GGTGGTGAAGAGGACAATTTTGCCATAGTCTTTGCTGCTATGGGAGTCAACATGGAAACA  726

QUERY  345   GCCCGGTTCTTCAAACAGGACTTCGAAGAGAACGGTTCTATGGAGAACGTGTGCCTGTTC  404
             || |  || |||||||  || || || || || || ||||||||||  |||   || |||
SBJCT  727   GCACAATTTTTCAAACGTGATTTTGAGGAAAATGGATCTATGGAGAGAGTGACACTTTTC  786

QUERY  405   TTGAACTTGGCCAATGACCCCACTATTGAGAGAATTATCACACCCCGTCTTGCTTTGAC  463
             || ||| |||||||||| || ||||| ||| | ||||| || ||| |  ||||| ||||
SBJCT  787   TTAAACCTGGCCAATGATCCTACTATAGAGCGTATTATTACTCCCAGGATTGCTCTGAC  845
```

FIG. 10B

IAP1-DS1_ _

SCORE	EXPECT	IDENTITIES	GAPS	STRAND	FRAME
37.4 BITS(40)	0.91()	22/23(96%)	0/23(0%)	PLUS/PLUS	

FEATURES:

```
QUERY  501    TCTCCCTATAGTGAGTCGTATTA  523
              || |||||||||||||||||||||
SBJCT  90683  TCGCCCTATAGTGAGTCGTATTA  90705
```

FIG. 10C

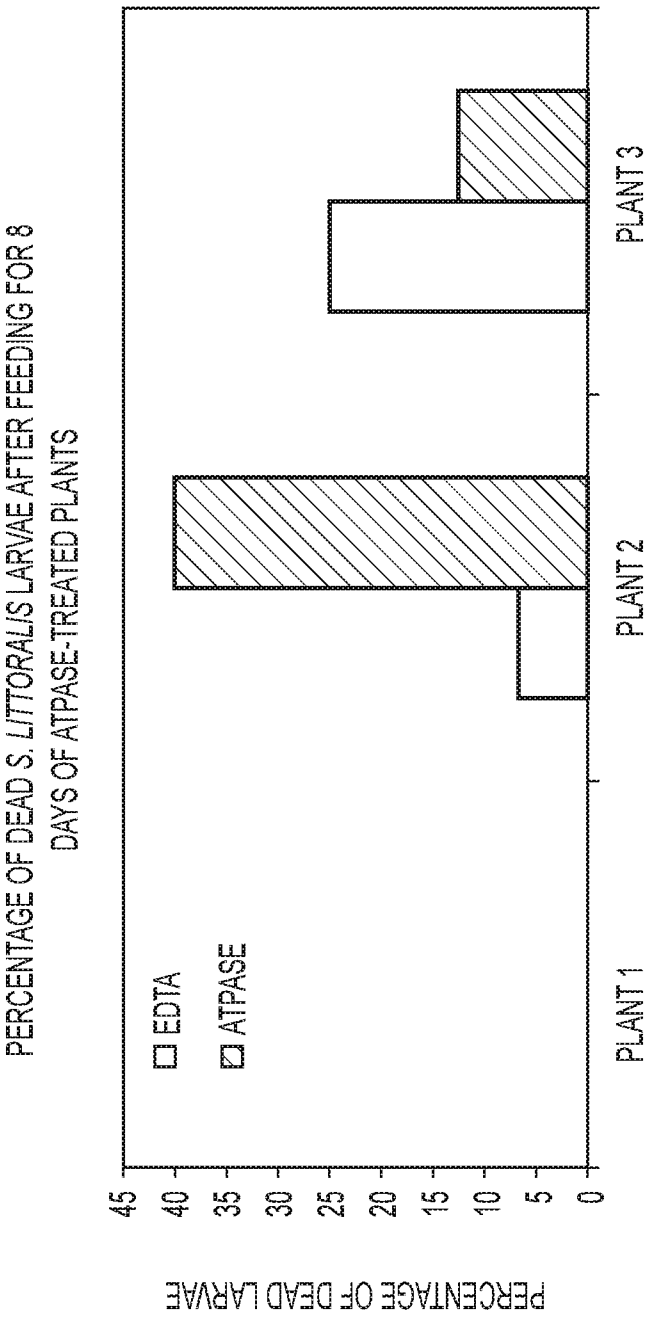

PERCENTAGE OF DEAD S. LITTORALIS LARVAE AFTER FEEDING FOR 8 DAYS OF ATPASE-TREATED PLANTS
EDTA
ATPASE
PLANT 1
PLANT 2
PLANT 3
PERCENTAGE OF DEAD LARVAE
45
40
35
30
25
20
15
10
5
0

FIG. 11A

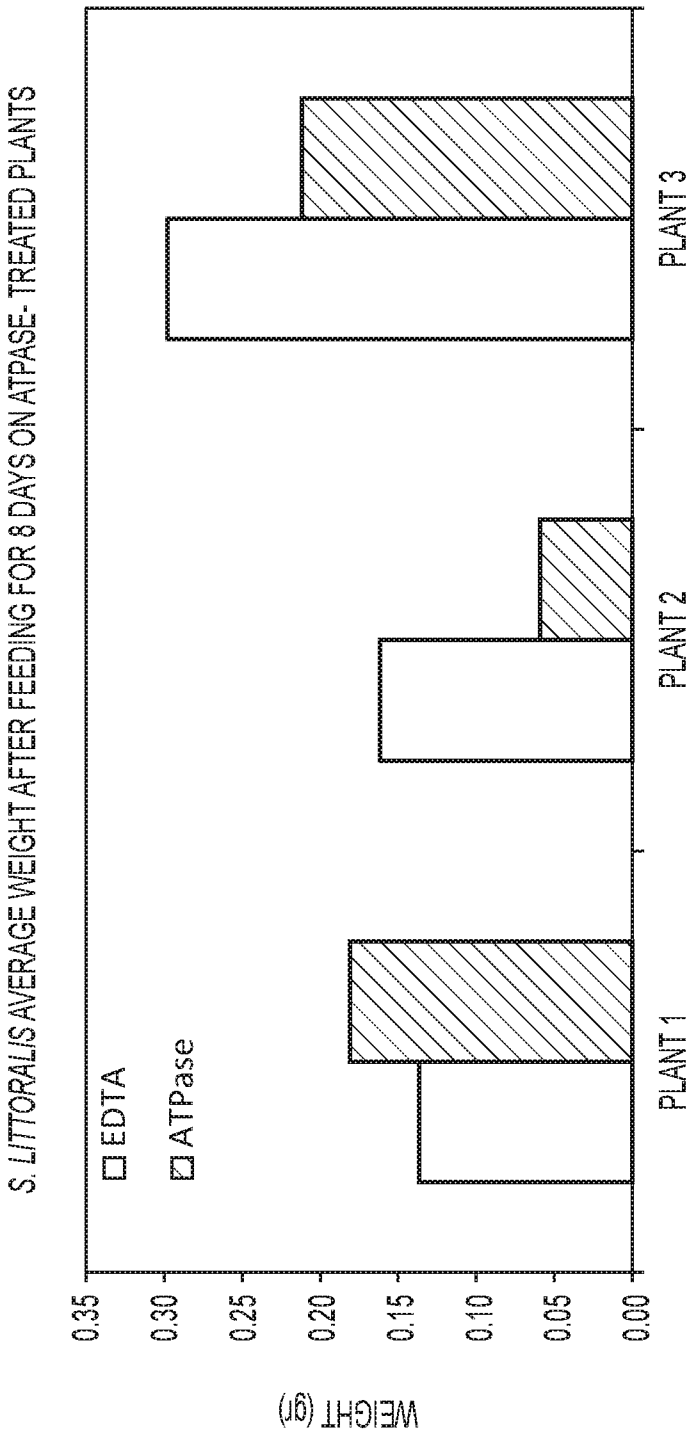

S. LITTORALIS AVERAGE WEIGHT AFTER FEEDING FOR 8 DAYS ON ATPASE- TREATED PLANTS
EDTA
ATPase
WEIGHT (gr)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
PLANT 1
PLANT 2
PLANT 3

FIG. 11B

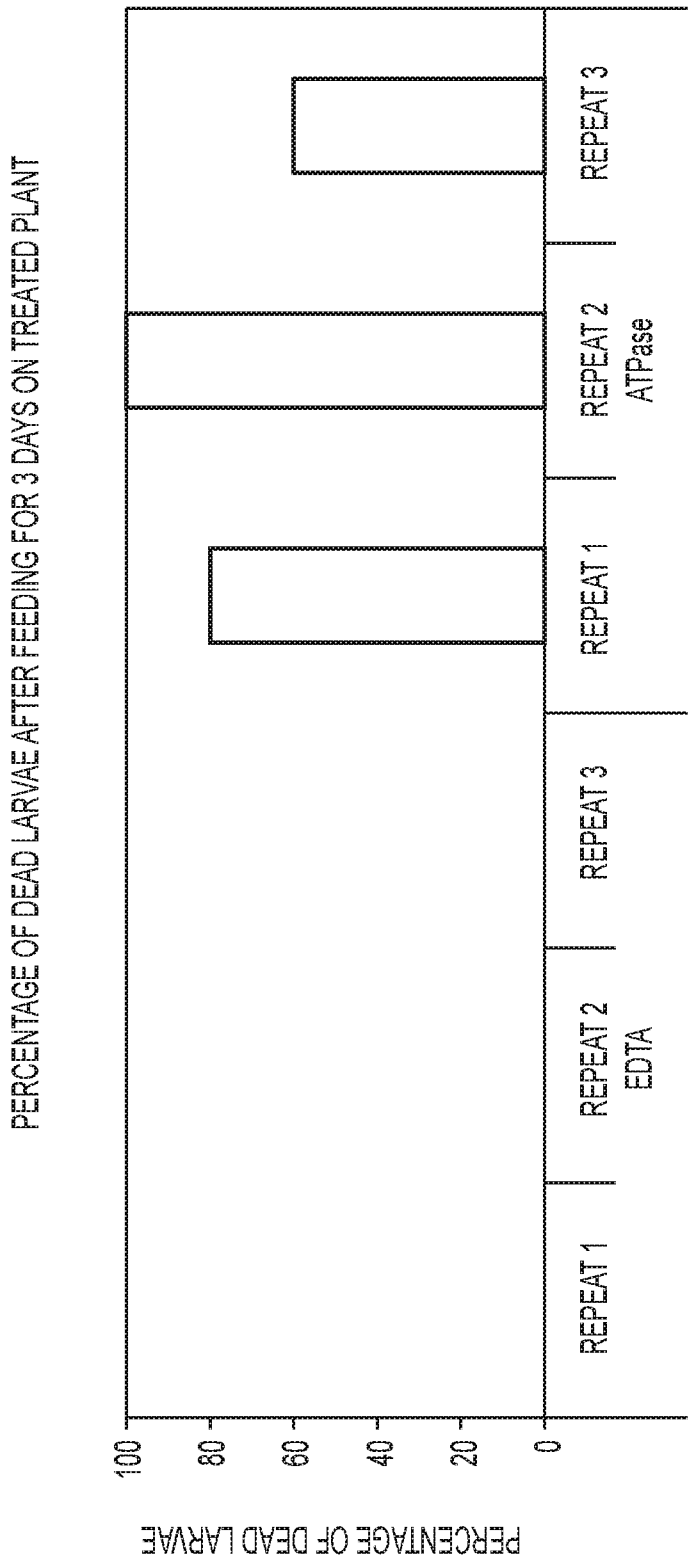

PERCENTAGE OF DEAD LARVAE AFTER FEEDING FOR 3 DAYS ON TREATED PLANT
PERCENTAGE OF DEAD LARVAE
100
80
60
40
20
0
REPEAT 1
REPEAT 2
EDTA
REPEAT 3
REPEAT 1
REPEAT 2
ATPase
REPEAT 3

FIG. 11C

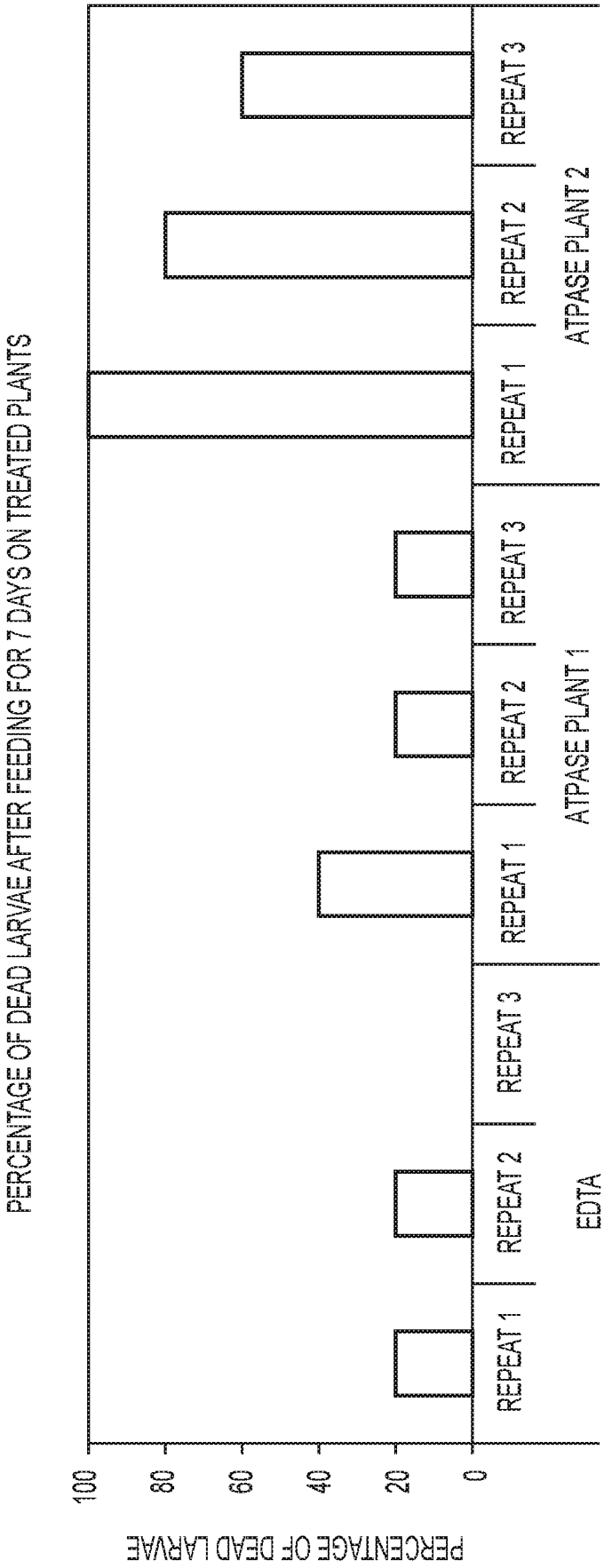

PERCENTAGE OF DEAD LARVAE AFTER FEEDING FOR 7 DAYS ON TREATED PLANTS
PERCENTAGE OF DEAD LARVAE
100
80
60
40
20
0
REPEAT 1
REPEAT 2
REPEAT 3
EDTA
REPEAT 1
REPEAT 2
REPEAT 3
ATPASE PLANT 1
REPEAT 1
REPEAT 2
REPEAT 3
ATPASE PLANT 2

FIG. 11D

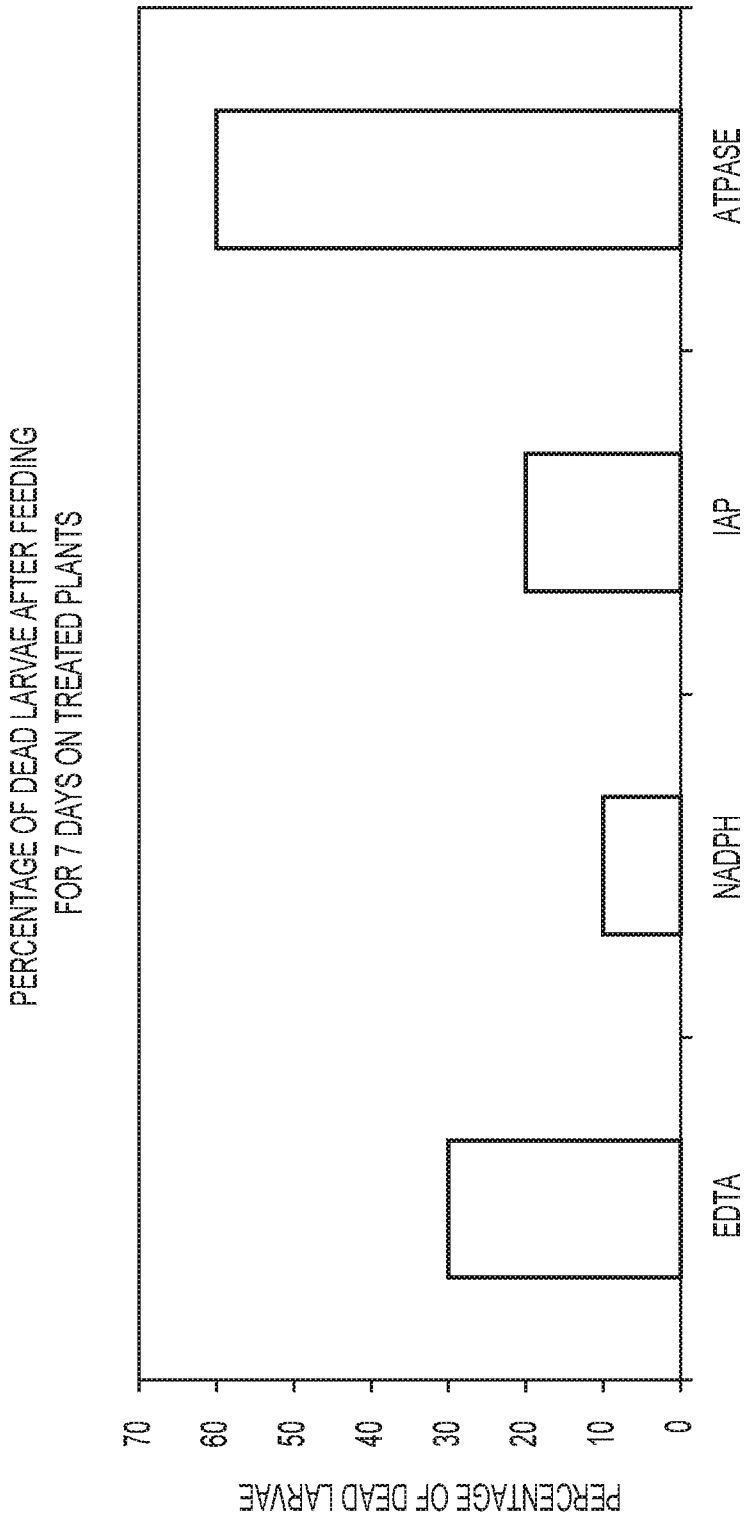

PERCENTAGE OF DEAD LARVAE AFTER FEEDING
FOR 7 DAYS ON TREATED PLANTS
PERCENTAGE OF DEAD LARVAE
70
60
50
40
30
20
10
0
EDTA
NADPH
IAP
ATPASE

FIG. 12

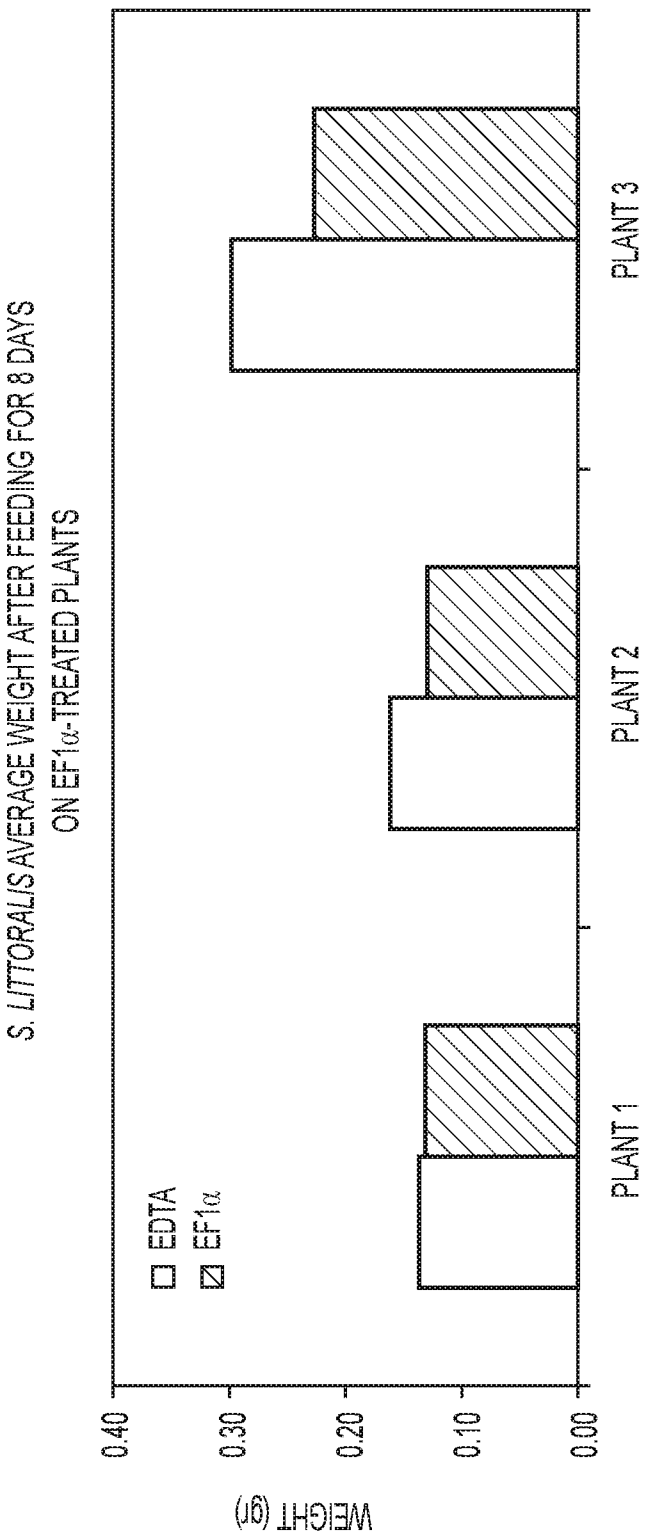

S. LITTORALIS AVERAGE WEIGHT AFTER FEEDING FOR 8 DAYS
ON EF1α-TREATED PLANTS
EDTA
EF1α
WEIGHT (gr)
0.40
0.30
0.20
0.10
0.00
PLANT 1
PLANT 2
PLANT 3

FIG. 13A

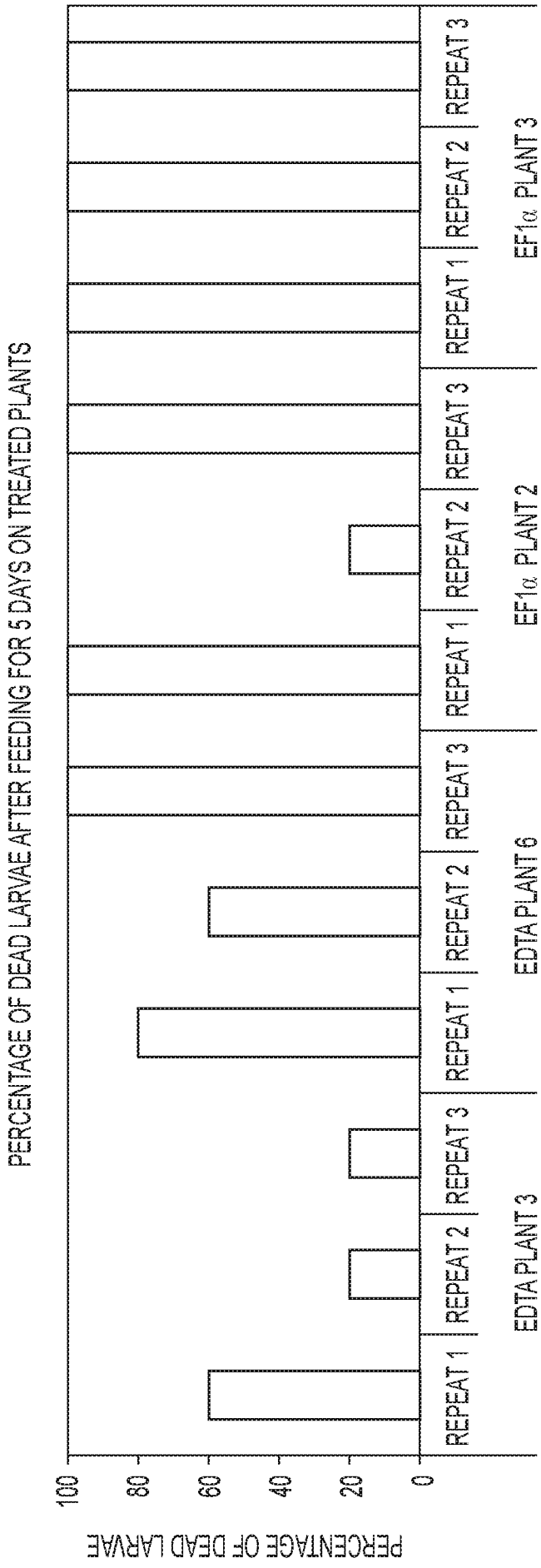

PERCENTAGE OF DEAD LARVAE AFTER FEEDING FOR 5 DAYS ON TREATED PLANTS
PERCENTAGE OF DEAD LARVAE
100
80
60
40
20
0
REPEAT 1
REPEAT 2
REPEAT 3
REPEAT 1
REPEAT 2
REPEAT 3
REPEAT 1
REPEAT 2
REPEAT 3
REPEAT 1
REPEAT 2
REPEAT 3
EDTA PLANT 3
EDTA PLANT 6
EF1α PLANT 2
EF1α PLANT 3

FIG. 13B

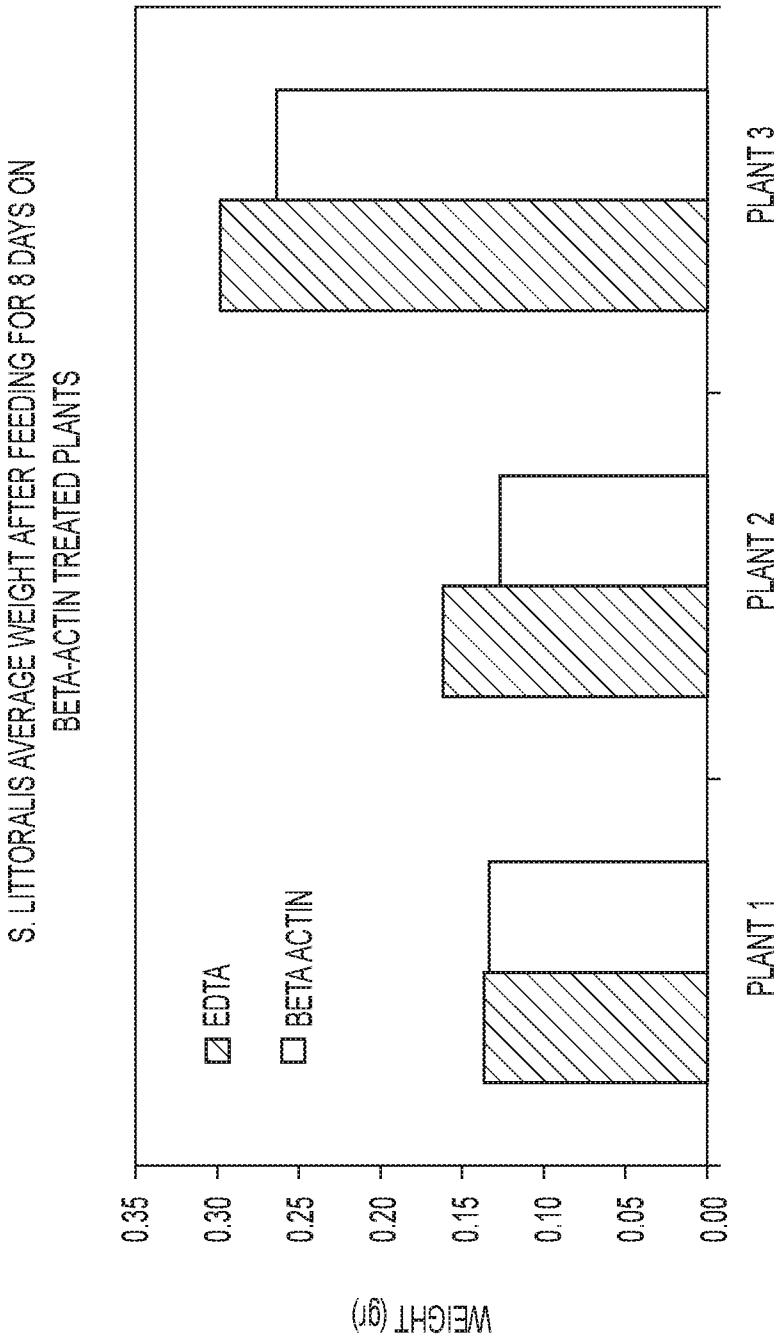

S. LITTORALIS AVERAGE WEIGHT AFTER FEEDING FOR 8 DAYS ON
BETA-ACTIN TREATED PLANTS
EDTA
BETA ACTIN
WEIGHT (gr)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
PLANT 1
PLANT 2
PLANT 3

FIG. 14

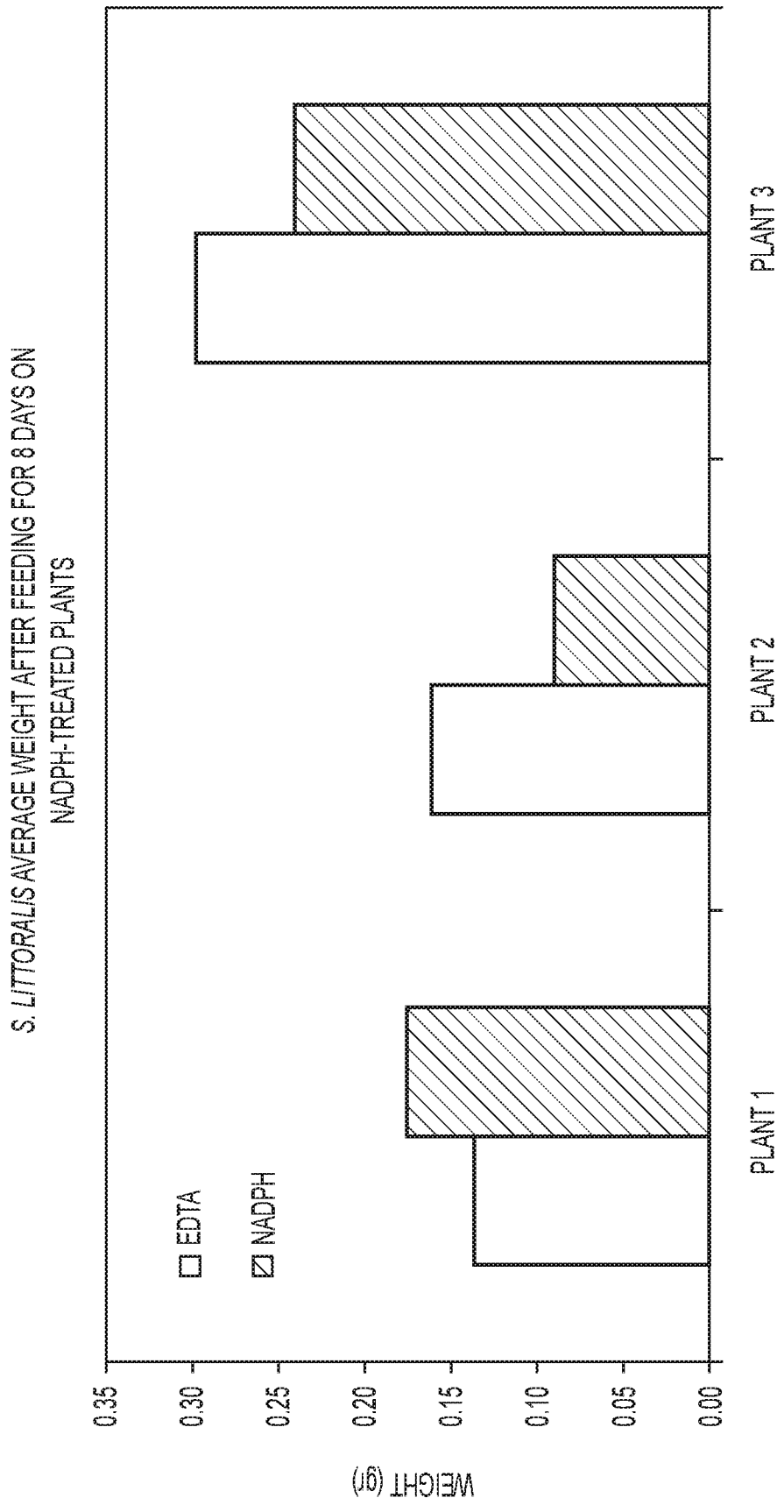

S. LITTORALIS AVERAGE WEIGHT AFTER FEEDING FOR 8 DAYS ON NADPH-TREATED PLANTS
EDTA
NADPH
WEIGHT (gr)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
PLANT 1
PLANT 2
PLANT 3

FIG. 15A

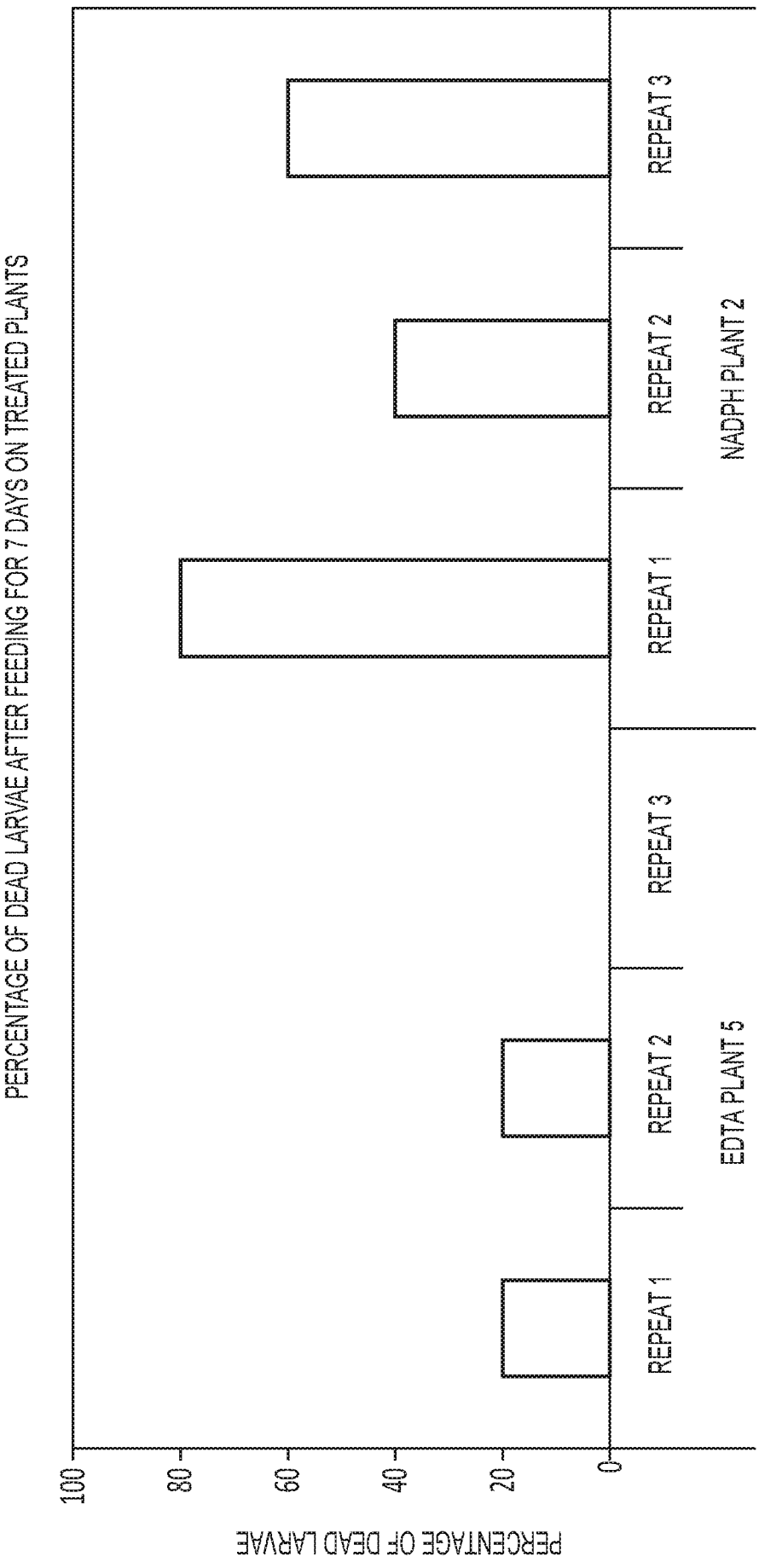

PERCENTAGE OF DEAD LARVAE AFTER FEEDING FOR 7 DAYS ON TREATED PLANTS
PERCENTAGE OF DEAD LARVAE
100
80
60
40
20
0
REPEAT 1
REPEAT 2
REPEAT 3
EDTA PLANT 5
REPEAT 1
REPEAT 2
REPEAT 3
NADPH PLANT 2

FIG. 15B

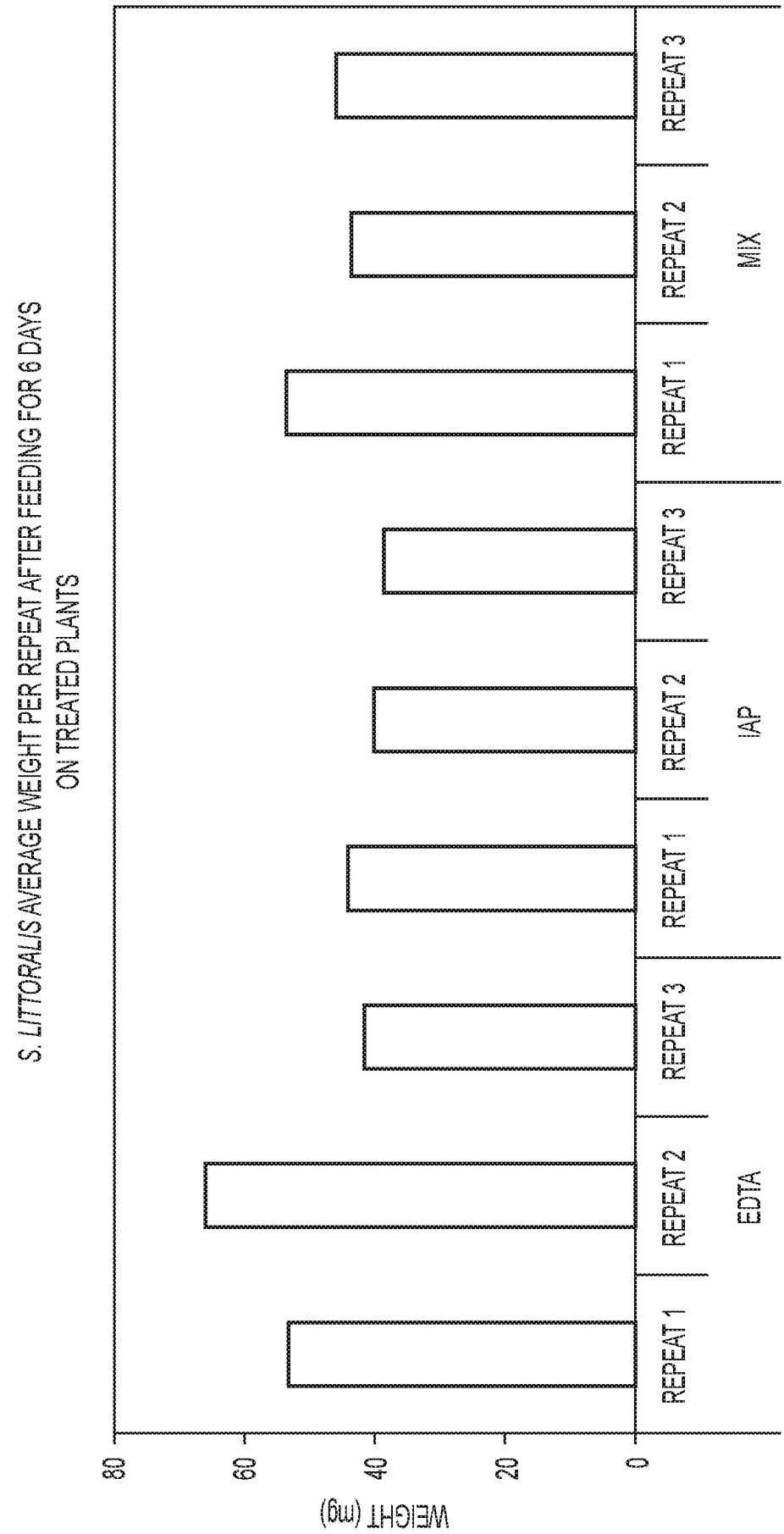

S. LITTORALIS AVERAGE WEIGHT PER REPEAT AFTER FEEDING FOR 6 DAYS
ON TREATED PLANTS
WEIGHT (mg)
0
20
40
60
80
REPEAT 1
REPEAT 2
REPEAT 3
EDTA
REPEAT 1
REPEAT 2
REPEAT 3
IAP
REPEAT 1
REPEAT 2
REPEAT 3
MIX

FIG. 16A

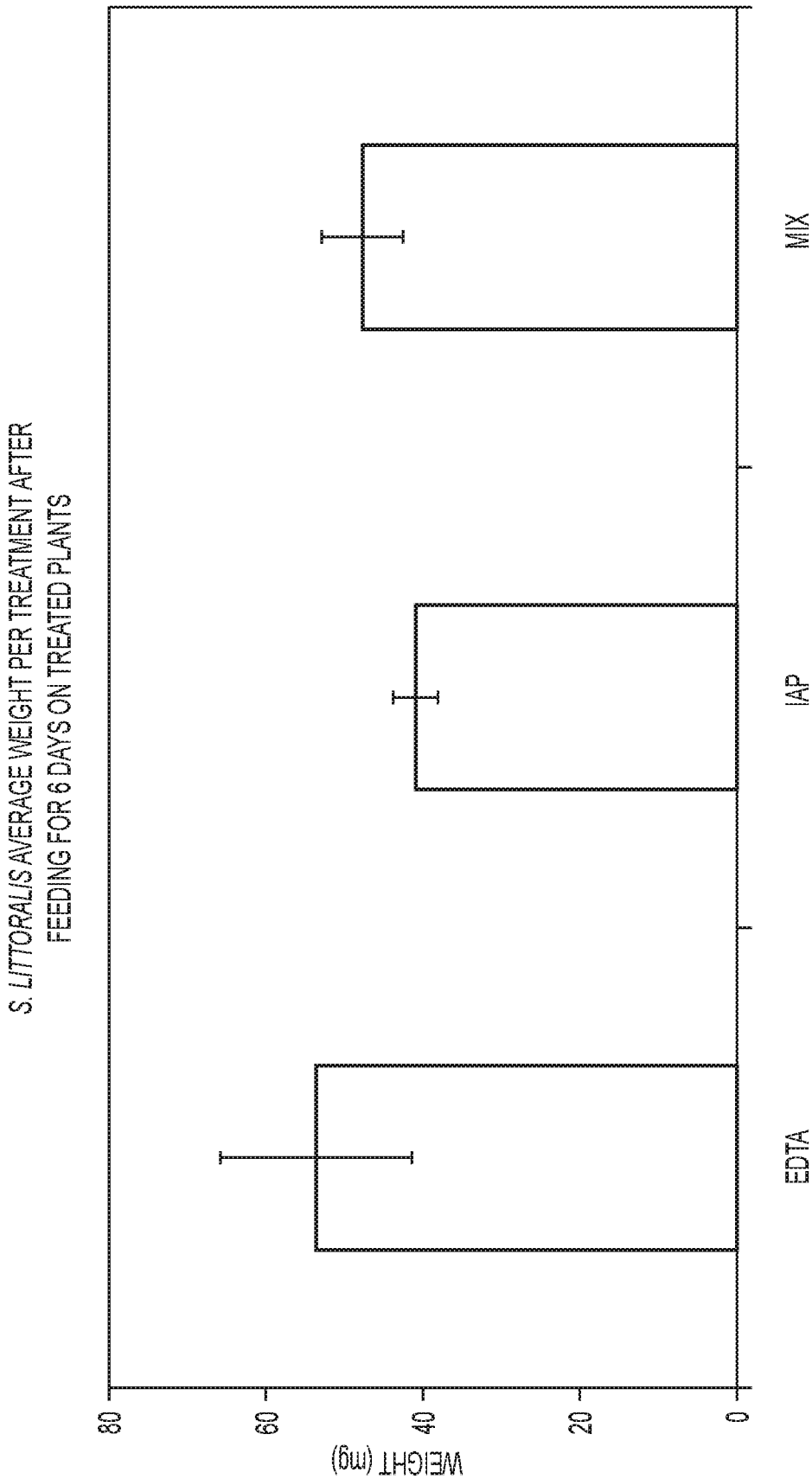

S. LITTORALIS AVERAGE WEIGHT PER TREATMENT AFTER FEEDING FOR 6 DAYS ON TREATED PLANTS
WEIGHT (mg)
0
20
40
60
80
EDTA
IAP
MIX

FIG. 16B

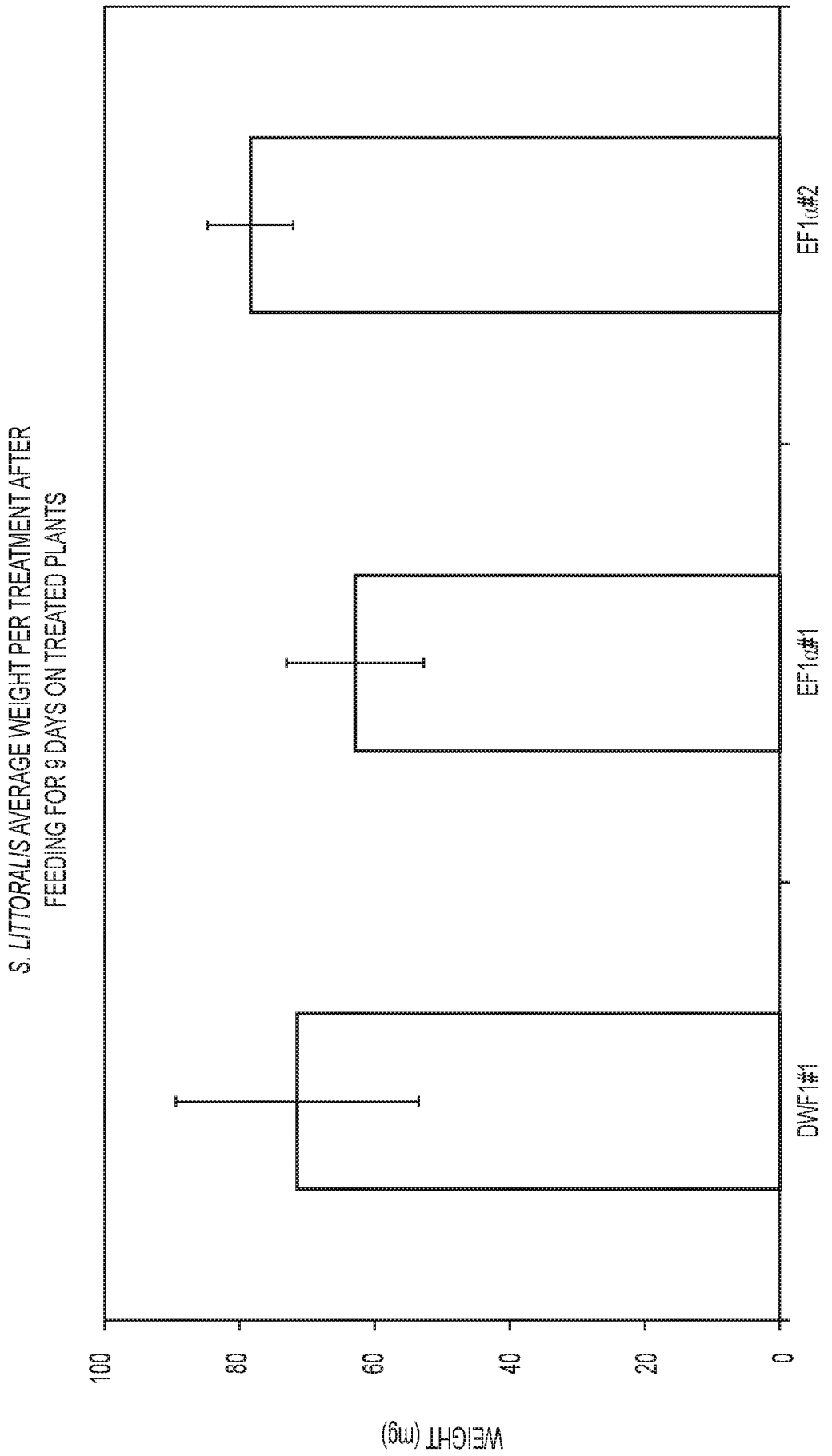

S. LITTORALIS AVERAGE WEIGHT PER TREATMENT AFTER FEEDING FOR 9 DAYS ON TREATED PLANTS
WEIGHT (mg)
100
80
60
40
20
0
DWF1#1
EF1α#1
EF1α#2

FIG. 17A

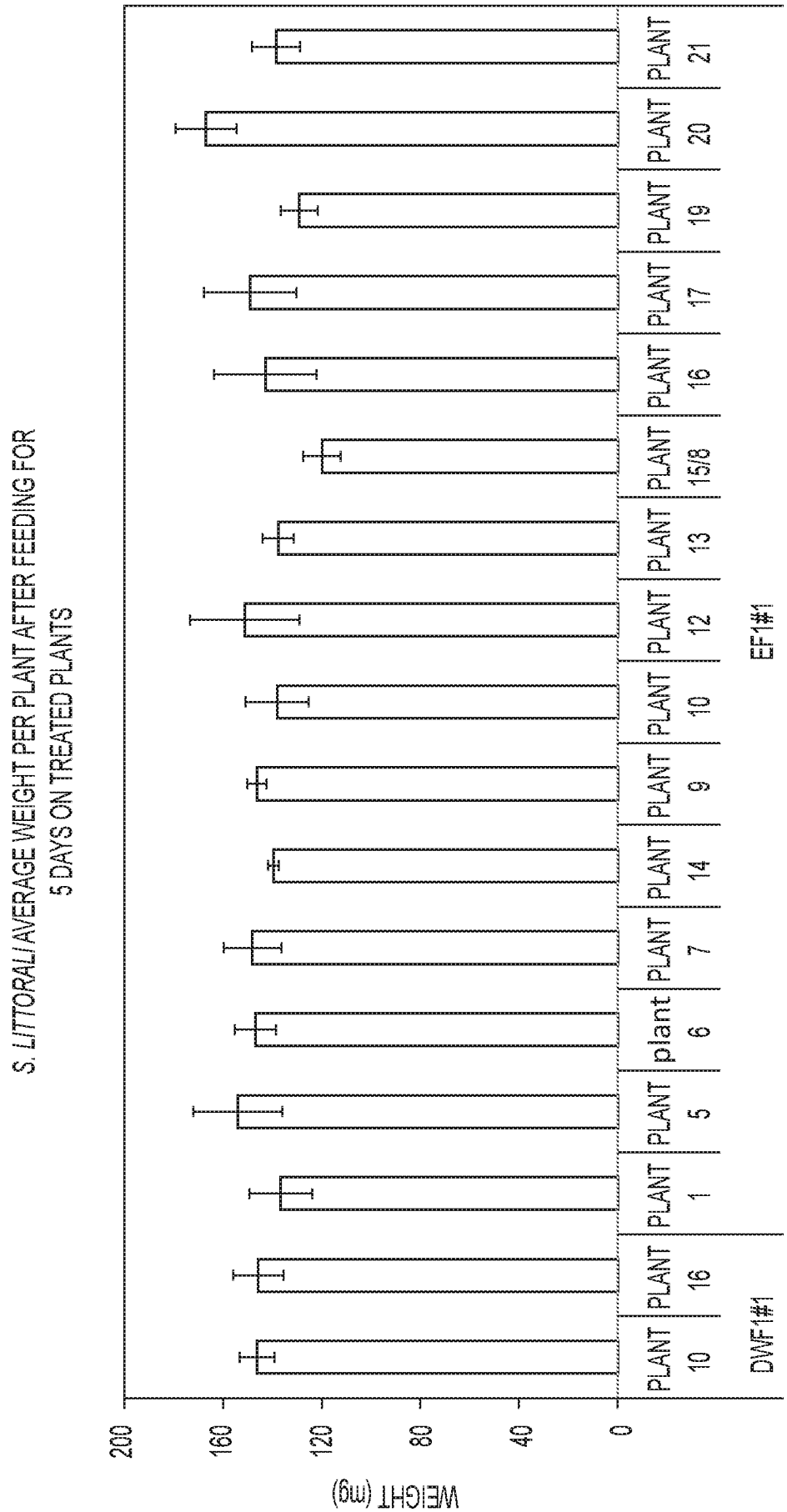

S. LITTORALI AVERAGE WEIGHT PER PLANT AFTER FEEDING FOR
5 DAYS ON TREATED PLANTS
WEIGHT (mg)
200
160
120
80
40
0
PLANT 10
PLANT 16
PLANT 1
PLANT 5
plant 6
PLANT 7
PLANT 14
PLANT 9
PLANT 10
PLANT 12
PLANT 13
PLANT 15/8
PLANT 16
PLANT 17
PLANT 19
PLANT 20
PLANT 21
DWF1#1
EF1#1

FIG. 17B

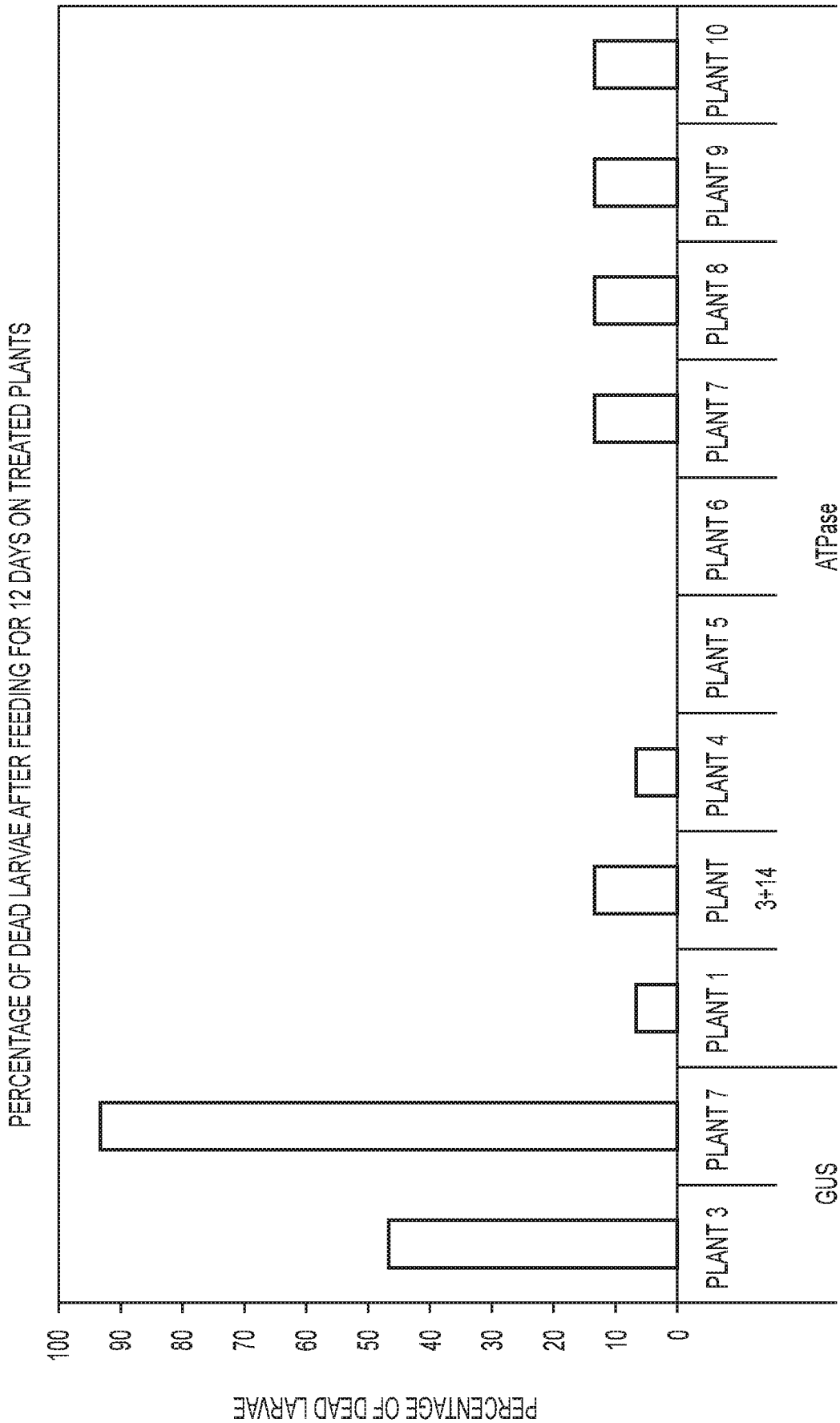

PERCENTAGE OF DEAD LARVAE AFTER FEEDING FOR 12 DAYS ON TREATED PLANTS
PERCENTAGE OF DEAD LARVAE
100
90
80
70
60
50
40
30
20
10
0
PLANT 3
PLANT 7
PLANT 1
PLANT 3+14
PLANT 4
PLANT 5
PLANT 6
PLANT 7
PLANT 8
PLANT 9
PLANT 10
GUS
ATPase

FIG. 18A

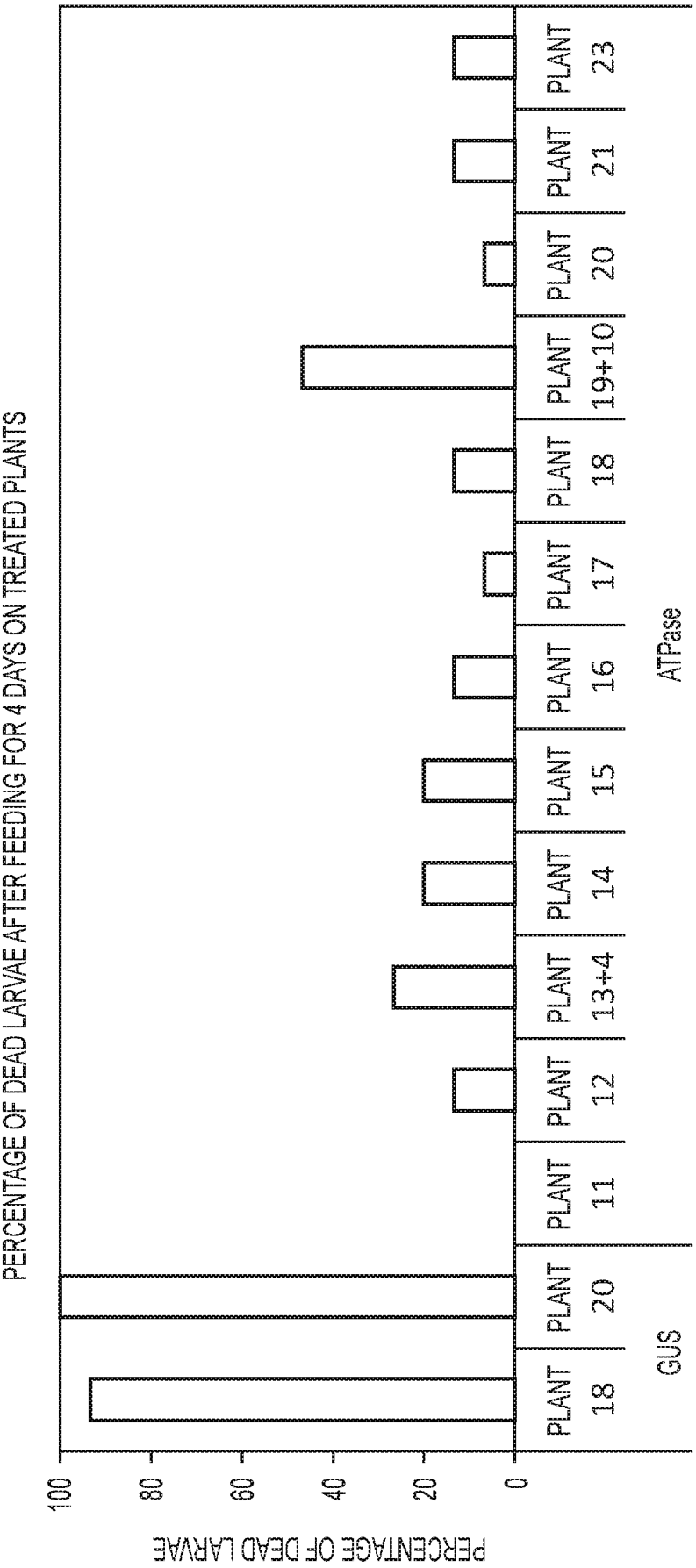

PERCENTAGE OF DEAD LARVAE AFTER FEEDING FOR 4 DAYS ON TREATED PLANTS
PERCENTAGE OF DEAD LARVAE
100
80
60
40
20
0
GUS
PLANT 18
PLANT 20
PLANT 11
PLANT 12
PLANT 13+4
PLANT 14
PLANT 15
PLANT 16
PLANT 17
PLANT 18
PLANT 19+10
PLANT 20
PLANT 21
PLANT 23
ATPase

FIG. 18B

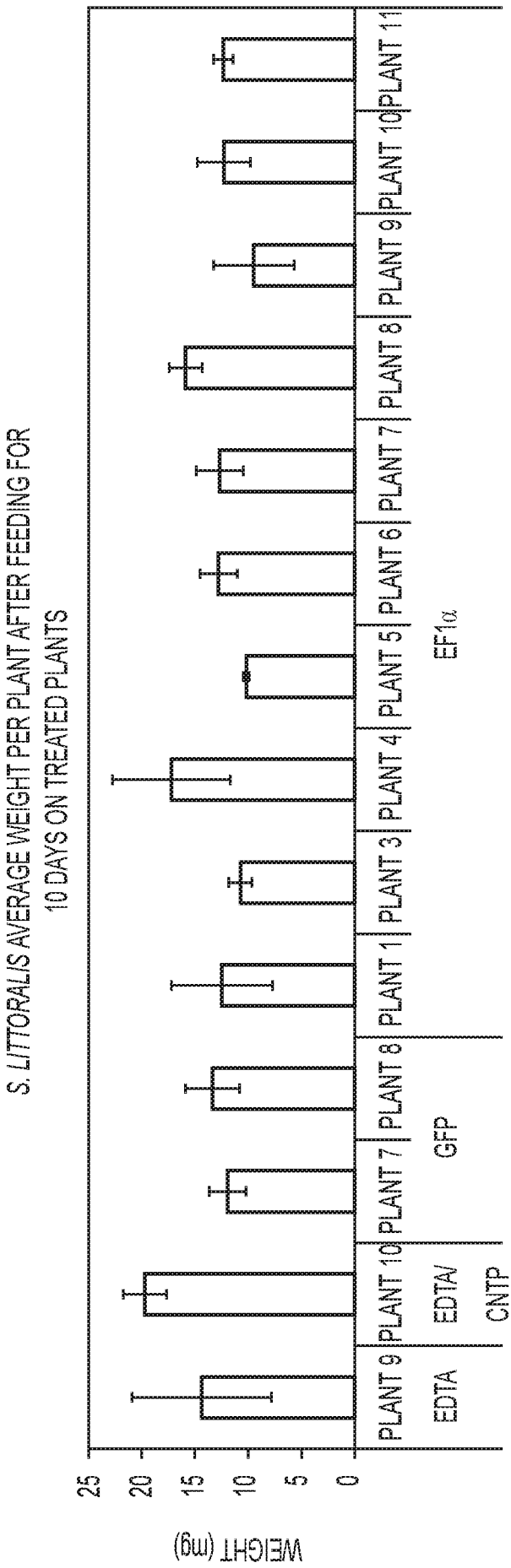

S. LITTORALIS AVERAGE WEIGHT PER PLANT AFTER FEEDING FOR 10 DAYS ON TREATED PLANTS
WEIGHT (mg)
0
5
10
15
20
25
PLANT 9
PLANT 10
PLANT 7
PLANT 8
PLANT 1
PLANT 3
PLANT 4
PLANT 5
PLANT 6
PLANT 7
PLANT 8
PLANT 9
PLANT 10
PLANT 11
EDTA
EDTA/ CNTP
GFP
EF1α

FIG. 19A

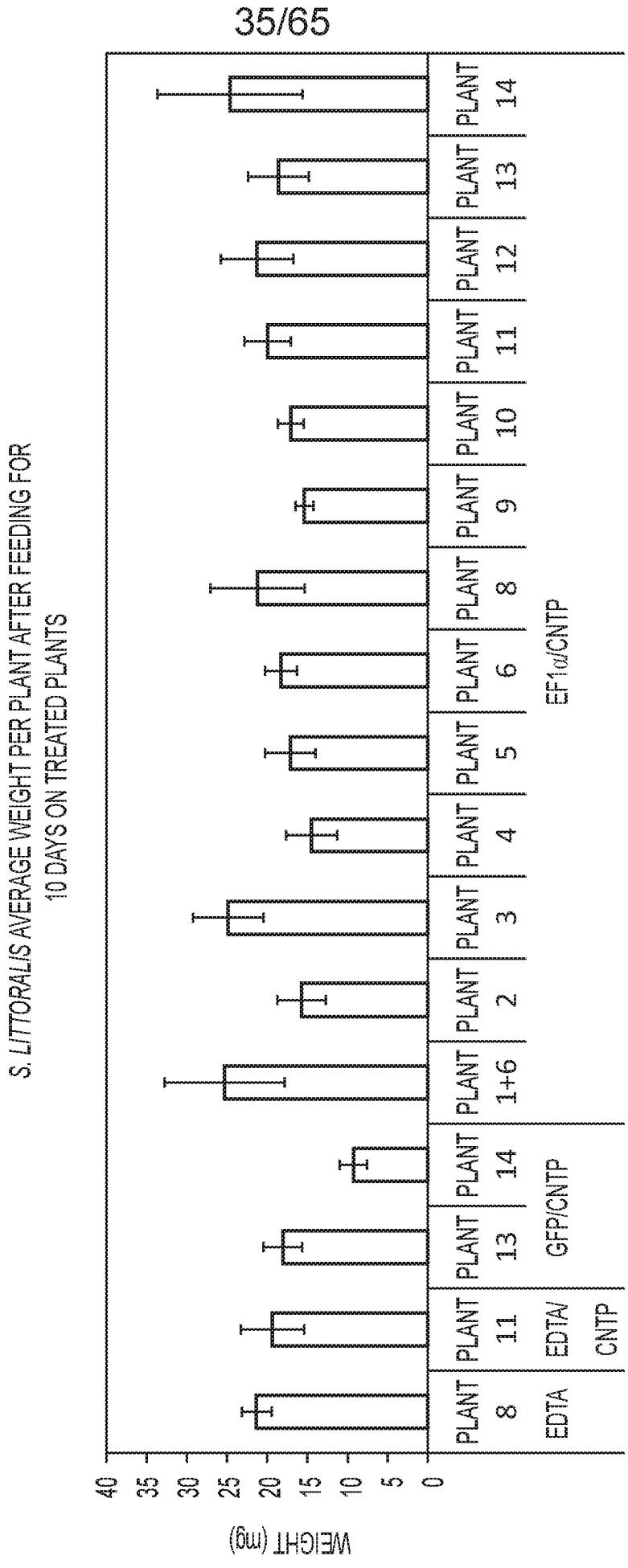

S. LITTORALIS AVERAGE WEIGHT PER PLANT AFTER FEEDING FOR 10 DAYS ON TREATED PLANTS
WEIGHT (mg)
40
35
30
25
20
15
10
5
0
PLANT 8
PLANT 11
PLANT 13
PLANT 14
PLANT 1+6
PLANT 2
PLANT 3
PLANT 4
PLANT 5
PLANT 6
PLANT 8
PLANT 9
PLANT 10
PLANT 11
PLANT 12
PLANT 13
PLANT 14
EDTA
EDTA/ CNTP
GFP/CNTP
EF1α/CNTP

FIG. 19B

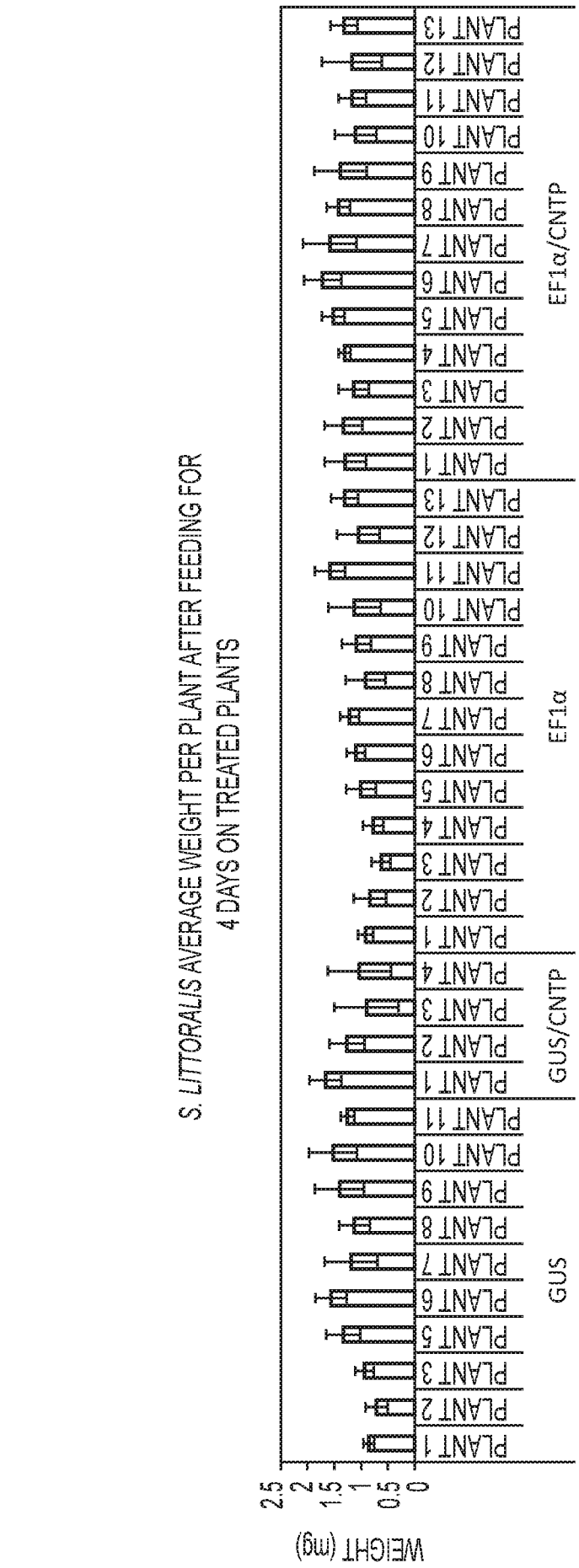

S. LITTORALIS AVERAGE WEIGHT PER PLANT AFTER FEEDING FOR 4 DAYS ON TREATED PLANTS
WEIGHT (mg)
2.5
2
1.5
1
0.5
0
GUS
GUS/CNTP
EF1α
EF1α/CNTP
PLANT 1
PLANT 2
PLANT 3
PLANT 4
PLANT 5
PLANT 6
PLANT 7
PLANT 8
PLANT 9
PLANT 10
PLANT 11
PLANT 12
PLANT 13

FIG. 20A

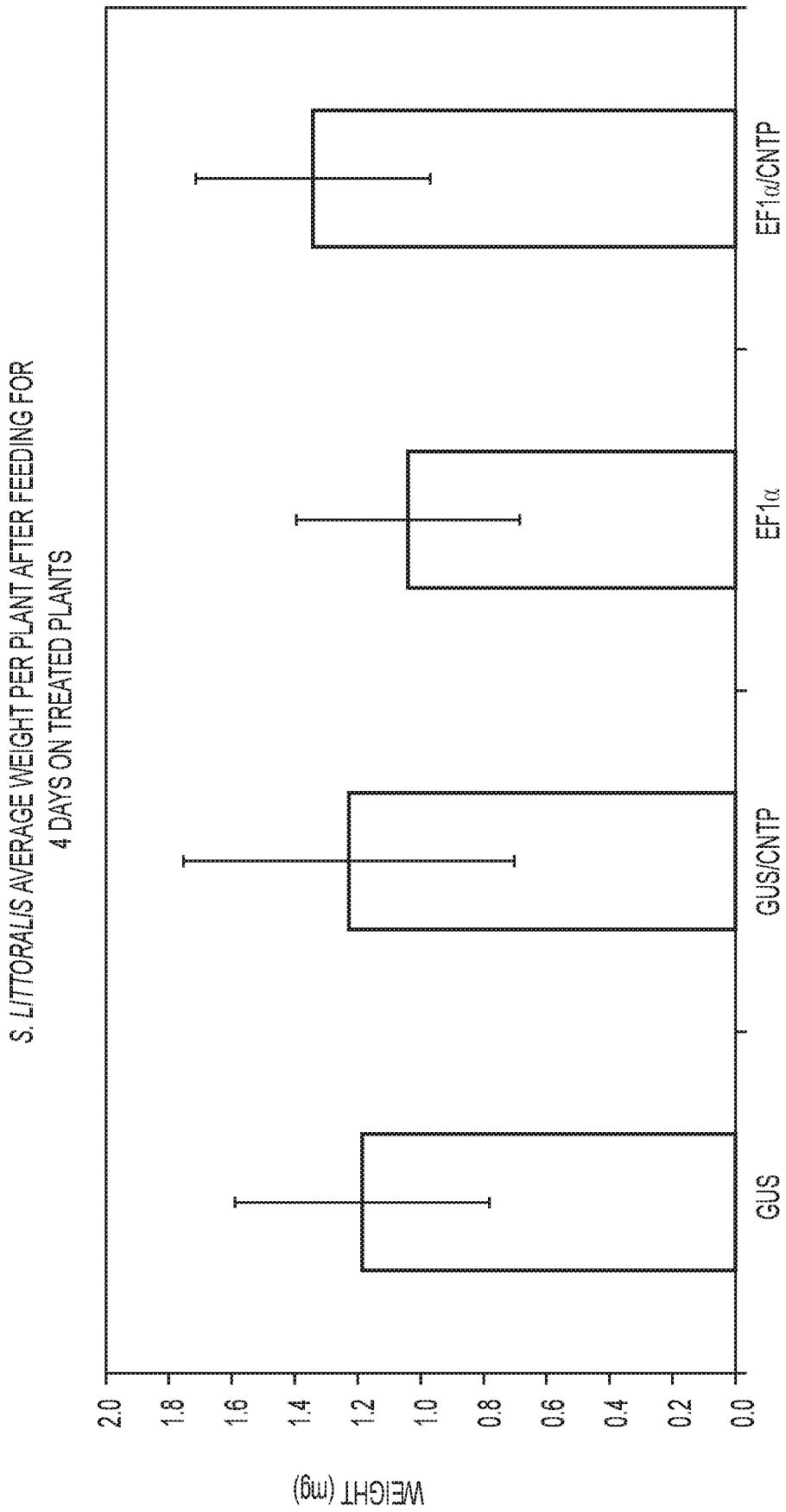

S. LITTORALIS AVERAGE WEIGHT PER PLANT AFTER FEEDING FOR 4 DAYS ON TREATED PLANTS
WEIGHT (mg)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
GUS
GUS/CNTP
EF1α
EF1α/CNTP

FIG. 20B

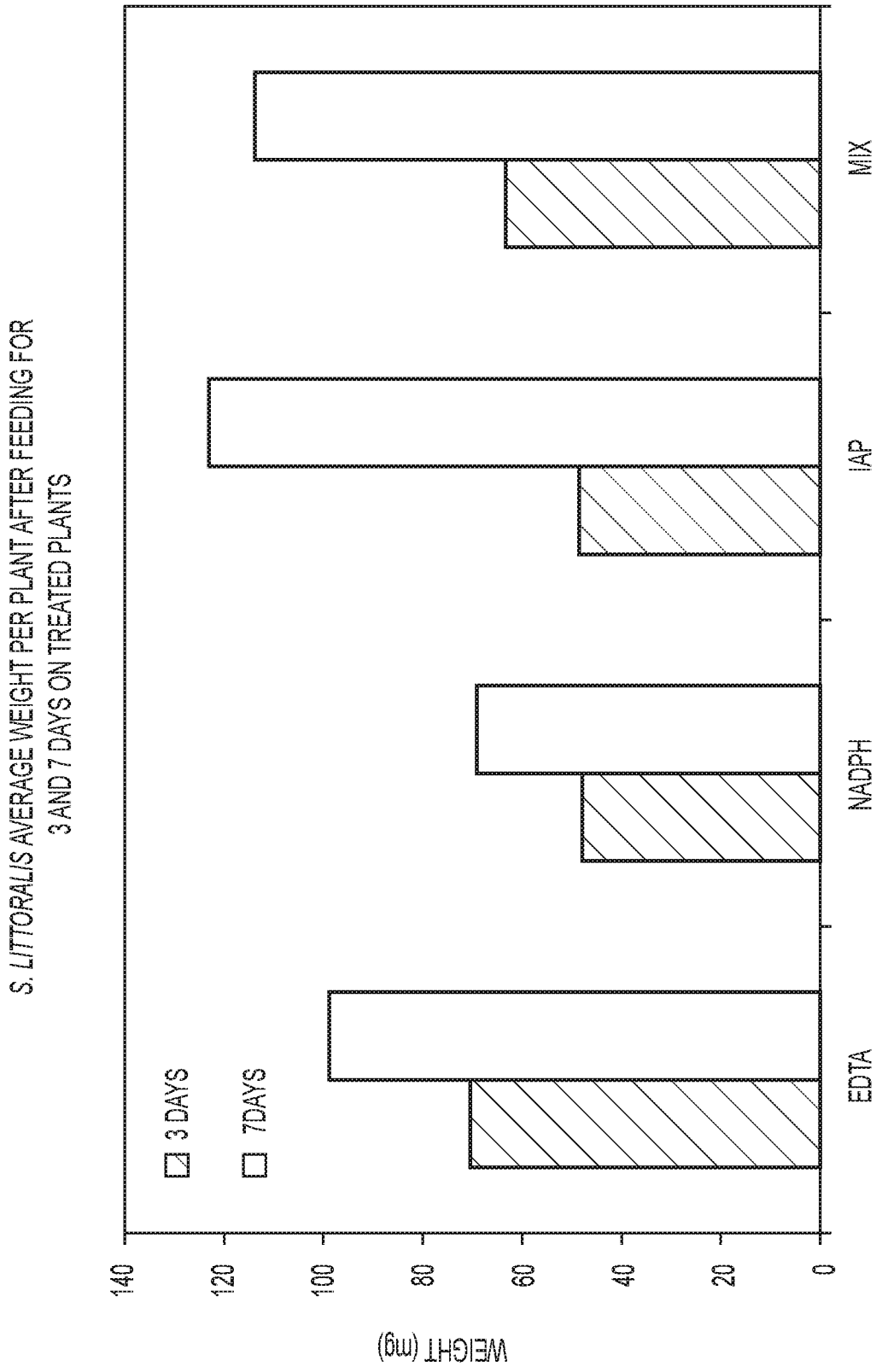

S. LITTORALIS AVERAGE WEIGHT PER PLANT AFTER FEEDING FOR
3 AND 7 DAYS ON TREATED PLANTS
WEIGHT (mg)
0
20
40
60
80
100
120
140
3 DAYS
7DAYS
EDTA
NADPH
IAP
MIX

FIG. 21

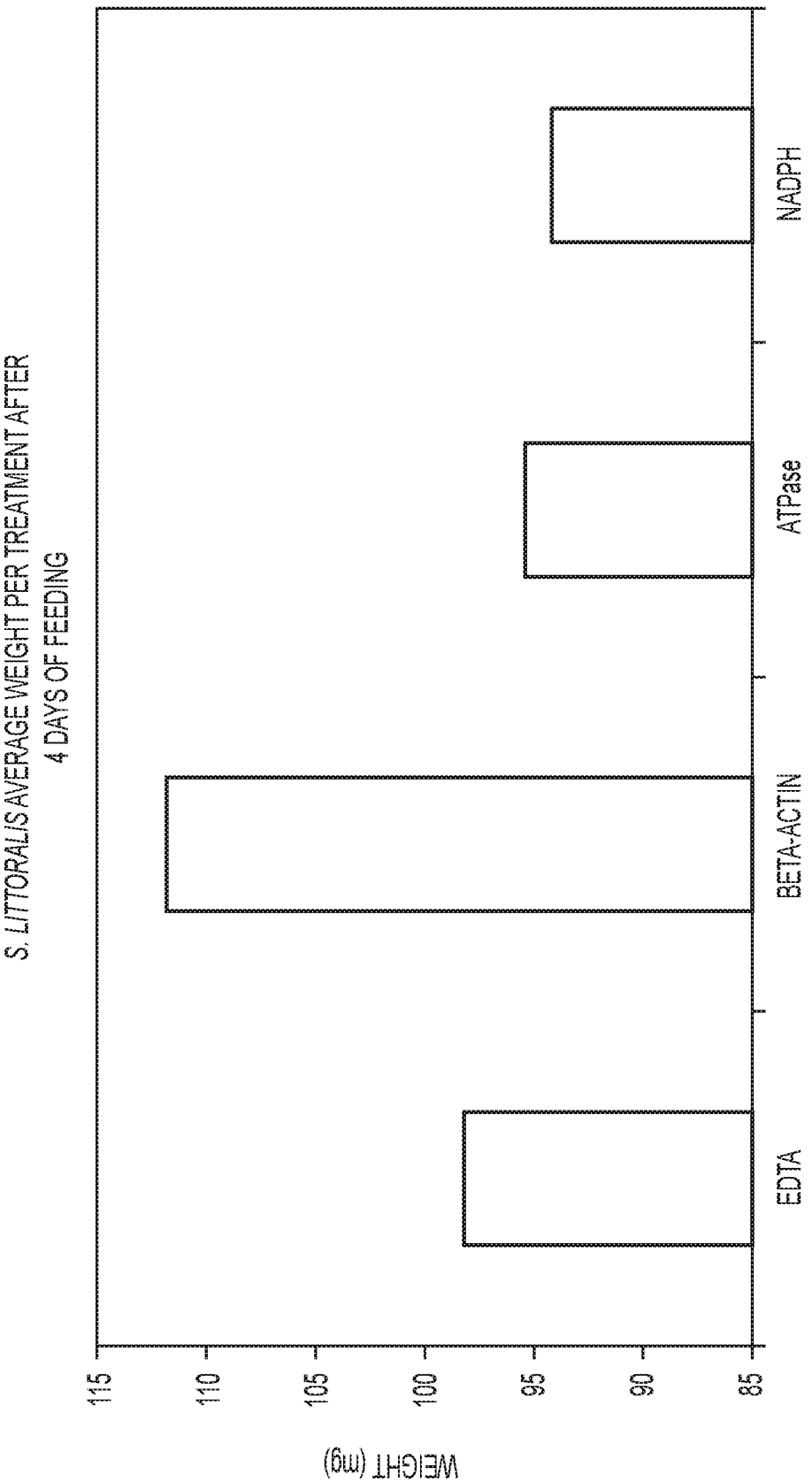

S. LITTORALIS AVERAGE WEIGHT PER TREATMENT AFTER
4 DAYS OF FEEDING
WEIGHT (mg)
115
110
105
100
95
90
85
EDTA
BETA-ACTIN
ATPase
NADPH

FIG. 22

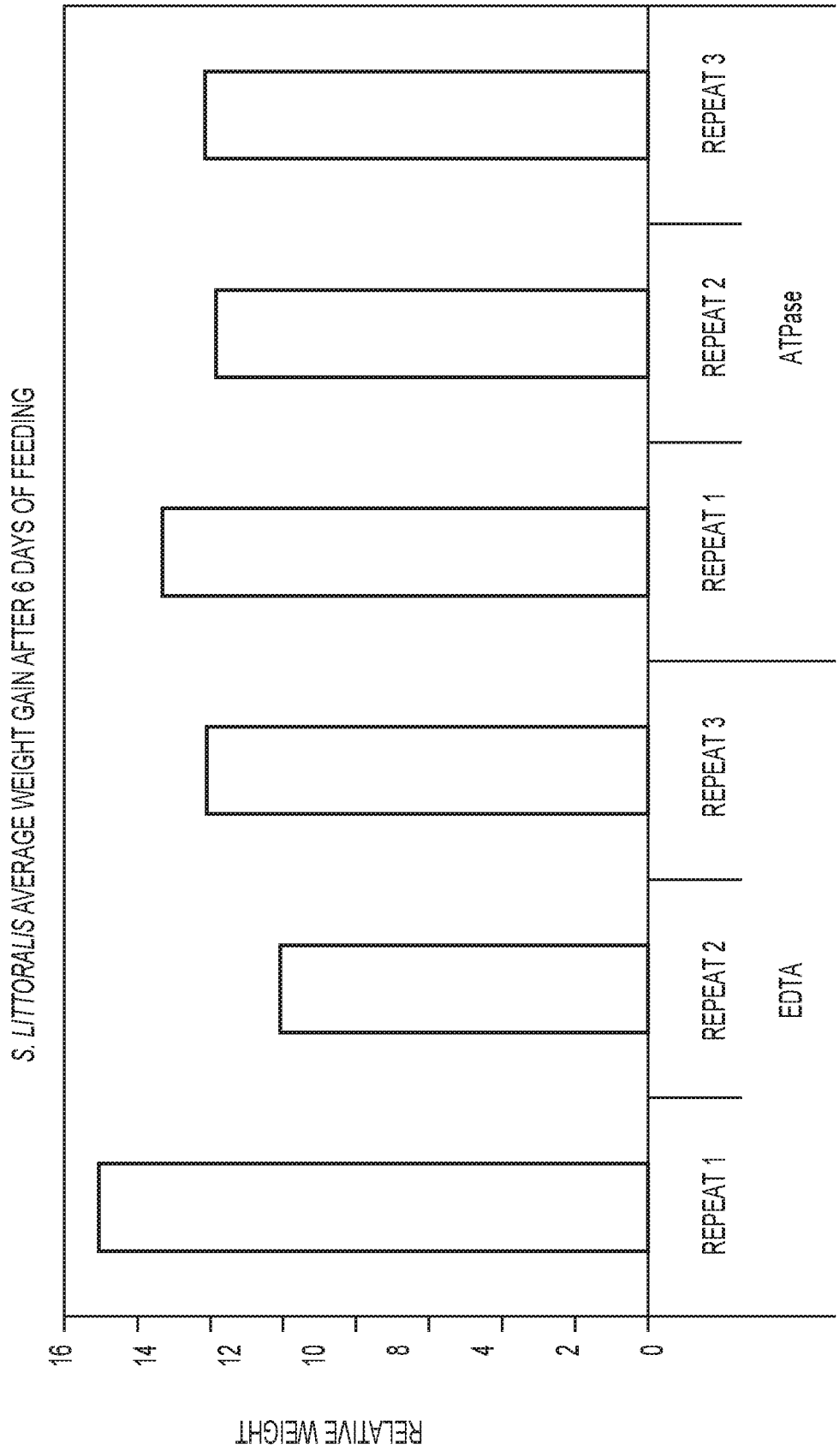

S. LITTORALIS AVERAGE WEIGHT GAIN AFTER 6 DAYS OF FEEDING
RELATIVE WEIGHT
16
14
12
10
8
4
2
0
REPEAT 1
REPEAT 2
REPEAT 3
EDTA
REPEAT 1
REPEAT 2
REPEAT 3
ATPase

FIG. 23A

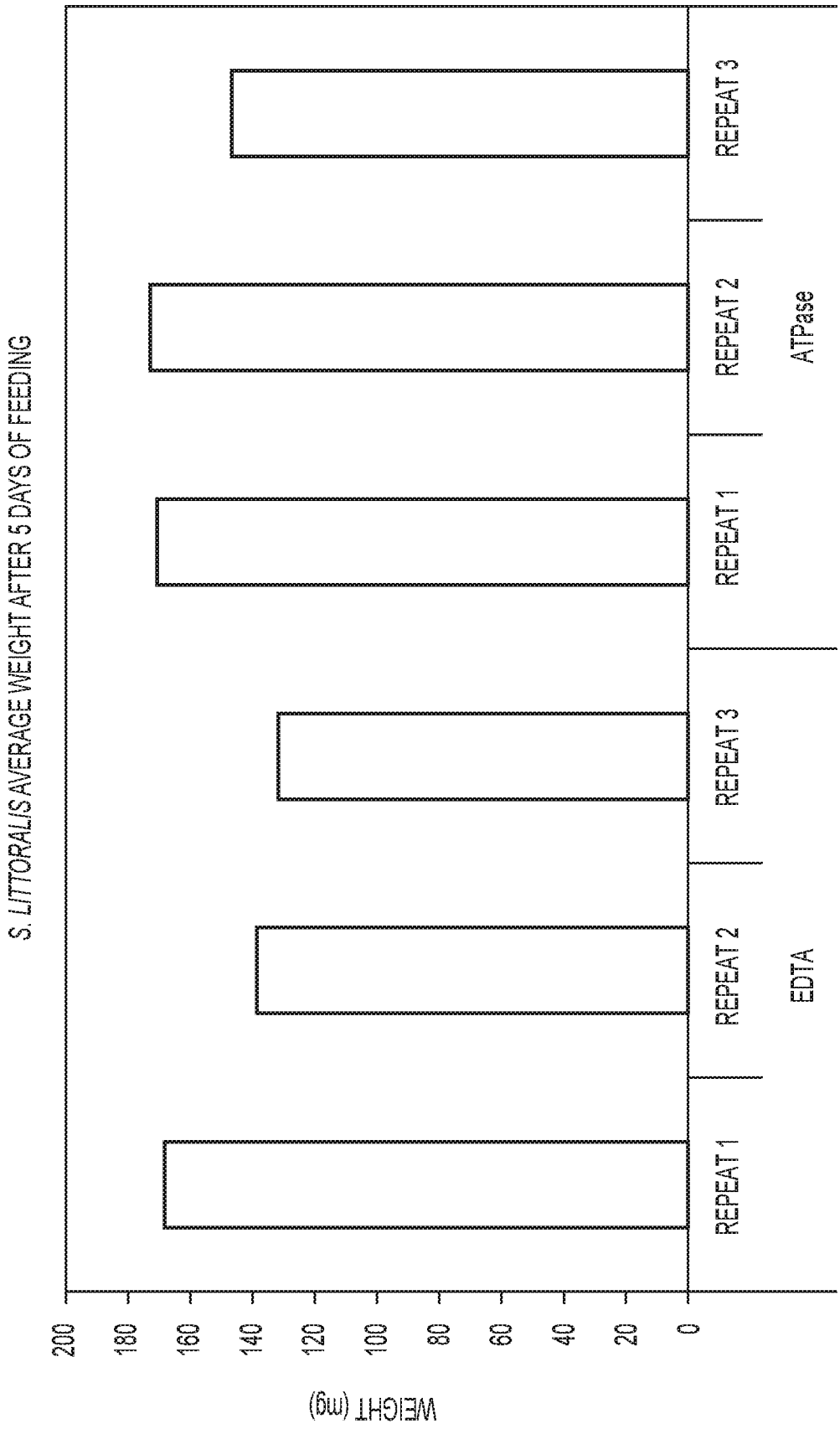

S. LITTORALIS AVERAGE WEIGHT AFTER 5 DAYS OF FEEDING
WEIGHT (mg)
200
180
160
140
120
100
80
60
40
20
0
REPEAT 1
REPEAT 2
REPEAT 3
EDTA
REPEAT 1
REPEAT 2
REPEAT 3
ATPase

FIG. 23B

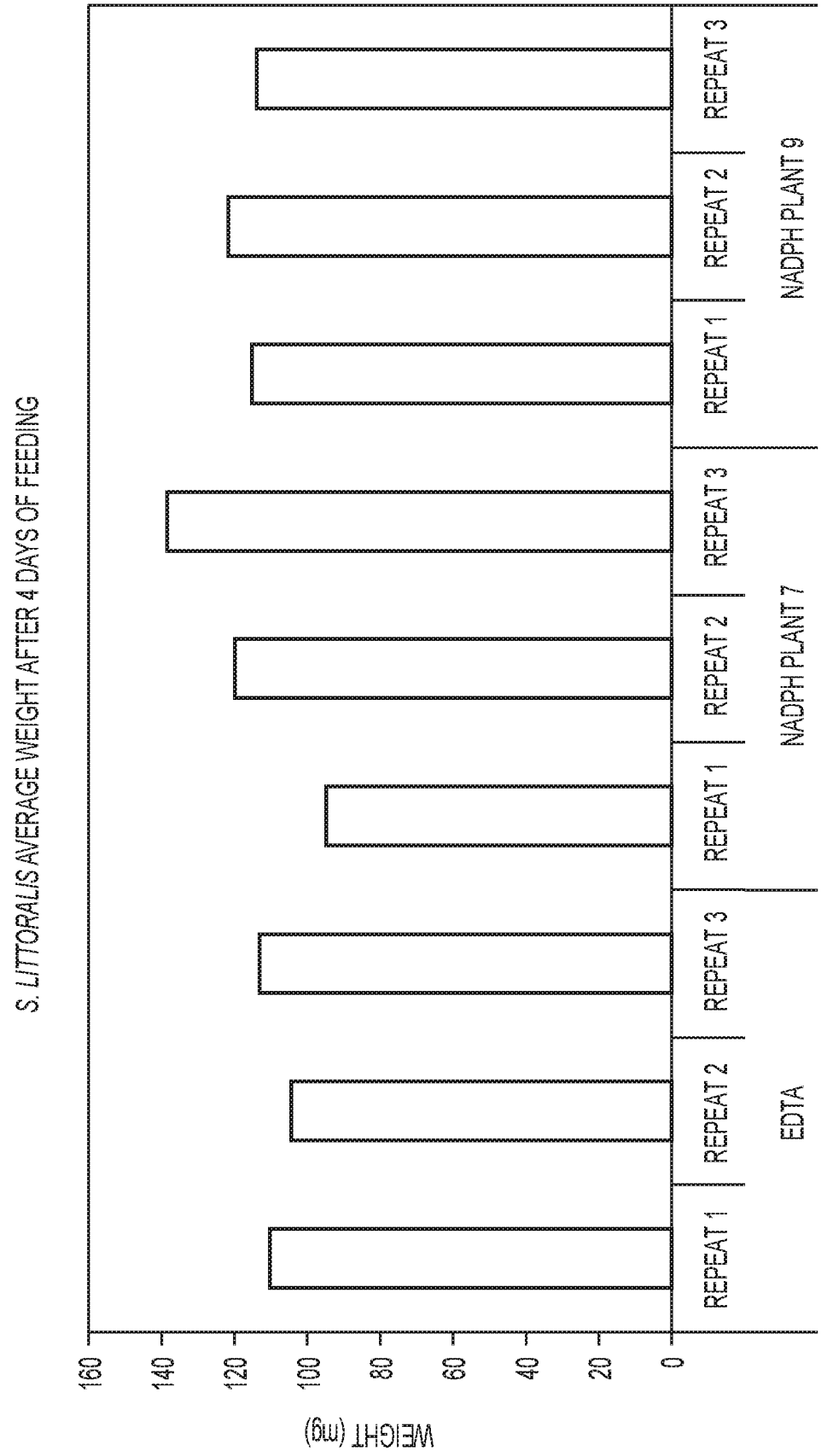

S. LITTORALIS AVERAGE WEIGHT AFTER 4 DAYS OF FEEDING
WEIGHT (mg)
160
140
120
100
80
60
40
20
0
REPEAT 1
REPEAT 2
REPEAT 3
EDTA
REPEAT 1
REPEAT 2
REPEAT 3
NADPH PLANT 7
REPEAT 1
REPEAT 2
REPEAT 3
NADPH PLANT 9

FIG. 24A

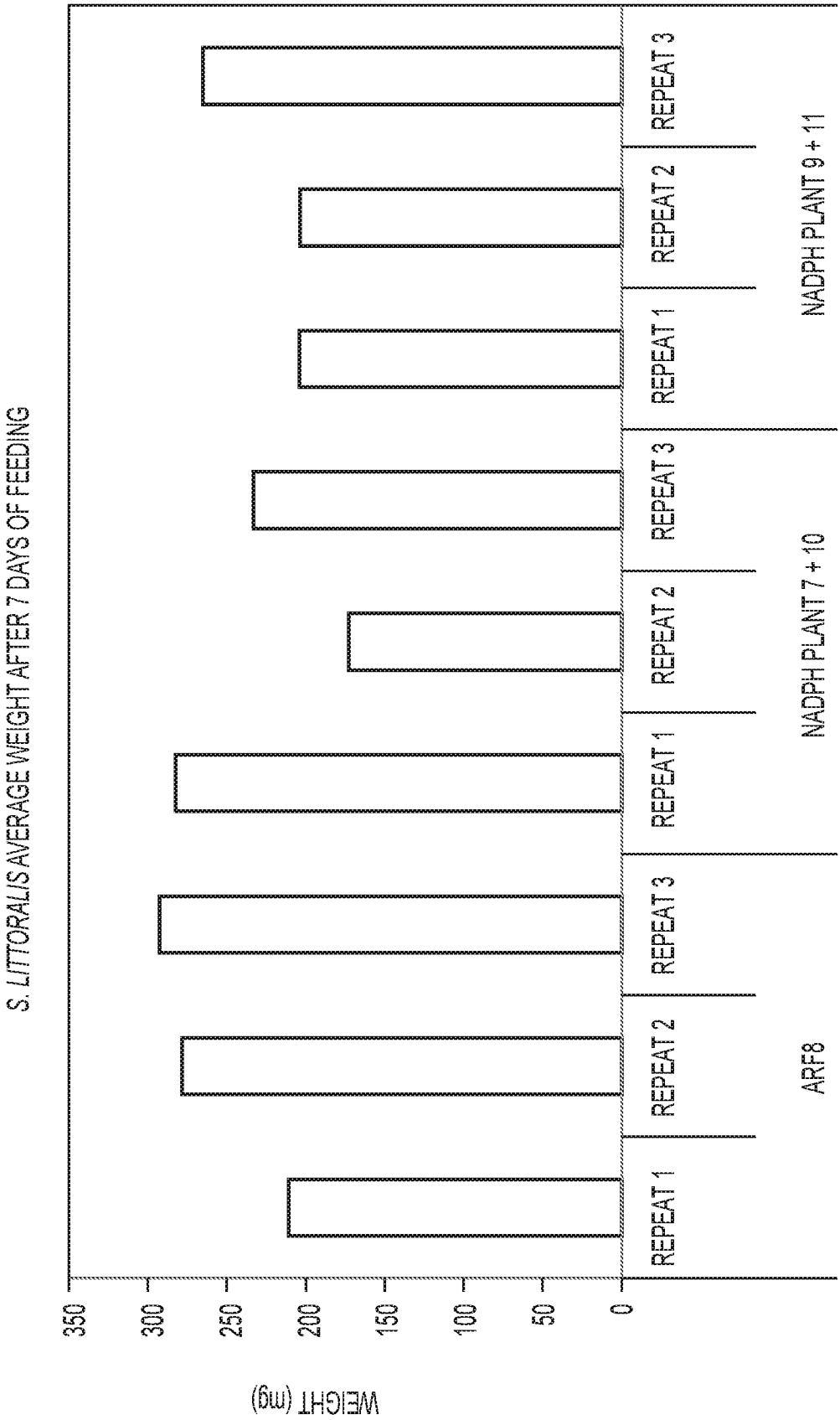

S. LITTORALIS AVERAGE WEIGHT AFTER 7 DAYS OF FEEDING
WEIGHT (mg)
350
300
250
200
150
100
50
0
REPEAT 1
REPEAT 2
REPEAT 3
ARF8
REPEAT 1
REPEAT 2
REPEAT 3
NADPH PLANT 7 + 10
REPEAT 1
REPEAT 2
REPEAT 3
NADPH PLANT 9 + 11

FIG. 24B

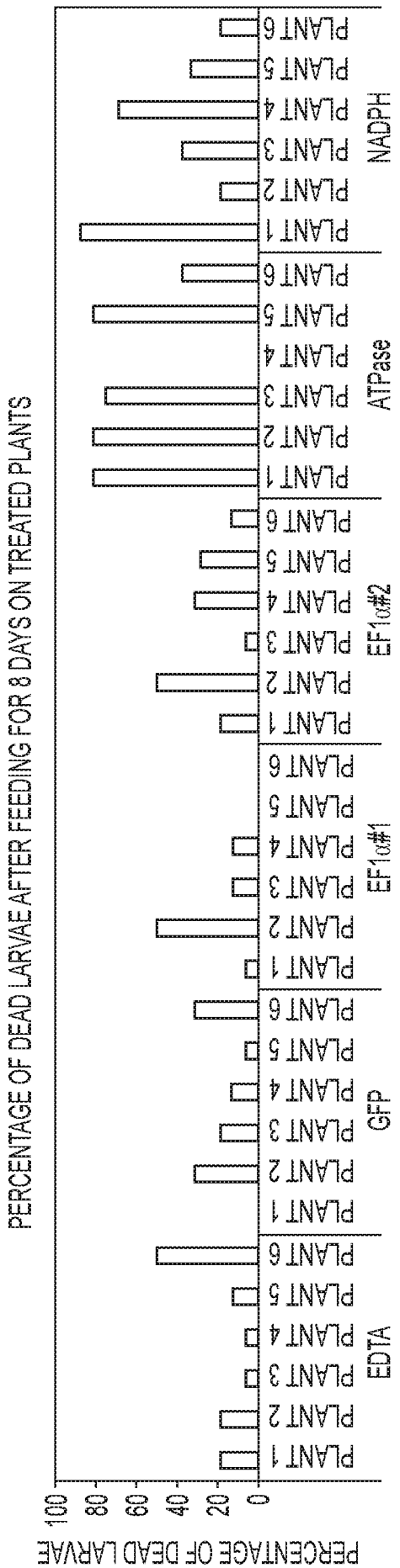

PERCENTAGE OF DEAD LARVAE AFTER FEEDING FOR 8 DAYS ON TREATED PLANTS
PERCENTAGE OF DEAD LARVAE
100
80
60
40
20
0
EDTA
GFP
EF1α#1
EF1α#2
ATPase
NADPH
PLANT 1
PLANT 2
PLANT 3
PLANT 4
PLANT 5
PLANT 6

FIG. 25A

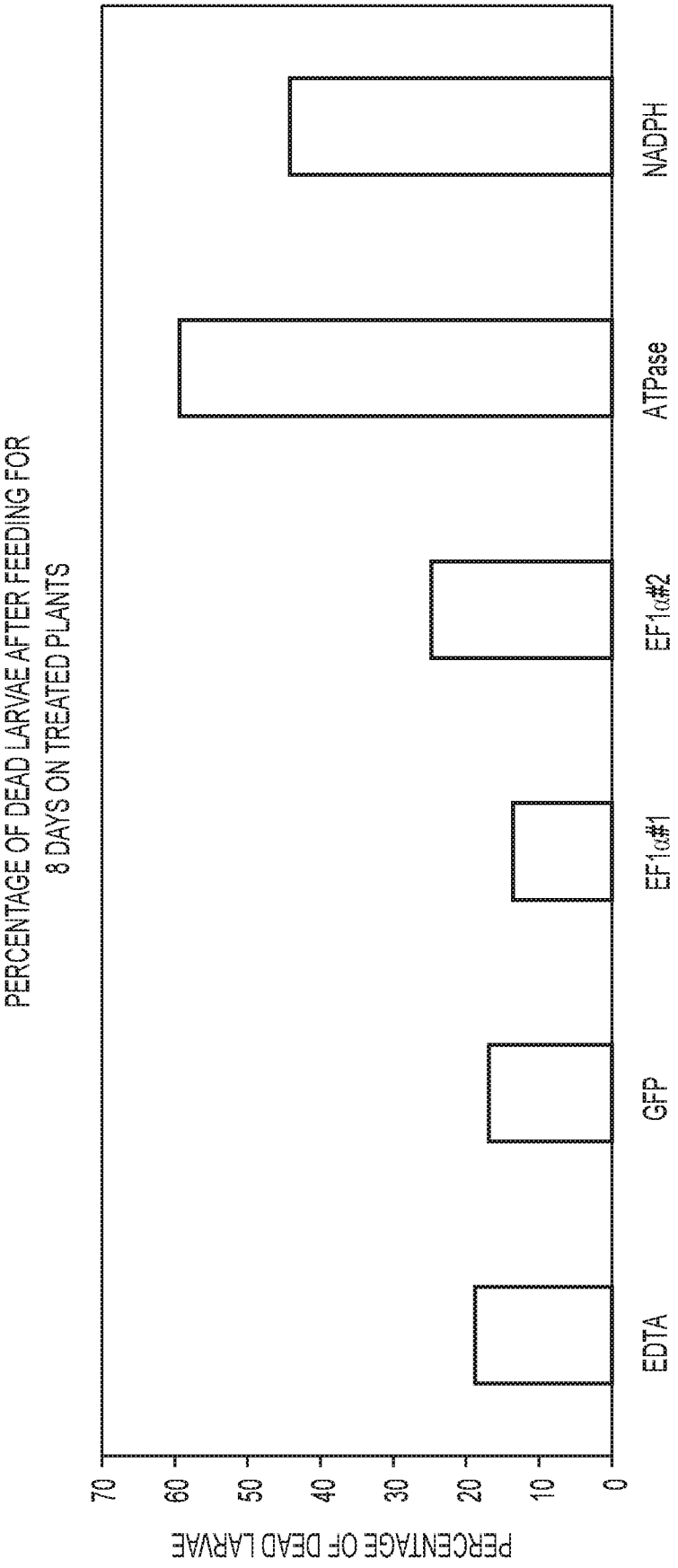

PERCENTAGE OF DEAD LARVAE AFTER FEEDING FOR 8 DAYS ON TREATED PLANTS
PERCENTAGE OF DEAD LARVAE
70
60
50
40
30
20
10
0
EDTA
GFP
EF1α#1
EF1α#2
ATPase
NADPH

FIG. 25B

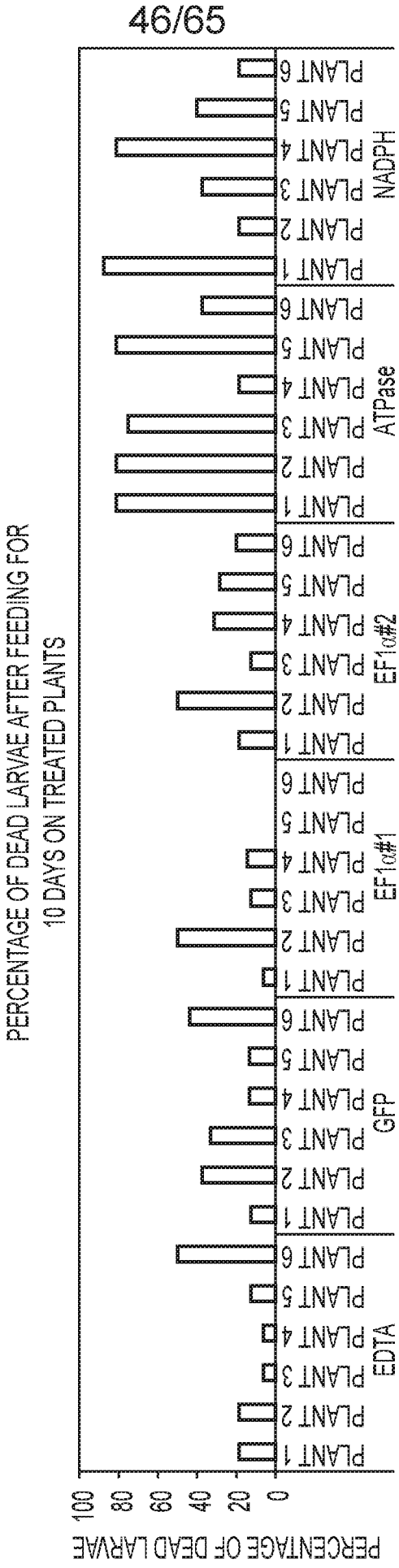

PERCENTAGE OF DEAD LARVAE AFTER FEEDING FOR
10 DAYS ON TREATED PLANTS
PERCENTAGE OF DEAD LARVAE
100
80
60
40
20
0
EDTA
GFP
EF1α#1
EF1α#2
ATPase
NADPH
PLANT 1
PLANT 2
PLANT 3
PLANT 4
PLANT 5
PLANT 6

FIG. 25C

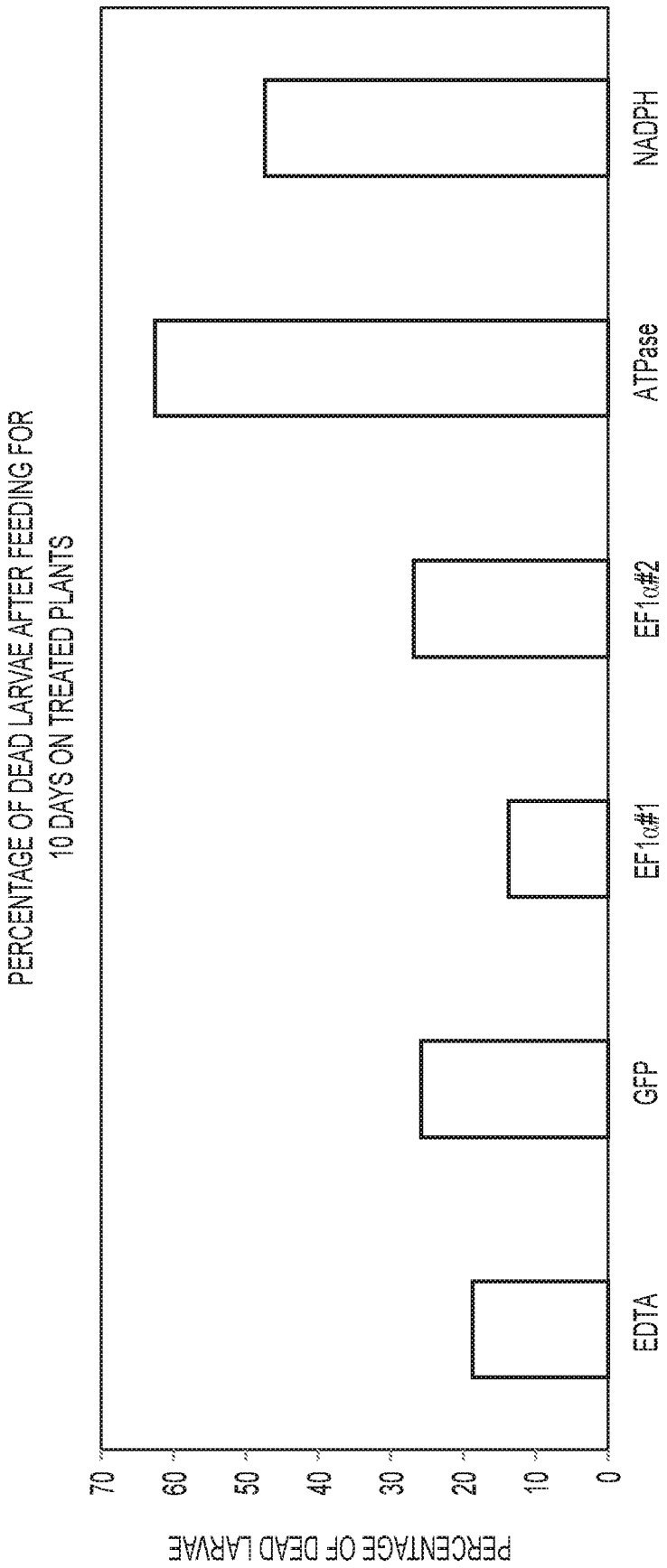

PERCENTAGE OF DEAD LARVAE AFTER FEEDING FOR
10 DAYS ON TREATED PLANTS
PERCENTAGE OF DEAD LARVAE
70
60
50
40
30
20
10
0
EDTA
GFP
EF1α#1
EF1α#2
ATPase
NADPH

FIG. 25D

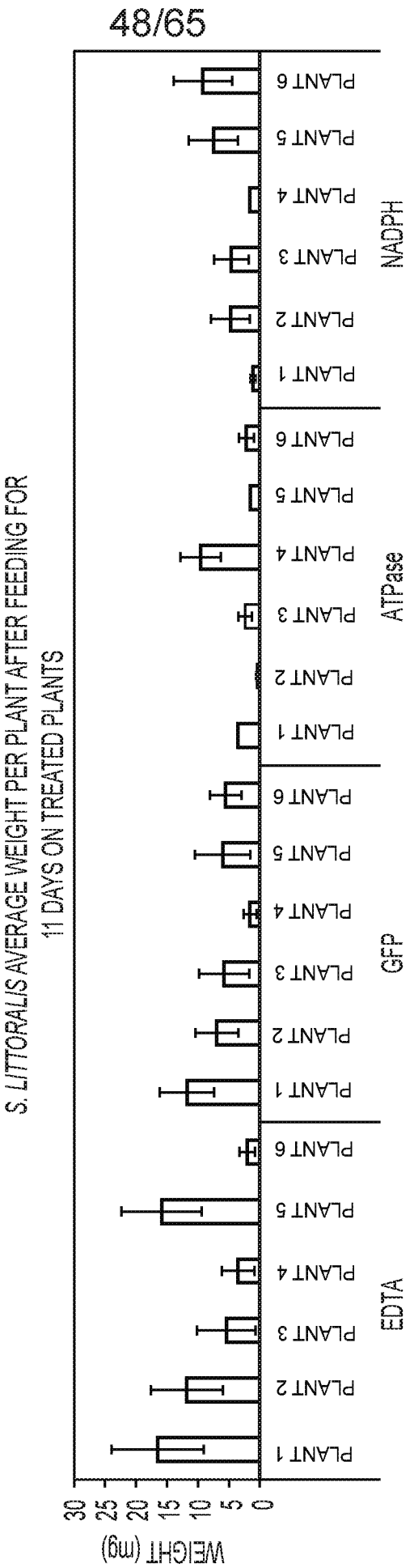

S. LITTORALIS AVERAGE WEIGHT PER PLANT AFTER FEEDING FOR 11 DAYS ON TREATED PLANTS
WEIGHT (mg)
30
25
20
15
10
5
0
EDTA
GFP
ATPase
NADPH
PLANT 1
PLANT 2
PLANT 3
PLANT 4
PLANT 5
PLANT 6

FIG. 25E

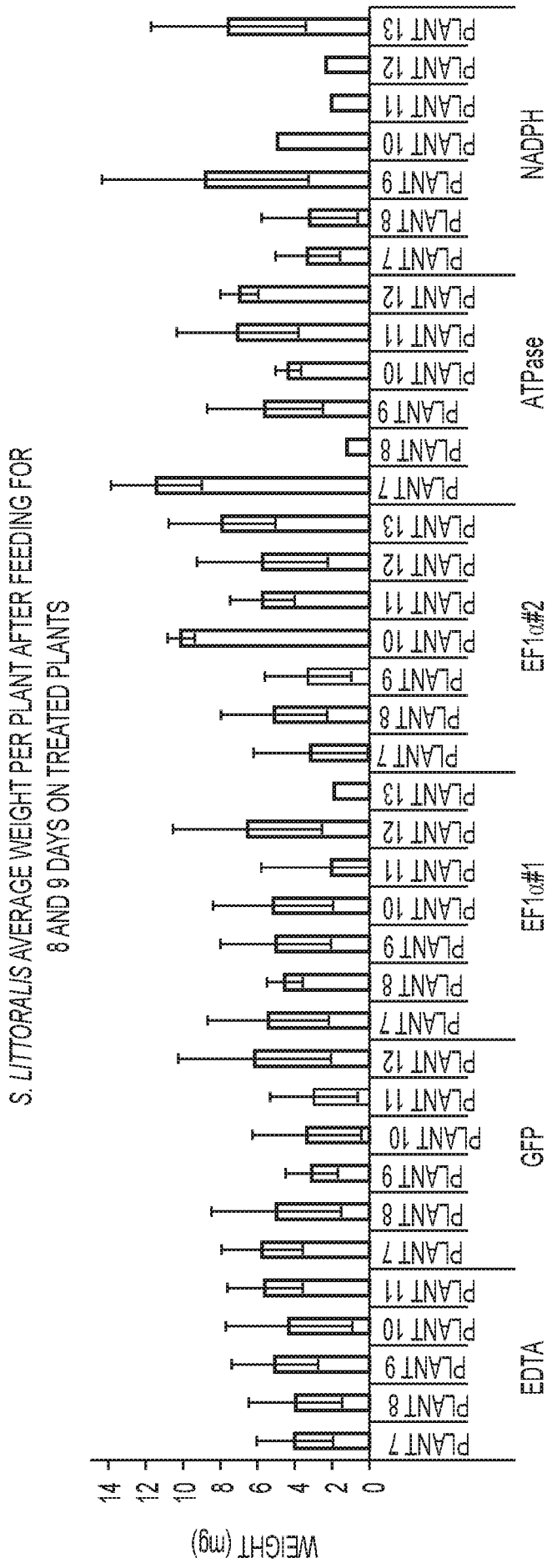

S. LITTORALIS AVERAGE WEIGHT PER PLANT AFTER FEEDING FOR 8 AND 9 DAYS ON TREATED PLANTS
WEIGHT (mg)
0
2
4
6
8
10
12
14
EDTA
GFP
EF1α#1
EF1α#2
ATPase
NADPH
PLANT 7
PLANT 8
PLANT 9
PLANT 10
PLANT 11
PLANT 12
PLANT 13

FIG. 25F

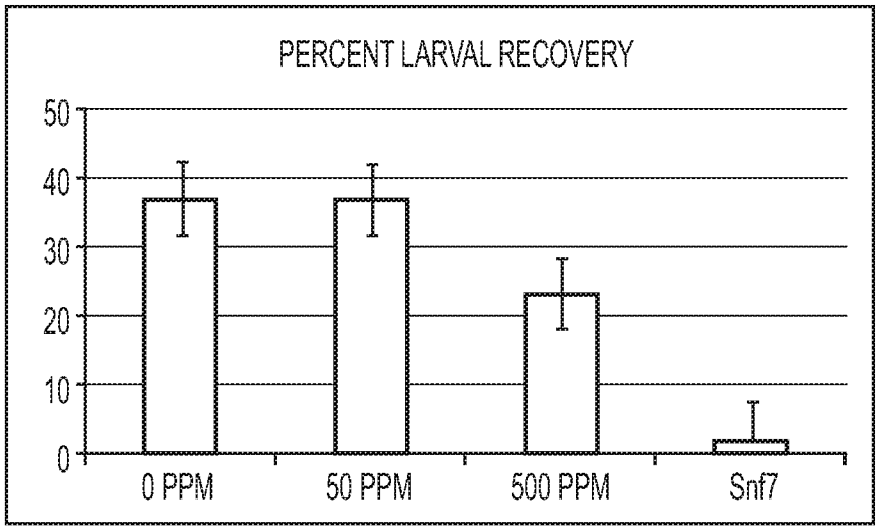


FIG. 26A

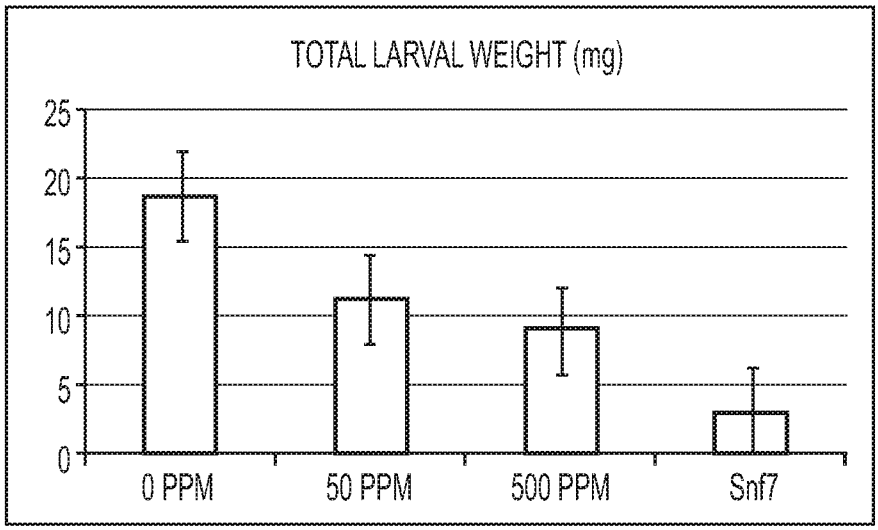


FIG. 26B

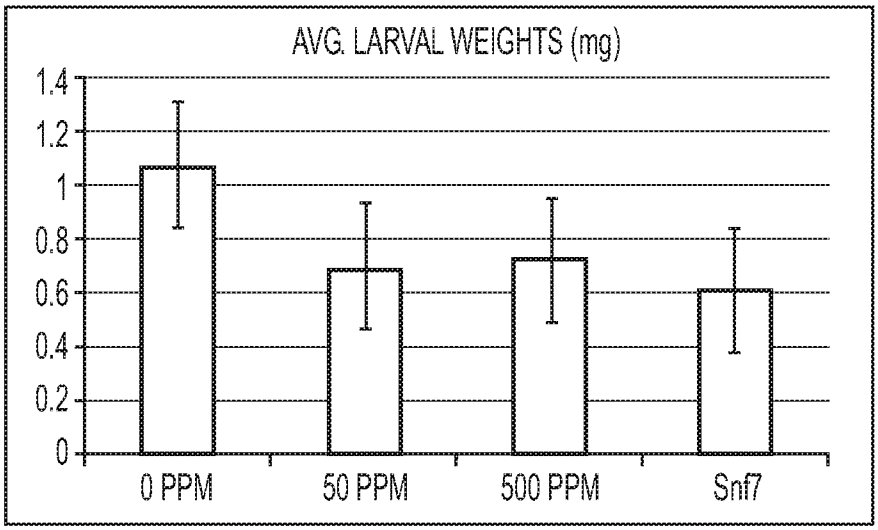


FIG. 26C

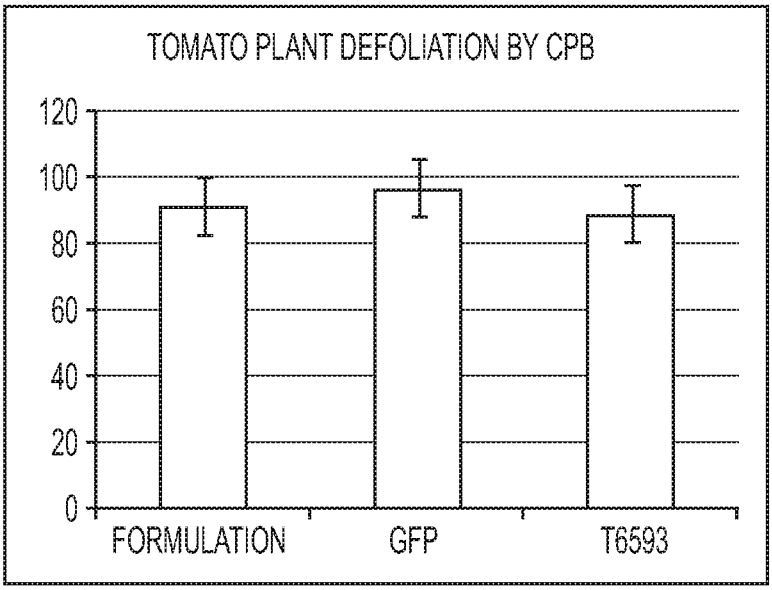


FIG. 27A

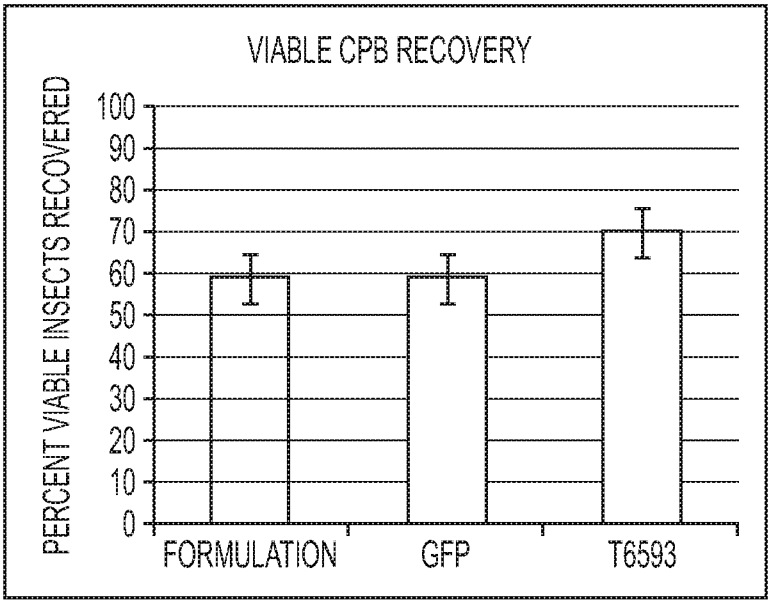


FIG. 27B

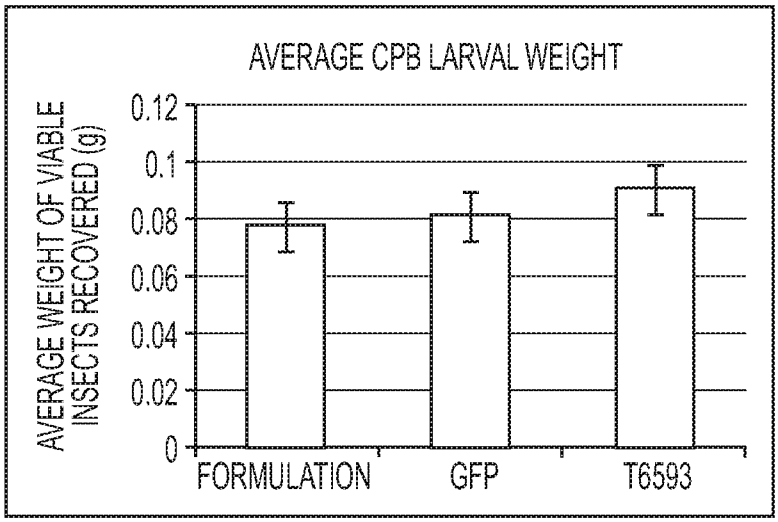


FIG. 27C

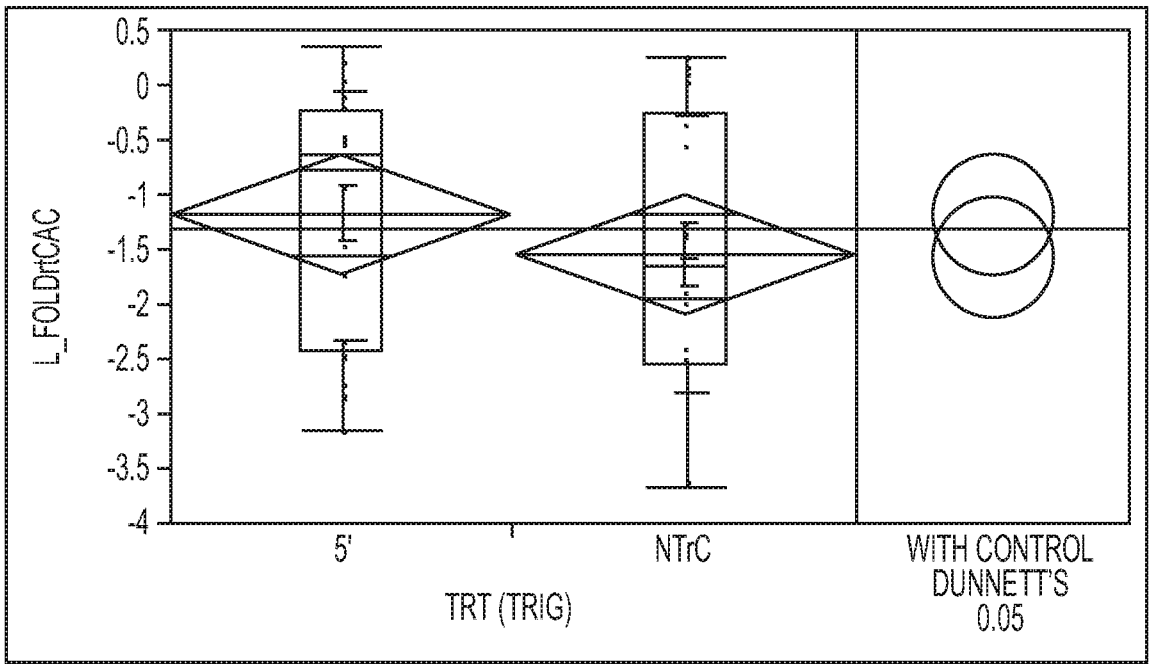


FIG. 28A

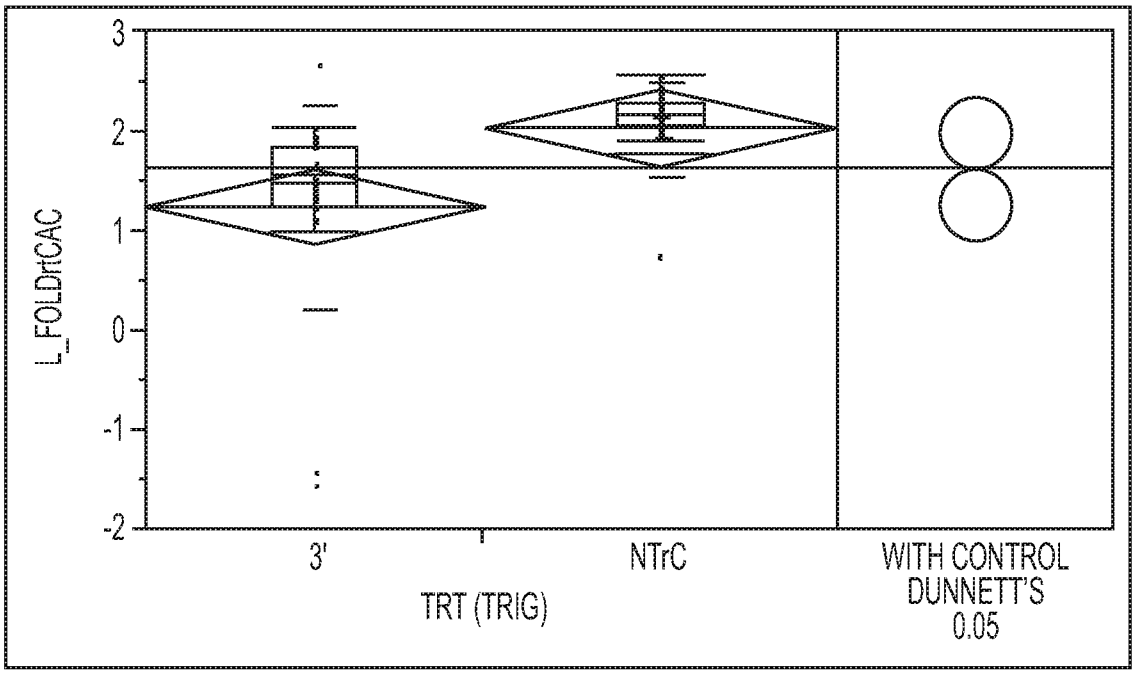


FIG. 28B

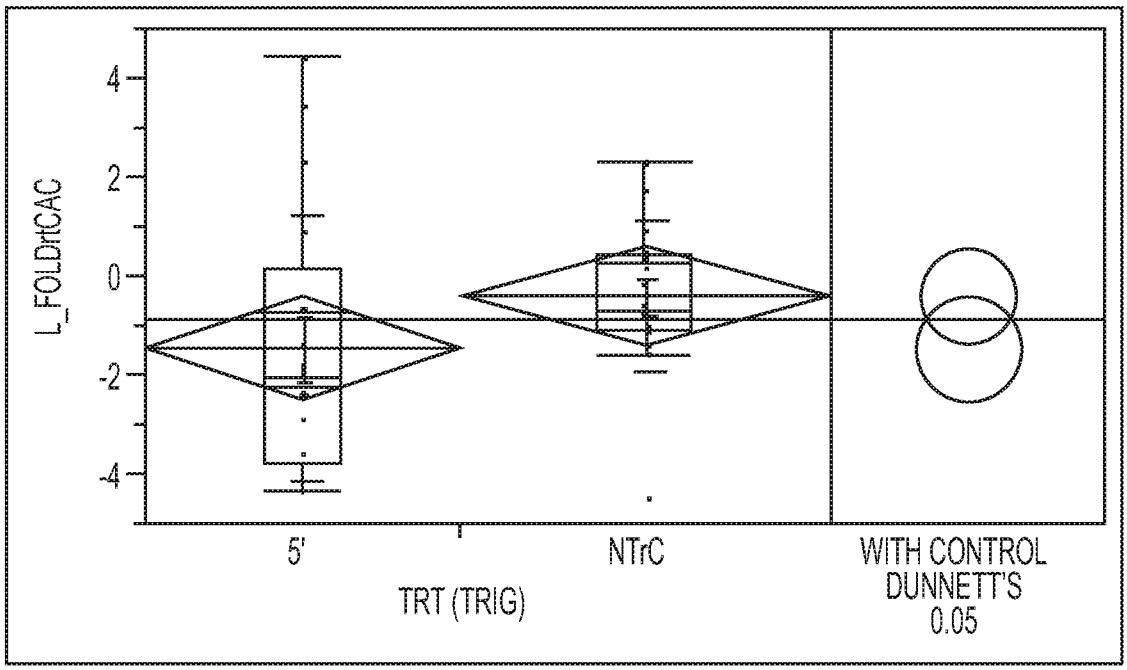


FIG. 29A

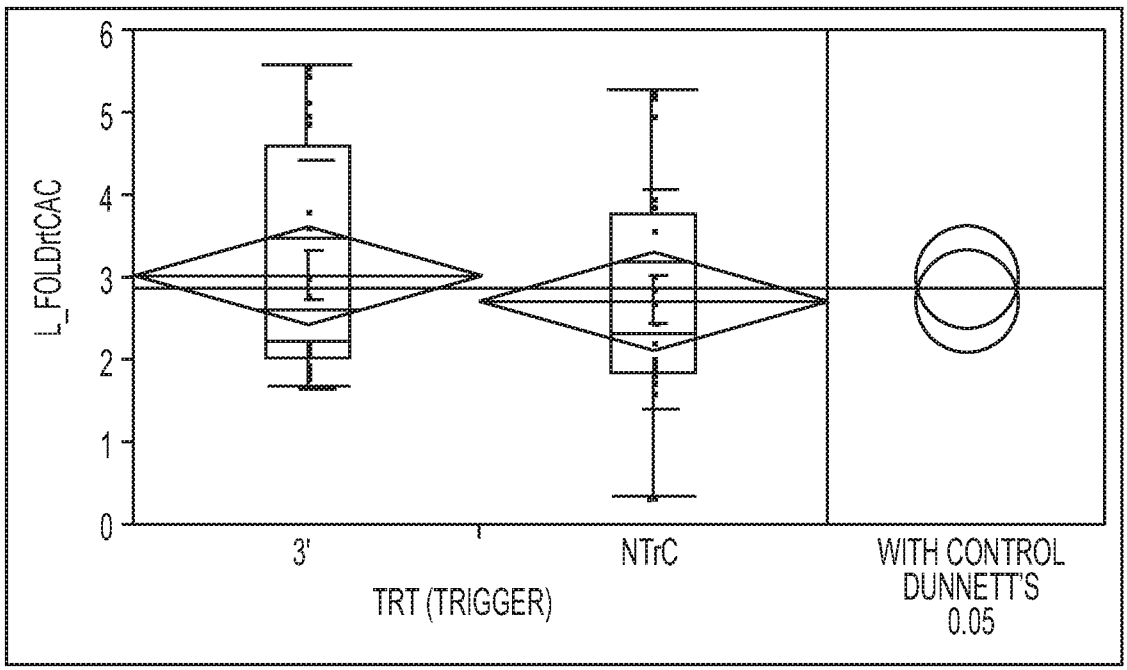


FIG. 29B

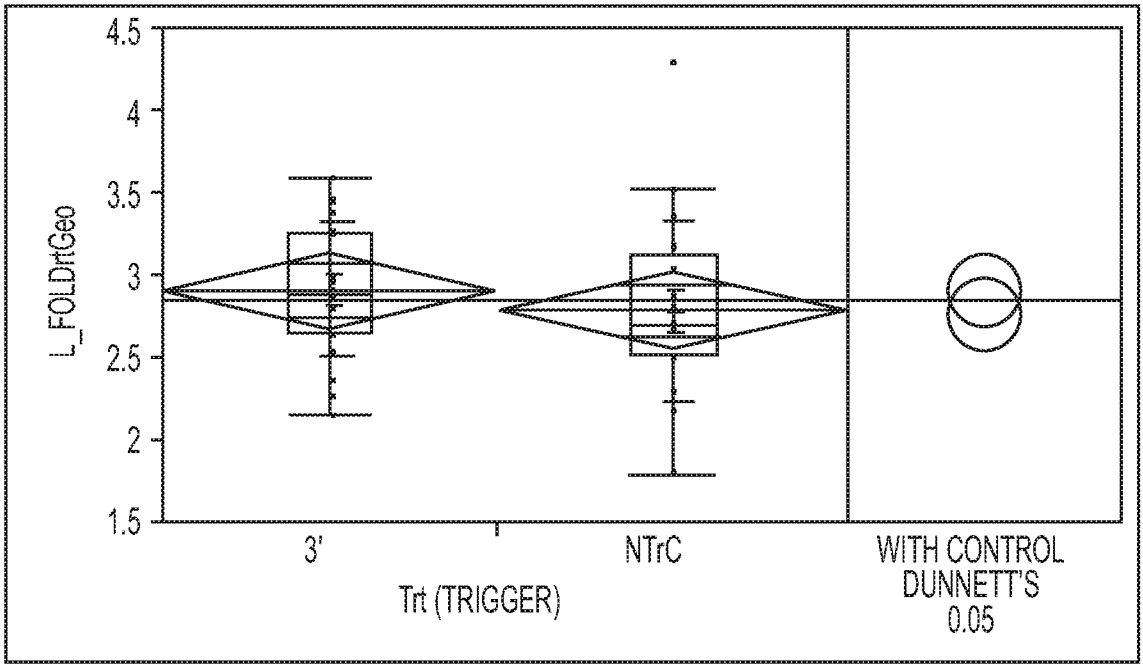

4.5
4
3.5
3
2.5
2
1.5
L_FOLDrtGeo
3'
NTrC
Trt (TRIGGER)
WITH CONTROL
DUNNETT'S
0.05

FIG. 30

SCORE	EXPECT	IDENTITIES	GAPS	STRAND
264 BITS (292)	2e-69	298/400(75%)	0/400(0%)	PLUS/PLUS

```
QUERY  124   CCCACAGACAAGCCCCTGCGTCTTCCCCTCCAGGACGTATACAAAATCGGTGGTATTGGT  183
             ||| | ||||||||||||||||| |||||||||| || ||||| || ||||||||||
SBJCT  837   CCCTCGGACAAGCCCCTGCGTCTCCCCCTCCAGGATGTGTACAAGATTGGTGGTATTGGA  896

QUERY  184   ACGGTGCCCGTAGGCAGAGTTGAAACTGGTATCCTCAAGCCTGGTACCATCGTCGTCTTC  243
             || ||||| || ||  | || || |||||| || ||||||||||||   | |||  |||
SBJCT  897   ACTGTGCCAGTTGGTCGTGTGGAGACTGGTGTCATCAAGCCTGGTATGGTTGTCACCTTT  956

QUERY  244   GCCCCCGCCAACATCACCACTGAAGTCAAGTCTGTGGAGATGCACCACGAAGCTCTCCAA  303
             |  ||  |   | | || ||||| || |||||||| |||||||||| || ||||| ||
SBJCT  957   GGTCCAACTGGCCTGACTACTGAGGTGAAGTCTGTTGAGATGCACCATGAGGCTCTTCAG  1016

QUERY  304   GAGGCCGTACCCGGTGACAACGTTGGTTTCAACGTAAAGAACGTTTCCGTCAAGGAGTTG  363
             |||||| | || |||||||| ||||| |||||||| ||||||||  | || |||||  |
SBJCT  1017  GAGGCCCTTCCTGGTGACAATGTTGGCTTCAACGTGAAGAACGTCGCTGTGAAGGATATC  1076

QUERY  364   CGTCGTGGTTACGTCGCTGGTGACTCCAAGAACAACCCACCCAAGGGCGCCGCCGATTTC  423
                ||||||||  || ||     ||||| || |  ||||  ||||||  || |||   |||
SBJCT  1077  AAGCGTGGTTATGTGGCCTCCAACTCCWAGGATGACCCTGCCAAGGAGGCTGCCAGCTTC  1136

QUERY  424   ACAGCACAGGTCATCGTGCTCAACCACCCTGGTCAAATCTCAAACGGATACACACCTGTG  483
             ||  | |||||||||  |  | ||||||||||| || |||   ||||| ||  | || |||
SBJCT  1137  ACCTCCCAGGTCATCATCATGAACCACCCTGGGCAGATCGGTAACGGTTATGCCCCAGTG  1196

QUERY  484   CTGGATTGCCACACAGCCCACATTGCCTGCAAGTTCGCTG  523
             |||||  ||||||||  ||||  |||||   ||||||  ||||
SBJCT  1197  CTGGACTGCCACACCTCCCATATTGCTGTCAAGTTTGCTG  1236
```

FIG. 31A

SCORE	EXPECT	IDENTITIES	GAPS	STRAND
300 BITS (332)	2e-80	335/446(75%)	6/446(1%)	PLUS/PLUS

```
QUERY  32   GAAATGGGTAAGGGTTCCTTCAAATACGCCTGGGTATTGGACAAACTGAAGGCTGAGCGT  91
SBJCT  218  GAAATGAATAAGCGGTCCTTCAAGTACGCGTGGGTGCTCGACAAGCTCAAGGCTGAGCGT  277
QUERY  92   GAACGTGGTATCACCATTGATATTGCTCTGTGGAAGTTCGAAACCGCTAAATACTATGTC  151
SBJCT  278  GAGAGAGGTATCACCATTGATATTGCTCTGTGGAAGTTTGAGACCACCAAGTACTACTGC  337
QUERY  152  ACCATTATTGACGCTCCCGGACACAGAGATTTCATCAAGAACATGATCACTGGAACCTCC  211
SBJCT  338  ACGGTCATTGATGCCCCTGGACACCGTGACTTCATCAAGAACATGATCACTGGTACCTCC  397
QUERY  212  CAGGCCGATTGCGCCGTAC---TCATTGTCGCCGCTGGTACCGGTGAATTCGAGGCTGGT  268
SBJCT  398  CAGGCTGACTGTGCTGTCCTTATCATTGACTCCAC---CACTGGTGGTTTTGAGGCTGGT  454
QUERY  269  ATCTCGAAGAACGGACAGACCCGTGAGCACGCTCTGCTCGCTTTCACACTCGGTGTCAAG  328
SBJCT  455  ATCTCCAAGGATGGCCAGACCCGTGAACATGCTCTCCTTGCGTTCACCCTTGGAGTGAAG  514
QUERY  329  CAGCTGATTGTGGGCGTCAACAAAATGGACTCCACTGAGCCCCCATACAGCGAATCCCGT  388
SBJCT  515  CAGATGATTTGCTGCTGCAACAAGATGGATGCAACCACTCCCAAATACTCCAAGGCACGT  574
QUERY  389  TTCGAGGAAATCAAGAAGGAAGTGTCCTCCTACATCAAGAAGATCGGTTACAACCCAGCT  448
SBJCT  575  TATGAAGAGATTGTGAAGGAAGTCTCATCCTACCTCAAGAAAGTTGGGTACAACCCTGAT  634
QUERY  449  GCTGTCGCTTTCGTACCCATTTCTGG  474
SBJCT  635  AAGATTGCCTTTGTTCCCATTTCTGG  660
```

FIG. 31B

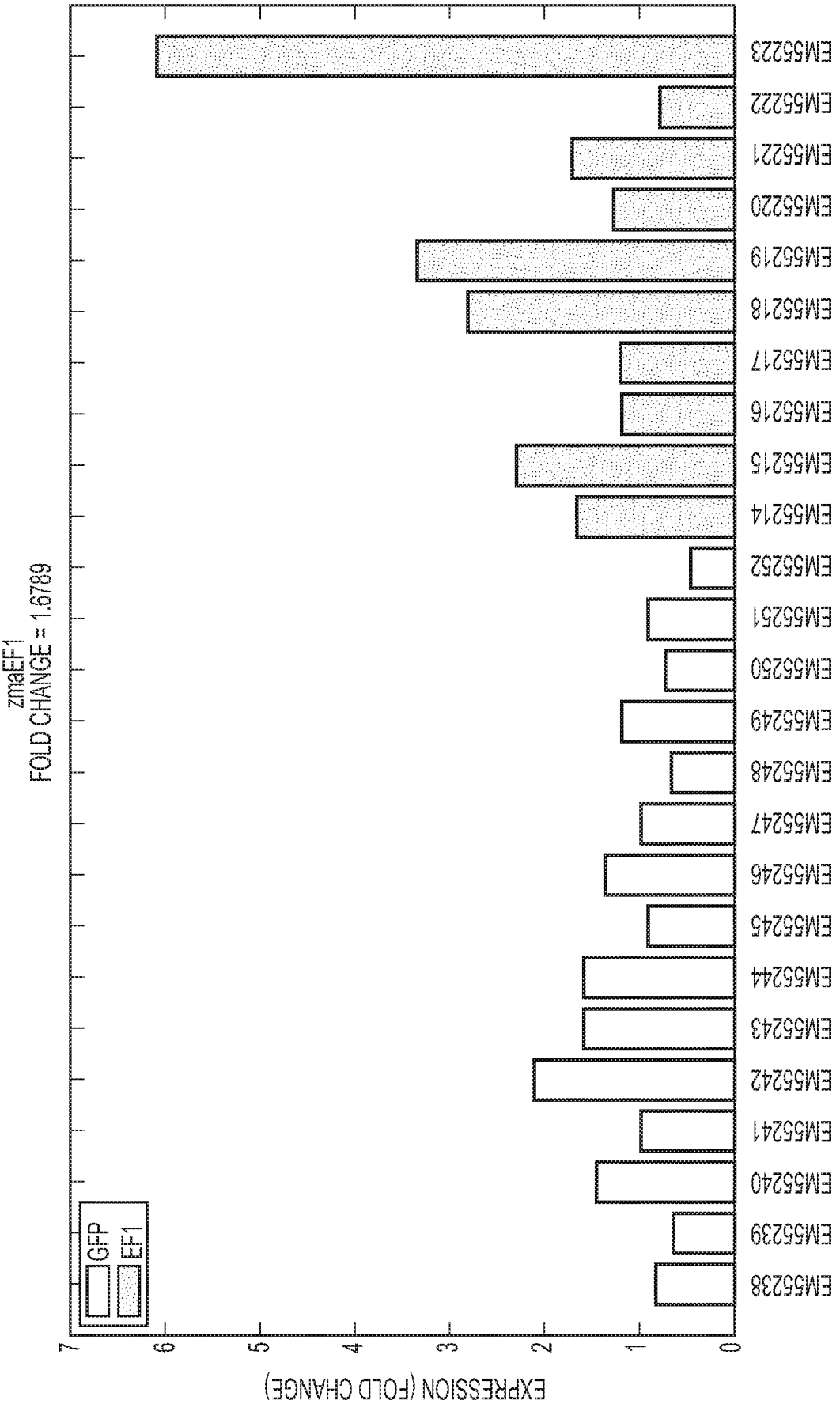

zmaEF1
FOLD CHANGE = 1.6789
GFP
EF1
EXPRESSION (FOLD CHANGE)
0
1
2
3
4
5
6
7
EM552238
EM552239
EM552240
EM552241
EM552242
EM552243
EM552244
EM552245
EM552246
EM552247
EM552248
EM552249
EM552250
EM552251
EM552252
EM552214
EM552215
EM552216
EM552217
EM552218
EM552219
EM552220
EM552221
EM552222
EM552223

FIG. 32A

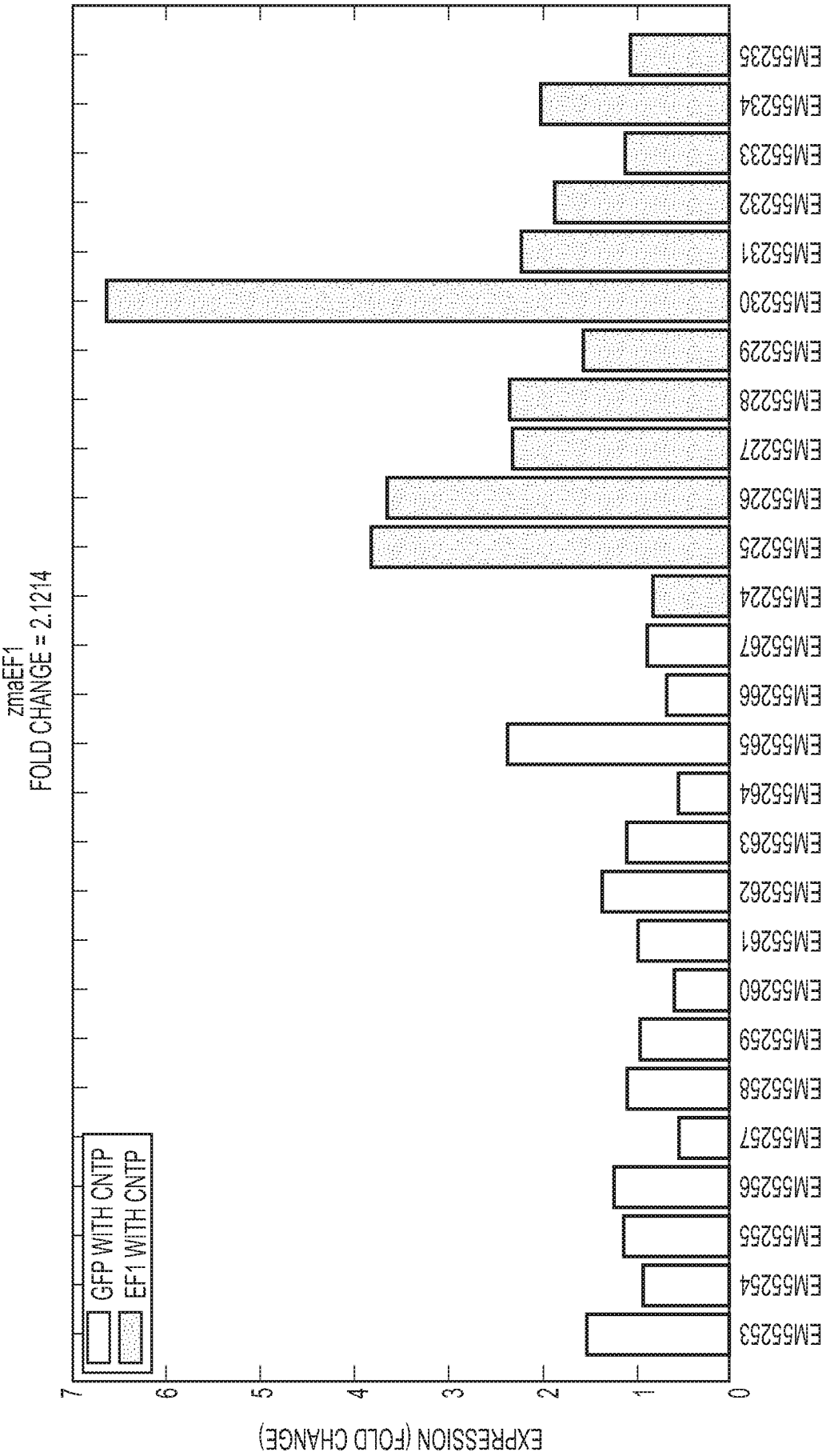

zmaEF1
FOLD CHANGE = 2.1214
GFP WITH CNTP
EF1 WITH CNTP
EXPRESSION (FOLD CHANGE)
0
1
2
3
4
5
6
7
EM552535
EM552534
EM552533
EM552532
EM552531
EM552530
EM552529
EM552528
EM552527
EM552526
EM552525
EM552524
EM552567
EM552566
EM552565
EM552564
EM552563
EM552562
EM552561
EM552560
EM552559
EM552558
EM552557
EM552556
EM552555
EM552554
EM552553

FIG. 32B

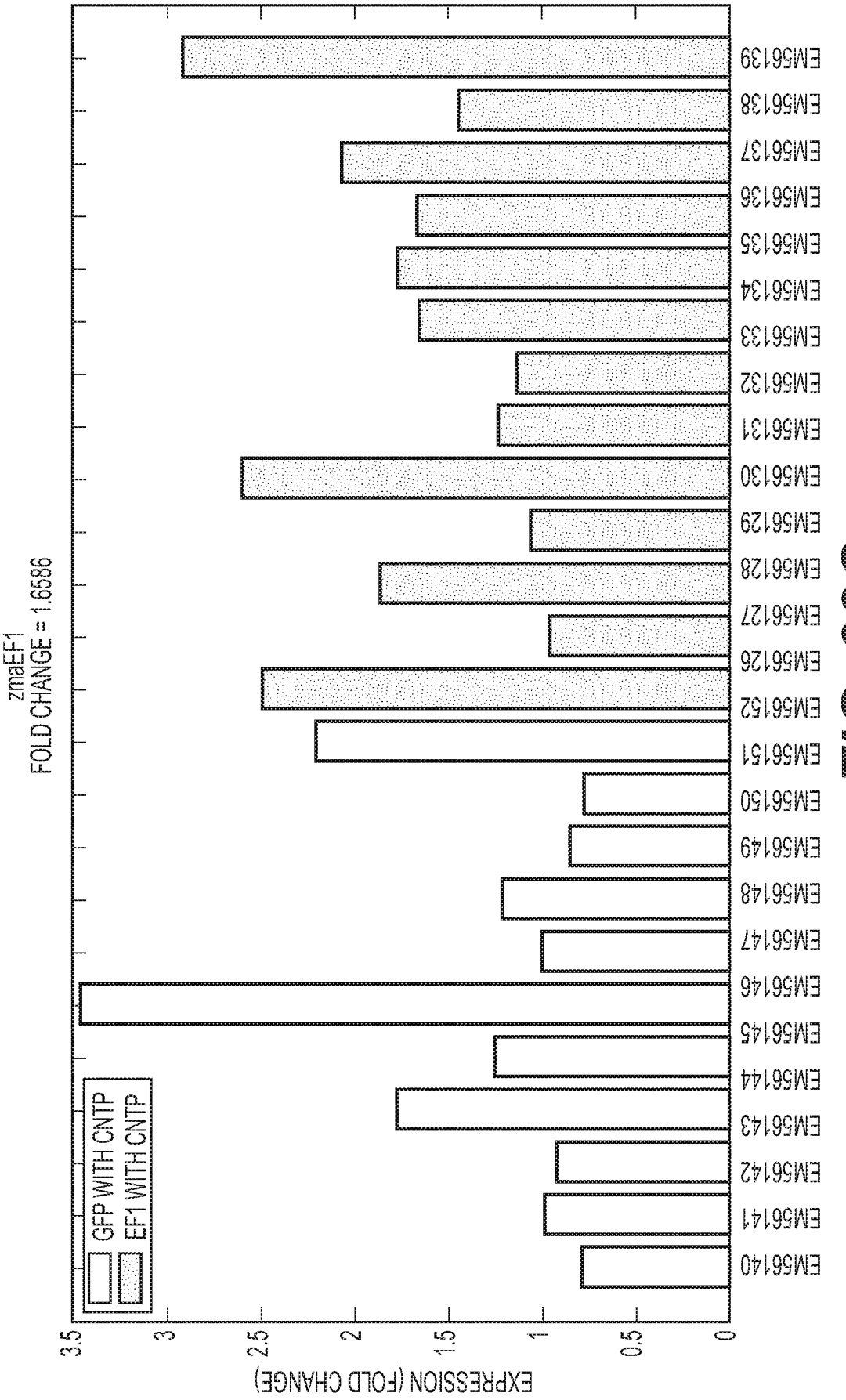
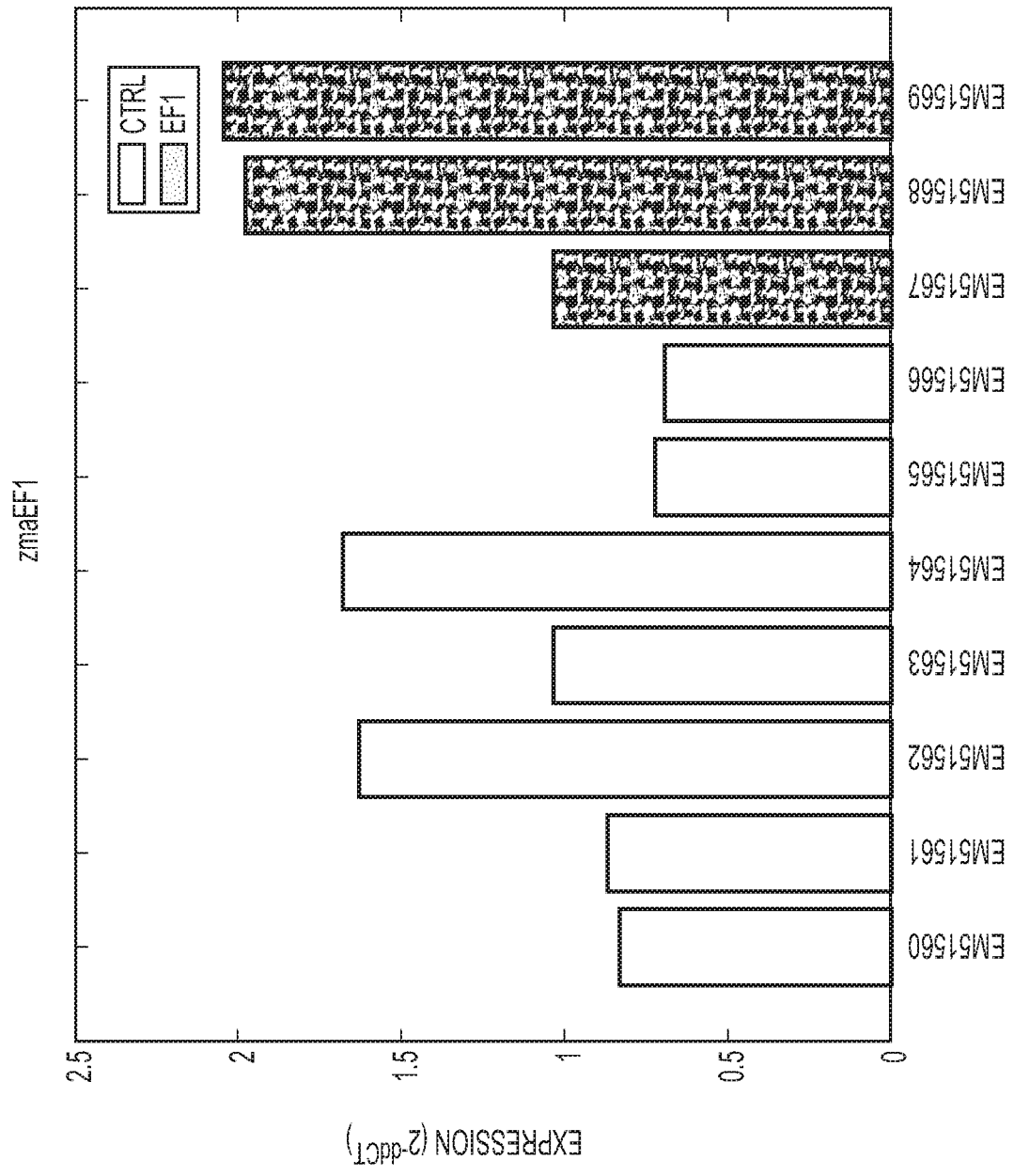

zmaEF1
FOLD CHANGE = 1.6586
GFP WITH CNTP
EF1 WITH CNTP
EXPRESSION (FOLD CHANGE)
3.5
3
2.5
2
1.5
1
0.5
0
EM561140
EM561141
EM561142
EM561143
EM561144
EM561145
EM561146
EM561147
EM561148
EM561149
EM561150
EM561151
EM561152
EM561126
EM561127
EM561128
EM561129
EM561130
EM561131
EM561132
EM561133
EM561134
EM561135
EM561136
EM561137
EM561138
EM561139

FIG. 32C

FIG. 33


zmaEF1
CTRL
EF1
EM51569
EM51568
EM51567
EM51566
EM51565
EM51564
EM51563
EM51562
EM51561
EM51560
0
0.5
1
1.5
2
2.5
EXPRESSION (2-ddCT)

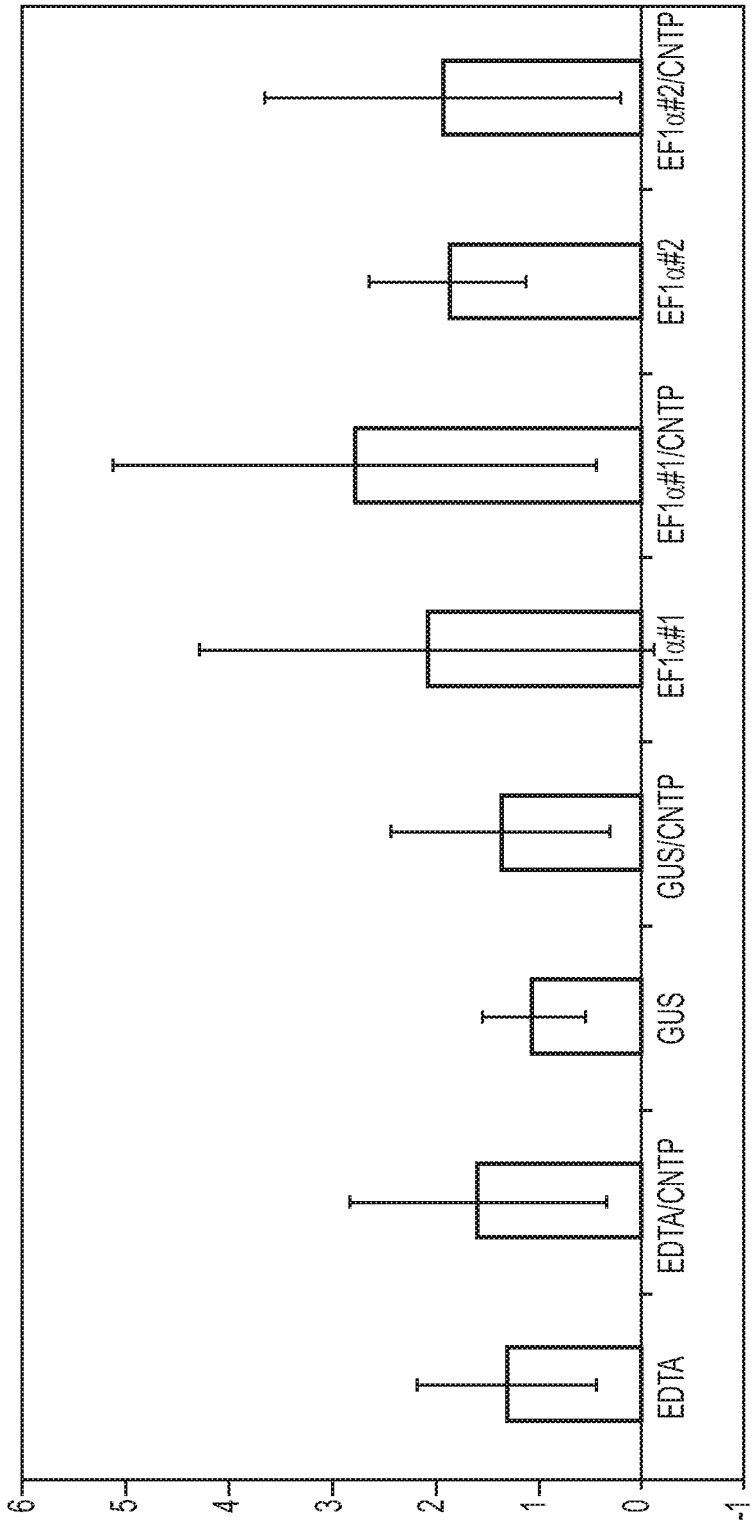

6
5
4
3
2
1
0
-1
EDTA
EDTA/CNTP
GUS
GUS/CNTP
EF1α#1
EF1α#1/CNTP
EF1α#2
EF1α#2/CNTP

FIG. 34A

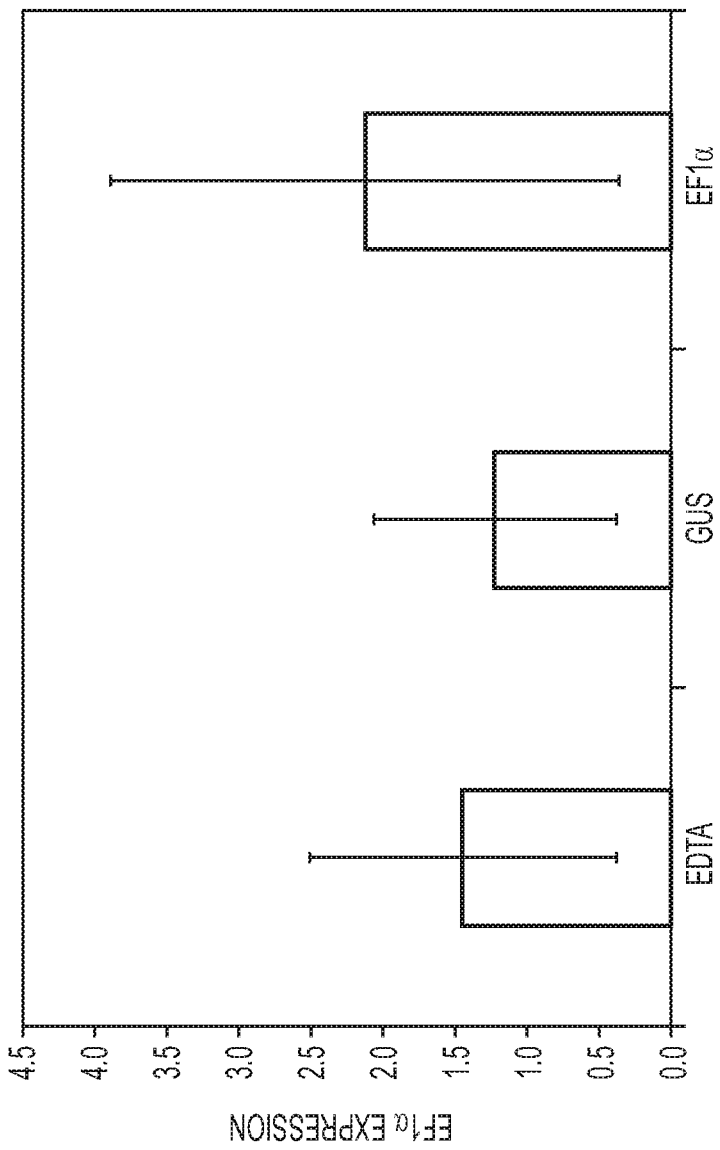


FIG. 34B

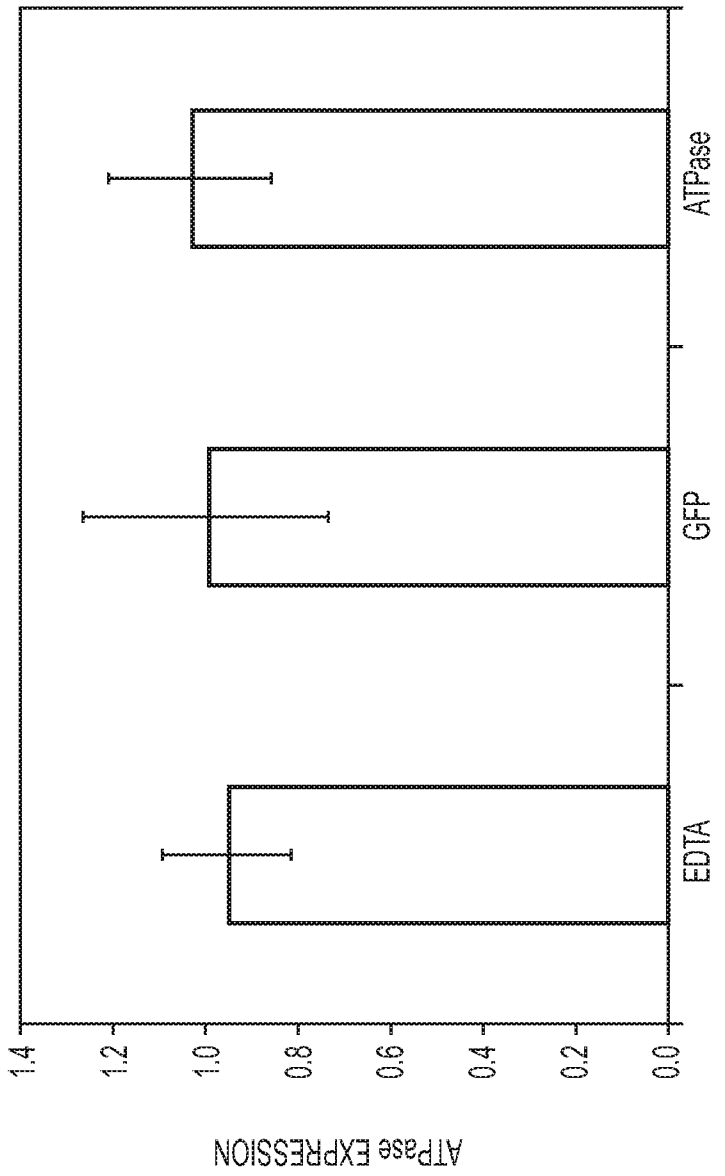


FIG. 35A

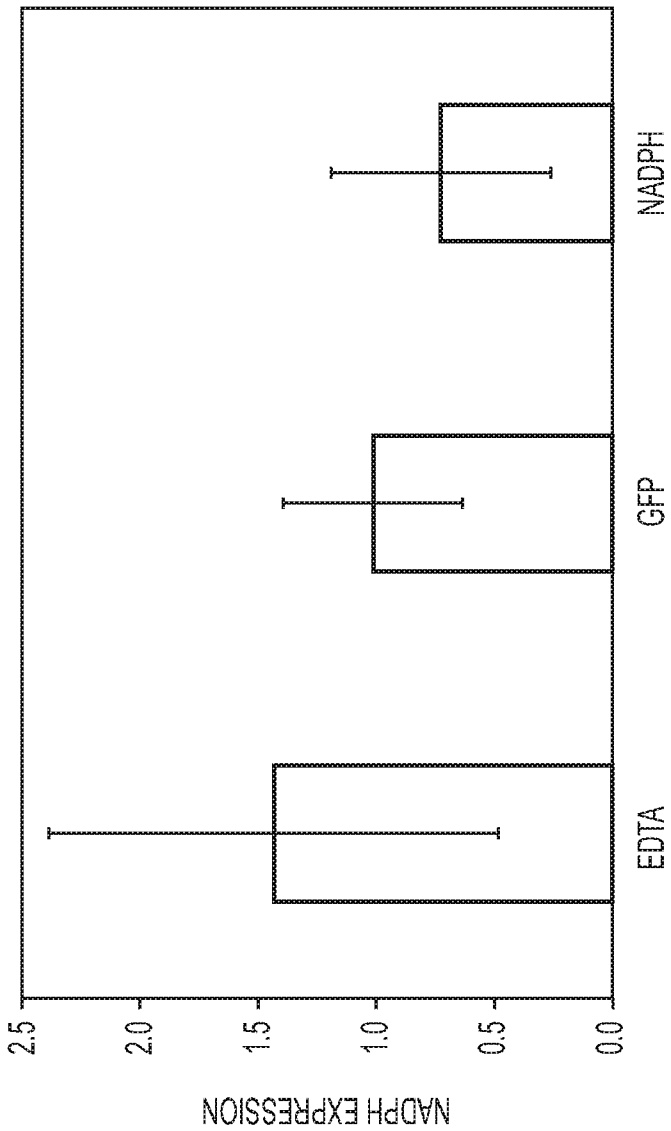


FIG. 35B

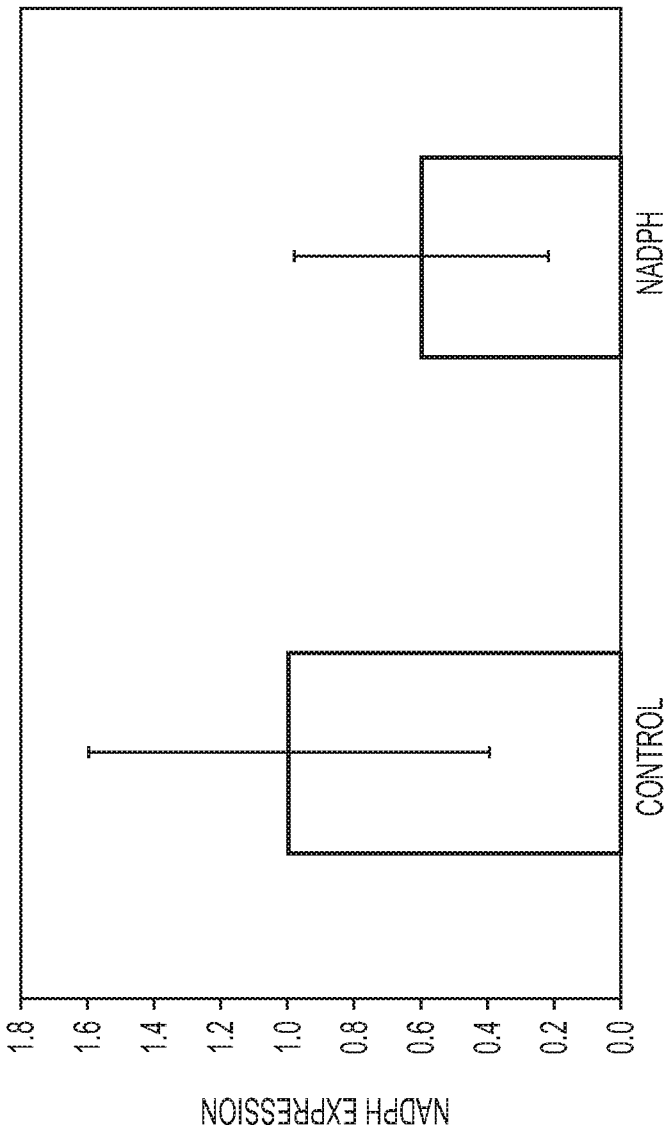


FIG. 35C