gene comprising the silencing specificity forms base complementation with the silencing molecule capable of recruiting the RdRp to produce the long dsRNA molecule capable of silencing the pest gene, thereby producing the long dsRNA molecule in the plant cell that is capable of silencing the pest gene.

According to an example of the present disclosure there is provided a method of producing a long dsRNA molecule in a plant cell that is capable of silencing a pest gene in a plant cell, the method comprising: (a) selecting in a genome of a plant a nucleic acid sequence encoding a silencing molecule having a plant gene as a target, the silencing molecule capable of recruiting RNA-dependent RNA Polymerase (RdRp); (b) modifying a nucleic acid sequence of the plant gene so as to impart a silencing specificity towards the pest gene, such that a transcript of the plant gene comprising the silencing specificity forms base complementation with the silencing molecule capable of recruiting the RdRp to produce the long dsRNA molecule capable of silencing the pest gene, thereby producing the long dsRNA molecule in the plant cell that is capable of silencing the pest gene in the plant cell.

According to some examples of the present disclosure there is provided a method of producing a long dsRNA molecule in a plant cell that is capable of silencing a pest gene, the method comprising: (a) selecting a nucleic acid sequence of a plant gene exhibiting a predetermined sequence homology to a nucleic acid sequence of the pest gene; (b) modifying a plant endogenous nucleic acid sequence encoding an RNA molecule so as to impart silencing specificity towards the plant gene, such that small RNA molecules capable of recruiting RNA-dependent RNA Polymerase (RdRp) processed from the RNA molecule form base complementation with a transcript of the plant gene to produce the long dsRNA molecule capable of silencing the pest gene, thereby producing the long dsRNA molecule in the plant cell that is capable of silencing the pest gene.

According to an aspect of some examples of the present disclosure there is provided a method of producing a long dsRNA molecule in a plant cell that is capable of silencing a pest gene, the method comprising: (a) selecting a nucleic acid sequence of a plant gene comprising a stretch of 20 of more nucleotides exhibiting 75-100% sequence identity to a nucleic acid sequence of the pest gene; (b) modifying a plant endogenous nucleic acid sequence encoding an RNA molecule so as to impart silencing specificity towards the plant gene, such that small RNA molecules capable of recruiting RNA-dependent RNA Polymerase (RdRp) processed from the RNA molecule form base complementation with a transcript of the plant gene to produce the long dsRNA molecule capable of silencing the pest gene, thereby producing the long dsRNA molecule in the plant cell that is capable of silencing the pest gene.

According to an aspect of some examples of the present disclosure there is provided a method of generating a pest tolerant or resistant plant, the method comprising producing a long dsRNA molecule in a plant cell capable of silencing a pest gene according to some examples of the disclosure.

According to some examples of the present disclosure there is provided a plant generated by the method of some examples of the disclosure.

According to some examples of the present disclosure there is provided a cell of the plant of some examples of the disclosure.

According to some examples of the present disclosure there is provided a seed of the plant of some examples of the disclosure.

According to an aspect of some examples of the present disclosure there is provided a method of producing a pest tolerant or resistant plant, the method comprising: (a) breeding the plant of some examples of the disclosure; and (b) selecting for progeny plants that express the long dsRNA molecule capable of suppressing the pest gene, and which do not comprise a DNA editing agent, thereby producing the pest tolerant or resistant plant.

According to some examples of the present disclosure there is provided a method producing a plant or plant cell of some examples of the disclosure comprising growing the plant or plant cell under conditions which allow propagation.

According to some examples of the disclosure, the silencing molecule capable of recruiting the RdRp comprises 21-24 nucleotides.

According to some examples of the disclosure, the silencing molecule capable of recruiting the RdRp comprises 21 nucleotides.

According to some examples of the disclosure, the silencing molecule capable of recruiting the RdRp comprises 22 nucleotides.

According to some examples of the disclosure, the silencing molecule capable of recruiting the RdRp comprises 23 nucleotides.

According to some examples of the disclosure, the silencing molecule capable of recruiting the RdRp comprises 24 nucleotides.

According to some examples of the disclosure, the silencing molecule capable of recruiting the RdRp consists of 21 nucleotides.

According to some examples of the disclosure, the silencing molecule capable of recruiting the RdRp consists of 22 nucleotides.

According to some examples of the disclosure, the silencing molecule capable of recruiting the RdRp consists of 23 nucleotides.

According to some examples of the disclosure, the silencing molecule capable of recruiting the RdRp consists of 24 nucleotides.

According to some examples of the disclosure, the silencing molecule capable of recruiting the RdRp is selected from the group consisting of: trans-acting siRNA (tasiRNA), phased small interfering RNA (phasiRNA), microRNA (miRNA), small interfering RNA (siRNA), short hairpin RNA (shRNA), Piwi-interacting RNA (piRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), ribosomal RNA (rRNA), small nucleolar RNA (snoRNA), extracellular RNA (exRNA), repeat-derived RNA, autonomous and non-autonomous transposable RNA.

WHAT IS CLAIMED IS:

1. A method of producing a long dsRNA molecule in a plant cell that is capable of silencing a pest gene, the method comprising:

(a) selecting in a genome of a plant a nucleic acid sequence encoding a silencing molecule having a plant gene as a target, said silencing molecule capable of recruiting RNA-dependent RNA Polymerase (RdRp);

(b) modifying a nucleic acid sequence of the plant gene so as to impart a silencing specificity towards the pest gene, such that a transcript of said plant gene comprising said silencing specificity forms base complementation with said silencing molecule capable of recruiting said RdRp to produce the long dsRNA molecule capable of silencing the pest gene,

thereby producing the long dsRNA molecule in the plant cell that is capable of silencing the pest gene.

2. The method of claim 1, wherein said silencing molecule capable of recruiting said RdRp comprises 21-24 nucleotides.

3. The method of any one of claims 1-2, wherein said silencing molecule capable of recruiting said RdRp is selected from the group consisting of: trans-acting siRNA (tasiRNA), phased small interfering RNA (phasiRNA), microRNA (miRNA), small interfering RNA (siRNA), short hairpin RNA (shRNA), Piwi-interacting RNA (piRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), ribosomal RNA (rRNA), small nucleolar RNA (snoRNA), extracellular RNA (exRNA), repeat-derived RNA, autonomous and non-autonomous transposable RNA, optionally wherein (i) said miRNA comprises a 22 nucleotides mature small RNA and/or (ii) said miRNA is selected from the group consisting of: miR-156a, miR-156c, miR-162a, miR-162b, miR-167d, miR-169b, miR-173, miR-393a, miR-393b, miR-402, miR-403, miR-447a, miR-447b, miR-447c, miR-472, miR-771, miR-777, miR-828, miR-830, miR-831, miR-831, miR-833a, miR-833a, miR-840, miR-845b, miR-848, miR-850, miR-853, miR-855, miR-856, miR-864, miR-2933a, miR-2933b, miR-2936, miR-4221, miR-5024, miR-5629, miR-5648, miR-5996, miR-8166, miR-8167a, miR-8167b, miR-8167c, miR-8167d, miR-8167e, miR-8167f, miR-8177, and miR-8182.

4. The method of any one of claims 1-3, wherein said plant gene is a non-protein coding gene, and/or wherein the plant gene encodes for a molecule having an intrinsic silencing activity towards a native plant gene.

5. The method of any one of claims 1-4, wherein said modifying of step (b) comprises introducing into the plant cell a DNA editing agent which redirects a silencing specificity of said plant gene towards said pest gene, said pest gene and a native plant gene being distinct.

6. The method of claim 4 or 5, wherein said plant gene having said intrinsic silencing activity is selected from the group consisting of trans-acting siRNA (tasiRNA), phased small interfering RNA (phasiRNA), microRNA (miRNA), small interfering RNA (siRNA), short hairpin RNA (shRNA), Piwi-interacting RNA (piRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), ribosomal RNA (rRNA), small nucleolar RNA (snoRNA), extracellular RNA (exRNA), autonomous and non-autonomous transposable RNA.

7. The method of any one of claims 4-6, wherein said plant gene having said intrinsic silencing activity encodes for a phased secondary siRNA-producing molecule, optionally wherein:

(i) modifying the gene encoding said phased secondary siRNA-producing molecule comprises modifying at least one secondary silencing RNA molecule formed by processing of said phased secondary siRNA-producing molecule; or

(ii) modifying the gene encoding said phased secondary siRNA-producing molecule comprises modifying all the secondary silencing RNA molecules formed by processing of said phased secondary siRNA-producing molecule,

or wherein said plant gene having said intrinsic silencing activity is a trans-acting-siRNA-producing (TAS) molecule.

8. The method of any one of claims 1-7, wherein:

(i) said silencing specificity of said plant gene is determined by measuring a transcript level of said pest gene; and/or

(ii) said silencing specificity of said plant gene is determined phenotypically, optionally wherein said determined phenotypically is effected by determination of pest resistance of said plant; and/or

(iii) said silencing specificity of said plant gene is determined genotypically, optionally wherein a plant phenotype is determined prior to a plant genotype, or wherein a plant genotype is determined prior to a plant phenotype.

9. A method of producing a long dsRNA molecule in a plant cell that is capable of silencing a pest gene, the method comprising:

(a) selecting a nucleic acid sequence of a plant gene comprising a stretch of 20 of more nucleotides exhibiting 75-100% sequence identity to a nucleic acid sequence of the pest gene;

(b) modifying a plant endogenous nucleic acid sequence encoding an RNA molecule so as to impart silencing specificity towards said plant gene, such that small RNA molecules capable of recruiting RNA-dependent RNA Polymerase (RdRp) processed from said RNA molecule form base complementation with a transcript of said plant gene to produce the long dsRNA molecule capable of silencing the pest gene,

thereby producing the long dsRNA molecule in the plant cell that is capable of silencing the pest gene.

10. The method of claim 9, wherein:

(i) said small RNA molecules capable of recruiting said RdRp comprise 21-24 nucleotides; and/or

(ii) said small RNA molecules capable of recruiting said RdRp are selected from the group consisting of microRNA (miRNA), small interfering RNA (siRNA), short hairpin RNA (shRNA), Piwi-interacting RNA (piRNA), trans-acting siRNA (tasiRNA), phased small interfering RNA (phasiRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), ribosomal RNA (rRNA), small nucleolar RNA (snoRNA), extracellular RNA (exRNA), repeat-derived RNA, autonomous and non-autonomous transposable RNA; and/or

(iii) said RNA molecule has an intrinsic silencing activity towards a native plant gene.

11. The method of claim 9 or 10, wherein:

(i) said modifying of step (b) comprises introducing into the plant cell a DNA editing agent which redirects a silencing specificity of said RNA molecule towards said plant gene, said plant gene and a native plant gene being distinct; and/or

(ii) said plant gene exhibiting said predetermined sequence homology to said nucleic acid sequence of the pest gene does not encode a silencing molecule; and/or

(iii) said silencing specificity of said RNA molecule is determined by measuring a transcript level of said plant gene or said pest gene; and/or

(iv) said silencing specificity of the RNA molecule is determined phenotypically, optionally wherein said determined phenotypically is effected by determination of pest resistance of said plant; and/or

(v) said silencing specificity of the RNA molecule is determined genotypically, optionally wherein a plant phenotype is determined prior to a plant genotype or wherein a plant genotype is determined prior to a plant phenotype.

12. The method of any one of claims 5-8 or 11, wherein:

(i) said DNA editing agent comprises at least one sgRNA,

optionally wherein said DNA editing agent comprises an endonuclease,

optionally wherein said DNA editing agent is of a DNA editing system selected from the group consisting of a meganuclease, a zinc finger nucleases (ZFN), a transcription-activator like effector nuclease (TALEN), CRISPR-endonuclease, dCRISPR-endonuclease and a homing endonuclease,

optionally wherein said endonuclease comprises Cas9; or

(ii) said DNA editing agent does not comprise an endonuclease.

13. The method of any one of claims 5-8, 11 or 12, wherein said DNA editing agent is applied to the cell as DNA, RNA or RNP.

14. The method of any one of claims 1-13, wherein said plant cell is a protoplast.

15. The method of any one of claims 1-14, wherein:

(i) said dsRNA molecule is processable by cellular RNAi processing machinery; and/or

(ii) said dsRNA molecule is processed into secondary small RNAs; and/or

(iii) said dsRNA and/or said secondary small RNAs comprise a silencing specificity towards a pest gene.

16. A method of generating a pest tolerant or resistant plant, the method comprising producing a long dsRNA molecule in a plant cell capable of silencing a pest gene according to any one of claims 1-15.

17. The method of claim 16, wherein said pest is an invertebrate and/or wherein said pest is selected from the group consisting of a virus, an ant, a termite, a bee, a wasp, a caterpillar, a cricket, a locust, a beetle, a snail, a slug, a nematode, a bug, a fly, a fruitfly, a whitefly, a mosquito, a grasshopper, a planthopper, an earwig, an aphid, a scale, a thrip, a spider, a mite, a psyllid, a tick, a moth, a worm, a scorpion and a fungus.

18. A method of producing a pest tolerant or resistant plant, the method comprising:

(a) breeding the plant generated in claim 16; and

(b) selecting for progeny plants that express the long dsRNA molecule capable of suppressing the pest gene, and which do not comprise a DNA editing agent,

thereby producing said pest tolerant or resistant plant.