SUMMARY

In one aspect, the present disclosure refers to a method of modifying a gene encoding or processed into an RNA silencing molecule to a target RNA in a plant cell, the method comprising introducing into the plant cell a DNA editing agent which redirects a silencing specificity of said RNA silencing molecule towards a second target RNA, said target RNA and said second target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule such that the original specificity of the RNA silencing molecule is abolished and a new specificity towards the second target RNA is gained.

In another aspect, the present disclosure refers to method of producing a plant with reduced expression of a target gene, the method comprising: (a) introducing into a plant cell a DNA editing agent which redirects a silencing specificity of said RNA silencing molecule towards a second target RNA, said target RNA and said second target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule such that the original specificity of the RNA silencing molecule is abolished and a new specificity towards the second target RNA is gained; (b) breeding a plant comprising the plant cell of part (a); and (c) selecting for progeny plants that have reduced expression of said target RNA of interest or said second target RNA, or progeny that comprises a silencing specificity in said non-coding RNA molecule towards a target RNA of interest, and which do not comprise said DNA editing agent, thereby producing said plant with reduced expression of a target gene.

In yet another aspect, the present disclosure refers to a method of generating a pathogen or pest tolerant or resistant plant, the method comprising modifying a gene encoding or processed into a non-coding RNA molecule or into a RNA silencing molecule in a plant cell as disclosed herein, wherein said second target RNA or said target RNA of interest is of a gene of the plant conferring sensitivity to said pathogen or said pest, or wherein the second target RNA or said target RNA of interest is a gene of said pathogen or said pest, thereby generating the pathogen or pest tolerant or resistant plant.

According to some examples of the present disclosure, there is provided a method of modifying a gene encoding or processed into a non-coding RNA molecule having no RNA silencing activity in a plant cell, the method comprising introducing into the plant cell a DNA editing agent conferring a silencing specificity of the non-coding RNA molecule towards a target RNA of interest, thereby modifying the gene encoding or processed into the non-coding RNA molecule.

According to some examples of the present disclosure, there is provided a method of modifying a gene encoding or processed into a non-coding RNA molecule having no RNA silencing activity in a plant cell, the method comprising introducing into the plant cell a DNA editing agent conferring a silencing specificity of the non-coding RNA molecule towards a target RNA of interest.

According to some examples of the present disclosure, there is provided a method of modifying a gene encoding or processed into a RNA silencing molecule to a target RNA in a plant cell, the method comprising introducing into the plant cell a DNA editing agent which redirects a silencing specificity of the RNA silencing molecule towards a second target RNA, the target RNA and the second target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule.

According to some examples of the present disclosure, there is provided a method of modifying a gene encoding or processed into a RNA silencing molecule to a target RNA in a plant cell, the method comprising introducing into the plant cell a DNA editing agent which redirects a silencing specificity of the RNA silencing molecule towards a second target RNA, the target RNA and the second target RNA being distinct.

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

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

According to some examples of the present disclosure, there is provided a method of producing a plant with reduced expression of a target gene, the method comprising: (a) breeding the plant of some examples of the disclosure; and (b) selecting for progeny plants that have reduced expression of the target RNA of interest or the second target RNA, or progeny that comprises a silencing specificity in the non-coding RNA molecule towards a target RNA of interest, and which do not comprise the DNA editing agent, thereby producing the plant with reduced expression of a target gene.

According to some examples of the present disclosure, there is provided a method of generating a plant with increased stress tolerance, increased yield, increased growth rate or increased yield quality, the method comprising modifying a gene encoding or processed into a non-coding RNA molecule or into a RNA silencing molecule in a plant cell according to some examples of the disclosure, wherein the target RNA of interest is of a gene of the plant conferring sensitivity to stress, decreased yield, decreased growth rate or decreased yield quality thereby generating the plant.

According to some examples of the present disclosure, there is provided a method of generating a pathogen tolerant or resistant plant, the method comprising modifying a gene encoding or processed into a non-coding RNA molecule or into a RNA silencing molecule in a plant cell according to some examples of the disclosure, wherein the target RNA of interest is of a gene of the plant conferring sensitivity to the pathogen, thereby generating the pathogen tolerant or resistant plant.

According to some examples of the present disclosure, there is provided a method of generating a pathogen tolerant or resistant plant, the method comprising modifying a gene encoding or processed into a non-coding RNA molecule or into a RNA silencing molecule in a plant cell according to some examples of the disclosure, wherein the target RNA of interest is of a gene of the pathogen, thereby generating the pathogen tolerant or resistant plant.

According to some examples of the present disclosure, there is provided a method of generating a pest tolerant or resistant plant, the method comprising modifying a gene encoding or processed into a non-coding RNA molecule or into a RNA silencing molecule in a plant cell according to some examples of the disclosure, wherein the target RNA of interest is of a gene of the pest, thereby generating the pest tolerant or resistant plant.

According to some examples of the present disclosure, there is provided a method of generating a pest tolerant or resistant plant, the method comprising modifying a gene encoding or processed into a non-coding RNA molecule or into a RNA silencing molecule in a plant cell according to some examples of the disclosure, wherein the target RNA of interest is of a gene of the plant conferring sensitivity to the pest, thereby generating the pest tolerant or resistant plant.

According to some examples of the present disclosure, there is provided a method of generating a herbicide resistant plant, the method comprising modifying a gene encoding or processed into a non-coding RNA molecule or into a RNA silencing molecule in a plant cell according to some examples of the disclosure, wherein the target RNA of interest is of a gene of the plant conferring sensitivity to the herbicide, thereby generating the herbicide resistant plant.

According to some examples of the present disclosure, there is provided a plant generated according to the method 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 some examples of the disclosure, the gene encoding or processed into the non-coding RNA molecule is endogenous to the plant cell.

According to some examples of the disclosure, the gene encoding the RNA silencing molecule is endogenous to the plant cell.

According to some examples of the disclosure, modifying the gene encoding or processed into the non-coding RNA molecule comprises imparting the non-coding RNA molecule with at least 45 % complementarity towards the target RNA of interest.

According to some examples of the disclosure, modifying the gene encoding the RNA silencing molecule comprises imparting the RNA silencing molecule with at least 45 % complementarity towards the second target RNA.

According to some examples of the disclosure, the silencing specificity of the non-coding RNA molecule is determined by measuring a RNA or protein level of the target RNA of interest.

According to some examples of the disclosure, the silencing specificity of the RNA silencing molecule is determined by measuring a RNA level of the second target RNA.

According to some examples of the disclosure, the silencing specificity of the non-coding RNA molecule or the RNA silencing molecule is determined phenotypically.

According to some examples of the disclosure, determined phenotypically is effected by determination of at least one plant phenotype selected from the group consisting of plant a leaf coloring, a flower coloring, a growth rate, a plant size, a crop yield, a fruit trait, a biotic stress resistance, and an abiotic stress resistance.

According to some examples of the disclosure, the silencing specificity of the non-coding RNA molecule is determined genotypically.

According to some examples of the disclosure, the plant phenotype is determined prior to a plant genotype.

According to some examples of the disclosure, the plant genotype is determined prior to a plant phenotype.

According to some examples of the disclosure, the non-coding RNA molecule or the RNA silencing molecule is processed from a precursor.

According to some examples of the disclosure, the non-coding RNA molecule or the

RNA silencing molecule is a RNA interference (RNAi) molecule.

According to some examples of the disclosure, the RNAi molecule is selected from the group consisting of a small interfering RNA (siRNA), a short hairpin RNA (shRNA), a microRNA (miRNA), a Piwi-interacting RNA (piRNA) and trans-acting siRNA (tasiRNA).

According to some examples of the disclosure, the non-coding RNA molecule is selected from the group consisting of a small nuclear RNA (snRNA), a small nucleolar RNA (snoRNA), a long non-coding RNA (lncRNA), a ribosomal RNA (rRNA), transfer RNA (tRNA), a repeat-derived RNA, and a transposable element RNA.

According to some examples of the disclosure, the RNA molecule or RNAi molecule is designed such that a sequence of the RNAi molecule is modified to preserve originality of structure and to be recognized by cellular RNAi factors.

According to some examples of the disclosure, modifying the gene is effected by a modification selected from the group consisting of a deletion, an insertion, a point mutation and a combination thereof.

According to some examples of the disclosure, the modification is in a stem region of the non-coding RNA molecule or the RNA silencing molecule.

According to some examples of the disclosure, the modification is in a loop region of the non-coding RNA molecule or the RNA silencing molecule.

According to some examples of the disclosure, the modification is in a non-structured region of the non-coding RNA molecule or the RNA silencing molecule.

According to some examples of the disclosure, the modification is in a stem region and a loop region of the non-coding RNA molecule or the RNA silencing molecule.

According to some examples of the disclosure, the modification is in a stem region and a loop region and in non-structured region of the non-coding RNA molecule or the RNA silencing molecule.

According to some examples of the disclosure, the modification is an insertion.

According to some examples of the disclosure, the modification is a deletion.

According to some examples of the disclosure, the modification is a point mutation.

According to some examples of the disclosure, the modification comprises a modification of at most 200 nucleotides.

According to some examples of the disclosure, the method further comprises introducing into the plant cell donor oligonucleotides.

According to some examples of the disclosure, the DNA editing agent comprises at least one gRNA operatively linked to a plant expressible promoter.

According to some examples of the disclosure, the DNA editing agent does not comprise an endonuclease.

According to some examples of the disclosure, the DNA editing agent comprises an endonuclease.

According to some examples of the disclosure, the 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) and CRISPR.

According to some examples of the disclosure, the endonuclease comprises Cas9.

According to some examples of the disclosure, the DNA editing agent is applied to the cell as DNA, RNA or RNP.

According to some examples of the disclosure, the DNA editing agent is linked to a reporter for monitoring expression in a plant cell.

According to some examples of the disclosure, the reporter is a fluorescent protein.

According to some examples of the disclosure, the target RNA of interest or the second target RNA is endogenous to the plant cell.

According to some examples of the disclosure, the target RNA of interest or the second target RNA is exogenous to the plant cell.

According to some examples of the disclosure, the plant cell is a protoplast.

According to some examples of the disclosure, the breeding comprises crossing or selfing.

According to some examples of the disclosure, the plant is non-genetically modified (non-GMO).

According to some examples of the disclosure, the plant is selected from the group consisting of a crop, a flower and a tree.

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 disclosure pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of examples of the disclosure, exemplary 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.

WHAT IS CLAIMED IS:

1. A method of modifying a gene encoding or processed into an RNA silencing molecule to a target RNA in a plant cell, the method comprising introducing into the plant cell a DNA editing agent which redirects a silencing specificity of said RNA silencing molecule towards a second target RNA, said target RNA and said second target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule such that the original specificity of the RNA silencing molecule is abolished and a new specificity towards the second target RNA is gained.

2. The method of claim 1, wherein the gene encoding the RNA silencing molecule is endogenous to the plant cell.

3. The method of any one of claims 1 or 2, wherein said modifying said gene encoding said RNA silencing molecule comprises imparting said RNA silencing molecule with at least 45 % complementarity towards said second target RNA.

4. The method of any one of claims 1-3, wherein said silencing specificity of said RNA silencing molecule is determined by measuring a RNA level of said second target RNA and/or is determined phenotypically and/or is determined genotypically.

5. The method of any one of claims 1-4, wherein said RNA silencing molecule or said non-coding RNA molecule is processed from a precursor,

optionally wherein said RNA silencing molecule is a RNA interference (RNAi) molecule selected from the group consisting of a small interfering RNA (siRNA), a short hairpin RNA (shRNA), a microRNA (miRNA), a Piwi-interacting RNA (piRNA) and trans-acting siRNA (tasiRNA),

optionally wherein said RNAi molecule is designed such that a sequence of said RNAi molecule is modified to preserve secondary RNA structure and to be recognized by cellular RNAi factors.

6. The method of any one of claims 1-5, wherein said modifying said gene is effected by a modification selected from the group consisting of a deletion, an insertion, a point mutation and a combination thereof,

optionally wherein said modification is in a stem region; in a loop region; in a stem region and a loop region; in a non-structured region; or in a stem region, a loop region and a non-structured region of said RNA silencing molecule or said non-coding RNA molecule,

optionally wherein said modification comprises a modification of at most 200 nucleotides.

7. The method of claim 6, wherein said method further comprises introducing into the plant cell a donor oligonucleotide sequence.

8. The method of any one of claims 1-7, wherein said DNA editing agent comprises an endonuclease,

optionally wherein the DNA editing agent comprises an endonuclease and a DNA targeting molecule,

optionally wherein the DNA targeting molecule is gRNA,

optionally wherein said endonuclease comprises Cas9.

9. The method of any one of claims 1-7, wherein said DNA editing agent is applied to the cell as DNA, RNA or RNP, wherein the DNA editing agent is introduced into the plant cell using DNA delivery methods or DNA-free methods,

optionally wherein the DNA editing agent is introduced into the plant cell using expression vectors,

optionally wherein said DNA editing agent comprises at least one gRNA operatively linked to a plant expressible promoter.

10. The method of any one of claims 1-7, 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) and CRISPR.

11. The method of any one of claims 1-10, wherein said second target RNA is exogenous to the plant cell.

12. A method of producing a plant with reduced expression of a target gene, the method comprising:

(a) introducing into a plant cell a DNA editing agent which redirects a silencing specificity of said RNA silencing molecule towards a second target RNA, said target RNA and said second target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule such that the original specificity of the RNA silencing molecule is abolished and a new specificity towards the second target RNA is gained;

(b) breeding a plant comprising the plant cell of part (a); and

(c) selecting for progeny plants that have reduced expression of said target RNA of interest or said second target RNA, or progeny that comprises a silencing specificity in said non-coding RNA molecule towards a target RNA of interest, and which do not comprise said DNA editing agent,

thereby producing said plant with reduced expression of a target gene.

13. A method of generating a pathogen or pest tolerant or resistant plant, the method comprising modifying a gene encoding or processed into a non-coding RNA molecule or into a RNA silencing molecule in a plant cell according to any one of claims 1-11, wherein said second target RNA or said target RNA of interest is of a gene of the plant conferring sensitivity to said pathogen or said pest, or wherein the second target RNA or said target RNA of interest is a gene of said pathogen or said pest, thereby generating the pathogen or pest tolerant or resistant plant.