Claims

What is claimed is:

1. A method of producing a plant or seed, the method comprising:

(a) providing a first plant comprising

(i) a gene of interest comprising a coding sequence and having a first allele that is a hypomorphic allele or a null allele, and

(ii) an expression cassette that encodes a RNA-guided endonuclease and at least four different guide RNAs (gRNAs), each gRNA containing a sequence that is complementary to a target sequence within a target region in a second allele of the gene of interest that is different from the first allele, wherein the target region is 0 to 5000 base pairs upstream of the 5' end of the coding sequence of the gene of interest or wherein the target region is 0 to 5000 base pairs downstream of the 3' end of the coding sequence of the gene of interest,

(b) providing a second plant comprising the second allele of the gene of interest,

(c) crossing the first plant to the second plant to produce a plurality of F1 hybrid plants, each F1 hybrid plant in the plurality comprising the first allele, the second allele and the expression cassette,

(d) maintaining the plurality of F1 hybrid plants under conditions that permit the gRNA/RNA-guided endonuclease to induce mutations within the target region of the second allele,

(e) selecting an F1 hybrid plant of step (d) having a phenotype of interest, and

(f) performing a cross with the F1 hybrid plant to produce a progeny plant or seed containing at least one gRNA/RNA-guided endonuclease-induced mutation.

2. A method of producing a plant or seed, the method comprising:

(a) providing a first plant comprising

(i) a gene of interest comprising a coding sequence and having a first allele that is a hypomorphic allele or a null allele, and

(ii) an expression cassette that encodes a RNA-guided endonuclease and at least four different guide RNAs (gRNAs), each gRNA containing a sequence that is complementary to a target

sequence within a target region in a second allele of the gene of interest that is different from the first allele, wherein the target region is 0 to 5000 base pairs upstream of the 5’ end of the coding sequence of the gene of interest or wherein the target region is 0 to 5000 base pairs downstream of the 3’ end of the coding sequence of the gene of interest,

(b) providing a second plant comprising the second allele of the gene of interest,

(c) crossing the first plant to the second plant to produce a plurality of F1 hybrid plants, each F1 hybrid plant in the plurality comprising the first allele, the second allele and the expression cassette,

(d) maintaining the plurality of F1 hybrid plants under conditions that permit the gRNA/RNA-guided endonuclease to induce mutations within the target region of the second allele,

(e) selecting an F1 hybrid plant of step (d) having a phenotype of interest, and

(f) performing a cross with the F1 hybrid plant to produce a progeny plant or seed that is homozygous for the second allele containing at least one gRNA/RNA-guided endonuclease - induced mutation.

3. A method of generating a commercially relevant allele or trait that can be used in plant breeding, comprising

(a) selecting an F1 hybrid plant, which is hemizygous for an expression cassette that encodes a RNA-guided endonuclease and at least two different gRNAs, each gRNA containing a sequence that is complementary to a target sequence within a target region of a gene of interest, and having a first allele of the gene of interest that is a null allele or a hypomorphic allele and a second allele of the gene of interest carrying a gRNA/endonuclease-induced mutation within the promotor region of the gene of interest; and

(b) fixing the second allele in a plant to produce a progeny plant or seed that is homozygous for that second allele.

4. A method for producing a crop plant or crop seed having a commercially relevant allele of a gene of interest, comprising using the method of claim 3 to produce a commercially relevant

allele of a gene of interest, introducing the allele into a crop plant, to produce a crop plant or crop seed containing the allele, and optionally further propagating or multiplying that crop plant or crop seed.

5. A method of generating a commercially relevant allele or trait that can be used in plant breeding, comprising

(a) selecting an F1 hybrid plant, which is hemizygous for an expression cassette that encodes a RNA guided endonuclease and at least two different gRNAs, each gRNA containing a sequence that is complementary to a target sequence within a target region of a gene of interest, and having a first allele of the gene of interest that is a null allele or a hypomorphic allele and a second allele of that gene carrying a gRNA/endonuclease induced mutation within the promotor region of that gene; and

(b) performing a cross of the F1 hybrid plant to produce a progeny plant or seed that is heterozygous for that second allele.

6. A method for producing a crop plant or crop seed having a commercially relevant allele of a gene of interest, comprising using the method of claim 5 to produce a commercially relevant allele of a gene of interest, introducing the allele into a crop plant, to produce a crop plant or crop seed containing the allele, and optionally further propagating or multiplying that crop plant or crop seed.

7. A method of generating a plant library comprising a plurality of F1 hybrid plants, the method comprising:

(a) providing a first plant comprising

(i) a gene of interest comprising a coding sequence and having a first allele that is a hypomorphic allele or a null allele, and

(ii) an expression cassette that encodes a RNA-guided endonuclease and at least four different guide RNAs (gRNAs), each gRNA containing a sequence that is complementary to a target sequence within a target region in a second allele of the gene of interest that is different from the first allele, wherein the target region is 0 to 5000 base pairs upstream of the 5’ end of the coding

sequence of the gene of interest or wherein the target region is 0 to 2000 base pairs downstream of the 3’ end of the coding sequence of the gene of interest,

(b) providing a second plant comprising the second allele of the gene of interest, and

(c) crossing the first plant to the second plant to produce a plurality of F1 hybrid plants, each F1 hybrid plant in the plurality comprising the first allele, the second allele and the expression cassette.

8. A method of generating a seed library comprising a plurality of F1 hybrid seeds, the method comprising:

(a) providing a first plant comprising

(i) a gene of interest comprising a coding sequence and having a first allele that is a hypomorphic allele or a null allele, and

(ii) an expression cassette that encodes a RNA-guided endonuclease and at least four different guide RNAs (gRNAs), each gRNA containing a sequence that is complementary to a target sequence within a target region in a second allele of the gene of interest that is different from the first allele, wherein the target region is 0 to 5000 base pairs upstream of the 5’ end of the coding sequence of the gene of interest or wherein the target region is 0 to 5000 base pairs downstream of the 3’ end of the coding sequence of the gene of interest,

(b) providing a second plant comprising the second allele of the gene of interest, and

(c) crossing the first plant to the second plant to produce a plurality of F1 hybrid seeds, each F1 hybrid seed in the plurality comprising the first allele, the second allele and the expression cassette.

9. A plant library comprising a plurality of F1 hybrid plants, each F1 hybrid plant in the plurality comprising:

(a) a gene of interest comprising a coding sequence and having a first allele that is a hypomorphic allele or a null allele and a second allele that is different from the first allele, and

(b) an expression cassette that encodes a RNA-guided endonuclease and at least four different guide RNAs (gRNAs), each gRNA containing a sequence that is complementary to a target sequence within a target region in the second allele of the gene of interest,

wherein the target region is 0 to 5000 base pairs upstream of the 5’ end of the coding sequence of the gene of interest or wherein the target region is 0 to 5000 base pairs downstream of the 3’ end of the coding sequence of the gene of interest.

10. A seed library comprising a plurality of F1 hybrid seeds, each F1 hybrid seed in the plurality comprising:

(a) a gene of interest comprising a coding sequence and having a first allele that is a hypomorphic allele or a null allele and a second allele that is different from the first allele, and

(b) an expression cassette that encodes a RNA-guided endonuclease and at least four different guide RNAs (gRNAs), each gRNA containing a sequence that is complementary to a target sequence within a target region in the second allele of the gene of interest,
wherein the target region is 0 to 5000 base pairs upstream of the 5’ end of the coding sequence of the gene of interest or wherein the target region is 0 to 5000 base pairs downstream of the 3’ end of the coding sequence of the gene of interest.

11. A method of selecting members of a library having a phenotype of interest, the method comprising:

(a) providing a plant or seed library of claims 9 or 10,

(b) selecting at least one member of the library that exhibits a phenotype of interest, and

(c) crossing the at least one member to at least one plant that does not contain the expression cassette.

12. A method comprising the steps of:

(a) providing a first plant comprising

(i) a gene of interest comprising a coding sequence and having a first allele that is a hypomorphic allele or a null allele, and

(ii) an expression cassette that encodes a RNA-guided endonuclease and at least four different guide RNAs (gRNAs), each gRNA containing a sequence that is complementary to a target sequence within a target region in a second allele of the gene of interest that is different from the first allele, wherein the target region is 0 to 5000 base pairs upstream of the 5’ end of the coding

sequence of the gene of interest or wherein the target region is 0 to 5000 base pairs downstream of the 3’ end of the coding sequence of the gene of interest,

(b) providing a second plant comprising the second allele of the gene of interest,

(c) crossing the first plant to the second plant to produce a plurality of F1 hybrid plants, each F1 hybrid plant in the plurality comprising the first allele, the second allele and the expression cassette,

(d) maintaining the plurality of F1 hybrid plants under conditions that permit the gRNA/RNA-guided endonuclease to induce mutations within the target region of the second allele,

(e) selecting an F1 hybrid plant of step (d) having a phenotype of interest, and

(f) performing a cross with the F1 hybrid plant to produce a progeny plant or seed that is homozygous for the second allele containing at least one gRNA/RNA-guided endonuclease - induced mutation.

13. The method of claim 12 further comprising the step of isolating a cell from the progeny plant or seed.

14. An isolated DNA molecule comprising a second allele of a gene of interest containing at least one gRNA/Cas9-induced mutation or a fragment of the second allele containing the target region containing the at least one gRNA/Cas9-induced mutation, the DNA molecule obtainable, or obtained by, a process comprising isolating a DNA molecule comprising the second allele, or the fragment thereof, from the progeny plant or seed obtained from the method of claim 12 or 13.

15. A method comprising the steps of:

(a) providing a first plant comprising

(i) a gene of interest comprising a coding sequence and having a first allele that is a hypomorphic allele or a null allele, and

(ii) an expression cassette that encodes a RNA-guided endonuclease and at least four different guide RNAs (gRNAs), each gRNA containing a sequence that is complementary to a target sequence within a target region in a second allele of the gene of interest that is different from the first allele, wherein the target region is 0 to 5000 base pairs upstream of the 5’ end of the coding

sequence of the gene of interest or wherein the target region is 0 to 5000 base pairs downstream of the 3’ end of the coding sequence of the gene of interest,

(b) providing a second plant comprising the second allele of the gene of interest, and

(c) crossing the first plant to the second plant to produce a plant library comprising a plurality of F1 hybrid plants, each F1 hybrid plant in the plurality comprising the first allele, the second allele and the expression cassette.

16. A method comprising the steps of:

(a) providing a first plant comprising

(i) a gene of interest comprising a coding sequence and having a first allele that is a hypomorphic allele or a null allele, and

(ii) an expression cassette that encodes a RNA-guided endonuclease and at least four different guide RNAs (gRNAs), each gRNA containing a sequence that is complementary to a target sequence within a target region in a second allele of the gene of interest that is different from the first allele, wherein the target region is 0 to 5000 base pairs upstream of the 5’ end of the coding sequence of the gene of interest or wherein the target region is 0 to 5000 base pairs downstream of the 3’ end of the coding sequence of the gene of interest,

(b) providing a second plant comprising the second allele of the gene of interest, and

(c) crossing the first plant to the second plant to produce a seed library comprising a plurality of F1 hybrid seeds, each F1 hybrid seed in the plurality comprising the first allele, the second allele and the expression cassette.

17. A nucleic acid comprising an expression construct encoding a RNA-guided endonuclease and at least four different guide RNAs (gRNAs), each gRNA containing a sequence that is complementary to a target sequence within a target region in an allele of a gene of interest in a plant, wherein the target region is 0 to 5000 base pairs upstream of the 5’ end of the coding sequence of the gene of interest or wherein the target region is 0 to 5000 base pairs downstream of the 3’ end of the coding sequence of the gene of interest.

18. Use of the library of claims 9 or 10, the DNA molecule of claim 14, the nucleic acid of claim 17, or the F1 hybrid plant of any one of claims 1-16 for the production of a crop plant or seed thereof.

19. A method for generating crop plants or a seed thereof with alleles that weakly affect one or more commercially relevant traits, comprising the use of the library of any one of claims 9 or 10, the DNA molecule of claim 14, the nucleic acid of claim 17, or the F1 hybrid plant of any one of claims 1-16.

20. A crop plant or seed thereof obtainable or obtained by the use or method of claims 18 or 19.

21. The method of any one of claims 1-2, 7-8, 12 and 15-16, wherein the method further comprises propagating or multiplying the plant obtained in step (c), or wherein the method further comprises producing a seed from the plant obtained in step (c).