PROGRAMMABLE EPIGENETIC CONTROL OF GENE EXPRESSION IN PLANTS

CROSS-REFERENCE

[0001] The present application claims the benefit of U.S. Provisional Application Serial No. 62/820,172, filed March 18, 2019, which is hereby incorporated by reference in its entirety.

SUMMARY

[0001a] The present disclosure provides a method of inducing an epigenetic modification in a plant organism, comprising applying a formulation to an agricultural object, the formulation comprising an excipient and a double stranded artificial nucleic acid construct comprising (a) a first strand and a second strand each having a length of ~~about~~ 10 to ~~about~~ 30 nucleotides or nucleosides or a combination thereof; and (b) a terminal overhang at each of the 3' ends of the first strand and second strand; wherein: one or both of the 3' terminal end overhangs comprise a ribose unit having a methoxy group (-O-Me) at the 2' position; wherein the agricultural object is a seed, a plant, a constituent of a plant, or any combination thereof, and wherein the applying causes methylation of at least one base of a nucleotide or nucleoside in a nucleic acid sequence of a gene of the agricultural object.

[0002] In some examples, disclosed herein is a nucleic acid construct, e.g., a polynucleotide. In some instances, the present disclosure provides an artificial nucleic acid construct, wherein the artificial nucleic acid construct comprises: (a) a ribose modified at a 2' or 3' position, or a deoxyribose modified at a 2' or 3' position, or a combination thereof; and (b) a terminal end overhang, wherein: the artificial nucleic acid construct is double stranded; at least one strand of the artificial nucleic acid construct independently comprises a length from at least: about 10 to about 30 nucleotides or nucleosides or a combination thereof; and when contacted with an organism, at least a portion of the artificial nucleic acid construct is configured to facilitate: i) an epigenetic modification of at least one base of a nucleotide or nucleoside in a nucleic acid sequence of the organism, ii) a silencing of a target mRNA sequence that is at least partially complementary to the at least one strand of the artificial nucleic acid construct, iii) a cleavage of the target mRNA sequence, or iv) any combination of i), ii), or iii). In some instances, the artificial nucleic acid construct comprises no epigenetic modification on any purine or pyrimidine base of a nucleotide or nucleoside. In some instances, the artificial nucleic acid construct facilitates the epigenetic modification through a system that at least in part comprises a DNA methyltransferase, a biologically active fragment thereof, or a derivative thereof. In some instances, the artificial nucleic acid construct facilitates the epigenetic modification through a system that at least in part comprises a DNA acetyltransferase, a biologically active fragment thereof, or a derivative thereof. In some

CLAIMS

WHAT IS CLAIMED IS:

1. A method of inducing an epigenetic modification in a plant organism, comprising applying a formulation to an agricultural object, the formulation comprising an excipient and a double stranded artificial nucleic acid construct comprising (a) a first strand and a second strand each having a length of ~~about~~ 10 to ~~about~~ 30 nucleotides or nucleosides or a combination thereof; and (b) a terminal overhang at each of the 3' ends of the first strand and second strand; wherein: one or both of the 3' terminal end overhangs comprise a ribose unit having a methoxy group (-O-Me) at the 2' position;

   wherein the agricultural object is a seed, a plant, a constituent of a plant, or any combination thereof, and

   wherein the applying causes methylation of at least one base of a nucleotide or nucleoside in a nucleic acid sequence of a gene of the agricultural object.

2. The method of claim 1, wherein both of the terminal end overhangs comprise a ribose unit having a 2' methoxy group at the terminal position.

3. The method of claim 2, wherein each strand of the nucleic acid construct comprises ~~about~~ 20 to ~~about~~ 30 nucleotides.

4. The method of claim 1, wherein one strand of the nucleic acid construct comprises at least ~~about~~ 80% sequence identity to a portion of a transcription regulatory region of the gene.

5. The method of claim 4, wherein the transcription regulatory region is a transcription start site, a TATA box, or an upstream activating sequence.

6. The method of claim 4, wherein the portion of the transcription regulatory region comprises at least ~~about~~ 30% guanine cytosine (GC) content.

7. The method of claim 1, wherein the formulation comprises an excipient and a plurality of double stranded artificial nucleic acid constructs.

8. The method of claim 1, wherein the at least one base is a cytosine.

9. The method of claim 1, wherein the applying causes methylation through a system that at least in part comprises a DNA methyltransferase, a biologically active fragment thereof~~, or a derivative thereof~~.

10. The method of claim 9, wherein the system comprises at least a portion of at least one component of an RNA directed DNA methylation pathway.

11. The method of claim 1, wherein at least ~~about~~ 80% of sugars of the artificial nucleic acid construct, based on a total number of the sugars, each independently comprises a deoxyribose, modified deoxyribose, or a combination thereof.

12. The method of claim 1, wherein each terminal end overhang is one nucleotide long.

13. The method of claim 12, wherein every sugar in the artificial nucleic acid construct is a deoxyribose except for the terminal 3' sugar of the first strand and the second strand.

14. The method of claim 1, wherein the agricultural object comprises a plant, a seed, a fruit, a leaf, a stalk, a root, a flower, a plant embryo, or any combination thereof.

15. The method of claim 1, wherein the method results in at least partial silencing or at least partial activation of the gene in the agricultural object.

16. The method of claim 15, wherein the gene is at least partially silenced for at least one reproduction cycle of the agricultural object.

17. The method of claim 1, wherein the applying results in one or more of the following: (a) preventing or reducing or delaying an enzymatic browning of the agricultural object; (b) increasing a growth rate, a yield, or a lifespan of the agricultural object; (c) decreasing a growth rate, a yield, or a lifespan of the agricultural object; (d) increasing a pest resistance, a salt tolerance, a heat tolerance, a heavy metal tolerance, a disease tolerance, or a drought resistance of the agricultural object; (e) increasing or at least partially decreasing an amount or a production of a molecule made by the agricultural object; (f) altering a color of at least a portion of the agricultural object; (g) increasing or at least partially decreasing a flowering rate of the agricultural object; (h) increasing a volume or a weight of the agricultural object; (i) improving a flavor or a texture of an edible product of the agricultural object; (j) increasing a shelf life of the agriculture product; (k) decreasing the number and size of seeds of the agriculture product; and (j) increasing a nutritional content of the agricultural object;

when the agricultural object is compared to a comparable agricultural object without application of the substance comprising the artificial nucleic acid construct.

18. The method of claim 1, wherein the plant organism is soybean, corn, rice, tomato, alfalfa, wheat, potato, pea, or green algae.

19. The method of claim 18, wherein the plant organism is soybean or corn.

20. The method of claim 1, wherein the formulation is a powder, granule, pellet, bead, or liquid.