3,7-dimethylxanthine (theobromine) (Figure 1, step 3), which is further methylated by DXMT to form 1,3,7-trimethylxanthine (caffeine) [Ogita, S., et al. (2005) *Plant Biotechnology* 22(5): 461-468].

Many groups have been researching caffeine biosynthesis in coffee in order to reduce caffeine accumulation within the plant. For example, the group of Ogita et al. [Ogita et al., Nature (2003) 423: 823; Ogita et al., Plant Molecular Biology (2004) 54(6): 931–941; and Ogita et al. (2005) supra], produced decaffeinated Arabica coffee plants through overexpression of a transgenic RNAi cassette. They designed their RNAi constructs to target the 3' – untranslated region (UTR) and the coding region of CaMXMT1. The overexpression of the CaMXMT1 RNAi constructs reduced the transcript levels of not only CaMXMT1 but also CaDXMT1 and CaXMT1. This is likely to be a result of the similarity shared between the coding regions of the methyltransferases (over 90%) where the primary small double-stranded RNAs (dsRNA) produces many secondary smaller dsRNA which target the mRNA sequences of CaXMT1 and CaDXMT1. Through this method they were able to reduce caffeine accumulation in the leaves by an average of 50 % with one example exhibiting a 70 % reduction.

Several patent applications relate to downregulation of genes involved in caffeine synthesis wherein downregulation is effected by ribozymes (U.S. Patent Application No. 2003/0014775) or by RNA interference (RNAi) using anti-sense molecules (U.S. Patent Application Nos. 2008/0127373 and 2002/0108143 and PCT publication No. WO 1998/036053).

Additional background art includes U.S. Patent Application No. 2017/0014449.

SUMMARY

In one aspect, the disclosure provides a *Coffea canephora* coffee plant comprising a genome comprising a loss of function mutation in a nucleic acid sequence encoding at least one component of a caffeine biosynthesis pathway, wherein said component is a *N*-methyltransferase: (i) encoded by a nucleic acid sequence at least 90% identical to Cc09_g06970 (SEQ ID NO: 9), Cc09_g06960 (SEQ ID NO: 7), Cc00_g24720 (SEQ ID NO: 1), Cc09_g06950 (SEQ ID NO: 5), Cc01_g00720 (SEQ ID NO: 3), or Cc02_g09350 (SEQ ID NO: 11); or (ii) at least 90% identical to Cc09_g06970 (SEQ ID NO: 10), Cc09_g06960 (SEQ ID NO: 8), Cc00_g24720 (SEQ ID NO: 2), Cc09_g06950 (SEQ ID NO: 6), Cc01_g00720 (SEQ ID NO: 4),Cc02_g09350 (SEQ ID NO: 12), BAC75663.1 (SEQ ID NO: 14), ABD90686.1 (SEQ ID NO: 16), BAB39215.1 (SEQ ID NO: 18), ABD90685.1 (SEQ ID NO: 20), BAB39216.1 (SEQ ID NO: 22), or BAC75664.1 (SEQ ID NO: 24); wherein said coffee plant comprises at least a 5% reduction in caffeine as compared to that of a coffee plant of the

same genetic background and developmental stage and growth conditions devoid of said loss of function mutation.

In another aspect, the disclosure provides a method of producing a *Coffea canephora* coffee plant or part thereof, the method comprising: (a) subjecting a *Coffea canephora* coffee plant cell to a DNA editing agent directed towards at least one nucleic acid sequence encoding at least one component of a caffeine biosynthesis pathway, wherein said component is a *N*-methyltransferase: (i) encoded by a nucleic acid sequence at least 90% identical to Cc09_g06970 (SEQ ID NO: 9), Cc09_g06960 (SEQ ID NO: 7), Cc00_g24720 (SEQ ID NO: 1), Cc09_g06950 (SEQ ID NO: 5), Cc01_g00720 (SEQ ID NO: 3), or Cc02_g09350 (SEQ ID NO: 11); or (ii) at least 90% identical to Cc09_g06970 (SEQ ID NO: 10), Cc09_g06960 (SEQ ID NO: 8), Cc00_g24720 (SEQ ID NO: 2), Cc09_g06950 (SEQ ID NO: 6), Cc01_g00720 (SEQ ID NO: 4),Cc02_g09350 (SEQ ID NO: 12), BAC75663.1 (SEQ ID NO: 14), ABD90686.1 (SEQ ID NO: 16), BAB39215.1 (SEQ ID NO: 18), ABD90685.1 (SEQ ID NO: 20), BAB39216.1 (SEQ ID NO: 22), or BAC75664.1 (SEQ ID NO: 24), to result in a loss of function mutation in said nucleic acid sequence; and (b) regenerating a coffee plant or part thereof from said coffee plant cell, wherein said coffee plant comprises at least a 5% reduction in caffeine as compared to that of a coffee plant of the same genetic background and developmental stage and growth conditions devoid of said loss of function mutation.

In another aspect, the disclosure provides a nucleic acid construct comprising a nucleic acid sequence encoding a DNA editing agent directed towards at least one nucleic acid sequence encoding a component of a caffeine biosynthesis pathway, wherein said component is a *N*-methyltransferase, operably linked to a plant promoter for expressing said DNA editing agent in a cell of a *Coffea canephora* coffee plant; wherein said *N*-methyltransferase is: (i) encoded by a nucleic acid sequence at least 90% identical to Cc09_g06970 (SEQ ID NO: 9), Cc09_g06960 (SEQ ID NO: 7), Cc00_g24720 (SEQ ID NO: 1), Cc09_g06950 (SEQ ID NO: 5), Cc01_g00720 (SEQ ID NO: 3), or Cc02_g09350 (SEQ ID NO: 11); or (ii) at least 90% identical to Cc09_g06970 (SEQ ID NO: 10), Cc09_g06960 (SEQ ID NO: 8), Cc00_g24720 (SEQ ID NO: 2), Cc09_g06950 (SEQ ID NO: 6), Cc01_g00720 (SEQ ID NO: 4),Cc02_g09350 (SEQ ID NO: 12), BAC75663.1 (SEQ ID NO: 14), ABD90686.1 (SEQ ID NO: 16), BAB39215.1 (SEQ ID NO: 18), ABD90685.1 (SEQ ID NO: 20), BAB39216.1 (SEQ ID NO: 22), or BAC75664.1 (SEQ ID NO: 24).

In another aspect, the disclosure provides a plant part of the *Coffea canephora* coffee plant of the disclosure.

In another aspect, the disclosure provides a method of producing *Coffea canephora* coffee beans with reduced caffeine content, the method comprising: (a) growing the *Coffea canephora* plant of the disclosure; and (b) harvesting beans from the plant.

In another aspect, the disclosure provides a method of producing *Coffea canephora* coffee with reduced caffeine content, the method comprising subjecting the *Coffea canephora* beans of claim 23 or 24 or produced by the method of claim 25 to extraction or dehydration; optionally further comprising roasting said coffee beans; further optionally wherein the method further comprises processing said roasted coffee beans to produce coarse-ground coffee, filter coffee, or instant coffee; further optionally wherein the method further comprises preparing a coffee beverage from said coarse-ground coffee, filter coffee, or instant coffee; further optionally wherein said coffee beverage further comprises natural or artificial flavouring substances, milk products, alcohol, foaming agents, and/or natural or artificial sweetening agents.

According to an aspect of some examples of the present disclosure there is provided a coffee plant comprising a genome comprising a loss of function mutation in a nucleic acid sequence encoding at least one component of a caffeine biosynthesis pathway.

According to an aspect of some examples of the present disclosure there is provided a method of producing a coffee plant or part thereof, the method comprising: (a) subjecting a coffee plant cell to a DNA editing agent directed at a nucleic acid sequence encoding at least one component of a caffeine biosynthesis pathway to result in a loss of function mutation in the nucleic acid sequence encoding the at least one component of the caffeine biosynthesis pathway; and (b) regenerating a coffee plant or part thereof from the coffee plant cell.

According to an aspect of some examples of the present disclosure there is provided a nucleic acid construct comprising a nucleic acid sequence encoding a DNA editing agent directed

towards at least one component of a caffeine biosynthesis pathway being operably linked to a plant promoter for expressing the DNA editing agent in a cell of a coffee plant.

According to an aspect of some examples of the present disclosure there is provided a plant part of the coffee 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 coffee beans with reduced caffeine content, the method comprising: (a) growing the plant of some examples of the disclosure; and (b) harvesting beans from the plant.

According to an aspect of some examples of the present disclosure there is provided a method of producing coffee with reduced caffeine content, the method comprising subjecting beans of some examples of the disclosure to extraction, dehydration and optionally roasting.

According to an aspect of some examples of the present disclosure there is provided coffee of the beans of some examples of the disclosure.

According to an aspect of some examples of the present disclosure there is provided coffee of the beans produced by the method of some examples of the disclosure.

According to some examples of the disclosure, the method further comprises harvesting beans from the coffee plant.

According to some examples of the disclosure, the method further comprises selfing or crossing the coffee plant.

According to some examples of the disclosure, the mutation occurs in at least one allele.

According to some examples of the disclosure, the mutation occurs in all alleles.

According to some examples of the disclosure, the coffee plant or progeny thereof of some examples of the disclosure, having been treated with a DNA editing agent directed to the nucleic acid sequence encoding the at least one component of the caffeine biosynthesis pathway.

According to some examples of the disclosure, the mutation is selected from the group consisting of a deletion, an insertion, an insertion/deletion (Indel), and a substitution.

According to some examples of the disclosure, the coffee plant is from a species *Coffea canephora.*

According to some examples of the disclosure, the coffee plant is from a species *Coffea arabica*.

According to some examples of the disclosure, the subjecting is to a nucleic acid construct encoding the DNA editing agent.

According to some examples of the disclosure, the subjecting is by a DNA-free delivery method.

According to some examples of the disclosure, the coffee plant comprises at least 5 % reduction in caffeine as compared to that of a coffee plant of the same genetic background and developmental stage and growth conditions devoid of the loss of function mutation.

According to some examples of the disclosure, the DNA editing agent is a non-integrated DNA editing agent.

According to some examples of the disclosure, the DNA editing agent comprises at least one sgRNA.

According to some examples of the disclosure, the sgRNA comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 51-78.

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 meganucleases, Zinc finger nucleases (ZFNs), transcription-activator like effector nucleases (TALENs), CRISPR-endonuclease, dCRISPR-endonuclease, and a homing endonuclease.

According to some examples of the disclosure, the DNA editing agent is of a DNA editing system comprising CRISPR-Cas.

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

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

According to some examples of the disclosure, the DNA editing agent is directed to a nucleic sequence that is at least 90 % identical between Cc09_g06970 (set forth in SEQ ID NO: 9), Cc09_g06960 (set forth in SEQ ID NO: 7), Cc00_g24720 (set forth in SEQ ID NO: 1), Cc09_g06950 (set forth in SEQ ID NO: 5), Cc01_g00720 (set forth in SEQ ID NO: 3) and Cc02_g09350 (set forth in SEQ ID NO: 11).

According to some examples of the disclosure, the DNA editing agent is directed to a nucleic acid segment comprised in a nucleic acid sequence as set forth in any one of SEQ ID NOs: 26-31, 33-36, 38-41, 43-45, 47-48 or 50.

According to some examples of the disclosure, the at least one component of a caffeine biosynthesis pathway is a methyltransferase.

According to some examples of the disclosure, the methyltransferase comprises a core SAM-binding domain.

According to some examples of the disclosure, the methyltransferase is a *N*-methyltransferase.

According to some examples of the disclosure, the *N*-methyltransferase is selected from the group consisting of a xanthosine methyltransferase (XMT), a 7-methyxanthine methyltrasferase (MXMT), and 3,7-dimethylxanthine methyltransferase (DXMT).

According to some examples of the disclosure, the *N*-methyltransferase is selected from the group consisting of Cc09_g06970 (set forth in SEQ ID NO: 10), Cc09_g06960 (set forth in SEQ ID NO: 8), Cc00_g24720 (set forth in SEQ ID NO: 2), Cc09_g06950 (set forth in SEQ ID NO: 6), Cc01_g00720 (set forth in SEQ ID NO: 4),Cc02_g09350 (set forth in SEQ ID NO: 12), BAC75663.1 (set forth in SEQ ID NO: 14), ABD90686.1 (set forth in SEQ ID NO: 16), BAB39215.1 (set forth in SEQ ID NO: 18), ABD90685.1 (set forth in SEQ ID NO: 20), BAB39216.1 (set forth in SEQ ID NO: 22), and BAC75664.1 (set forth in SEQ ID NO: 24).

According to some examples of the disclosure, the coffee plant is non-transgenic.

According to some examples of the disclosure, the plant part being a bean.

According to some examples of the disclosure, the bean is dry.

According to some examples of the disclosure, the coffee being in a powder form.

According to some examples of the disclosure, the coffee being in a granulated form.

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.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS 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.

In the drawings:

FIG. 1 shows the caffeine biosynthetic pathway in coffee plants. The first (1), third (3), and fourth (4) steps feature methyl group transfer, and the second step (2) involves ribose removal.

WHAT IS CLAIMED IS:

1. A *Coffea canephora* coffee plant comprising a genome comprising a loss of function mutation in a nucleic acid sequence encoding at least one component of a caffeine biosynthesis pathway, wherein said component is a *N*-methyltransferase:

(i) encoded by a nucleic acid sequence at least 90% identical to Cc09_g06970 (SEQ ID NO: 9), Cc09_g06960 (SEQ ID NO: 7), Cc00_g24720 (SEQ ID NO: 1), Cc09_g06950 (SEQ ID NO: 5), Cc01_g00720 (SEQ ID NO: 3), or Cc02_g09350 (SEQ ID NO: 11); or

(ii) at least 90% identical to Cc09_g06970 (SEQ ID NO: 10), Cc09_g06960 (SEQ ID NO: 8), Cc00_g24720 (SEQ ID NO: 2), Cc09_g06950 (SEQ ID NO: 6), Cc01_g00720 (SEQ ID NO: 4),Cc02_g09350 (SEQ ID NO: 12), BAC75663.1 (SEQ ID NO: 14), ABD90686.1 (SEQ ID NO: 16), BAB39215.1 (SEQ ID NO: 18), ABD90685.1 (SEQ ID NO: 20), BAB39216.1 (SEQ ID NO: 22), or BAC75664.1 (SEQ ID NO: 24);

wherein said coffee plant comprises at least a 5% reduction in caffeine as compared to that of a coffee plant of the same genetic background and developmental stage and growth conditions devoid of said loss of function mutation.

2. A method of producing a *Coffea canephora* coffee plant or part thereof, the method comprising:

(a) subjecting a *Coffea canephora* coffee plant cell to a DNA editing agent directed towards at least one nucleic acid sequence encoding at least one component of a caffeine biosynthesis pathway, wherein said component is a *N*-methyltransferase:

(i) encoded by a nucleic acid sequence at least 90% identical to Cc09_g06970 (SEQ ID NO: 9), Cc09_g06960 (SEQ ID NO: 7), Cc00_g24720 (SEQ ID NO: 1), Cc09_g06950 (SEQ ID NO: 5), Cc01_g00720 (SEQ ID NO: 3), or Cc02_g09350 (SEQ ID NO: 11); or

(ii) at least 90% identical to Cc09_g06970 (SEQ ID NO: 10), Cc09_g06960 (SEQ ID NO: 8), Cc00_g24720 (SEQ ID NO: 2), Cc09_g06950 (SEQ ID NO: 6), Cc01_g00720 (SEQ ID NO: 4),Cc02_g09350 (SEQ ID NO: 12), BAC75663.1 (SEQ ID NO: 14), ABD90686.1 (SEQ ID NO: 16), BAB39215.1 (SEQ ID NO: 18), ABD90685.1 (SEQ ID NO: 20), BAB39216.1 (SEQ ID NO: 22), or BAC75664.1 (SEQ ID NO: 24),

to result in a loss of function mutation in said nucleic acid sequence ; and

(b) regenerating a coffee plant or part thereof from said coffee plant cell, wherein said coffee plant comprises at least a 5% reduction in caffeine as compared to that of a coffee plant of the

same genetic background and developmental stage and growth conditions devoid of said loss of function mutation.

3. The method of claim 2, further comprising harvesting beans from said coffee plant.

4. The method of claim 2 or 3, further comprising selfing or crossing the coffee plant.

5. The coffee plant of claim 1, or method of any one of claims 2 to 4, wherein said mutation occurs in at least one allele.

6. The coffee plant of claim 1, or method of any one of claims 2 to 4, wherein said mutation occurs in all alleles.

7. The coffee plant of claim 1, 5 or 6 or progeny thereof, having been treated with a DNA editing agent directed to said nucleic acid sequence.

8. The coffee plant of any one of claims 1 and 5 to 7, or the method of any one of claims 2 to 6, wherein said mutation is selected from the group consisting of a deletion, an insertion, an insertion/deletion (Indel), and a substitution.

9. The method of any one of claims 2 to 6 and 8, wherein said subjecting is to a nucleic acid construct encoding said DNA editing agent.

10. The method of any one of claims 2 to 6 and 8, wherein said subjecting is by a DNA-free delivery method.

11. A nucleic acid construct comprising a nucleic acid sequence encoding a DNA editing agent directed towards at least one nucleic acid sequence encoding a component of a caffeine biosynthesis pathway, wherein said component is a *N*-methyltransferase, operably linked to a plant promoter for expressing said DNA editing agent in a cell of a *Coffea canephora* coffee plant; wherein said *N*-methyltransferase is:

(i) encoded by a nucleic acid sequence at least 90% identical to Cc09_g06970 (SEQ ID NO: 9), Cc09_g06960 (SEQ ID NO: 7), Cc00_g24720 (SEQ ID NO: 1), Cc09_g06950 (SEQ ID NO: 5), Cc01_g00720 (SEQ ID NO: 3), or Cc02_g09350 (SEQ ID NO: 11); or

(ii) at least 90% identical to Cc09_g06970 (SEQ ID NO: 10), Cc09_g06960 (SEQ ID NO: 8), Cc00_g24720 (SEQ ID NO: 2), Cc09_g06950 (SEQ ID NO: 6), Cc01_g00720 (SEQ ID NO: 4),Cc02_g09350 (SEQ ID NO: 12), BAC75663.1 (SEQ ID NO: 14), ABD90686.1 (SEQ ID NO: 16), BAB39215.1 (SEQ ID NO: 18), ABD90685.1 (SEQ ID NO: 20), BAB39216.1 (SEQ ID NO: 22), or BAC75664.1 (SEQ ID NO: 24).

12. The coffee plant of claim 7 or 8, method of any one of claims 2 to 6 and 8 to 10, or nucleic acid construct of claim 11, wherein said DNA editing agent comprises at least one sgRNA.

13. The coffee plant, method, or nucleic acid construct of claim 12, wherein said sgRNA comprises a nucleic acid sequence selected from the group consisting of SEQ ID Nos: 51-78.

14. The coffee plant of any one of claims 7, 8, 12 and 13, method of any one of claims 2 to 6, 8 to 10, 12 and 13, or nucleic acid construct of any one of claims 11 to 13, wherein said DNA editing agent does not comprise an endonuclease.

15. The coffee plant of any one of claims 7, 8, 12 and 13, method of any one of claims 2 to 6, 8 to 10, 12 and 13, or nucleic acid construct of any one of claims 11 to 13, wherein said DNA editing agent comprises an endonuclease.

16. The coffee plant of any one of claims 7, 8, 12 and 13 to 15 , method of any one of claims 2 to 6, 8 to 10, and 12 to 15, or nucleic acid construct of any one of claims 11 to 15, wherein said DNA editing agent is of a DNA editing system selected from the group consisting of meganucleases, Zinc finger nucleases (ZFNs), transcription-activator like effector nucleases (TALENs), CRISPR-endonuclease, dCRISPR-endonuclease, and a homing endonuclease.

17. The coffee plant of any one of claims 7, 8, 12 and 13 to 15, method of any one of claims 2 to 6, 8 to 10, and 12 to 15, or nucleic acid construct of any one of claims 11 to 15, wherein said DNA editing agent is of a DNA editing system comprising CRISPR-Cas.

18. The coffee plant of any one of claims 7, 8, 12 and 13 to 17, method of any one of claims 2 to 6, 8 to 10, and 12 to 17, or nucleic acid construct of any one of claims 11 to 17, wherein said DNA editing agent is linked to a reporter for monitoring expression in a cell.

19. The coffee plant, method, or nucleic acid construct of claim 18, wherein said reporter is a fluorescent protein.

20. The coffee plant of any one of claims 7, 8, 12 and 13 to 19, method of any one of claims 2 to 6, 8 to 10, and 12 to 19, or nucleic acid construct of any one of claims 11 to 19, wherein said DNA editing agent is directed to a nucleic acid segment comprised in a nucleic acid sequence as set forth in any one of SEQ ID NOs: 26-31, 33-36, 38-41, 43-45, 47-48, and 50.

21. The coffee plant of any one of claims 1, 5 to 8 and 12 to 20, method of any one of claims 2 to 6, 8 to 10 and 12 to 20, wherein the coffee plant is non-transgenic.

22. A plant part of the *Coffea canephora* coffee plant of any one of claims 1, 5 to 8 and 12 to 21.

23. The plant part of claim 22, wherein said plant part is a bean.

24. The bean of claim 23, wherein said bean is dry.

25. A method of producing *Coffea canephora* coffee beans with reduced caffeine content, the method comprising:

(a) growing the *Coffea canephora* plant of any one of claims 1, 5 to 8 and 12 to 21; and

(b) harvesting beans from the plant.

26. A method of producing *Coffea canephora* coffee with reduced caffeine content, the method comprising subjecting the *Coffea canephora* beans of claim 23 or 24 or produced by the method of claim 25 to extraction or dehydration;

optionally further comprising roasting said coffee beans;

further optionally wherein the method further comprises processing said roasted coffee beans to produce coarse-ground coffee, filter coffee, or instant coffee;

further optionally wherein the method further comprises preparing a coffee beverage from said coarse-ground coffee, filter coffee, or instant coffee;

further optionally wherein said coffee beverage further comprises natural or artificial flavouring substances, milk products, alcohol, foaming agents, and/or natural or artificial sweetening agents.

27. *Coffea canephora* coffee of the beans of claim 23 or 24.

28. *Coffea canephora* coffee of the beans produced by the method of claim 25 or 26.

29. The coffee of claim 27 or 28, wherein said coffee is in a powder form.

30. The coffee of claim 27 or 28, wherein said coffee is in a granulated form.