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United States Patent Application Publication 20250261603
Kind Code A1
Publication Date August 21, 2025
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METHODS OF MAKING FEMINIZED CANNABIS SATIVA SEED AND COMPOSITIONS THEREOF

Abstract

The present disclosure relates to methods of producing viable *Cannabis sativa* seeds by inducing male flowers on a genetically female *Cannabis sativa* plant. The pollen produced by the induced flowers of such plant can be used to pollinate female flowers from the same plant or another female plant to produce seed. In certain embodiments, feminized *Cannabis sativa* seed is produced on a production scale.

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Family ID: **1000008423379**

Appl. No.: **19/048458**

Filed: **February 07, 2025**

Related U.S. Application Data

parent US continuation 17274369 20210308 ABANDONED US continuation PCT/US2020/056694 20201021 child US 19048458
us-provisional-application US 62924456 20191022

Publication Classification

Int. Cl.: **A01H1/02** (20060101); **A01H5/02** (20180101); **A01H6/28** (20180101); **C12Q1/6895** (20180101)

U.S. Cl.:

CPC **A01H1/026** (20210101); **A01H5/02** (20130101); **A01H6/28** (20180501); **C12Q1/6895** (20130101); C12Q2600/13 (20130101); C12Q2600/158 (20130101)

Background/Summary

BACKGROUND

[0001] Plant breeding is the process of manipulating and selecting plant species in order to create improved genotypes and phenotypes for specific purposes in agriculture. This process often involves controlled pollinations to produce elite genetic combinations which are then selected for their unique traits or ability to solve key production challenges. Reproductive biology differs across cultivated crops and breeders require specific technologies in each crop to enable advanced breeding methods that are used for genetic improvement. *Cannabis* is emerging as a valuable new crop for medicinal, recreational, and industrial purposes and there is a need to improve breeders' ability to develop improved strains and seed production systems.

[0002] *Cannabis sativa* is one of the oldest cultivated plants, as it has many uses. The stem is a source of the strong, hemp fiber which is used to make rope, cloth, and other textiles. Oil extracts from the seed are used in food products, paint, varnishes, and lubricants. Female plants produce flowers with dense trichomes that accumulate oil that contains terpenes and cannabinoids with medicinal and euphoria-inducing properties.

[0003] *Cannabis sativa* is described as a wind-pollinated dioecious plant (an individual plant has only male or female flowers and only the flowers from female plants have value as a product themselves or extracts thereof) with gender being determined by heteromorphic chromosomes (X and Y): the male plant is heterogametic (XY) and produces male reproductive structures and the female is homogametic (XX) and produces female reproductive structures. This reproductive system makes breeding challenging for two main reasons.

[0004] First, crossing a genetically male plant to a genetically female plant produces seed in which approximately 50% of the progeny is male and approximately 50% is female, due to Mendelian segregation of the sex chromosomes. Since the most valuable cannabinoid compounds come from the unfertilized flower on female plants, male progeny must be identified and rogued out of any cultivation system before any pollen is formed and released. This requirement creates a range of logistical challenges that makes large-scale production expensive and inefficient. Establishment of male-free cultivation areas is the main reason that clonal propagation of female plants is the primary form of plant production today, even after decades of cultivation. Clonal selection and propagation has led to short-term gains in cannabis phenotypes but for large-scale production, cloned plants have a range of production problems from cost, to pathogens, loss of genetics, and inability to scale. Thus, advanced seed-based breeding and production methods appear essential to the cannabis industry's forecasted growth and long-term sustainability.

[0005] Second, because cannabis is dioecious, it is biologically impossible to make inbred lines through sequential self-pollination, as has been commonly done in most row and vegetable crops. Advanced breeding methods often require development of near-isogenic inbred lines. Inbred lines are made to be homozygous through multiple generations of self-pollination (inbreeding) which allows for the selection of desired traits to be genetically fixed. Development of inbred lines simplifies the identification of markers and associations between phenotypes and genotypes, and allows for the precision breeding for traits, such as maturity adaptation, disease resistance, composition, and yield. Development of inbred lines also allows for the eventual selection and production of hybrid genetics which have a range of benefits for farmers and agricultural production.

[0006] Though *Cannabis sativa* is genetically dioecious, the species has shown some flexibility in

gender phenotype and there are described examples of individual plants with both male and female inflorescences. Spinach is another dioecious crop showing hermaphrodite floral phenotypes and many species of cucurbits show a broad range of gender expression in flower morphology. Modification of sex phenotype may be induced by environmental triggers such as stress, light intensity, photoperiod, temperature or a combination of several environmental signals making it difficult to reproduce the effect. Sex expression can also be influenced by plant growth hormones, including auxins, ethylene, gibberellic acid, and cytokinins. The mode of action of phenotypic sex expression is thought to be caused by endogenous plant hormones. Thus, the modification of sex expression in plants is caused by a complex and difficult to predict interaction of environmental signals, hormones, and genetics. This complexity has made discovering and developing treatments to produce reliable changes in gender needed for large-scale seed production historically very challenging.

[0007] Ethylene inhibitors are thought to influence sex expression in a variety of plant genus. For example, ethylene inhibitors are used to induce male flowers in cucumbers, but in some watermelon types ethylene inhibitor has the opposite effect and hastens the appearance of female or pistillate flowers (Rudich and Zamski, 1985; Sugiyama et al., 1998). Ethylene inhibitors, such as silver thiosulfate (STS) (Lubell 2018; Ram 1982) and AVG (Ram and Sett, 1981) have been used to induce male flowers in *Cannabis sativa*, but not through a method that can be scaled for large-scale production. Additionally, there is no reported evidence of the production of viable seeds.

[0008] In general, seeds produced by using pollen from a female plant induced to produce male flowers onto female flowers of the same plant or female flowers from another female plant will result in exclusively female plants. Such seed are termed “feminized”. While *Cannabis sativa* seeds advertised as feminized seeds can be purchased in small quantities ($20-50/seed for indoor cultivation strains), the methods of producing such seeds have not been disclosed. Further, purchasers of such seed frequently report contamination of purported female seeds with male seeds. The controlled environment *Cannabis sativa* industry relies exclusively on vegetative clonal propagation for *Cannabis sativa* flower production from female plants. It is most desirable to grow *Cannabis sativa* from seed, rather than from clones to prevent the spread of disease from donor mother plants to clonal offspring. Clones have highly variable vigor and growth, with significant mortality. Based on the reports of variability of success of STS induction of male flowers, the lack of evidence for feminized seed production methods in the literature, the high price and limited availability of purportedly feminized seed to purchase, and the fact that the controlled environment *Cannabis* industry is still reliant on clonal propagation, there appears to be currently no production-scale method of inducing male flowers on female plants that successfully produces feminized seed.

[0009] Thus, there remains a need for methods, including high-throughput methods, of producing large numbers of feminized *Cannabis sativa* seeds free of contaminating male seeds.

SUMMARY

[0010] Certain aspects of this disclosure are directed to a method of producing a genetically female *Cannabis sativa* seed comprising the steps of: a) inducing one or more male flowers on a genetically female *Cannabis sativa* plant by applying a chemical ethylene inhibitor to the plant, wherein the female plant forms one or more pollen-producing fertile male flowers; and b) producing the seed by pollinating a female flower with pollen from the induced one or more male flowers, wherein the seed produced is genetically female.

[0011] Certain aspects are drawn to a method of inducing male flowers on a genetically female *Cannabis sativa* plant comprising applying a chemical ethylene inhibitor to a genetically female *Cannabis sativa* plant, wherein the female plant forms one or more pollen-producing fertile male flowers.

[0012] Certain aspects provide for a method of producing an inbred variety of *Cannabis sativa*, the method comprising: (i) producing a genetically female *Cannabis sativa* plant with induced-male flowers, wherein the female plant comprises one or more pollen-producing fertile male flowers, by

a method disclosed herein; (ii) producing seed by: (a) self-pollination of pollen from an induced-male flower onto a female flower of the same plant, or (b) cross-pollination of pollen from induced-male flowers onto female flowers of a separate but genetically identical female plant; and (iii) growing a genetically female *Cannabis sativa* plant from a seed produced in (ii); and optionally, (iv) repeating steps (i), (ii), and (iii) for at least two, three, four, five, six, or seven generations. Certain embodiments provide for an inbred *Cannabis sativa* seed, an inbred variety of *Cannabis sativa*, or a plant of the variety, and/or a population of inbred *Cannabis sativa* plants produced according to or derived from the above method.

[0013] Certain aspects provide for a method of producing reproducible hybrid *Cannabis sativa* seed comprising crossing two distinct inbred *Cannabis sativa* varieties, wherein at least one inbred variety has been produced by the above method. Certain embodiments provide for a hybrid *Cannabis sativa* seed, a hybrid *Cannabis sativa* plant, and/or a population of hybrid *Cannabis sativa* plants produced according to or derived from the above method.

[0014] Certain aspects provide for a method of producing a population of *Cannabis sativa* plants comprising: i) producing a genetically female *Cannabis sativa* plant with induced-male flowers, wherein the female plant comprises one or more pollen-producing fertile male flowers, produced by a method disclosed herein; (ii) producing seed by pollinating a female flower with pollen from the induced one or more male flowers; and (iii) growing a population of *Cannabis sativa* plants from a plurality of seed produced in (ii).

[0015] Certain aspects provide for a method of producing inbred *Cannabis sativa* seed comprising producing a plurality of genetically female *Cannabis sativa* plants with induced-male flowers, wherein the female plants comprise one or more pollen-producing fertile male flowers, produced by the method disclosed herein, wherein the female plants with induced-male flowers are grown in isolation or in coincidence with non-induced female plants of the same genotype.

[0016] Certain aspects provide for a method of producing hybrid *Cannabis sativa* seed comprising producing a plurality of genetically female *Cannabis sativa* plants with induced-male flowers, wherein the female plants comprise one or more pollen-producing fertile male flowers, produced by a method disclosed herein, wherein the female plants with induced-male flowers are grown in isolation or in coincidence with non-induced female plants having a different genotype.

[0017] Certain aspects provide for a method of screening *Cannabis sativa* germplasm to identify genotypes responsive to the induction of male flowers on genetically female plants comprising applying a chemical ethylene inhibitor to a genetically female plant and determining the response of the plant with respect to the induction of male flowers. In certain embodiments, the method further comprises selecting a plant based on its response to the induction of male flowers. In certain embodiments, the method further comprises genotyping the selected plant. In certain embodiments, the method further comprises identifying an association between the genotype of the selected plant and its response to the induction of male flowers. Certain aspects provide for a method of introgressing a genotype associated with responsiveness to the induction of male flowers into plant germplasm using a genotype identified by the above method. Certain aspects provide for a method of establishing inbred breeding pools of *Cannabis sativa* germplasm based on the genotypes identified by the above method.

[0018] Certain aspects provide for a method of processing pollen from a genetically female plant with induced-male flowers produced by a method disclosed herein, the method of pollen processing comprising: (i) removing an inflorescence-containing male flower from the female plant after mature male flowers or anthers are formed; (ii) drying the male flowers or anthers for 1 to 5 days; and then (iii) sifting to isolate pollen.

Description

DETAILED DESCRIPTION

[0019] To the extent necessary to provide descriptive support, the subject matter and/or text of the appended claims is incorporated herein by reference in their entirety.

[0020] Further, it will be understood by all readers of this written description that the exemplary aspects and embodiments described and claimed herein may be suitably practiced in the absence of any recited feature, element or step that is, or is not, specifically disclosed herein.

Definitions

[0021] It is to be noted that the term “a” or “an” entity refers to one or more of that entity; for example, “a plant” is understood to represent one or more plants. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

[0022] Furthermore, “and/or” where used herein is to be taken as specific disclosure of each of the specified features or components with or without the other. Thus, the term and/or” as used in a phrase such as “A and/or B” herein is intended to include “A and B,” “A or B,” “A” (alone), and “B” (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0023] It is understood that wherever aspects are described herein with the language “comprising” or “comprises” otherwise analogous aspects described in terms of “consisting of,” “consists of,” “consisting essentially of,” and/or “consists essentially of,” and the like are also provided.

[0024] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related.

[0025] Numeric ranges are inclusive of the numbers defining the range. Even when not explicitly identified by “and any range in between,” or the like, where a list of values is recited, e.g., 1, 2, 3, or 4, unless otherwise stated, the disclosure specifically includes any range in between the values, e.g., 1 to 3, 1 to 4, 2 to 4, etc.

[0026] For purposes of this disclosure, “flowering” in a female plant is defined by the visible formation of an inflorescence at one or more nodes.

[0027] For purposes of this disclosure, the “stem diameter” of a *Cannabis sativa* plant is a measurement taken 1 cm above the soil line.

[0028] Unless otherwise specified, reference to an exclusively female population of *Cannabis sativa* plants refers to a population of plants grown from seeds and not a clonal population of female plants.

[0029] The headings provided herein are solely for ease of reference and are not limitations of the various aspects or embodiments of the disclosure, which can be had by reference to the specification as a whole.

Overview

[0030] *Cannabis sativa* has 2 copies of 10 chromosomes (2n=20) and sex is primarily determined by sex chromosomes, with XX being female and XY being male.

[0031] *Cannabis sativa* plants are generally grown vegetatively under long days and after transition to natural or artificial short day conditions, flowering is induced. Male plants produce staminate floral buds that open to release pollen that is naturally wind-distributed. Female plants produce flowers in a cluster, called a cola or an inflorescence. Each flower contains two stigmas and an ovule enclosed by bracts, which have the highest density of resin-containing trichomes.

2-aminoethoxyvinylglycine

[0032] 2-aminoethoxyvinylglycine (AVG) is an inhibitor of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, a key enzyme involved in ethylene biosynthesis. In contrast, silver ions (Ag) and the gaseous compound 1-methylcyclopropene (1-MCP) have a different mode of action, and are both inhibitors of the ethylene receptors.

[0033] AVG not only inhibits ACC synthase involved in ethylene biosynthesis but also inhibits

tryptophan aminotransferase involved in auxin biosynthesis, an enzyme that shares a similar pyridoxal-phosphate-dependent mechanism to ACC synthase (Schaller G. E., Binder B. M. (2017) Inhibitors of Ethylene Biosynthesis and Signaling. In: Binder B., Eric Schaller G. (eds.) Ethylene Signaling. Methods in Molecular Biology, vol. 1573. Humana Press, New York, NY). In 1981, Ram et al., report delivering AVG to the apical meristem through a cotton wick to induce male flowers on the apical node of female *Cannabis sativa* plants, but failed to provide any evidence of the production of viable, feminized seed. In the past 30 plus years, there have been no reports of AVG being used to induce male structures or pollen, or seed produced using such pollen in *cannabis* plants. Thus, given that AVG has a different mode of action on the ethylene biosynthesis pathway and that it affects the biosynthesis of another key plant hormone, auxin, it was unknown if AVG could result in conversion of female *cannabis* flowers to male flowers that produced viable pollen that could be used to produce feminized seed. It was also unknown if AVG applied as a foliar spray or as a root drench would result in the movement of AVG to developing meristems to produce a robust male response required for large-scale seed production.

Feminized *Cannabis sativa* Seed

[0034] Provided herein are methods of producing a genetically female *Cannabis sativa* seed and/or embryo as well as a genetically female *Cannabis sativa* seed and/or embryo obtained by such methods. For the purposes of brevity, it will be understood that unless otherwise specified, reference to genetically female *Cannabis sativa* "seed" is shorthand encompassing genetically female *Cannabis sativa* "seed and/or embryo." In certain embodiments, the genetically female *Cannabis sativa* seed is inbred. In certain embodiments, the genetically female *Cannabis sativa* seed is a hybrid. Certain embodiments provide for a *Cannabis sativa* plants grown from such a seed. Certain embodiments provide for a population of *Cannabis sativa* plants grown from a plurality of such seed. Likewise, as large *Cannabis sativa* operations rely on clonal propagation, certain embodiments provide for a population of exclusively female *Cannabis sativa* plants grown from seed. In certain embodiments, the population of *Cannabis sativa* plants comprises at least about 6 plants, 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, 50,000 plants, 100,000 plants, 500,000 plants, or 1,000,000 plants. In certain embodiments, the population of *Cannabis sativa* plants comprises from any of about 6 plants, 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, 50,000 plants, 100,000 plants, or 500,000 plants to any of about 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, 50,000 plants, 100,000 plants, 500,000 plants or 1,000,000 plants. In certain embodiments anywhere herein, greater than 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 99.99% of the plants in the population are genetically female plants. In certain embodiments anywhere herein, all of the plants in the population are genetically female plants. Certain embodiments provide for a floral bud/inflorescence of the *Cannabis sativa* plant disclosed above or of a *Cannabis sativa* plant of a population of *Cannabis sativa* plants disclosed above. Further, certain embodiments provide for a method of growing a *Cannabis sativa* plant that comprises growing a *Cannabis sativa* plant from a genetically female *Cannabis sativa* seed disclosed anywhere herein including methods of producing inbred and hybrid seeds.

[0035] In certain embodiments, the method of producing a genetically female *Cannabis sativa* seed comprises inducing one or more male flowers on a genetically female *Cannabis sativa* plant by applying a chemical ethylene inhibitor to the plant. A number of chemical ethylene inhibitors are known. Representative examples of ethylene inhibitors include aminoethoxyvinylglycine (AVG), silver thiosulfate, and 1-Methylcyclopropene (1-MCP). In certain embodiments, the ethylene inhibitor is aminoethoxyvinylglycine (AVG). Application of the chemical ethylene inhibitor causes the female plant to form one or more pollen-producing fertile male flowers. Seed can then be produced by pollinating a female flower with pollen from the induced one or more male flowers. In certain embodiments, some (<50%), most (50%-99%), or all (100%) of the seed is genetically

female. In certain embodiments, the seed is produced by self-pollination of pollen from the induced one or more male flowers onto a female flower of the same plant. In certain other embodiments, the seed is produced by cross-pollination of pollen from the induced one or more male flowers onto a female flower of a separate female plant. The separate female plant used for cross-pollination can be genetically identical to the plant with the induced male flowers or the separate female plant used for cross-pollination can have a genotype that is distinct from the plant with the induced male flowers. In certain embodiments, the separate female plant used having a genotype that is distinct from the plant with the induced male flowers is from a distinct inbred strain or variety. Thus, in certain embodiments, cross-pollination creates inbred seed and in certain embodiments cross-pollination creates hybrid seed. Pollination can be natural (e.g., wind-blown), assisted, or done manually. In certain embodiments, pollination is done manually, such as by transferring the pollen to the female flower with a brush. In certain embodiments, pollination can be assisted by dispersing the pollen such as by shaking or whacking the plant and/or by creating air movement. In certain embodiments, pollen from the induced one or more male flowers is disbursed for pollination by an ultrasonic dehiscence system.

[0036] In certain embodiments, one application of the ethylene inhibitor is sufficient. It has also been discovered that multiple applications of the ethylene inhibitor can be used. In certain embodiments, the ethylene inhibitor is applied to the plant in two, three, four, five, or six separate applications. In certain embodiments, the ethylene inhibitor is applied to the plant in at least two, three, four, five, or six separate applications. In certain embodiments, the ethylene inhibitor is applied to the plant in 5 or more, 10 or more, 15 or more, or 20 or more separate applications.

[0037] Certain aspects of the methods of this disclosure involve different modes of ethylene inhibitor application. In certain embodiments, the ethylene inhibitor is applied in at least one application by root drench to the plant. In certain embodiments, the ethylene inhibitor is applied by root drench in all applications. In certain embodiments, the ethylene inhibitor is applied in at least one application by foliar spray to the plant. In certain embodiments, the ethylene inhibitor is applied by foliar spray in all applications. In certain embodiments, the ethylene inhibitor is applied in at least one application by root drench and also in at least one application by foliar spray. In certain embodiments involving at least one application by root drench and at least one application by foliar spray, the first application of the ethylene inhibitor to the plant between the two is by root drench. In certain embodiments involving at least one application by root drench and at least one application by foliar spray, the first application of the ethylene inhibitor to the plant between the two is by foliar spray.

[0038] It will be understood that in certain embodiments, certain applications of the ethylene inhibitor (e.g., AVG) comprise root drench, i.e., root drench can be in combination with application by foliar spray but does not require combination with foliar spray, while other applications comprising root drench exclude foliar spray. Likewise, certain applications comprise foliar spray, i.e., foliar spray can be in combination with application by root drench but does not require combination with root drench, while other applications comprising foliar spray exclude root drench. For example, in certain embodiments the ethylene inhibitor is applied to the plant: [0039] (i) wherein the application comprises root drench in all applications; [0040] (ii) wherein the application comprises root drench but not foliar spray in any applications; [0041] (iii) wherein the application comprises foliar spray in all applications; [0042] (iv) wherein the application comprises foliar spray but not root drench in any applications; [0043] (v) wherein the application comprises root drench combined with foliar spray in all applications; [0044] (vi) wherein the application comprises root drench combined with foliar spray in at least one application and root drench but not foliar spray in at least one separate application; [0045] (vii) wherein the application comprises root drench combined with foliar spray in at least one application and foliar spray but not root drench in at least one separate application; and/or [0046] (viii) wherein the application comprises root drench but not foliar spray in at least one application and also comprises foliar spray but not

root drench in at least one separate application.
[0047] In certain embodiments, the first application of the ethylene inhibitor to the plant comprises application by root drench. In certain embodiments, the first application of the ethylene inhibitor to the plant comprises root drench application but not foliar spray. In certain embodiments, the first application of the ethylene inhibitor to the plant comprises application by foliar spray. In certain embodiments, the first application of the ethylene inhibitor to the plant comprises foliar spray application but not root drench.
[0048] Certain aspects of the methods of this disclosure involve the timing of the ethylene inhibitor application. In certain embodiments, the ethylene inhibitor is applied to the plant prior to flowering. In certain embodiments, the ethylene inhibitor is first applied to the plant prior to flowering. In certain embodiments, the ethylene inhibitor is only applied to the plant prior to flowering. In certain embodiments, the ethylene inhibitor is applied to the plant in at least two, three, four, five, or six separate applications prior to flowering. In certain embodiments, the ethylene inhibitor is applied to the plant in 5 or more, 10 or more, 15 or more, or 20 or more separate applications prior to flowering. In certain embodiments, the ethylene inhibitor is applied to the plant after flowering is initiated. In certain embodiments, the ethylene inhibitor is only applied to the plant after flowering is initiated. In certain embodiments, the ethylene inhibitor is applied to the plant in at least two, three, four, five, or six separate applications after flowering is initiated. In certain embodiments, the ethylene inhibitor is applied to the plant in 5 or more, 10 or more, 15 or more, or 20 or more separate applications after flowering is initiated. In certain embodiments, the ethylene inhibitor is re-applied 2, 3, 4, 5, 6, 7, 8, or 9 days after the initial application. Further, in certain embodiments, the ethylene inhibitor is re-applied 2, 3, 4, 5, 6, 7, 8, or 9 days after the application that is 2, 3, 4, 5, 6, 7, 8, or 9 days after the initial treatment. In certain embodiments, the ethylene inhibitor is re-applied 10, 11, 12, 13, 14, 15, 16, 17, or 18 days after the initial treatment.
[0049] In certain of any of these methods, the ethylene inhibitor can be applied to the plant prior to flowering and/or after flowering is initiated and can be applied by root drench and/or foliar spray. For example, in certain embodiments, the ethylene inhibitor is applied by root drench to the plant prior to flowering and the ethylene inhibitor is separately applied by root drench to the plant after flowering is initiated. For example, in certain embodiments, the ethylene inhibitor is applied by root drench to the plant prior to flowering and the ethylene inhibitor is separately applied by foliar spray to the plant after flowering is initiated. For example, in certain embodiments, the ethylene inhibitor is applied by foliar spray to the plant prior to flowering and the ethylene inhibitor is separately applied by root drench to the plant after flowering is initiated. For example, in certain embodiments, the ethylene inhibitor is applied by foliar spray to the plant prior to flowering and the ethylene inhibitor is separately applied by foliar spray to the plant after flowering is initiated.
[0050] As noted above, it will be understood that in certain embodiments, certain applications of the ethylene inhibitor (e.g., AVG) comprise root drench, i.e., root drench can be in combination with application by foliar spray but does not require combination with foliar spray, while other applications comprising root drench exclude foliar spray. Likewise, certain applications comprise foliar spray, i.e., foliar spray can be in combination with application by root drench but does not require combination with root drench, while other applications comprising foliar spray exclude root drench. Thus, in certain embodiments: [0051] (i) The ethylene inhibitor application comprises root drench prior to flowering and separately comprises root drench after flowering is initiated. In certain embodiments, the ethylene inhibitor application comprises at least one root drench application without foliar spray prior to flowering. Further, in certain embodiments, the ethylene inhibitor application comprises root drench but not foliar spray prior to flowering. In certain embodiments, the ethylene inhibitor application separately comprises at least one root drench application without foliar spray after flowering is initiated. Further, in certain embodiments, the ethylene inhibitor application comprises root drench but not foliar spray after flowering is initiated;
[0052] (ii) The ethylene inhibitor application comprises root drench prior to flowering and

separately comprises foliar spray after flowering is initiated. In certain embodiments, the ethylene inhibitor application comprises at least one root drench application without foliar spray prior to flowering. Further, in certain embodiments, the ethylene inhibitor application comprises root drench but not foliar spray prior to flowering. In certain embodiments, the ethylene inhibitor application separately comprises at least one foliar spray application without root drench after flowering is initiated. Further, in certain embodiments, the ethylene inhibitor application comprises foliar spray but not root drench after flowering is initiated; [0053] (iii) The ethylene inhibitor application comprises foliar spray prior to flowering and separately comprises root drench after flowering is initiated. In certain embodiments, the ethylene inhibitor application comprises at least one foliar spray application without root drench prior to flowering. Further, in certain embodiments, the ethylene inhibitor application comprises foliar spray but not root drench prior to flowering. In certain embodiments, the ethylene inhibitor application separately comprises at least one root drench application without foliar spray after flowering is initiated. Further, in certain embodiments, the ethylene inhibitor application comprises root drench but not foliar spray after flowering is initiated; or [0054] (iv) The ethylene inhibitor application comprises foliar spray prior to flowering and separately comprises foliar spray after flowering is initiated. In certain embodiments, the ethylene inhibitor application comprises at least one foliar spray application without root drench prior to flowering. Further, in certain embodiments, the ethylene inhibitor application comprises foliar spray but not root drench prior to flowering. In certain embodiments, the ethylene inhibitor application separately comprises at least one foliar spray application without root drench after flowering is initiated. Further, in certain embodiments, the ethylene inhibitor application comprises foliar spray and not root drench after flowering is initiated.

[0055] Certain aspects of the methods of this disclosure are drawn to inducing flowering of the plants. In certain embodiments, one the same day of or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 days after the initial application of the ethylene inhibitor to the plant, the plant is exposed to environmental conditions known to induce flowering in *Cannabis sativa*. In certain embodiments, one the same day of or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days after the initial application of the ethylene inhibitor to the plant, the plant is exposed to environmental conditions known to induce flowering in *Cannabis sativa*. In certain embodiments, prior to at least the initial application of the ethylene inhibitor to the plant, the plant is grown under vegetative growth conditions for *Cannabis sativa*. In certain embodiments, the plant is grown under vegetative growth conditions for *Cannabis sativa* up until 1, 2, 3, 4, 5, 6, or 7 days after the initial application of the ethylene inhibitor to the plant, at which time the plant is switched to environmental conditions that induce flowering in *Cannabis sativa.*

[0056] Certain aspects of the methods of this disclosure are drawn to the rate at which the ethylene inhibitor is applied. In certain embodiments, the ethylene inhibitor is applied in one or more applications by root drench in a solution containing from any of about 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1.0 mg, 1.5 mg, 2.0 mg, 2.5 mg, 3.0 mg, 3.5 mg, 4.0 mg, or 5.0 mg to any of about 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1.0 mg, 1.5 mg, 2.0 mg, 2.5 mg, 3.0 mg, 3.5 mg, 4.0 mg, 5.0 mg, or 10 mg of the ethylene inhibitor per millimeter of stem diameter. In certain embodiments, the ethylene inhibitor is applied at such amount in a volume of at least 25 mL, 50 mL, 100 mL, 150 mL, 200 mL, 400 mL, 500 mL, or 1 L of solution. In certain embodiments, the ethylene inhibitor is applied in one or more applications by root drench in a solution containing from any of about 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1.0 mg, 1.5 mg, 2.0 mg, 2.5 mg, or 3.0 mg to any of about 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1.0 mg, 1.5 mg, 2.0 mg, 2.5 mg, 3.0 mg, or 3.5 mg of the ethylene inhibitor per millimeter of stem diameter. In certain embodiments, the ethylene inhibitor is applied at such amount in a volume of at least 25 mL, 50 mL, 100 mL, 150 mL, 200 mL, 400 mL, 500 mL, or 1 L of solution. In certain embodiments, the ethylene inhibitor is applied at such amount in a volume of solution that is sufficient to come in contact of majority of root area. In certain embodiments, the

volume of solution is sufficient to contact at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% of the root area. In certain embodiments, the ethylene inhibitor is applied in one or more applications by root drench in a solution at a rate of any of about 5 mg/100 ml, 10 mg/100 ml, 15 mg/100 ml, 20 mg/100 ml, 25 mg/100 ml, 30 mg/100 ml, 35 mg/100 ml, 40 mg/100 ml, 45 mg/100 ml, 50 mg/100 ml, 55 mg/100 ml, 60 mg/100 ml, or 65 mg/100 ml to any of about 10 mg/100 ml, 15 mg/100 ml, 20 mg/100 ml, 25 mg/100 ml, 30 mg/100 ml, 35 mg/100 ml, 40 mg/100 ml, 45 mg/100 ml, 50 mg/100 ml, 55 mg/100 ml, 60 mg/100 ml, 65 mg/100 ml, or 75 mg/100 ml of the ethylene inhibitor in the solution. In certain embodiments, the ethylene inhibitor is applied by root drench in a solution at a rate of about 15 mg/100 ml to 50 mg/100 ml, 15 mg/100 ml to 30 mg/100 ml, or 40 mg/100 ml to 50 mg/100 ml of the ethylene inhibitor in the solution. In certain embodiments, the foliar spray is applied to apical and/or axillary nodes of the plant. In certain such embodiments, the foliar spray is applied to apical and/or axillary nodes of the plant at a rate of any of about 0.10 mg, 0.11 mg, 0.12 mg, 0.15 mg, 0.2 mg, 0.3 mg, or 0.4 mg to any of about 0.11 mg, 0.12 mg, 0.15 mg, 0.2 mg, 0.3 mg, 0.4 mg, or 0.5 mg ethylene inhibitor per ml of solution. In certain such embodiments, the foliar spray is applied to apical and/or axillary nodes of the plant at a rate of any of about 0.12 mg, 0.15 mg, 0.2 mg, or 0.3 mg to any of about 0.15 mg, 0.2 mg, 0.3 mg, or 0.4 mg ethylene inhibitor per ml of solution. In certain embodiments, the solution for foliar spray comprises 0.01% of Tween 80 solution. In certain embodiments, the above rates apply to AVG.

[0057] Certain aspects of the methods of this disclosure result in the production of *Cannabis sativa* seed, and in certain embodiments, large numbers, such on a production and/or commercial scale. In certain embodiments, at least about 5, 10, 25, 50, 100, 125, 150, 200, 250, 500, 750, 1,000, 1,250, 1,500, 2,000, 3,000, 4,000, or 5,000 genetically female *Cannabis sativa* seeds are produced on a self-pollinated plant. In certain embodiments, any of about 5, 10, 25, 50, 100, 125, 150, 200, 250, 500, 750, 1,000, 1,250, 1,500, 2,000, 3,000, or 4,000, to any of about 10, 25, 50, 100, 125, 150, 200, 250, 500, 750, 1,000, 1,250, 1,500, 2,000, 3,000, 4,000, or 5,000 genetically female *Cannabis sativa* seeds are produced on a self-pollinated plant. In certain embodiments, all the seeds produced on the self-pollinated plant are genetically female. In certain embodiments, at least about 5, 10, 25, 50, 100, 125, 150, 200, 250, 500, 750, 1,000, 1,250, 1,500, 2,000, 3,000, 4,000, or 5,000 genetically female *Cannabis sativa* seeds are produced on a cross-pollinated plant. In certain embodiments, any of about 5, 10, 25, 50, 100, 125, 150, 200, 250, 500, 750, 1,000, 1,250, 1,500, 2,000, 3,000, or 4,000 to any of about 10, 25, 50, 100, 125, 150, 200, 250, 500, 750, 1,000, 1,250, 1,500, 2,000, 3,000, 4,000, or 5,000 genetically female *Cannabis sativa* seeds are produced on a cross-pollinated plant. In certain embodiments, greater than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9% or 99.99% of the seeds produced on a cross-pollinated plant are genetically female. In certain embodiments, all the seeds produced on the cross-pollinated plant are genetically female. When the method is applied to a plurality or population of plants, genetically female *Cannabis sativa* seed can be produced on a production and/or commercial scale. In certain embodiments, any of about 5,000, 10,000, 20,000, 50,000, 100,000, or 500,000 to any of about 10,000, 20,000, 50,000, 100,000, 500,000, or 1,000,000 genetically female *Cannabis sativa* seeds are produced. In certain embodiments, at least about 5,000, 10,000, 20,000, 50,000, 100,000, 500,000, or 1,000,000 genetically female *Cannabis sativa* seeds are produced. In certain embodiments, the seeds produced are exclusively genetically female. In certain embodiments, the genetically female seed can be packaged into bags, containers, seed lots, commercial seed lots, and the like having any of about 5,000, 10,000, 20,000, 50,000, 100,000, or 500,000, to any of about 10,000, 20,000, 50,000, 100,000, 500,000 or 1,000,000 genetically female *Cannabis sativa* seeds wherein greater than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9% or 99.99% of the seeds in the bags, containers, seed lots, commercial seed lots and the like are genetically female *Cannabis sativa* seeds. In certain embodiments, the genetically female seed can be packaged into bags, containers, seed lots, commercial seed lots, and the like having at least 5,000,

10,000, 20,000, 50,000, 100,000, 500,000, or 1,000,000 exclusively genetically female *Cannabis sativa* seeds. In certain embodiments, the genetically female seed can be packaged into bags, containers, seed lots, commercial seed lots, and the like having any of about 5,000, 10,000, 20,000, 50,000, 100,000, or 500,000, to any of about 10,000, 20,000, 50,000, 100,000, 500,000 or 1,000,000 genetically female *Cannabis sativa* seeds wherein greater than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9% or 99.99% of the seeds in the bags, containers, seed lots, commercial seed lots and the like are genetically female *Cannabis sativa* seeds. In certain embodiments, the genetically female seed can be packaged into bags, containers, seed lots, commercial seed lots, and the like having any of about 5,000, 10,000, 20,000, 50,000, 100,000, or 500,000, to any of about 10,000, 20,000, 50,000, 100,000, 500,000 or 1,000,000 exclusively genetically female *Cannabis sativa* seeds. Thus, certain embodiments provide for a method of packaging bags, containers, seed lots, and/or commercial seed lots according to the above and for bags, containers, seed lots, and/or commercial seed lots of seeds according to the above. Certain embodiments provide for methods including the steps of producing and further packaging genetically female *Cannabis sativa* seed incorporating the methods of producing genetically female *Cannabis sativa* seed disclose anywhere herein.

[0058] As noted, the genetically female *Cannabis sativa* seed produced by the methods of this disclosure can be grown into *Cannabis sativa* plants. Thus, certain embodiments comprise or further comprise growing a *Cannabis sativa* plant from the genetically female *Cannabis sativa* seed produced. It will be understood that the party generating the feminized seed can further grow that seed, or, they can transfer the seed they produce to another party to grow the seed. Both are specifically contemplated in this disclosure. Certain embodiments comprise growing from a plurality of seeds a population of *Cannabis sativa* plants. The population may be small, such as two or three or four or five plants, such as for personal use or the population may be large, such as on a commercial scale, such as hundreds, or thousands, or tens of thousands, or hundreds of thousands, or millions of genetically female plants. For example, in certain embodiments, the population comprises at least about 6 plants, 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, 50,000 plants, or 100,000 genetically female plants. In certain embodiments, the population has greater than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9% or 99.99% genetically female plants. In certain embodiments, the population is exclusively composed of genetically female.

[0059] The *Cannabis sativa* plant can be grown for its floral bud/inflorescence which is generally considered the main consumable portion of the plant. Certain embodiments are drawn to a floral bud/inflorescence of a plant grown from a seed produced by a method of this disclosure.

[0060] Certain embodiments are drawn to a seed of a *Cannabis sativa* plant grown from a seed produced by a method of this disclosure. In certain embodiments, the seed is part of a plurality of seeds and/or a seed lot such as a commercial seed lot. Certain embodiments are drawn to a *Cannabis sativa* plant grown from such a seed or a progeny thereof. Certain embodiments are drawn to a population of *Cannabis sativa* plants grown from a plurality of such seed. In certain embodiments, the population comprises at least about 6 plants, 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, 50,000 plants, or 100,000 plants. In certain embodiments, greater than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9% or 99.99% of the plants in the population are genetically female plants. In certain embodiments, all of the plants in the population are genetically female plants.

[0061] Also provided for herein are methods of inducing male flowers on a genetically female *Cannabis sativa* plant by applying a chemical ethylene inhibitor (as described elsewhere herein) to a genetically female *Cannabis sativa* plant as well as a genetically female *Cannabis sativa* plant comprising induced fertile male flowers produced by such methods. In certain embodiments, the chemical ethylene inhibitor is aminoethoxyvinylglycine (AVG). In certain embodiments, the female plant forms one or more pollen-producing fertile male flowers. Consistent with methods described

in greater detail elsewhere herein (which are incorporate in the methods of inducing male flowers), in certain embodiments, one application of the ethylene inhibitor is sufficient. And, in certain embodiments, the ethylene inhibitor is applied to the plant multiple time, e.g., in at least two, three, four, five, or six separate applications. In certain embodiments, the ethylene inhibitor is applied to the plant in 5 or more, 10 or more, 15 or more, or 20 or more separate applications.

[0062] In certain embodiments, the ethylene inhibitor is applied in at least one application by root drench to the plant. In certain embodiments, the ethylene inhibitor is applied in at least one application by foliar spray to the plant. In certain embodiments, the ethylene inhibitor is applied to the plant: by root drench in all applications; by foliar spray in all applications; or by root drench in at least one application and also by foliar spray in at least one separate application. In certain embodiments where the ethylene inhibitor is applied by root drench in at least one application and also by foliar spray in at least one separate application; the first application between the two of the ethylene inhibitor to the plant is by root drench. In certain embodiments where the ethylene inhibitor is applied by root drench in at least one application and also by foliar spray in at least one separate application; the first application between the two of the ethylene inhibitor to the plant is by foliar spray.

[0063] It will be understood that in certain embodiments, certain applications of the ethylene inhibitor (e.g., AVG) comprise root drench, i.e., root drench can be in combination with application by foliar spray but does not require combination with foliar spray, while other applications comprising root drench exclude foliar spray. Likewise, certain applications comprise foliar spray, i.e., foliar spray can be in combination with application by root drench but does not require combination with root drench, while other applications comprising foliar spray exclude root drench. For example, in certain embodiments the ethylene inhibitor is applied to the plant: [0064] (i) wherein the application comprises root drench in all applications; [0065] (ii) wherein the application comprises root drench but not foliar spray in any applications; [0066] (iii) wherein the application comprises foliar spray in all applications; [0067] (iv) wherein the application comprises foliar spray but not root drench in any applications; [0068] (v) wherein the application comprises root drench combined with foliar spray in all applications; [0069] (vi) wherein the application comprises root drench combined with foliar spray in at least one application and root drench but not foliar spray in at least one separate application; [0070] (vii) wherein the application comprises root drench combined with foliar spray in at least one application and foliar spray but not root drench in at least one separate application; and/or [0071] (viii) wherein the application comprises root drench but not foliar spray in at least one application and also comprises foliar spray but not root drench in at least one separate application.

[0072] In certain embodiments, the first application of the ethylene inhibitor to the plant comprises application by root drench. In certain embodiments, the first application of the ethylene inhibitor to the plant comprises root drench application but not foliar spray. In certain embodiments, the first application of the ethylene inhibitor to the plant comprises application by foliar spray. In certain embodiments, the first application of the ethylene inhibitor to the plant comprises foliar spray application but not root drench.

[0073] Certain aspects of the methods of this disclosure involve the timing of the ethylene inhibitor application. In certain embodiments, the ethylene inhibitor is applied to the plant prior to flowering. In certain embodiments, the ethylene inhibitor is first applied to the plant prior to flowering. In certain embodiments, the ethylene inhibitor is only applied to the plant prior to flowering. In certain embodiments, the ethylene inhibitor is applied to the plant in at least two, three, four, five, or six separate applications prior to flowering. In certain embodiments, the ethylene inhibitor is applied to the plant in 5 or more, 10 or more, 15 or more, or 20 or more separate applications prior to flowering. In certain embodiments, the ethylene inhibitor is applied to the plant after flowering is initiated. In certain embodiments, the ethylene inhibitor is only applied to the plant after flowering is initiated. In certain embodiments, the ethylene inhibitor is applied to the plant in at least two,

three, four, five, or six separate applications after flowering is initiated. In certain embodiments, the ethylene inhibitor is applied to the plant in 5 or more, 10 or more, 15 or more, or 20 or more separate applications after flowering is initiated. In certain embodiments, the ethylene inhibitor is re-applied 2, 3, 4, 5, 6, 7, 8, or 9 days after the initial application. Further, in certain embodiments, the ethylene inhibitor is re-applied 2, 3, 4, 5, 6, 7, 8, or 9 days after the application that is 2, 3, 4, 5, 6, 7, 8, or 9 days after the initial treatment. In certain embodiments, the ethylene inhibitor is re-applied 10, 11, 12, 13, 14, 15, 16, 17, or 18 days after the initial treatment.

[0074] In certain of any of these methods, the ethylene inhibitor can be applied to the plant prior to flowering and/or after flowering is initiated and can be applied by root drench and/or foliar spray. For example, in certain embodiments, the ethylene inhibitor is applied by root drench to the plant prior to flowering and the ethylene inhibitor is separately applied by root drench to the plant after flowering is initiated. For example, in certain embodiments, the ethylene inhibitor is applied by root drench to the plant prior to flowering and the ethylene inhibitor is separately applied by foliar spray to the plant after flowering is initiated. For example, in certain embodiments, the ethylene inhibitor is applied by foliar spray to the plant prior to flowering and the ethylene inhibitor is separately applied by root drench to the plant after flowering is initiated. For example, in certain embodiments, the ethylene inhibitor is applied by foliar spray to the plant prior to flowering and the ethylene inhibitor is separately applied by foliar spray to the plant after flowering is initiated.

[0075] As noted above, it will be understood that in certain embodiments, certain applications of the ethylene inhibitor (e.g., AVG) comprise root drench, i.e., root drench can be in combination with application by foliar spray but does not require combination with foliar spray, while other applications comprising root drench exclude foliar spray. Likewise, certain applications comprise foliar spray, i.e., foliar spray can be in combination with application by root drench but does not require combination with root drench, while other applications comprising foliar spray exclude root drench. Thus, in certain embodiments: [0076] (i) The ethylene inhibitor application comprises root drench prior to flowering and separately comprises root drench after flowering is initiated. In certain embodiments, the ethylene inhibitor application comprises at least one root drench application without foliar spray prior to flowering. Further, in certain embodiments, the ethylene inhibitor application comprises root drench but not foliar spray prior to flowering. In certain embodiments, the ethylene inhibitor application separately comprises at least one root drench application without foliar spray after flowering is initiated. Further, in certain embodiments, the ethylene inhibitor application comprises root drench but not foliar spray after flowering is initiated; [0077] (ii) The ethylene inhibitor application comprises root drench prior to flowering and separately comprises foliar spray after flowering is initiated. In certain embodiments, the ethylene inhibitor application comprises at least one root drench application without foliar spray prior to flowering. Further, in certain embodiments, the ethylene inhibitor application comprises root drench but not foliar spray prior to flowering. In certain embodiments, the ethylene inhibitor application separately comprises at least one foliar spray application without root drench after flowering is initiated. Further, in certain embodiments, the ethylene inhibitor application comprises foliar spray but not root drench after flowering is initiated; [0078] (iii) The ethylene inhibitor application comprises foliar spray prior to flowering and separately comprises root drench after flowering is initiated. In certain embodiments, the ethylene inhibitor application comprises at least one foliar spray application without root drench prior to flowering. Further, in certain embodiments, the ethylene inhibitor application comprises foliar spray but not root drench prior to flowering. In certain embodiments, the ethylene inhibitor application separately comprises at least one root drench application without foliar spray after flowering is initiated. Further, in certain embodiments, the ethylene inhibitor application comprises root drench but not foliar spray after flowering is initiated; or (iv) The ethylene inhibitor application comprises foliar spray prior to flowering and separately comprises foliar spray after flowering is initiated. In certain embodiments, the ethylene inhibitor application comprises at least one foliar spray application without root

drench prior to flowering. Further, in certain embodiments, the ethylene inhibitor application comprises foliar spray but not root drench prior to flowering. In certain embodiments, the ethylene inhibitor application separately comprises at least one foliar spray application without root drench after flowering is initiated. Further, in certain embodiments, the ethylene inhibitor application comprises foliar spray and not root drench after flowering is initiated.

[0079] In certain embodiments, the ethylene inhibitor is re-applied 2, 3, 4, 5, 6, 7, 8, or 9 days after the initial treatment. In certain embodiments, the ethylene inhibitor is further re-applied 2, 3, 4, 5, 6, 7, 8, or 9 days after the application that is 2, 3, 4, 5, 6, 7, 8, or 9 days after the initial treatment. In certain embodiments, the ethylene inhibitor is re-applied 10, 11, 12, 13, 14, 15, 16, 17, or 18 days after the initial treatment. In certain embodiments, on the same day of or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 days after the initial application of the ethylene inhibitor to the plant, the plant is exposed to environmental conditions that induce flowering in *Cannabis sativa*. In certain embodiments, on the same day of or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days after the initial application of the ethylene inhibitor to the plant, the plant is exposed to environmental conditions that induce flowering in *Cannabis sativa*. In certain embodiments, prior to the initial application of the ethylene inhibitor to the plant, the plant is grown under vegetative growth conditions for *Cannabis sativa*. In certain embodiments, the plant is grown under vegetative growth conditions for *Cannabis sativa* up until 1, 2, 3, 4, 5, 6, or 7 days after the initial application of the ethylene inhibitor to the plant, at which time the plant is switched to environmental conditions that induce flowering in *Cannabis sativa*. In certain embodiments, the ethylene inhibitor is applied by root drench and/or foliar spray at a rate described in detail elsewhere herein.

[0080] Certain aspects of this disclosure are drawn to methods of producing an inbred variety of *Cannabis sativa*. In certain embodiments, the method comprises first producing a genetically female *Cannabis sativa* plant with induced-male flowers, wherein the female plant comprises one or more pollen-producing fertile male flowers, by any of the methods described elsewhere herein. The method next comprises producing seed by self-pollination of pollen from an induced-male flower onto a female flower of the same plant or by cross-pollination of pollen from an induced-male flower onto a female flower of a separate but genetically identical female plant. In certain embodiments, the genetically identical female plant is produced through clonal propagation. Certain embodiments comprise growing a genetically female *Cannabis sativa* plant from a seed produced above. In certain embodiments, these steps can be repeated, for example for at least two, three, four, five, six, or seven or more generations. Certain embodiments provide for an inbred *Cannabis sativa* seed produced by such methods. In certain embodiments, the seed is part of a plurality of seeds and/or seed lot, such as a commercial seed lot and/or comprising a number of seeds and/or seed composition as disclosed elsewhere herein. Certain embodiments provide for an inbred variety of *Cannabis sativa*, or a plant of the variety, produced by such methods of producing an inbred variety of *Cannabis sativa*. Certain embodiments provide for a population of inbred *Cannabis sativa* plants comprising an inbred variety produced by such methods of producing an inbred variety of *Cannabis sativa*. In certain embodiments, the population of inbred *Cannabis sativa* plants comprises at least about 6 plants, 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, 50,000 plants, or 100,000 plants. In certain embodiments, the population of inbred *Cannabis sativa* plants comprises from any of about 6 plants, 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, or 50,000 plants to any of about 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, 50,000 plants, or 100,000 plants. In certain embodiments of a population of inbred *Cannabis sativa* plants, all of the plants in the population are genetically female plants. Certain embodiments provide for a floral bud/inflorescence of an inbred *Cannabis sativa* plant or of an inbred *Cannabis sativa* plant of a population of plants described above. Certain embodiments provide for a seed from the inbred *Cannabis sativa* variety or inbred plant described above. The seed could be inbred or could be the

result of hybridization. In certain embodiments, the seed is part of a plurality of seeds and/or seed lot as described elsewhere herein.

[0081] Certain embodiments are also drawn to methods of producing reproducible hybrid *Cannabis sativa* seed comprising crossing two distinct inbred *Cannabis sativa* varieties, wherein at least one inbred variety has been produced by a method of producing an inbred variety disclosed herein. In certain embodiments, both inbred varieties have been produced by a method of producing an inbred variety disclosed herein. Certain embodiments also provide for a hybrid *Cannabis sativa* seed produced by a method of producing an inbred variety disclosed herein. In certain embodiments, the seed is part of a plurality of seeds and/or seed lot as described elsewhere herein. Certain embodiments also provide for a hybrid *Cannabis sativa* plant grown from such seed and also a population of hybrid *Cannabis sativa* plants grown from a plurality such seeds. In certain embodiments, the population of hybrid *Cannabis sativa* plants comprises at least about 6 plants, 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, 50,000 plants, or 100,000 plants. In certain embodiments, the population of hybrid *Cannabis sativa* plants comprises from any of about 6 plants, 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, or 50,000 plants to any of about 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, 50,000 plants, or 100,000 plants. In certain embodiments of a population of hybrid *Cannabis sativa* plants, all of the plants in the population are genetically female plants. Certain embodiments provide for a floral bud/inflorescence of a hybrid *Cannabis sativa* plant or of a hybrid *Cannabis sativa* plant of a population of plants described above.

[0082] Certain aspects of methods of this disclosure provide for producing a population of *Cannabis sativa* plants. In certain embodiments, the method comprises the steps of: i) producing a genetically female *Cannabis sativa* plant with induced-male flowers, wherein the female plant comprises one or more pollen-producing fertile male flowers, as described in detail elsewhere herein; (ii) producing seed by pollinating a female flower with pollen from the induced one or more male flowers; and (iii) growing a population of *Cannabis sativa* plants from a plurality of seed produced in (ii). In certain embodiments, the seed produced in (ii) is produced by self-pollination of pollen from the induced one or more male flowers onto a female flower of the same plant. In certain embodiments, the seed produced in (ii) is produced by cross-pollination of pollen from the induced one or more male flowers onto a female flower of a separate female plant. In certain embodiments, the separate female plant used for cross-pollination is genetically identical to the plant with the induced male flowers. In certain embodiments, the separate female plant used for cross-pollination that is genetically identical is a clone of the plant with the induced flowers. In certain other embodiments, the separate female plant used for cross-pollination has a genotype that is distinct from the plant with the induced male flowers. In certain embodiments, the separate female plant used having a genotype that is distinct from the plant with the induced male flowers is from a distinct inbred variety. In certain embodiments, the population comprises at least about 6 plants, 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, 50,000 plants, 100,000 plants, or 500,000. In certain embodiments, the population comprises from any of about 6 plants, 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, 50,000 plants, 100,000, or 500,000 plants to any of about 12 plants, 24 plants, 48 plants, 72 plants, 96 plants, 200 plants, 500 plants, 1,000 plants, 5,000 plants, 10,000 plants, 50,000 plants, 100,000, 500,000 plants, or 1,000,000 plants. In certain embodiments, at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9% or 99.99% of the plants in the population are genetically female plants. In certain embodiments, all of the plants in the population are genetically female plants.

[0083] Certain aspects of this disclosure provide for producing inbred *Cannabis sativa* seed by producing one or a plurality of genetically female *Cannabis sativa* plants with induced-male flowers by the methods described in detail elsewhere herein, wherein the female plant(s) comprise

one or more pollen-producing fertile male flowers. In certain embodiments, the female plant(s) with induced-male flowers are grown in isolation or in coincidence with non-induced female plants of the same genotype. In certain embodiments, the method is used to produce seed on a commercial or production scale. For example, in certain embodiments, the method is used to produce at least about 100 inbred seeds, 500 inbred seeds, 1,000 inbred seeds, 5,000 inbred seeds, 10,000 inbred seeds, 50,000 inbred seeds, 100,000 inbred seed, 500,000 inbred seeds, 1,000,000 inbred seeds. In certain embodiments, the method is used to produce any of about 100 inbred seeds, 500 inbred seeds, 1,000 inbred seeds, 5,000 inbred seeds, 10,000 inbred seeds, 50,000 inbred seeds, 100,000 inbred seed, or 500,000 inbred seeds to any of about 500 inbred seeds, 1,000 inbred seeds, 5,000 inbred seeds, 10,000 inbred seeds, 50,000 inbred seeds, 100,000 inbred seed, 500,000 inbred seeds, or 1,000,000 inbred seeds. Pollen generated from the induced-male flowers can be used in a variety of ways. In certain embodiments, the pollen from the induced-male flowers naturally dehiscesses. In certain embodiments, the pollen from the induced-male flowers is collected for immediate application to female flowers. In certain embodiments, the pollen from the induced-male flowers is stored for later application. And, in certain embodiments, the pollen from the induced-male flowers is disbursed for pollination by an ultrasonic dehiscence system as described elsewhere herein.

[0084] Certain aspects of this disclosure provide for producing hybrid *Cannabis sativa* seed by producing one or a plurality of genetically female *Cannabis sativa* plant(s) with induced-male flowers as described in detail elsewhere herein, wherein the female plant(s) comprise one or more pollen-producing fertile male flowers. In certain embodiments, the female plant(s) with induced-male flowers are grown in isolation or in coincidence with non-induced female plants having a different genotype. In certain embodiments, the non-induced female plant having a different genotype is an inbred plant. In certain embodiments, the hybrid seed is produced on a production or commercial scale. For example, in certain embodiments, the method is used to produce at least about 100 hybrid seeds, 500 hybrid seeds, 1,000 hybrid seeds, 5,000 hybrid seeds, 10,000 hybrid seeds, 50,000 hybrid seeds, 100,000 hybrid seed, 500,000 hybrid seeds, 1,000,000 hybrid seeds. In certain embodiments, the method is used to produce any of about 100 hybrid seeds, 500 hybrid seeds, 1,000 hybrid seeds, 5,000 hybrid seeds, 10,000 hybrid seeds, 50,000 hybrid seeds, 100,000 hybrid seed, or 500,000 hybrid seeds to any of about 500 hybrid seeds, 1,000 hybrid seeds, 5,000 hybrid seeds, 10,000 hybrid seeds, 50,000 hybrid seeds, 100,000 hybrid seed, 500,000 hybrid seeds, or 1,000,000 hybrid seeds. In certain embodiments, the pollen from the induced-male flowers naturally dehiscesses. In certain embodiments, the pollen from the induced-male flowers is collected for immediate application to female flowers. In certain embodiments, the pollen from the induced-male flowers is stored for later application. And, in certain embodiments, the pollen from the induced-male flowers is disbursed for pollination by an ultrasonic dehiscence system as described elsewhere herein.

[0085] Certain aspects of this disclosure also provide for screening *Cannabis sativa* germplasm to identify genotypes responsive to the induction of male flowers on genetically female plants by applying a chemical ethylene inhibitor (e.g., AVG) to a genetically female plant and determining the response of the plant with respect to the induction of male flowers. In certain embodiments, the induction of male flowers is done as described in detail elsewhere herein. In certain embodiments, a plant is selected based on its response to the induction of male flowers. In certain embodiments, one or more plants is genotyped. In certain embodiments, a plant selected based on its response to the induction of male flowers is genotyped and, or otherwise, in certain embodiments an association between the genotype of the selected plant and its response to the induction of male flowers is identified. In certain embodiments, the genotype is associated with responsiveness to the induction of male flowers such as the production of pollen-producing fertile male flowers. In certain embodiments, the genotype is associated with a lack of responsiveness to the induction of male flowers. One of ordinary skill in the art will recognize that in any of these method and/or embodiments, a plant is selected for use, treatment, pollination, crossing, etc. based on knowledge

of its genotype. One of ordinary skill in the art will also recognize that the steps of applying the inhibitor, genotyping, selecting plants, and/or identifying an association can be performed by a single party or may be performed by multiple parties, such as those working together to identify genotypes responsive to the induction of male flowers. Further, in certain embodiments, a genetically female *Cannabis sativa* plant comprising a genotype associated with responsiveness to the induction of male flowers is treated to induce male flowers and/or produce seed as described elsewhere herein. In certain embodiments, a *Cannabis sativa* plant comprising a genotype associated with responsiveness to the induction of male flowers is self-pollinated. In certain embodiments, a *Cannabis sativa* plant comprising a genotype associated with responsiveness to the induction of male flowers is crossed to a second *Cannabis sativa* plant. In certain embodiments, the second *Cannabis sativa* plant does not comprise the same genotype associated with responsiveness to the induction of male flowers as the first plant. In certain embodiments, a population of *Cannabis sativa* plants segregating for a genotype associated with responsiveness to the induction of male flowers is generated. Further provided for is the introgression into the genome of a *Cannabis sativa* plant of a genotype associated with responsiveness to the induction of male flowers into plant germplasm using a genotype identified as associated with responsiveness to the induction of male flowers.

[0086] Certain aspects of this disclosure provide for a method of establishing inbred breeding pools of *Cannabis sativa* germplasm based on the genotypes identified by the methods disclosed herein. In certain embodiments, those genotypes are associated with responsiveness to the induction of male flowers on female plants. In certain embodiments, those genotypes are associated with a lack of responsiveness to the induction of male flowers on female plants. Once established, such breeding pools can be used, for example, in hybrid *Cannabis sativa* plant development.

[0087] Certain aspects provide for a method of processing pollen from a genetically female plant with induced-male flowers produced as described elsewhere herein. In certain embodiments, the method comprises removing an inflorescence-containing male flower from the female plant after mature male flowers or anthers are formed and drying the male flowers or anthers for about 1, 2, 3, 4, 5, 6, or 7 days. The dried male flowers or anthers are then sifted to isolate pollen. The pollen can then be used immediately or it can be storage for later use.

[0088] Representative, non-limiting strains of *Cannabis sativa* on which male-flower induction and seed production may occur include those referred to in the field as Wily Jack, Birthday Cake, *Mimosa*, Gorilla Glue, Capstone, Sour Strawberry, Strawanna, and BioDiesel.

EXAMPLES

Example 1. Ethylene Inhibitor Treatment of *Cannabis sativa* Plants

[0089] Two replicate clones of five separate strains/genotypes were selected to test the effects of multiple treatments of 2-aminoethoxyvinylglycine (AVG) prior to initiation of flowering. Clones were transplanted into 4 L pots with peat-based substrate. The plants were grown outside under natural long day conditions (16 hour day) for 39-49 days (depending on strain), when they received their first AVG treatment. The stems of the plants ranged from 9-11 mm at the time of treatment, which was correlated with plant size and vigor. AVG was applied as a root drench by pouring 200 ml of AVG solution (AVG in water) near the base of the plant (aka “root drench”). Strains A and B had more biomass and each plant was treated with 100 mg of the product ReTain, which contains 15% AVG (15 mg AVG), which was dissolved in 200 ml of water. Strains C, D and E were smaller and treated with 12 mg AVG dissolved in 200 ml of water (Table 1).

TABLE-US-00001 TABLE 1 Plant size and treatment rates. Plant Plant Stem Plant Height Width Diameter Archi- (cm) (cm) (mm) AVG Strain tecture Rep 1 Rep 2 Rep 1 Rep 2 Rep 1 Rep 2 mg/vol A tall and 77.5 74.5 41 38 11 10 15 mg/ lanky 200 ml B medium, 59 55.5 30 35 9 10 15 mg/ branching 200 ml C small, 46.5 42 18 20 9 9 12 mg/ compact 200 ml D smaller, 44 37.5 33 29 10 9 12 mg/ compact 200 ml E smaller, 37.5 36.6 30 29 9 9 12 mg/ compact 200 ml

[0090] Five days after the first AVG treatment, the plants were transferred to a controlled

environment with a 13 h day and an 11 h night, to induce flowering. The initiation of flowering was observed 4 days after the transition to short days (13 h). Seven and 14 days after the first treatment, the same AVG treatment, as above, was applied, for a total of three treatments. By three days after the first AVG treatment, the apical and axillary meristems showed signs of chlorosis, indicating the AVG chemistry was successfully translocated through the roots into aerial parts of the plants. The amount of chlorosis observed was strain dependent, with strain A showing the strongest chlorosis, strain B showing mild chlorosis and strains C, D and E showing intermediate chlorosis. All plants grew out of the chlorosis, and plant health was not impacted.

Example 2. Production of Male Structures, Pollen Collection, and Seed Harvest

[0091] Strains A, C, D, and E started producing chemically induced male flowers 14-22 days after being transferred to short days to induce flowering, which was 19-27 days after the first AVG treatment. Strain B did not develop chemically induced male flowers. Overall, strain A produced the most chemically induced male flowers, with large inflorescences being exclusively male, although female structures were formed on other branches, but always with male structures, as well. Apical portions of branches were more highly converted toward maleness, while interior portions were more likely to remain partially female. High quality pollen was produced from male flowers over the course of a week. Strain D was the most productive, with high quality pollen produced over 15 days. Strain C produced pollen over 11 days. Strain E produced a small amount of pollen over 3 days. Strain B never produced pollen.

[0092] To obtain pollen for controlled pollinations, extruded anthers or whole male flowers were collected using fine forceps. These tissues were then 1) immediately gently sieved with a 355 micron sieve or 2) allowed to dry for approximately 24 h, 36 h, or 48 h, and then sieved to release pollen. Pollen quality was assessed under a microscope (10× magnification). Clear, round pollen was scored as high quality. Clear, angular pollen was scored as moderate quality. Angular and cloudy pollen was scored as low quality. Pollen was applied to female flowers using a small paintbrush.

[0093] To achieve efficient, whole plant pollinations, since chemically induced male flowers do not dehisce as readily as natural males, an ultrasonic dehiscence system was used to mobilize clouds of airborne pollen from chemically induced male flowers onto an adjacent female plant for pollination. Ultrasonic devices (˜100-500 Hz) were applied directly to chemically induced male inflorescences, branch stems or the main stem to mobilize pollen from mature male flowers.

[0094] Approximately 30-45 days after pollination, individual inflorescences, or stems containing multiple inflorescences, as appropriate, were removed from the plant and placed in a fine mesh bag and allowed to fully dry out for at least 7 days. After this desiccation step, the seeds were removed from the dried inflorescences through manual manipulation. Seeds were then counted and placed in seed tubes for storage.

[0095] Alternatively, approximately 30-45 days after pollination, the plants were allowed to dry out fully in their pots for about 3 days. Inflorescences or branches with marked controlled crosses were collected individually, allowed to dry at approximately 75-80 degrees Fahrenheit for at least 7 days, then seeds were harvested, counted and stored. Remaining plants or branches were removed and dried similarly, and seed produced through ultrasonic vibration pollination was harvested, counted and stored.

Example 3. Production of *Cannabis sativa* Selfed Seed Through Controlled Pollinations

[0096] Because female flowers and chemically induced male flowers were present on responsive strains, it was possible to create selfed seed. To obtain a small quantity of selfed seed, pollen was collected from chemically induced male structures (yellow stamen and whole male flowers) of Strain D, plant rep number 1 (D1). This pollen was used to immediately pollinate female inflorescences on the same plant, D1, as described in Example 2. Some harvested chemically induced male structures were allowed to dry for 24 h at room temperature (70-79 degrees Fahrenheit), after which they were sieved to release and collect pollen. The pollen was applied to

female inflorescences on the genetically identical clone, plant D2, demonstrating an alternative method to obtain the equivalent of selfed seed. Seed counts are reported in Table 2.

TABLE-US-00002 TABLE 2 Summary of self-crosses and resulting seed counts. CROSS Pollination Female × Seed # Type Induced Male Comment Count 21 Self E2 × E2 24 h dried anthers, one small 3 inflorescence pollinated 40 Self D1 × D1 Fresh pollen 44 42 Sib Cross D2 × D1 24 h dried anthers 35 43 Sib Cross D2 × D1 24 h dried anthers 20 44 Sib Cross D2 × D1 Fresh pollen used on multiple 48 small inflorescences 45 Sib Cross D2 × D1 36 h dried anthers used on 63 multiple inflorescences 46 Sib Cross A1 × A2 Fresh pollen, but small amount 19 48 Sib Cross A1 × A2 24 h dried anthers, good 37 amount of pollen 49 Sib Cross A1 × A2 36 h dried anthers 8 50 Sib Cross A1× A2 24 h dried anthers, 2 small 4 flowers pollinated 51 Self C2 × C2 20 h dried anthers, moderate 3 quality pollen grains, but good amount

[0097] Similarly, male structures (yellow stamen and whole male flowers) were harvested from induced male inflorescences from Strain C, plant rep number 2 (C2). The male structures were dried at room temperature (70-79 degrees Fahrenheit) and 20-40% humidity for 20 h. Pollen was extracted, as described in Example 2, and applied to non-induced female flowers on the same plant, C2, to produce selfed seed. Final seed counts are reported in Table 2.

Example 4. Production of *Cannabis sativa* Seed from Untreated Female Plants Pollinated with Pollen from Chemically Induced Male Flowers

[0098] Controlled pollinations between AVG treated and untreated strains were made, as shown in Table 3, to demonstrate utility to create novel genetic combinations in a breeding program or to produce hybrid seed. Two individuals from two different untreated strains, Strains F and G, were used as female recipients for crosses from various pollen collections off of strains with chemically induced male flowers. Individual female inflorescences from an untreated female plant from Strain G, plant G2, received controlled pollinations from 5 distinct pollen collections from Strain D, plant D1, as shown in Table 3. Plant G2 was also the recipient of two controlled crosses from Strain C, plant C2, Table 3. Strain G, plant G3, received controlled pollinations on female inflorescences from three distinct pollen collections from Strain D, plant D1, as shown in Table 3. Plant G3 also received a controlled pollination from Strain E, plant E2, which produced a small amount of pollen. Seed was harvested from each cross and the seed return is summarized in Table 3.

TABLE-US-00003 TABLE 3 Summary of crosses and resulting seed counts from untreated female plants pollinated with pollen from chemically induced male flowers. Pollination Female × Seed CROSS # Type Induced Male Comment Count 29 Cross G2 × D1 Fresh pollen, high quality, large 145 amount used on multiple inflorescences 30 Cross G2 × D1 Fresh pollen, high quality, large amount 49 31 Cross G2 × C2 20 h dried anthers, good quality pollen 61 32 Cross G2 × D2 20 h dried anthers, good quality 94 33 Cross G2 × D1 24 h dried anthers, good quality 12 34 Cross G2 × D1 48 h dried anthers, pollen quality good 4 35 Cross G2 × C2 24 h dried anthers, small amount no data applied to small inflorescence- Could not find at harvest 36 Cross G3 × D1 Fresh pollen, high quality used on 245 multiple inflorescences 37 Cross G3 × D1 Fresh pollen, high quality used on 142 multiple inflorescences 38 Cross G3 × E2 Fresh pollen, small amount and 7 moderate quality 39 Cross G3 × D1 20 h dried anthers, good quality 20 52 Cross F2 × D1 36 h dried anthers, used on multiple 145 inflorescences 53 Cross F2 × D1 24 h dried anthers, small amount 9 used on one small inflorescence 54 Cross F2 × C1 48 h dried anthers, low quality 1 pollen 55 Cross F2 × M1 24 h dried anthers, low quality pollen 1 and very little 56 Cross F2 × D1 24 h dried anthers, small amount 1 applied to one inflorescence 57 Cross F2 × D2 24 h dried anthers, good quality 28 pollen 58 Cross F3 × D1 Fresh pollen, high quality 79 59 Cross F3 × D1 36 h dried anthers, large volume 147 applied to multiple inflorescences 60 Cross F3 × D1 24 h dried anthers, good quality 41 61 Cross F3 × D1 24 h dried anthers, moderate quality 7 pollen

[0099] Individual female inflorescences from an untreated female plant from Strain F, plant F2, received controlled pollinations from 3 distinct pollen collections from Strain D, plant D1, as shown in Table 3. Plant F2 also received pollen from Strain D, plant D2, Strain C, plant C2 and

Strain E, plant E1, as shown in Table 3. Plant F3 received controlled pollinations from four distinct pollen collections from Strain D, Plant D1, as shown in Table 3.

Example 5. Production of *Cannabis sativa* Seed from Controlled Crosses Using Pollen from Chemically Induced Flowers and Applied to Non-Converted Female Flowers on the Sample Treated Plant

[0100] To demonstrate that the creation of novel genetic combinations for a breeding program or hybrid seed can be produced between two chemically induced strains, numerous crosses were performed. Controlled pollinations between AVG treated male donors and inflorescences that remained female on AVG treated plants were made, as shown in Table 4. Additionally, the crosses were made over time to determine the duration of viability of pollen produced from chemically induced male flowers and the receptivity of non-induced female flowers on plants treated with AVG chemistry. Pollen was collected and applied, as described in Example 2. Seed was harvested from each cross and the seed return is summarized in Table 4.

TABLE-US-00004 TABLE 4 Summary of crosses and resulting seed counts from using pollen from chemically induced flowers and applied to non-converted female flowers on the sample treated plant. CROSS Pollination Female × Seed # Type Induced Male Comment Count 1 Cross B1 × A2 Fresh pollen, good quality 44 2 Cross B1 × A2 36 h dried anthers, small amount 30 3 Cross B1 × C2 36 h dried anthers, good amount of 35 pollen 4 Cross B1 × D1 Fresh pollen, good quality and 75 quantity 5 Cross B1 × A2 Fresh pollen, low quantity 49 6 Cross B1 × A2 Fresh pollen, high quality pollen 67 8 Cross B1 × C2 Fresh pollen, high quality pollen, 339 many inflorescences pollinated 9 Cross B1 × D1 Fresh, high quality pollen 51 10 Cross B2 × C2 Fresh, high quality pollen 79 11 Cross B2 × A2 Fresh, low quantity pollen 26 12 Cross B2 × D1 36 h dried anthers, low quality 19 pollen 13 Cross B2 × D2 Fresh, high quality pollen 67 14 Cross E2 × A2 Fresh pollen, moderate quality, 16 small inflorescence pollinated 15 Cross E2 × A2 48 h dried anthers, low quality 32 pollen 16 Cross E2 × A2 48 h dried anthers, very low 3 volume pollen 17 Cross E2 × C2 36 h dried anthers, poor quality 4 pollen 18 Cross E2 × C2 24 h dried anthers, moderate 17 quality pollen 19 Cross E2 × D1 Fresh, high quality pollen 49 20 Cross E2 × D2 20 h dried anthers, high quality 57 pollen 22 Cross E1 × C2 Fresh pollen, moderate quality 13 pollen 23 Cross E1 × C2 48 h dried anthers, low quality 8 pollen 24 Cross E1 × D1 Fresh, high quality pollen 42 25 Cross E1 × C1 Fresh, high quality pollen 9 26 Cross E1 × C2 24 h dried anthers, moderate 1 quality pollen used on multiple inflorescences 27 Cross El × D1 24 h dried anthers, large volume of 20 pollen 28 Cross El × D1 Fresh, high quality pollen 47

Example 6. Large Scale Production of *Cannabis sativa* Hybrid Seed Using an Ultrasonic Dehiscence System

[0101] To demonstrate the ability to produce hybrid seed efficiently, an ultrasonic dehiscence system was developed to release the pollen from chemically induced males in a cloud, while in proximity of the recipient plants. Plants D1 and D2 of the highly responsive Strain D were placed in proximity of plants B1 and B2 of Strain B that remained female, as they were unresponsive to the AVG chemistry. Ultrasonic devices (˜100-500 Hz) were used to mobilize clouds of pollen that drifted onto the female Strain B plants to pollinate them. The device was applied to the main stem, branch stems, and/or inflorescences to induce pollen release. Seed was harvested from each cross and the seed return is summarized in Table 5.

TABLE-US-00005 TABLE 5 Summary of crosses and resulting seed counts from crosses using an ultrasonic vibration system. CROSS Pollination Female × Seed # Type Induced Male Comment Count 7 Ultrasonic B1 × C2 Pollen visibly streaming off plant 225 vibe cross with ultrasonic vibration 63 Ultrasonic B2 × D1 Pollen visibly streaming off plant 348 vibe cross with ultrasonic vibration

Example 7. Large Scale Production of Self Seed Using an Ultrasonic Dehiscence System on *Cannabis sativa* Plants

[0102] To demonstrate the ability to obtain self seed efficiently over an entire plant, an ultrasonic

dehiscence system was developed to easily release pollen from the chemically induced males in a cloud around the plant. An ultrasonic device (˜100-500 Hz) was used to vibrate out clouds of pollen from plant A1 from Strain A, by touching the device to the stem, branches and induced male inflorescences. The plant was isolated from other plants to ensure purity. Some female inflorescences on plant A1 were used for controlled crosses of A2 pollen onto A1, and these inflorescences were protected and excluded from the seed count for selfing through the ultrasonic dehiscence system. Seed was harvested and the seed return is summarized in Table 6.

TABLE-US-00006 TABLE 6 Summary of crosses and resulting seed counts from self crosses using an ultrasonic vibration system. Pollination CROSS # Type Female × Induced Male Comment Seed Count 64 Ultrasonic D2 × D2 Pollen visibly streaming off plant 253 vibe self with ultrasonic vibration 65 Ultrasonic C2 × C2 Pollen visibly streaming off plant 139 vibe self with ultrasonic vibration 66 Ultrasonic C1 × C1 Pollen visibly streaming off plant 27 vibe self with ultrasonic vibration 67 Ultrasonic A2 × A2 Pollen visibly streaming off plant 41 vibe self with ultrasonic vibration 68 Ultrasonic D1 × D1 Pollen visibly streaming off plant 171 vibe self with ultrasonic vibration 69 Ultrasonic A1 × A1 Pollen visibly streaming off plant 41 vibe self with ultrasonic vibration

Example 8. Validation of the Production of Feminized *Cannabis sativa* Seed

[0103] To demonstrate that the seed produced from the pollinations described in examples 3-8 was feminized, meaning only female plants were derived from crosses, a subset of seeds were planted from some crosses and the resulting plants were scored for gender. Plants were grown under a vegetative photoperiod (18 h day, 6 h night) until the first true leaves were formed, at which time a sample cotyledon was taken and sent for molecular sex testing to verify the progeny were exclusively female. Results are summarized in Table 7. Alternatively, plants are grown under a vegetative photoperiod (18 h day, 6 h night) until the 7th palmate leaf is observed and are then shifted to a reproductive photoperiod (12 h day and 12 h night) to induce flowering. Plants are scored for gender 7 days after the switch to a floral inductive photoperiod. Similarly, seedlings derived from the remaining crosses are scored for sex.

TABLE-US-00007 TABLE 7 Molecular sex test results for progeny. Cross Cross # Description Progeny ID Sex (F/M) 1 B1 × A2 1.1 F 1.2 F 1.3 F 1.4 F 1.5 F 2 B1 × A2 2.1 F 2.2 F 2.3 F 3 B1 × C2 3.1 F 3.2 F 3.3 F 4 B1 × D1 4.1 F 5 B1 × A2 5.1 F 5.2 F 5.3 F 5.4 F 5.5 F 6 B1 × A2 6.1 F 6.2 F 6.3 F 6.4 F 6.5 F 7 B1 × C2 7.1 F

Example 9. Treatment of Genetically Female *Cannabis sativa* Plants with AVG as a Nodal Spray, Apical Drip or Root Drench to Induce Male Flowers

[0104] To compare delivery methods of AVG onto genetically female *Cannabis sativa* plants for induction of male flowers, three different modes of delivery were tested on two different strains, L and M. Plants were grown in 4 L pots outdoors under long days (˜15.5 h) until they were approximately 90 cm tall and were then transferred to a floral inductive photoperiod (12 h day/12 h night). Soon after the onset of flowering the plants were treated with 1) 200 ml root drench to the base of the plant, 2) 50 ml spray (AVG dissolved in 0.01% Tween-80) focused on the nodes with developing inflorescences or 3) 2 ml of AVG solution (AVG dissolved in 0.01% Tween-80) dripped solely on the apex of the plant with gentle massage for coverage of all tissues. For each strain and treatment, one plant was treated only one time and the other was treated 3 times, with 6 to 7 days between treatments. For the nodal spray treatment, one branch was covered during the spray application (untreated control, UTC) to test if direct contact with the solution was necessary for conversion, or if the chemistry was translocated from treated tissues to untreated tissues. Treatment descriptions are summarized in Table 8. Male structures developed, but the plants matured before pollen could form.

TABLE-US-00008 TABLE 8 Treatments in comparison of AVG delivery methods. Strain Plant Treatment Number of AVG Code ID Type Treatments mg/vol/Plant L Ll Drench 1 12 mg/200 ml L2 Drench 3 12 mg/200 ml L3 Spray 1 12 mg/50 ml L4 Spray 3 12 mg/50 ml L5 Apex Drip 1 0.5

g/2 ml L6 Apex Drip 3 0.5 g/2 ml M M1 Drench 1 12 mg/200 ml M2 Drench 3 12 mg/200 ml M3 Spray 1 12 mg/50 ml M4 Spray 3 12 mg/50 ml M5 Apex Drip 1 0.5 g/2 ml M6 Apex Drip 3 0.5 g/2 ml

Example 10. Effects of Different Delivery Modes of AVG on *Cannabis sativa* Plants

[0105] When AVG was applied as a spray or a drip directly to apical or axillary nodes, chemically induced male flowers were only observed on treated tissues, except in one instance which was due to a known contamination, demonstrating that AVG is not mobile when applied to the surface of the plant. Interestingly, when AVG is applied as a root drench, it is easily mobilized to rapidly growing sink tissues, as evidenced by localized chlorosis and the formation of chemically induced male flowers at all nodes. The root drench delivery of AVG was most efficient and had the most penetrant response. To quantitatively assess the response to the three different delivery methods of AVG, mature plants we cut down and every node on a given branch was scored for having 1) only female flowers, 2) both male and female flowers or 3) exclusively male flowers and is summarized in Tables 9 to 20.

TABLE-US-00009 TABLE 9 Scoring of flower sex at each node of Strain L treated with AVG on the apical node a single time. Strain L: Single Apical Treatment Nodes Nodes with Nodes with Branch with Male Male & Female Female Vegetative # Flowers Flowers Flowers Nodes 1 0 0 25 0 2 0 0 28 0 3 0 0 25 0 4 0 0 24 0 5 0 0 27 0 6 0 0 24 0 7 0 0 25 0 8 0 0 27 0 9 0 0 22 0 10 0 0 20 0 11 0 0 16 0 12 0 0 15 0 13 0 0 14 0 Apical 0 5 22 0

TABLE-US-00010 TABLE 10 Scoring of flower sex at each node of Strain M treated with AVG on the apical node a single time. Strain M: Single Apical Treatment Nodes Nodes with Nodes with Branch with Male Male & Female Female Vegetative # Flowers Flowers Flowers Nodes 1 0 0 40 0 2 0 0 39 0 3 0 0 25 0 4 0 0 21 0 5 0 0 23 0 6 0 0 17 0 7 0 0 18 0 8 0 0 17 0 9 0 0 14 0 10 0 0 9 0 11 0 0 9 0 12 0 0 5 0 13 0 0 5 0 Apical 0 12 4 0

TABLE-US-00011 TABLE 11 Scoring of flower sex at each node of Strain L treated with AVG on the apical node three times. Strain L: Three Apical Treatments Nodes Nodes with Nodes with Branch with Male Male & Female Female Vegetative # Flowers Flowers Flowers Nodes 1 0 0 18 0 2 0 0 22 0 3 0 0 13 0 4 0 0 25 0 5 0 0 24 0 6 0 0 21 0 7 0 0 26 0 8 0 0 20 0 9 0 0 16 0 10 0 0 17 0 11 0 0 14 0 12 0 0 14 0 13 0 0 13 0 Apical 0 21 2 0

TABLE-US-00012 TABLE 12 Scoring of flower sex at each node of Strain M treated with AVG on the apical node three times. Strain M: Three Apical Treatments Nodes Nodes with Nodes with Branch with Male Male & Female Female Vegetative # Flowers Flowers Flowers Nodes 1 3 0 23 0 2 0 0 18 0 3 0 0 21 0 4 0 0 17 0 5 0 0 23 0 6 0 0 22 0 7 0 0 17 0 8 0 0 7 0 9 0 0 6 0 10 0 0 9 0 11 0 0 11 0 12 0 0 7 0 Apical 0 11 3 0

TABLE-US-00013 TABLE 13 Scoring of flower sex at each node of Strain L treated with AVG as a single spray. Strain L: Single Plant Spray Nodes Nodes with Nodes with Branch with Male Male & Female Female Vegetative # Flowers Flowers Flowers Nodes 1 0 33 3 0 2 0 9 27 0 3 0 15 11 0 4 0 16 14 0 5 0 8 16 0 6 0 14 12 0 7 0 11 11 0 8 UTC 0 3 20 0 9 0 6 9 0 10 0 6 8 0 11 0 6 5 0 12 0 3 6 0 Apical 0 12 4 0

TABLE-US-00014 TABLE 14 Scoring of flower sex at each node of Strain M treated with AVG as a single spray. Strain M: Single Plant Spray Nodes with Nodes with Nodes with Branch Male Male & Female Female Vegetative # Flowers Flowers Flowers Nodes 1 0 21 4 0 2 0 24 8 0 3 0 19 8 0 4 0 19 10 0 5 0 5 8 0 6 0 7 10 0 7 0 0 17 0 UTC 8 0 8 7 0 9 0 5 7 0 10 0 3 7 0 11 0 4 8 0 12 0 6 0 0 Apical 0 12 6 0

TABLE-US-00015 TABLE 15 Scoring of flower sex at each node of Strain L treated with AVG as a spray three times. Strain L: Three Plant Sprays Nodes with Nodes with Nodes with Branch Male Male & Female Female Vegetative # Flowers Flowers Flowers Nodes 1 0 37 10 0 2 0 15 4 0 3 0 11 15 0 4 0 25 8 0 5 0 26 2 0 6 0 21 7 0 7 0 22 6 0 8 0 0 22 0 UTC 9 0 17 4 0 10 0 21 4 0 11 0 14 0 0 12 0 22 0 0 13 0 13 1 0 14 0 11 0 0 Apical 0 23 0 0

TABLE-US-00016 TABLE 16 Scoring of flower sex at each node of Strain M treated with AVG as

a spray three times. Strain M: Three Plant Sprays Nodes with Nodes with Nodes with Branch Male Male & Female Female Vegetative # Flowers Flowers Flowers Nodes 1 0 52 0 0 2 0 23 3 0 3 0 28 0 0 4 0 16 3 0 5 0 11 5 0 6 0 17 0 0 7 0 0 12 0 UTC 8 0 13 0 0 9 0 10 0 0 10 0 6 4 0 11 0 6 0 0 Apical 0 14 0 0

TABLE-US-00017 TABLE 17 Scoring of flower sex at each node of Strain L treated with AVG as a single root drench. Strain L: Single Root Drench Nodes with Nodes with Nodes with Branch Male Male & Female Female Vegetative # Flowers Flowers Flowers Nodes 1 0 18 7 0 2 6 18 6 0 3 11 8 29 0 4 0 14 8 0 5 0 16 7 0 6 0 30 1 0 7 0 13 20 0 8 0 6 20 0 9 0 1 19 0 10 0 2 17 0 11 0 4 14 0 12 0 8 10 0 13 0 2 14 0 Apical 0 3 17 0

TABLE-US-00018 TABLE 18 Scoring of flower sex at each node of Strain M treated with AVG as a single root drench. Strain M: Single Root Drench Nodes with Nodes with Nodes with Branch Male Male & Female Female Vegetative # Flowers Flowers Flowers Nodes 1 2 25 5 0 2 0 33 0 0 3 0 31 4 0 4 0 17 4 0 5 0 21 2 0 6 0 20 6 0 7 0 19 4 0 8 0 19 2 0 9 0 15 1 0 10 0 13 2 0 11 0 12 2 0 12 0 18 2 0 13 0 6 1 0 Apical 0 23 0 0

TABLE-US-00019 TABLE 19 Scoring of flower sex at each node of Strain M treated with AVG with three root drenches. Strain L: Three Root Drenches Nodes with Nodes with Nodes with Branch Male Male & Female Female Vegetative # Flowers Flowers Flowers Nodes 1 14 54 0 0 2 9 24 0 0 3 2 33 0 0 4 6 30 3 0 5 0 38 2 0 6 3 23 7 0 7 0 23 8 0 8 0 24 12 0 9 0 23 10 0 10 0 10 14 0 11 0 10 13 0 12 0 8 8 0 13 0 10 8 0 Apical 0 32 5 0

TABLE-US-00020 TABLE 20 Scoring of flower sex at each node of Strain M treated with AVG with three root drenches. Strain M: Three Root Drenches Nodes with Nodes with Nodes with Branch Male Male & Female Female Vegetative # Flowers Flowers Flowers Nodes 1 0 35 0 0 2 0 34 0 0 3 0 34 3 0 4 0 32 4 0 5 0 34 0 0 6 0 26 0 0 7 0 26 0 0 8 0 23 0 0 9 0 18 0 0 10 0 13 0 0 11 0 12 0 0 12 0 15 0 0 13 0 14 0 0 14 0 12 0 0 15 0 10 0 0 Apical 0 29 0 0

Example 11. Treatment of *Cannabis sativa* Plants with AVG at Two Rates

[0106] Two replicate clones of three separate strains/genotypes, H, I and J, were selected to test the effects of two different rates of multiple treatments of AVG initiated prior to the transition to flowering. Clones were transplanted into 2.2 L pots with peat-based substrate. The plants were grown under long day conditions (19 h day and 5 h night) for 35 days, when they received their first AVG treatment. The stems of the plants ranged from 6-7 mm at the time of treatment. As stem diameter is an indicator of plant size and vigor, a reduced amount of chemistry was applied compared to the experiment described in Example 1. For each strain, one replicate clone received 4.5 mg of AVG and the other received 9 mg AVG. AVG was applied as a root drench by pouring 200 ml of AVG solution near the base of the plant. Five days after the first AVG treatment, the plants were transferred to a controlled environment with a 13 h day and an 11 h night, to induce flowering. Within a week after the transition to short days (13 h), the initiation of flowering was observed. Seven and 14 days after the first treatment, the same AVG treatment, as above, was applied, for a total of three treatments.

[0107] The day after the first AVG treatment, the apical and axillary nodes showed signs of chlorosis, indicating the AVG chemistry was successfully translocated through the roots to apical parts of the plants. The amount of chlorosis observed was dose dependent, with plants treated with 9 mg of AVG showing more chlorosis than those treated with 4.5 mg of AVG. The amount of chlorosis was also strain dependent. The 4.5 mg treatment produced the strongest chlorosis in Strain J, a moderate chlorosis in strain I, and Strain H had almost no response. However, Strain H had a strong chlorosis response with the 9 mg treatment, similar to Strain I. Strain J had the most chlorosis. The chlorosis response was apparent after the first treatment only, except for strain J that had chlorosis with additional 9 mg treatments.

TABLE-US-00021 TABLE 21 Treatment of three *Cannabis sativa* strains with two rates of AVG and the male conversion rating. Strain Male Conversion Code Plant ID mg AVG/vol/plant Rating H H1 4.5 mg/200 ml Moderate H2 9 mg/200 ml Complete I I1 4.5 mg/200 ml Mild I2 9 mg/200

ml Moderate J J1 4.5 mg/200 ml Mild J2 9 mg/200 ml Moderate

Example 12. Production of Male Structures, Pollen Collection and Seed Harvest on *Cannabis sativa* Plants

[0108] Induction of male flowers was dose dependent, with more male flowers induced on plants that received 9 mg AVG than those that received 4.5 mg AVG. Strain H was most responsive overall, and with the 9 mg treatment there was almost complete conversion to maleness. Strain I had moderate chemical induction of male flowers and strain J had mild chemical induction of male flowers. 4.5 mg of AVG induced male flowers minimally for strain J, mildly for strain I and moderately for strain H. To obtain pollen for controlled pollinations, extruded anthers or whole male flowers were collected using fine forceps. These tissues were then 1) immediately gently sieved with a 355 micron sieve or 2) allowed to dry for approximately 24 h, 36 h, or 48 h, and then sieved to release pollen. Pollen quality was assessed under a microscope (10× magnification) and clear, round pollen was scored as high quality. Clear, angular pollen was scored as moderate quality. Angular and cloudy pollen was scored as low quality. Pollen was applied to female flowers using a small paintbrush.

[0109] In addition to controlled pollinations, individual plants treated with 9 mg of AVG which had a stronger male response, were subjected to the ultrasonic dehiscence method in isolation to generate self seed. Strain H, plant 2 (H2) and strain I, plant 2 (12) were treated with the ultrasonic dehiscence method.

[0110] Approximately 30-45 days after pollination, individual inflorescences, or stems containing multiple inflorescences, as appropriate, are removed from the plant and placed in a fine mesh bag and allowed to fully dry out for at least 7 days. After this desiccation step, the seeds are removed from the dried inflorescences through manual manipulation. Seeds are then counted and placed in tubes for storage.

[0111] Alternatively, approximately 30-45 days after pollination, the plants are allowed to dry out fully in their pots for about 3 days. Inflorescences or branches with marked controlled crosses are collected individually, allowed to dry at approximately 75-80 degrees Fahrenheit and 20-40% humidity for at least 7 days, then seeds are harvested, counted and stored. Remaining plants or branches are removed and dried similarly, and seed produced through ultrasonic vibration pollinations are harvested, counted and stored.

Example 13. Production of *Cannabis sativa* Selfed Seed Through Controlled Pollinations

[0112] Because female flowers and chemically induced male flowers were present on responsive strains, it is possible to create feminized selfed seed. To obtain a small quantity of selfed seed, pollen was collected from chemically induced male structures (yellow stamen and whole male flowers) of Strain I, plant rep number 1 (11). This pollen was used to pollinate female inflorescences on the same plant, 11, as described in Example 2. Pollen was similarly collected from Strain I, plant rep number 2 (12). The pollen was applied to female inflorescences on the genetically identical clone, plant 11, demonstrating an alternative method to obtain the equivalent of selfed seed. Seed was collected, counted and stored.

TABLE-US-00022 TABLE 22 Female × CROSS Pollination Induced Seed # Type Male Comment Count 12 sib cross I1 × I2 24 h dried anthers, very 0 little pollen, moderate quality on 1 small inflorescence 14 self I1 × I1 24 h dried anthers from a few 0 flowers, small amount of pollen applied to small branch

Example 14. Production of *Cannabis sativa* Seed from Untreated Female Plants Pollinated with Pollen from Chemically Induced Male Flowers

[0113] To further demonstrate that viable male pollen can be produced on genetically female plants that are chemically induced to produce male flowers, pollen was collected as described in Example 2. Controlled pollinations were made between AVG treated male donors from strains H, I and J onto naturally female flowers of an untreated plant of strain K, (K1) as shown in Table 23. Seed was collected, counted, and stored.

TABLE-US-00023 TABLE 23 Female × CROSS Pollination Induced Seed # Type Male Comment Count 1 cross K1 × J2 18 h dried anthers, small amount 0 of low quality pollen 2 cross K1 × H2 18 h dried anthers, small amount 0 of low quality pollen 3 cross K1 × I2 18 h dried anthers, small amount 0 of low quality pollen 4 cross K1 × H2 42 h dried anthers, a lot of 0 moderate quality pollen 7 cross K1 × I2 42 h dried anthers, small amount 0 of low quality pollen 10 cross K1 × H2 24 h dried anthers, large amount 0 of good quality pollen put on apex 13 cross K1 × I1 24 h dried anthers from a few 0 flowers, small amount of pollen applied to small inflorescence

Example 15. Production of *Cannabis sativa* Seed from Controlled Crosses (Pollen on Female Flowers of Induced Plants)

[0114] To demonstrate that the creation of novel genetic combinations for a breeding program or hybrid seed can be produced between two chemically induced strains, several crosses were performed as described in Table 24. Controlled pollinations between AVG treated male donors and inflorescences that remained female on AVG treated plants were performed. Seed was harvested as described in Example 2.

TABLE-US-00024 TABLE 24 Female × CROSS Pollination Induced Seed # Type Male Comment Count 15 cross J1 × I1 24 h dried anthers from a few 32 flowers, small amount of pollen applied to small branch 5 cross I1 × H2 42 h dried anthers, a lot of 0 moderate quality pollen 6 cross J1 × H2 42 h dried anthers, a lot of 0 moderate quality pollen 8 cross J1 × I2 42 h dried anthers, small 0 amount of low quality pollen 9 cross I1 × H2 24 h dried anthers, large 0 amount of good quality pollen put on 2 branches 11 cross J1 × H2 24 h dried anthers, large amount 0 of good quality pollen put on 2 branches 16 cross I1 × H2 24 h dried anthers, large volume 1 of high quality pollen applied to all available flowers. Previous crosses covered to avoid contamination. 17 cross J1 × H2 24 h dried anthers, large volume 0 of high quality pollen applied to all available flowers. Previous crosses covered to avoid contamination.

Example 16. Induction of Male Flowers on Larger Genetically Female *Cannabis sativa* Plants

[0115] To demonstrate that seed could be produced using pollen generated from induced male flowers on a larger genetically female plant, plants are grown under vegetative conditions until the plants are greater than 85 cm tall, with stem diameters greater than 12 mm. Plants are grown in 8 liter pots. Pollen produced from the induced male plants is used to pollinate untreated female plants or flowers that remain female on AVG treated plants, as described in Examples 2-5. Larger plants require more active ingredient and in a larger volume to contact roots, so the dose of AVG provided is more than 15 mg active ingredient dissolved in at least 300 ml of water. Strains D, M and N are grown in a long day, vegetative photoperiod (18 h day, 6 h night), until they are greater than 85 cm tall. The first treatment of AVG is delivered by root drench about 5 days prior to transition to a short day, floral inductive photoperiod (13 h day, 11 h night). Additional AVG treatments are applied after the transition to a floral inductive photoperiod. Chemically induced male flowers and anthers are collected as described in Example 2. Pollen is collected and applied to untreated female plant or female flowers on chemically treated plants that do not completely convert to maleness. Alternatively, the ultrasonic dehiscence method is used to mobilize pollen from chemically induced male flowers to female flowers for pollination. Resulting seed are collected, counted and stored.

Example 17. Foliar Application of AVG to Induce Male Flowers and Viable Pollen on *Cannabis sativa* Plants

[0116] Genetically female plants derived from seed produced in previous examples were used to test the ability of AVG to induce the production of male flowers. Plants from two different genetic backgrounds (L and M) were grown vegetatively under 18 h of light per day for 79 days and then transferred to a floral inductive environment with 12 h of light per day. Apical portions of the plants were spray treated with 8 ml of 6.3 mg of AVG in 25 ml water per plant using a small atomizer on day 79 and day 85 after seeding. Flowering was first observed 88 days after seeding and viable male structures were observed 11 days later. Pollen was used for self and cross pollinations 110 days after seeding. One plant was highly converted and most flowers were male. Pollen from that

plant was used to pollinate some of the remaining female structures on other treated plant and on an untreated plant of genotype N. Table 25 summarizes the results obtained in Example 17.

TABLE-US-00025 TABLE 25 CROSS Pollination Female × Seed # Type Induced Male Count 123 Cross N1 × L1 5 124 Cross N1 × L1 7 125 Self M1 × M1 29 126 Self M1 × M1 19 127 Cross M1 × M1 25 128 Cross M1 × M1 62 129 Cross M1 × M1 31 130 Cross N1 × L1 0 131 Cross N1 × L1 0 132 Cross M1 × L1 29 133 Ultrasonic L1 × L1 161 Self 134 Self M1 × M1 15 135 Cross M1 × L1 6 136 Cross M2 × L1 discard plant 137 Ultrasonic M1 × M1 36 Self

Example 18. Chemically Induced Male Flowers on Genetically Female *Cannabis sativa* with Auto-Flower Trait

[0117] *Cannabis sativa* plants harboring the auto-flower trait are expected to flower under long day photoperiods and should not require a short-day reproductive signal to flower. To demonstrate the efficacy of AVG to induce male flowers on genetically female plants, two plants containing the auto-flower trait were grown under 18 h days. Treatments were performed at 32 (signs of first flower), 35 and 39 days after seeding with 8 ml of 4.2 mg AVG in 25 ml water per plant under 18-hour day conditions.

[0118] The first plant (Auto-1) was sprayed with AVG a fourth time with 10 ml of 6.3 mg AVG in 25 ml of water at 44 days after seeding and then moved to a 13 h day to increase the flowering response 48 days after seeding. Male flowers were first observed at 48 days after seedings and pollen was collected at 62 days after seeding. Pollen from Auto-1 was used for controlled pollinations on other plants.

[0119] The second plant (Auto-2) was moved 44 days after seeding to a 16 h day environment to slightly reduce the vegetative signal, as the flowering response was not strong. This plant was sprayed with AVG a fourth time with 10 ml of 6.3 mg AVG in 25 ml of water per plant at 44 days after seeding. This plant had a more vegetative phenotype and was moved into 13-hour day 83 days after seeding. Relatively few male structures were observed, and pollen was used for a controlled self-pollination and was allowed to naturally self-pollinate female flowers on the same plant. Seed results are summarized in Table 26.

[0120] Three additional auto-flower seeds were germinated to further demonstrate the ability of AVG to induce male structures on plants harboring the auto-flower trait. These plants were designated Auto-3, Auto-4, and Auto-5. Plants were germinated and initially grown under 18 h days. All plants received a foliar spray of 8 ml of 4.2 mg AVG in 25 ml water per plant at 25 and 29 days after seeding. Flowering was first observed at 33 days after seeding and a third foliar spray of 8 ml of 4.2 mg AVG in 25 ml water per plant was delivered 34 days after seeding. Auto-3 and Auto-5 plant both died due to root disease.

[0121] Auto-4 was moved to a 16 h day at 35 days after seeding and sprayed a fourth time with 10 ml of 6.3 mg AVG in 25 ml of water. Auto-4 then received one root drench treatment of 100 mL solution with 3.75 mg AVG 62 days after seeding. Auto-4 was then moved into 13-hour day 73 days after seeding and male flowers were observed 80 days after seeding. Auto-4 produced a significant number of male flowers with viable pollen that was used in a controlled cross and to self-pollinate, resulting in seed set. Seed results are summarized in Table 26.

TABLE-US-00026 TABLE 26 CROSS Pollination Female Induced Seed # Type Recipient Male Comment Count 169 Self Auto-4 — Pollen mobilized to self 331 remaining open flowers 172 Controlled Auto-2 self Pollen applied to 8 Self marked nodes 176 Cross Auto-2 Auto-1 Pollen applied to 8 marked nodes 177 Cross Auto-2 Auto-4 Pollen applied to 1 marked nodes 178 Cross Auto-2 Auto-4 Pollen applied to 113 marked nodes 183 Controlled Auto-4 — Pollen applied to 508 Self marked nodes 185 Self Auto-2 — Pollen mobilized to self 20 remaining open flowers

Example 19. Chemically Induced Male Flowers and Seed Production with a 1-MCP Root Drench

[0122] To determine if the ethylene inhibitor 1-Methylcyclopropene (1-MCP) can induce male flowers on genetically female *Cannabis* ssp plants, 1-MCP was applied at two different rates to 3 different varieties, with 2 replicates per treatment. The plants were treated with 1-MCP three times,

with the first treatment 51 days after seeding, which was on the day plants were transitioned from a vegetative, long day photoperiod (18 h day) to a reproductive, short day photoperiod (12 h day), the second treatment was applied 7 days later and the third treatment was 8 days after the second. For the first two treatments the low rate was 0.27 mM 1-MCP and 25 ml solution per plant was immediately applied as a root drench. The high rate was 0.54 mM and similarly, 25 ml of solution was applied as a root drench. For the third treatment, the dose was doubled by applying 50 ml of the solutions described above, as the plants were larger. While mild chlorosis of growing apices was observed, which is an expected response with the application of an ethylene inhibitor, no clear or consistent male structures were observed in any of the treatments.

Example 20. Chemically Induced Male Flowers and Seed Production: Treatment Number

[0123] To demonstrate that a single AVG root drench treatment delivered after a shift to a reproductive environment was sufficient to induce a female cannabis clone to produce male flowers, a clone of variety Q was given a single treatment 1 week after being moved to a reproductive 12 hour day environment. The Q clone was grown under vegetative conditions (18 h day) until the stem was about 10 mm in diameter, at which time it was transferred to floral inductive conditions (12 h day). After 7 days of growth in a floral inductive environment, and after signs of flowering were present, the main stem diameter was 11 mm. A single drench treatment containing 19 mg of AVG in 300 ml water was delivered to the roots at this time. Approximately 95% of the flowers were converted to male flowers that produced significant pollen one month after the treatment. An untreated female plant of another variety (O) was used as a recipient for Q pollen, using the ultrasonic dehiscent system two times (one day in between pollination events). This system was highly effective at mobilizing the pollen to completely pollinate the female plant in a matter of seconds. Pollen was also manually collected an applied to 2 other recipients, P and L. These data demonstrate a single treatment of AVG can be sufficient to convert a genetically female plant almost completely to a male plant phenotypically. Seed counts are reported in Table 27.

TABLE-US-00027 TABLE 27 Female × CROSS Pollination Induced Seed # Type Pollination Method Male Count 153 Cross Ultrasonic Dehiscence O1 × Q1 1984 System 154 Cross Limited, controlled P1 × Q1 26 pollination 155 Cross Limited, controlled L1 × Q1 123 pollination

Example 21. 2-Aminoisobutyric Acid (AIB)

[0124] To determine if the ethylene inhibitor 2-Aminoisobutyric acid (AIB) can induce male flowers on genetically female *Cannabis* ssp plants, three different rates of 2-Aminoisobutyric acid (AIB) were applied to plants as a root drench, with two applications per rate. The first application occurred 79 days after seeding, which was on the day plants were transitioned from a vegetative, long day photoperiod (18 h day) to a reproductive, short day photoperiod (12 h day), and the second treatment was applied 5 days later. The stem diameter of the plants on the day of the first treatment were 9 to 10 mm and the plants ranged from 37 to 54 cm in height. The three rates tested were 50, 150 and 300 mM AIB in water, and 150 ml of solution was applied to the roots as a soil drench. Mild phytotoxicity was observed in leaf and meristematic tissues within 2 days of the first treatment. After the second treatment, more severe phototoxicity was observed, particularly in the highest rate, of which the plant ultimately died. The plants treated with 50 and 150 mM AIB survived and produced small male structures that did not produce viable pollen.

Example 22. Chemically Induced Male Flowers and Seed Production: 2-Aminoisobutyric Acid (AIB)

[0125] To determine the effect of treatment number and rate of AVG on the ability to induce male flowers on genetically female *Cannabis sativa* plants. Cloned plants of genotype P were treated 1, 3 or 8 times with an AVG drench, at correspondingly high, medium or low rates. The plants were 45-54 cm tall with a stem diameter of 9 mm at the time of the first treatment day, Day 1. The plant treated 8 times (8×) received its first treatment on Day 1, and subsequent treatments occurred every other day until 8 treatments were delivered over a 15 day period. The 8× plant was switched to 13-hour day on day 7 of the treatment time course. The plant that received 3 treatments (3×) received

its first treatment on Day 2, with following treatments one week apart, finishing on Day 22. The 3× plant was switched to 13-hour day on day 6 of the treatment time course. The single treatment (1×) was delivered on Day 6, which was the day before plants were switched from a vegetative photoperiod (16 h day) to a reproductive photoperiod (13 h day). Plants treated 8 times, received a low dose of 11 ppm 2.25 mg AVG in 200 ml water on each treatment, plants treated 3 times received 9 mg AVG in 300 ml water on each treatment, and the plant treated one time received 21 mg AVG in 300 ml water. On Day 25, all plants showed some signs of maleness, with the 1× and 3× plants showing a stronger response, with ultimately about 80% conversion. The 8× treated plant had about 50% conversion, with fewer viable male structures. Pollen from treated plants was used to pollinate female plants not treated with AVG of germplasm Q or P. Seed results are summarized in Table 28.

TABLE-US-00028 TABLE 28 Cross Pollination Female Induced Seed # Type Recipient Male Comment Count 91 Controlled Q7 P-3X AVG Little pollen 0 Cross 93 Controlled Q7 P-3X AVG 2 inflorescences 6 Cross pollinated 95 Controlled Q7 P-8X AVG Young flowers 3 Cross 96 Controlled Q7 P-1X AVG 1 inflorescence 3 Cross pollinated 97 Controlled Q7 P-3X AVG 2 inflorescences 5 Cross pollinated 101 Controlled Q9 P-3X AVG Many flowers 37 Cross pollinated 102 Controlled Q8 P-3X AVG Many flowers 9 Cross pollinated 103 Controlled Q8 P-1X AVG Female flowers 0 Cross young 105 Controlled Q9 P-3X AVG 72 h pollen 16 Cross 106 Controlled Q9 P-1X AVG 4 day-old pollen 8 Cross 111 Controlled Q9 P-3X AVG Fresh pollen 15 Cross 112 Controlled P1 P-3X AVG Small inflorescences 3 Cross 113 Controlled P1 P-3X AVG Small inflorescences 1 Cross

[0126] The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents.

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Claims

1. A method of producing a genetically female *Cannabis sativa* seed, the method comprising: a) inducing one or more male flowers on a genetically female *Cannabis sativa* plant by applying the chemical ethylene inhibitor aminoethoxyvinylglycine (AVG) in at least one application by root drench to the plant, wherein the female plant forms one or more pollen-producing fertile male flowers; and b) producing the seed by pollinating a female flower with pollen from the induced one or more male flowers, wherein the seed produced is genetically female.

2. The method of claim 1, wherein the seed is produced by self-pollination of pollen from the

induced one or more male flowers onto a female flower of the same plant.
3. The method of claim 1, wherein the seed is produced by cross-pollination of pollen from the induced one or more male flowers onto a female flower of a separate female plant.
4. The method of claim 3, wherein the separate female plant used for cross-pollination is genetically identical to the plant with the induced male flowers.
5. The method of claim 3, wherein the separate female plant used for cross-pollination has a genotype that is distinct from the plant with the induced male flowers.
6-14. (canceled)
15. The method of claim 1, wherein AVG is applied to the plant prior to flowering.
16-22. (canceled)
23. The method of claim 1, wherein AVG is applied in one or more applications by root drench in a solution containing 0.3 mg to 3.5 mg of AVG per millimeter of stem diameter.
24-27. (canceled)
28. The method of claim 1, wherein exclusively genetically female *Cannabis sativa* seed is produced on a commercial scale.
29. The method of claim 1, further comprising growing a *Cannabis sativa* plant from the genetically female *Cannabis sativa* seed produced.
30-37. (canceled)
38. A method of inducing male flowers on a genetically female *Cannabis sativa* plant, the method comprising applying the chemical ethylene inhibitor aminoethoxyvinylglycine (AVG) in at least on application by root drench to a genetically female *Cannabis sativa* plant, wherein the female plant forms one or more pollen-producing fertile male flowers.
39-44. (canceled)
45. The method of claim 38, wherein the AVG is applied to the plant prior to flowering.
46-52. (canceled)
53. The method of claim 38, wherein AVG is applied in one or more applications by root drench in a solution containing 0.3 mg to 3.5 mg of AVG per millimeter of stem diameter.
54. A method of producing an inbred variety of *Cannabis sativa*, the method comprising: (i) producing a genetically female *Cannabis sativa* plant with induced-male flowers, wherein the female plant comprises one or more pollen-producing fertile male flowers, by the method of claim 38; (ii) producing seed by: (a) self-pollination of pollen from an induced-male flower onto a female flower of the same plant, or (b) cross-pollination of pollen from induced-male flowers onto female flowers of a separate but genetically identical female plant; (iii) growing a genetically female *Cannabis sativa* plant from a seed produced in (ii); and (iv) repeating steps (i), (ii), and (iii) for at least two, three, four, five, six, or seven generations.
55-61. (canceled)
62. A method of producing reproducible hybrid *Cannabis sativa* seed, the method comprising crossing two distinct inbred *Cannabis sativa* varieties, wherein at least one inbred variety has been produced by the method of claim 54.
63. The method of claim 62, wherein both inbred varieties have been produced by the method of claim 54.
64-69. (canceled)
70. A method of producing a population of at least 500 *Cannabis sativa* plants, the method comprising: i) producing a genetically female *Cannabis sativa* plant with induced-male flowers, wherein the female plant comprises one or more pollen-producing fertile male flowers, by the method of claim 38; (ii) producing seed by pollinating a female flower with pollen from the induced one or more male flowers; and (iii) growing the population of at least 500 *Cannabis sativa* plants from a plurality of seed produced in (ii).
71-73. (canceled)
74. The method of claim 70, wherein all of the plants in the population are genetically female

plants.

75. A method of producing inbred *Cannabis sativa* seed, the method comprising producing a plurality of genetically female *Cannabis sativa* plants with induced-male flowers, wherein the female plants comprise one or more pollen-producing fertile male flowers, by the method of claim 38, wherein the female plants with induced-male flowers are grown in isolation or in coincidence with non-induced female plants of the same genotype.

76-**78**. (canceled)

79. A method of producing hybrid *Cannabis sativa* seed, the method comprising producing a plurality of genetically female *Cannabis sativa* plants with induced-male flowers, wherein the female plants comprise one or more pollen-producing fertile male flowers, by the method of claim 38, wherein the female plants with induced-male flowers are grown in isolation or in coincidence with non-induced female plants having a different genotype.

80-**103**. (canceled)

104. A method of growing a *Cannabis sativa* plant, the method comprising growing a *Cannabis sativa* plant from a genetically female *Cannabis sativa* seed of claim 1.

105-**106**. (canceled)