Cannabis sativa: Dioecious into Monoecious Plants influencing Sex Determination

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Abstract: This review paper highlights about sex determination and conversion of dioecious into monoecious plants by applying exogenous growth regulators or chemicals. *Cannabis sativa* L. (Cannabaceae) is a dioecious plant, producing male and female flowers on separate unisexual individuals. Although both male and female plants are capable of producing cannabinoids in equal concentrations, female plants produce greater floral biomass than male plants and thus are exclusively used in commercial Medical *Cannabis sativa* (drug or marijuana) production facilities. In commercial production, marijuana plants are all genetically unfertilized female plants and, male plants are destroyed as seed formation reduces flower quality. One male Cannabis plant can ruin the entire female plant crop due to uncontrolled pollination and crop is designated as contaminated. Moreover, after pollination, female plants alter their relative investment in phytochemicals by reducing the production of secondary metabolites like cannabinoids, flavonoids, and terpenoids. Therefore, early diagnosis of sex is very important to both breeders and farmers for Cannabis crop improvement or production purposes. Cannabis sex determination could be modified by applying exogenous growth regulators or chemicals, which can influence the ratio of endogenous hormones and hence the incidence of sex organs. Silver compounds such as silver nitrate ($AgNO_3$) or silver thiosulfate ($Ag_2S_2O_3$; STS) have been found to have masculine effects in many plant species including Cannabis. A gap in the literature highlighting Cannabis sex determination has been updated in this review paper.

Key Words: Cannabis sativa, Dioecious, Feminized seeds, Hermaphrodites, Phytocannabinoids, Sex determination, Silver compounds

I. Introduction

The important characteristic of *Cannabis sativa* L. is the production of phytocannabinoids in abundance which are present in their acidic form in plant tissue (1-17). Phytocannabinoids are produced as a part of defensive mechanism in the trichomes on flower bracts of female inflorescences (1-17, 61). In recent years, the medicinal applications of *Cannabis sativa* L. have gained wider attention worldwide (1-20, 61-62). *Cannabis sativa* L., is classified into two types as Industrial *Cannabis sativa*, hemp or Medical *Cannabis sativa* (drug or marijuana) based on its THC content (1-45). Medical *Cannabis sativa* (drug or marijuana) contains very high levels of THC (1 to 35% of dry weight). On the other hand Industrial *Cannabis sativa* (Hemp) contains very low levels of THC (0 to 0.3% of dry weight) (1-40, 61, 62). However, due to the presence of psychoactive molecules, Λ9-tetrahydrocannabinol (Λ9-THC) and Λ8-tetrahydrocannabinol (Λ8-THC), Cannabis cultivation and its use is restricted/regulated in many countries (1-17, 61, 62). Medical *Cannabis sativa* (drug or marijuana) plants are grown commercially for their psychoactive compounds, which are produced in the trichomes that develop on flower bracts in female inflorescences (1-35, 61-62). Although the classification between hemp and marijuana is based on legal convention, different studies indicated that the discrimination between these varieties is not limited to Cannabinoid biosynthesis but can be monitored across the whole genome (1-17). *Cannabis sativa* L. (hemp, marijuana) produces male and female inflorescences on different plants (dioecious) (1-40, 61-62). Therefore, the plants are obligatory out-crossers. In commercial production, marijuana plants are all genetically female. The male plants are destroyed as seed formation reduces flower quality (1-35, 61, 62). Spontaneously occurring hermaphroditic inflorescences, in which pistillate flowers are accompanied by the formation of anthers, leads to undesired seed formation, the mechanism for this is poorly understood (1-50, 61-62).

Currently, Cannabis is used for the treatment of several diseases, such as epilepsy, Parkinson's disease, chronic pain, and rheumatoid arthritis due to its therapeutic safety and efficacy based on confirmed recent clinical trials (1-47, 61, 62). Legal restrictions in the last decades have prevented the progress of academic research involving *Cannabis sativa* L (1-46, 61, 62). These conditions have resulted in a scarcity of science based information on *Cannabis sativa* L (1-30, 61-62). International Narcotic Conventions and associated legislation have constrained the establishment, characterization, and use of Cannabis genetic resource collections (1-35, 61). This has resulted in the underutilization of gene-pool variability in cultivar development (1-36, 61). Prohibitionist legislation has discouraged scientists from studying Cannabis production, the main factor responsible for the current lack of research (1-25, 61). In several countries, legislation is changing due to recognition of the medicinal and agricultural value of Cannabis plants (1-25, 61, 62). The United Nations (UN) recently removed Cannabis species from the highly dangerous substances list and of low medicinal application, and this change recognizes the Cannabis plants medicinal value, which may be allowed additional scientific advances (1-36, 61-62). The use of the Cannabis plant as a source of therapeutic compounds is gaining great importance since restrictions on its growth and use are gradually reduced throughout the world (1-37, 61-62).

In the following section, role of external applications of chemicals on Cannabis female plants inducing male plants has been discussed and updated. This will result in the conversion of dioecious into monoecious plants.

II. Cannabis sativa; Sexual Dimorphism and Determination

Sexual dimorphism is a characteristic nature of animal species and it is generally easy to predict the sex of animal embryos once diagnostic gendered features appeared in juveniles (53, 54-58). On the other hand, confirming sexual identity to a plant prior to sexual maturity is difficult because diagnostic, gendered morphological features are not well described in juvenile plants (53, 54-58). Some dioecious species are economically important, including, but not limited to: Asparagus (*Asparagus officinalis* L.), hemp (*Cannabis sativa* L.), Mulberry (*Morus* spp.), Nutmeg (*Myristica fragrans* Houtt), Date palm (*Phoenix dactylifera* L.), Rattan (*Calamus* spp.), Betel (*Piper betle* L.), Pistachio (*Pistacia vera* L.), Kiwifruit (*Actinidia deliciosa*), and yam (*Dioscorea* spp.) (53, 54-58). Some of these dioecious plants have evolved sex chromosomes, as is the case with *Cannabis sativa*, a species which also displays lability in sexual expression, wherein unisexual plants can develop co-sexual sex expression in response to environmental or chemical triggers (53, 54).

Sex determination is accomplished in dioecious plants via three broad mechanistic categories: genetic control, epigenetic control and hormonal control (20-45; 50-61). Therefore, understanding the sexual dimorphism in juvenile plants may provide key economic, ecological, and evolutionary insights into dioecious plant species in addition to improving the process of crop cultivation (53-58). Many researchers have developed genetic tools to pre-emptively diagnose plant sex. However, there are problems with relying on molecular markers for predicting sex (53-58). The major problem is the diagnostic tools are very expensive which are unaffordable for the most growers and breeders. Second one is labile sex expression is predicted to be adaptive in variable environments (53-58, 61). Therefore, morphological markers in seedlings of future sex expression may be more accurate predictors of plant sex, given the environmental context in which plants are grown, and more affordable (53-58). Furthermore, sexual dimorphism increases with body size when males are the larger sex and decreases when females are (53-58, 61).

Early diagnosis of sex is very important to both breeders and farmers for Cannabis crop improvement or production purposes (53, 54-58). Cannabis is anemophilous (wind-pollinated). For instance, in *Cannabis sativa*, if pollen is released near un-pollinated female plants, the crop of exclusively female plants will be pollinated and is considered by federal regulators in Canada as Contaminated (53). Therefore, unfertilized female plants were only used for the commercial production of Medical *Cannabis sativa* (drug or marijuana) (53). In Canada, pollinated Cannabis can only be used for oil extract, not raw floral material, limiting market capacity (53). Moreover, pollinated Cannabis has lower cannabinoid content, making it a less efficient way to produce oil extracts (53, 54). Moreover, after pollination, female plants alter their relative investment in phytochemicals by reducing the production of secondary metabolites like cannabinoids, flavonoids, and terpenoids (60). In the absence of pollen, stigmas on female plants continue to grow and thus produce more surface area on which cannabinoids can be produced (60). Because of this **negative impact of pollination** on cannabinoid yield, Cannabis industrial growers rarely maintain male plants in production facilities, and instead propagate their stock of female plants by vegetative cloning (60). Early morphological differences may reflect divergent life history strategies and underlying differences in the genetic architecture among male and female plants (53-58, 61). Since pollination has significant negative consequences for cannabinoid production, a lack of knowledge of sex can be costly for the farmer in this expanding agricultural business, providing practical reasons for exploring this information (53-61, 62). The study conducted by Campbell et al., 2021 (53) reported that males grew significantly longer hypocotyls than females by week 2, but this difference depended on the cultivar investigated (53-58). Preliminary evidence suggested that co-sexual plants

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may be distinguished from male and female plants using short hypocotyl length and seedling height, although this relationship required more study since sample sizes of co-sexual plants were small (53-58). In one of the cultivars, two-week old male plants tend to produce longer hypocotyls than other plants, which may help to identify these plants prior to anthesis (53-58). The preliminary data suggests that short hypocotyl length may be an indicator of co-sexuality (53-58, 61).

Male and Female, Pollination, and Hermaphroditic inflorescences

Cannabis is a herbaceous, annual, dioecious species that can produce monoecious plants (Figure-3). Cannabis is a 4 month crop which can be grown throughout the year (1-33, 34). In dioecious plants, sex determination is governed by several factors: sex-determining genes and sex chromosomes, epigenetic control by DNA methylation and micro RNA's, and physiological regulation by phytohormones (33, 34, 53-60). Sexual dimorphism is expressed at very early stages of organ initiation or specification with differential expression of genes in male and female tissues (1-33, 34, 51). On occasion, it has been observed that hermaphroditic inflorescences can develop spontaneously (1-33, 34). These plants produce predominantly female inflorescences, but anthers (ranging from a few to many) may develop within the leaf axils or in pistillate flower buds (1, 20-60). These hermaphroditic inflorescences can be induced by exogenous applications of different chemicals suggesting that external triggers and epigenetic factors may play a role (1-33, 34, 53-60).

Hermaphroditism (Figure-3) occurs widely in plants (51). Hermaphrodites occur abundantly throughout the plant kingdom with both stamens and carpels within the same flower (51) (Figure-3). Nevertheless, 10% of flowering plants have separate unisexual flowers, either in different locations of the same individual (Monoecy) or on different individuals (Dioecy) (51). Despite their rarity, dioecious plants provide an excellent opportunity to investigate the mechanisms involved in sex expression and the evolution of sex-determining regions (SDRs) and sex chromosomes (33, 34, 51-60). However, even though dioecy is considered rare among flowering plants, its occurrence has been reported in several phylogenetic taxa (around 15,600 species spread over 175 families and 987 genera), suggesting that its evolution occurred independently hundreds if not thousands of times (1-51). Dioecy is proposed to have evolved from a hermaphrodite ancestor in angiosperms and is found in about 6% of all angiosperm plant species (1-17, 33-34, 51). It has been proposed that dioecy is a basic evolutionary mechanism to ensure cross-fertilization and, as a consequence, results in maintenance of high genetic diversity and heterozygosity (1-34, 51-50). This diversity of sexual systems in plants has an important impact on evolutionary biology as well as importance for seed and fruit production (51-62).

Morphologically, the inflorescences of male dioecious plants are characterized by hanging panicles with few or no leaves, and inflorescences of female plants bear racemes with leafy bracts (1-17, 33-51). Female plants have the highest phytocannabinoid production due to a higher density of glandular trichomes, where these compounds are synthesized and stored (1-17, 33-40, 51-60). In addition, male and hermaphroditic plants have reduced floral biomass and, thus, reduced phytocannabinoid yield (1-51-60, 61. 62). Pollination prevention stimulates the formation of new flowers, and increasing phytocannabinoid production (1-17, 33-40, 51). Although male plants tend to be larger and bloom before female plants, it is difficult to distinguish them during the vegetative phase (1-50). The most common way to differentiate female plants from male plants is by analyzing the anatomy of the inflorescences, although some genotypes develop solitary internode flowers at early stages of development, making it possible for early sexual differentiation (1-17, 33-51). To avoid pollination, one option is to remove male plants as they appear. Another alternative is to prevent the presence of male plants by using vegetative propagation, ensuring that the mother plant is female (1-16, 33-45-60). When cultivation is carried out under ideal conditions, it is unlikely that there is a change in the sexual expression of clones from the mother plant (1-16, 17-51). However, care should be taken with the prolonged life of the mother plant due to the occurrence of mutations and somaclonal variations that can decline the vigour and phytocannaboid content in the clones compared to the original mother plants (1-51). Another option is to carry out the seminiferous propagation with feminized seeds (1-17, 34). However, it should be noted that parthenocarpy consists of the growth of the ovary into seedless fruit without the occurrence of pollination (1-34). The structures produced by this complex floral structures (such as stigmas) that support their identification as flowers (1-60). It should be considered that, when the flower reaches the ideal harvest point it presents an advanced stage of senescence it does not mature (1-17-60).

According to the literature, male and female plants of some Cannabis varieties may have similar concentrations of cannabinoids (1-17). However, Cannabis cultivation for medicinal purposes is carried out with unfertilized female plants (1-35). Furthermore, sowing male plants for phytocannabinoid production is uncommon due to the female plants pollination, diverting phytocannabinoid production to seed development (1-19, 20-51). There is a misunderstanding that cross-pollination changes the chemotype of the plant (1-2). However, this change only appears in seeds resulting from cross-pollination and not in the pollinated plant (1-16, 17-51). The problem with pollination occurring in crops intended for the production of phytocannabinoids is that the energy is shifted to seed production, not cannabinoid production. Feder et al. (2021) (2) observed that pollination resulted in a significant decrease in the overall total phytocannabinoid concentration in inflorescences (1-2, 3-51-62). The THC-

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rich chemovar female exhibited an average of 75% decrease, while CBD-rich females showed a 60% decrease in phytocannabinoid content after fertilization (1-16, 17-35, 61, 62).

III. How to Identify Male and Female Cannabis Plants

Male Cannabis

Male plants essentially produce pollen which is needed for Cannabis plants to naturally reproduce (1-60). Male weed plants grow "**Balls**" that open up to let their pollen out, ending up looking like a small bunch of flowers. They can take up to three weeks to burst and male flowers do not have any pistils on them at all (1-51). Although several monoecious varieties have been developed for agronomical purposes and in nature, *Cannabis sativa* is a dioecious plant characterized by unisexual flowers confined to separate individuals (1-50) (Figure-1).

The male flowers are pale green, carried on axillary branched cymose panicles. The panicle flowers are solitary or alternative and occur in clusters or three flowered cymules. Each flower is composed of five tepals and as many stamens, and a thin pedicel. The tepals are ovate-oblong, 2–4 cm in length, yellowish or whitish-green, scattered, with tiny hairs. The stamens hang and consist of thin oblong and greenish filaments and anthers. The pollen grains are released through the terminal pores of the anthers (1-40).

1) **Look for thicker, sturdier stalks with fewer leaves on male plants:** A male plant, compared to a female plant of the same strain, generally has a thicker stalk. This is because it gets taller than female plants and needs to be able to support the weight. They also have fewer leaves than female plants (1-51) (Figure-1).

2) **Check plants regularly to sex them:** If male plants are allowed to pollinate, they will severely lower the potential crop of female plants (1-51). This is because, once fertilized, female plants spend energy producing seeds instead of THC, leading to a smaller harvest (1-51). For indoor cultivation, regular observation of plants is very much needed and check every plant to determine if it is male or female, as one rogue male can wreck harvest. In general, male plants showed their sex 7-10 days (indoor) or 3 weeks (outdoor) before female plants (1-51) (Figure-1).

3) **Check the joints on the stalk for male flowers:** The little balls that grow on the joints of the stalk (where the other branches meet the main stalk) are the main indicators of male plants (1-51). These flowers release pollen and need to be removed for a better crop. If the cultivator is trying to create new plants or reproduce, then need to leave these balls undisturbed (1-51). Female plants will have these bulbs too, but will also have long, translucent hairs on them. If 1-2 balls on a plant, one has to wait and see if more balls develop before cutting them (1-51) (Figure-1, 2).

4) **Know that hermaphroditic (both sexes) plants exist, and should be treated as males (Figure-3):** Marijuana plants can grow both sex organs. Tall male plants should be recognized and trimmed too (1-51). They will still release pollen that can ruin crop. "Hermies" are generally undesirable plants, and they can ruin a small crop with their pollen (1-51) (Figure-3).

5) **Remove male plants unless specifically want seeds:** Male plant should be identified and need to get rid of it or it will ruin crop (1-51). Do not try and remove the buds by hand, as missing even a few will significantly decrease crop. While the most of the growers simply throw the plants out, a few keep them around for breeding purposes (1-51). Keep them in a separate room from the females. Track pollen from the male room to the female room on clothes or hands (1-51).

6) **Plants grow for roughly six weeks before sexing them:** Marijuana plants, male and female will be identical in the first 6 weeks of life (1-51). It is only after they have begun developing their sex organs later on that will be able to differentiate them. Purchase "feminized" seeds as well, which usually create close to 100% female plants (1-51). However, there are occasional errors, and should still keep a close eye on plants to make sure there are no rogue males (1-51).

7) **Note fuller bodies of leaves, when compared to males, on a grown female plant:** Male plants have thicker, sturdier stalks and very few leaves (1-51). A female of the same strain will be shorter and bushier, with more leaves, especially near the top. If cultivator is trying to sex mature plants, one of the easiest indicators is how bushy they get (1-51) (Figure-1).

8) **Check the joints for of the stalk for small, translucent hairs.** Once the plant has matured enough, a female will begin flowering (1-51). At the joints where the branches meet the main stalk, small, **translucent hairs, known as pistils**, coming out of a small, tear-shaped bud tucked in the joint (1-51). Frequently, there will also be "growth tips," which are new branches and groups of leaves, growing as well (1-51). Male plants will have the small buds (pollen sacs) but will not have the associated hair growing out of it. Plants can grow both **pollen sacs and pistils**. If it does, it is hermaphroditic and should be treated like a male (1-51).

9) **Separate females from any males, as only females create buds.** Only female plants will produce enough THC to be used as medicine, but they will not create much if they become fertilized (1-51). The **pistil** (**hair like structure**) is meant to attract

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pollen. If it gets it, it will create a seed, and all the plants energy and nutrients will be spent making seeds, not making big, THC-full buds. Female plants are the only ones that will produce a crop but only if they stay away from the males (1-51).

10) Cannabis sex can be observed visually once pre-flowers have formed next to the stipule at the base of each node. Female cannabis plants will grow a **pair of white hairs**, while males will form little balls on stalks.

Figure-1: Male Marijuana plants with ball like structures with short nodal region.

Female Cannabis

Female plants are basically the ones that make buds, which is the part of the plant that contains the most of THC (1-51). With just one male plant and a minuscule amount of pollen, might end up filling their flowers with seeds. If the **male and female plants** in the same growing area, the buds grown there will only produce seeds so can not be used to smoke any of it (1-50). Furthermore, females apart due to the fact that their flowers do not fully close, they are actually quite open and they produce little **hairs called pistils**. They are incredibly easy to recognize, as the first thing they produce their pistils, **which male plants do not have at all** (1-40) (Figure-2, 3).

Female flowers, which are dark green, subsessile and carried in pairs are closely aggregated at the apex of inflorescences, which are prevalently formed at the upper axes of branches. Every single flower is constituted of an ovary with a style that terminates in a pair of long, thin feathered stigmas at the apex, a membranous perianth surrounding the ovary and a bract (1-40). The perianth is transparent and can be smooth or partially frayed, and when mature, it covers approximately two-thirds of the ovary. The bracts are green and rough, with overlapping edges, which enclose the female flower (1-40) (Figure-2, 3)

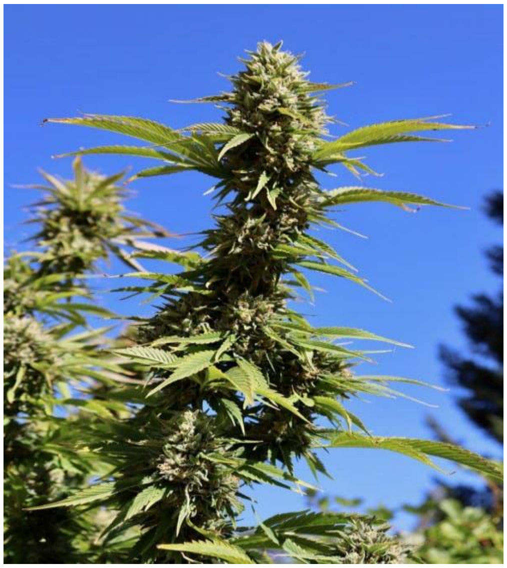

Figure-2: Female Marijuana plant with inflorescence

Hermaphrodite Cannabis

Hermaphrodites are a type of plant that contains **both male and female flowers** (Figure-3). Therefore, they will produce buds but they will also pollinate those buds and the rest of plants (1-51). Plants may naturally become hermaphrodites or be turned into one due to stress. Both female and male plants can turn due to stress (1-51). Thai strains are **more genetically inclined to become hermaphrodites**, although any strain can turn when stressed enough (1-51). There are many factors that can stress out plants and end up turning them, such as extra light when they are supposed to be in the night cycle, too much or not enough water, certain insects or pathogens, watering with cold water, or even a badly done transplant (1-51, 63). **Hermaphrodites are not the best type of plants** to keep around, as they can produce buds but it is definitely a risk because they might pollinate the rest of plants (1-51).

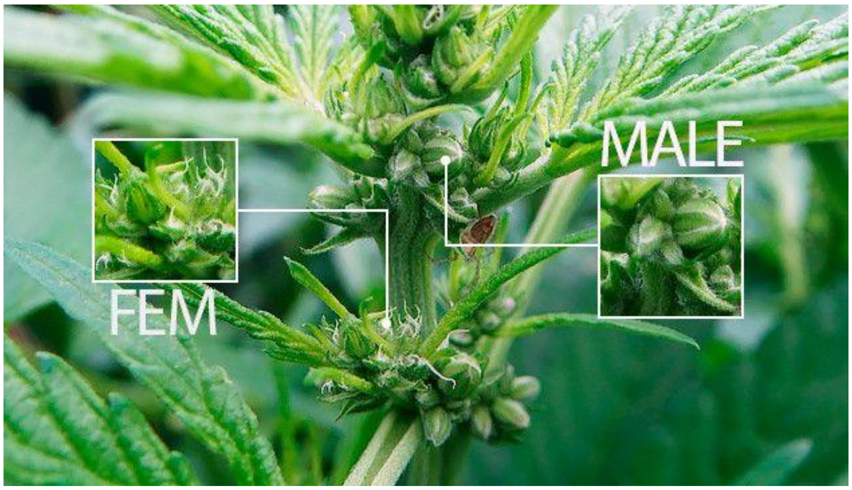

Figure-3: Hermaphrodite Cannabis plant showing male and female flowers on same plant at different locations (Monoecious). Male flowers showed closed Ball like structures. Female flowers showing balls with hairs. **Hairy like structures are female flower identity**.

Manipulation of Sex Expression: **Dioecious into Monoecious**

Manipulation of sex expression is of paramount importance in breeding medical Cannabis, since only genetically, phenotypically and unfertilized female plants are used in commercial cultivation (1-51). It enables self-pollination and crossing of female plants for obtaining pure lines and feminized seeds (1-51). Upon germination, the latter produce entirely female progeny that is used for the production of female flowers (1-51). Most of the Cannabis sex manipulation studies are performed on fiber-type hemp, and knowledge about the efficiency of various exogenous factors and application methods for inducing sex conversion in medical Cannabis is needed (1-51). Sex of *Cannabis sativa* L. (2n = 20) is genetically determined by one pair of sex chromosomes X and Y, where male gender of dioecious plants is determined by heterogametic XY chromosomes, while dioecious female and monoecious or hermaphrodite plants exhibit homogametic chromosomes XX (1-51). Due to instability of the sexual phenotypes across generations of XX plants, and the quantitative nature of sex expression, it was hypothesized that sex expression is a polygenic trait (1-51). Several studies with hormonal manipulation confirmed gender reversal in *Cannabis. sativa* L. and proved bi-potency of sexually predetermined dioecious Cannabis plants (1-51). It has been shown that **gibberellins** induce **maleness** in plants, while ethylene, cytokinins, and auxins stimulated the formation of female flowers on genetically male plants (1-51).

Galoch (1978) (27) showed that indole-3-acetic acid (IAA), kinetin (up to 100 mg/plant), and ethylene-releasing compound ethrel (up to 500 mg/plant) enhanced the feminization of male plants (1-27, 29-51). Abscisic acid (ABA) was completely ineffective in sexing both male and female hemp when used alone (1-51). GA_3 (up to 100 mg/plant) promoted masculinization of female plants while having no effect on sex change in male plants (1-51). Similarly, Ram and Jaiswal (1972) (37) earlier found that male plants showed no change in sex expression when treated with gibberellins (up to 100 mg/plant), but female plants developed male flowers with normal stamens and viable pollen grains (1-51). Besides, environmental factors such as temperature, photoperiod, light conditions, nutrient deficiency, and mechanical stresses (e.g., damages) can influence sex expression and induce monoecism (35-39). As reviewed in Truta et al. (2007) (18) and Petit et al. (2020) (33), the ratio of different phytohormones played a crucial role in the sex expression of hemp (1-51). External treatment of GA_3 to spinach, for example, affects the expression of the GAI gene, which is a transcription factor of the DELLA family (1-51). It is highly expressed in female inflorescences and acts as a repressor of the expression of B-class homeotic genes, which are masculinizing

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factors (1-51). **B-class genes** stimulated male organ formation and simultaneously suppress the development of female organs in the flowers (1-30, 33. 34-51).

Cannabis sex determination could be modified by applying exogenous growth regulators or chemicals, which can influence the ratio of endogenous hormones and hence the incidence of sex organs (15-51). Silver compounds such as silver nitrate ($AgNO_3$) or silver thiosulfate ($Ag_2S_2O_3$; STS) have been found to have masculine effects in many plant species, e.g., in *Coccinia grandis* (19), *Cucumis sativus* (20), *Silene latifolia* (29), *Cucumis melo* (32), and also *Cannabis sativa* (1-15-51).

In one of the experiment reported by Ram and Sett (1982) (35) applied 50, 100, and 150 mg of silver nitrate and 25, 50, and 100 mg of STS to shoot tips of female Cannabis plants (1-15-51). Both silver compounds successfully evoked the formation of male flowers, but STS was more effective than $AgNO_3$ (1, 35-51). 100 mg of STS caused the highest number of fully altered male flowers, which was significantly higher than the number of reduced male, intersexual, and female flowers (1-15, 35-51). On the other hand, the treatment of shoot tip with 100 mg of $AgNO_3$ resulted in more than half the lower number of male flowers, with the highest amount of $AgNO_3$ (150 mg) being ineffective in altering sex expression (1, 35-50). Furthermore, pollen from all induced male flowers was viable in vitro and also successfully induced seed set (1,2, 17-51). Lubell and Brand (2018) (30) published the results of using 3 and 0.3 mM silver thiosulfate (STS) to induce male flowers in genetically female hemp plants of four strains (1, 2, 30). They sprayed three times at 7-day intervals and counted flowers (male and female) on terminal buds, not whole plants (1, 2, 30). They determined the percentage of male flowers to all flowers and the masculinization rate (1, 2, 17-44, 45-51). They also confirmed the successful induction of male flowers in hemp strains (1-51). Regarding the percentage of inflorescences with male flowers, their best two hemp strains yielded up to 15% no male inflorescences, regardless of the STS concentration used (1-51). In one of the experimental study (46, 47), the authors suggested a method for making 0.3mM silver thiosulfate (STS) and spraying the entire female plant until the solution drips from the plant (1-45, 46, 47-51). There is no quantitative evidence of the success of the method used (46, 47). More recently, two other studies have also successfully used silver thiosulfate (STS) to induce male flowers (1-51).

Di-Matteo et al. (2020) (24) sprayed 3 mM of silver thiosulfate (STS) until runoff three times at 7- day intervals after exposing the plants to short-day conditions for 12 h (1-51). Adal et al. (2021) (52) applied 20 ml of silver thiosulfate (STS) (2.5 mg/ml) to whole plants on the first and third day after the start of 12-h lighting and fertilization on a foliar basis (52). Foliar spraying of male Cannabis plants with 960 ppm 2- chloroethanephosphonic acid caused the highest formation of the fertile female flower (1-52). A total of 100 mg/plant of cobalt chloride applied to the shoot tip triggers male sex expression in the female plants of Cannabis. The mode of action of these chemicals in plants is not yet entirely deciphered (1-52).

Intensification of medical (drug type) Cannabis production stimulated breeding activities aimed at developing new, improved cultivars with precisely defined, and stable cannabinoid profiles (1-17, 18-52). The effects of several exogenous substances, known to be involved in sex expressions, such as silver thiosulfate (STS), gibberellic acid (GA_3), and colloidal silver, were analyzed (17). In one of the experimental study reported by Flajšman et al. (2021) (17), various concentrations of silver thiosulfate (STS), gibberellic acid (GA_3), and colloidal silver were tested within 23 different treatments on two high cannabidiol (CBD) breeding populations (1-17, 18-52). Experimental results of Flajšman et al. (2021) (17) showed that spraying whole plants with Silver thiosulfate (STS), once is more efficient than the application of silver thiosulfate (STS) on shoot tips while spraying plants with 0.01% GA_3 and intensive cutting is ineffective in stimulating the production of male flowers (1-17, 18-52). Additionally, spraying whole plants with colloidal silver was also shown to be effective in the induction of male flowers on female plants, since it produced up to 379 male flowers per plant (17-51).

The viability and fertility of the induced male flowers were confirmed by fluorescein diacetate (FDA) staining of pollen grains in vitro and in vivo germination tests of pollen. Further counting the number of seeds developed after hybridization, and evaluating germination rates of developed seeds (17, 18-52). Finally, one established protocol was implemented for crossing selected female plants (1-51). The cannabinoid profile of the progeny was compared with the profile of the parental population and an improvement in the biochemical profile of the breeding population was confirmed (17-52). The progeny had a higher and more uniform total CBD (tCBD) to total tetrahydrocannabinol (tTHC) ratio (up to 29.6; average 21.33 ± 0.39) compared with the original population (up to 18.8; average 7.83 ± 1.03). Flajšman et al. (2021) (17) reported the first comprehensive report on the induction of fertile male flowers on female plants from dioecious Medical Cannabis sativa (drug or marijuana) (17-52).

IV. Conclusion

Cannabis sativa L. (hemp, or marijuana) produces male and female inflorescences on different plants (dioecious). Therefore the Cannabis plants are obligatory out-crossers. In commercial production, marijuana plants are all genetically female. Male plants are destroyed as seed formation reduces flower quality. Spontaneously occurring hermaphroditic inflorescences, in which pistillate flowers are accompanied by formation of anthers, leads to undesired seed formation, the mechanism for this is poorly understood. One male plant can ruin the entire female plant crop due to uncontrolled pollination and crop is designated as contaminated. Therefore, early diagnosis of sex is very important to both breeders and farmers for Cannabis crop improvement or production purposes.

Over the last few years, a renewed interest in *Cannabis sativa* and its products has occurred worldwide due to the easing of legislation. The more recognised active ingredients are the psychoactive, Δ9 -tetrahydrocannabinol (Δ9-THC) and the therapeutic Cannabidiol (CBD). The legal status of *Cannabis* is changing, fuelling an increasing diversity of *Cannabis* derived products. Medical Cannabis *sativa* (Marijuana-type)' lineages are used for human consumption (recreational and medical), while the Industrial *Cannabis sativa* 'hemp' lineages are used in industry settings for fibre or oil extraction. *Cannabis sativa* L. is grown and marketed under a large number of named strains. Strains are often associated with phenotypic traits of interest to consumers, such as aroma and cannabinoid content.

Manipulation of sex expression is of paramount importance in breeding medical Cannabis, since only genetically and phenotypically, unfertilized female plants are used in commercial cultivation. Cannabis sex determination could be modified by applying exogenous growth regulators or chemicals, which can influence the ratio of endogenous hormones and hence the incidence of sex organs. Silver compounds such as silver nitrate ($AgNO_3$) or silver thiosulfate ($Ag_2S_2O_3$; STS) have been found to have masculine effects in many plant species including Cannabis. This review paper has updated the sex determination and also highlights about the conversion of dioecious species that can produce monoecious Cannabis plants.

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