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Review Article

Development and Maintenance of Parthenocarpic Gynoecious Cucumber (*Cucumis sativus* L.) via the Induction of Male Flowers through Different Chemicals

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ABSTRACT

Chemical induction of male flowers in parthenocarpic gynoecious cucumber (*Cucumis sativus* L.) lines is essential for maintaining high-yielding genotypes through self-pollination. This review evaluates the efficacy of three primary chemical agents for sex manipulation in cucumber breeding programs. Silver nitrate ($AgNO_3$) demonstrated optimal results at concentrations of 400-600 ppm for inducing male flower development. Gibberellic acid (GA_3) proved most effective at 1500-2000 ppm, while silver thiosulfate (STS) induced favorable male flower ratios at 200-250 mg/L. These chemicals function as ethylene inhibitors or gibberellin promoters, hormonally mediating the shift from female to male flower formation. The successful application of these treatments enables efficient maintenance and improvement of parthenocarpic gynoecious cucumber lines, supporting the development of high-quality, disease-resistant cultivars with superior yield potential while optimizing resource utilization and reducing production costs.

Keywords: Gibberellic acid, seedless cucumber, sex expression, silver nitrate, silver thiosulphate

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a trailing vine vegetable crop from the Cucurbitaceae family, commonly known as the melon family. *Cucumis sativus* is the second most cultivated vegetable in its family, following watermelon. In recent years, the demand and production of cucumbers have surged significantly, leading to their widespread cultivation in fields and greenhouses worldwide. Cucumber is an annual plant based on three main varieties (slicing, pickling, and seedless). Most species are distributed in warmer areas. It grows in the summer and winter. It is an annual plant with a diploid chromosome number of 14 (2n = 14) (Kadi *et al.*, 2018).

The Cucurbitaceae family contains more than 130 genera and 900 species. Plant sex determination has evolved through complex biological processes. Hermaphroditic plants are considered the ancestral form, with monoecious and dioecious species developing later (Tanurdzic & Banks, 2004). Most angiosperms are hermaphroditic, producing flowers with both male and female reproductive organs. Cucurbits exhibit diverse sex types, and some species have even developed sex chromosomes (Ming *et al.*, 2011). Plant sex is typically determined by unisexual flowers, where male flowers contain only stamens and female flowers contain only pistils (Bai, 2015). Unisexual flowers in plants offer increased heterosis benefit and better genetic diversity (Chen *et al.*, 2016). Researchers have conducted extensive research to specifically understand plant sex determinants while studying evolutionary factors that affect sex variations (Diggle *et al.*, 2011). The flowering types in Cucumber include hermaphroditic plants as well as gynoecious and monoecious specimens, and both androecious and andro-monoecious forms. The reproductive system of dioecy exists typically in animals and only affects approximately 5–6% of flowering plant species, as well as 7% of plant genera. The occurrence of dioecious species extends to 43% of plant families that generate unisexual flowers (Guo *et al.*, 2020). The Cucurbitaceae family includes up to 96 genera and 1000 species, which produce flowers whose male and female organs are usually in separate

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structures, although they have both monoecious and dioecious forms of species. Unisexual flowers, hence developed independently of their hermaphroditic roots since they occurred in dioecious and monoecious plants (Zhang *et al.*, 2006). Sex differentiation is closely related to the production of cucumbers and is repeated in such a complicated chain. During the early stages of development, cucumber flowers are bisexual, and they possess both anther and a pistil primordia. Nevertheless, the process of sex determination is regulated by the selective inhibition of either male or female organs (M. E. Pawełkowicz *et al.*, 2019a; Z. Li *et al.*, 2022). Being a model plant concerning sex determination studies (M. E. Pawełkowicz *et al.*, 2019a; Z. Li *et al.*, 2022), cucumber has been determined in detail in an atomic, anatomical, and genomic distinction. Nevertheless, its genetic variability is restricted, and the expression of its sex is very variable and complicated (Grumet *et al.*, 2023). The process of flowering in plants is complex, and there are morphological and physiological changes that stimulate a plant to flower; this is controlled by the internal and external factors. The hormones in plants play a crucial role in sex expression, with ethylene and auxins influencing the development of females and gibberellins influencing the growth of males (Yamasaki *et al.*, 2000). The biosynthesis of ethylene occurs in the cycle of Yang, according to which 1-aminocyclopropane-1-carboxylic acid (ACC) is transformed under the influence of ACC synthase (ACS) and ACC oxidase (ACO) (M. Pawełkowicz *et al.*, 2019b). ACS is encoded by the F locus in cucumber, and this locus determines (Mibus & Tatlioglu, 2004). The ACS gene family of cucumbers is made up of six members: CsACS1, CsACS1G, CsACS2, CsACS3, CsACS4, and CsACS11 (Trebitsh *et al.*, 1997). Out of these, CsACS1G controls the female characteristics, whereas CsACS2 controls the male characteristics. Research demonstrates node number on the primary stem to be correlated with the CsACS2 expression and to be less expressed in the monoecious cucumbers than in the gynoecious (Kahana *et al.*, 1999; Perl-Treves, 2004). The genes that control sex differentiation are the ACS and ACO, which adjust the levels of ethylene that stimulate the growth of female flowers. As well, the ETR (Ethylene Receptor) gene contributes to suppression of stamen (Wang *et al.*, 2010), and the mutations in ETR1 and ETR2 are negatively involved in ethylene response (Yamasaki *et al.*, 2000). One more important gene, CsWIP1, is a negative regulator of femaleness and an ortholog to Cucumis melo WIP (Chen *et al.*, 2016). Although in cucumbers, plant hormones determine the sex expression, several genes act cooperatively in regulating this mechanism.

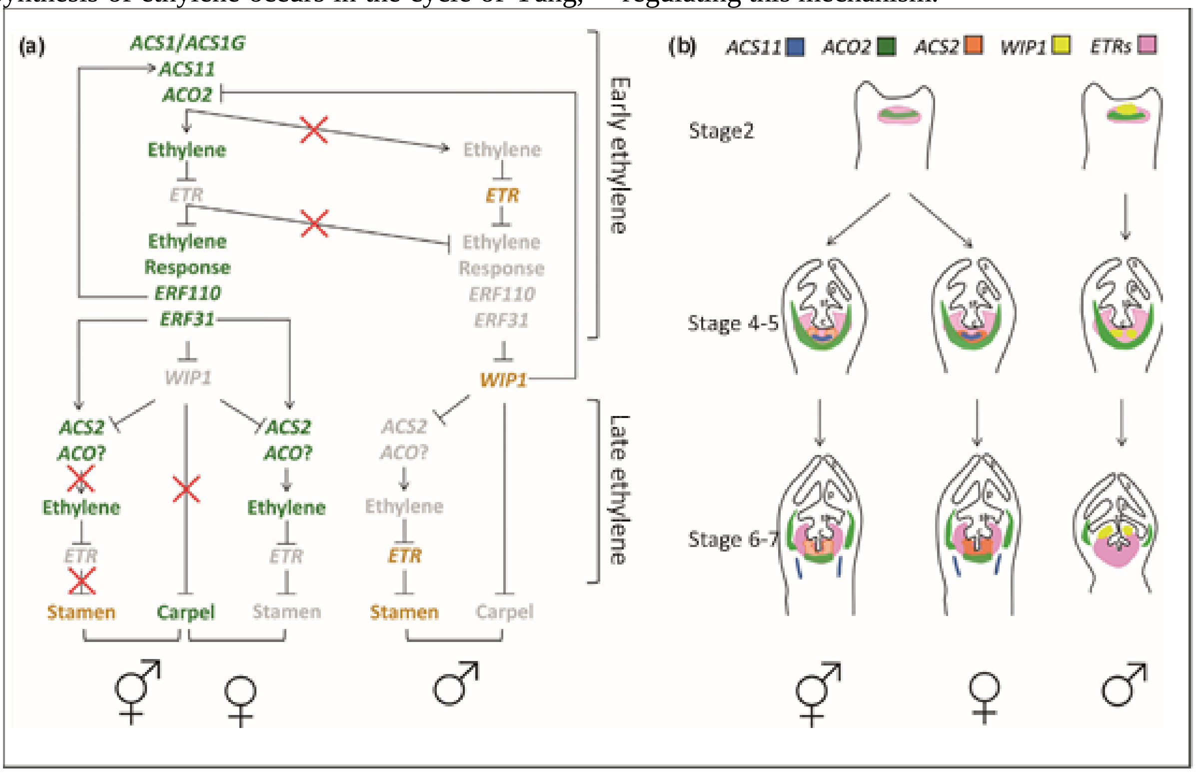

Figure 1. *Sex determination through genetic networks in cucurbit species. This figure illustrates the genetic network of sex determination in cucurbit species. Panel (a) shows how* ***ethylene biosynthesis genes*** *such as* ***ACS (1-aminocyclopropane-1-carboxylic acid synthase)*** *and* ***ACO (1-aminocyclopropane-1-carboxylic acid oxidase)*** *regulate ethylene production, while* ***ETR (Ethylene Receptor)*** *and downstream transcription factors* ***ERF (Ethylene Response Factor)*** *mediate the ethylene response.* ***WIP1 (WIP Domain Protein 1)*** *plays a*

critical role in repressing carpel or stamen development depending on ethylene signaling. Panel (b) depicts floral organ development stages (2, 4–5, 6–7), showing spatial expression of ***ACS11, ACO2, ACS2, WIP1, and ETRs****, which collectively direct whether the flower develops as male, female, or hermaphroditic.*

(a) A gene interaction model (Figure 1) shows how the stamen and carpel growth stoppage leads to the creation of hermaphrodite and female or male reproductive flowers. Identification of genes promoting carpel development uses green coloration, while stamen development genes utilize yellow coloration, and non-active or mutated genes receive a gray categorization. An ✕ sign indicates mutation sites in the presented diagram, while the gene names follow those identified in cucumber.

A gene interaction model exhibiting the developmental blocking, which leads to stamen and carpel arrest for hermaphrodite and female or male bloom formation. The genes that support carpel formation appear green, but stamen developing genes show themselves as yellow, while any inactive or mutated genes maintain a grey color. A mutation symbolized by an appears in the model. The authors propose names of genes within the cucumber, which are marked with an ✕.

Ethylene Genes Switched ON Lead to Femaleness

Gynoecious plants generate increased amounts of ethylene through ACS1 and ACS1G gene expression (described only in cucumber) to produce female flowers. The ethylene biosynthesis genes ACS11+ACO2 (green) speed up ethylene release within the FM of monoecious plants, which enhances carpel development. Early ethylene activates ethylene receptors (ETR), as these receptors act as negative controllers of ER expression when no ethylene exists. The activated ER function blocks WIP1 (grey) transcription elements. The late-acting ethylene biosynthesis production occurs because WIP1 transcription factor expression stays inhibited by ethylene receptors. The identical ETR genes detect late ethylene signals that activate ER proteins (green) until they cause stamen abortion to result in female flowers.

Ethylene Genes Switched OFF and WIP1 ON Lead to Maleness

Early ethylene production and response failure allow WIP1 (yellow) to be activated since it functions to stop carpel development. WIP1 represses early and late ethylene production by suppressing ACO2 expression together with ACS2 (grey) to prevent stamen arrest and determine the flower as male.

During the study of mutants in the scientific field, researchers exposed the functions of sex-determining genes. Mutation of ACO2, ACS11 or ETR (✕) genes prevent early Ethylene Response, which leads to the development of androecy. The WIP1 mutations activate ACS2 to generate late-emitting ethylene because they prevent carpel arrest, triggering gynoecy development. Mutations in both ETR and ACS2 genes (✕) lead to the blockage of early-acting ethylene formation or response processes. The process leads to female flower conversion into hermaphrodite type and subsequently results in the transformation of monoecious flowers into andromonoecious types. FM, floral meristem; ER, Ethylene Response

(b) Spatio-temporal expression of sex-determining genes at the developmental stages of flower 2, 4-5, and 6-7 (Bai *et al.*, 2004). ACS11 and ACS2 genes are expressed specifically in female-determined flowers in the FM tissue, and WIP1 shows its main expression in the female tissue that becomes destined to be male. All determining genes have their expression in both female and male flowers.

Female and Hermaphrodite Floral Buds

Early detection of ACO2 and ETRs begins to occur on carpel primordia during stage 2. During stage 4-5, the expression of ACO2 expands toward the base of petals, and ETRs move to the stamen primordia. The carpel primordia show transcriptomic overlap between ACO2 and ACS11 during stage 4, which allows them to coactively produce ethylene that drives carpel development. ACS2 transcripts build up in carpel primordia starting from stage 4, but ACS2 enzymatic activity does not appear in the orange-striped hermaphrodite flowers. The genes are expressed at stage 6-7.

Male Floral Buds

ETRs, as well as ACO2 and WIP1 transcripts, start becoming detectable within carpel primordia during stage 2. The expression patterns of ETRs and ACO2 show similarity to female floral buds during stages 4-5 and 6-7. The WIP1 transcript builds up within the carpel primordia at developmental stages 4-5 and in stamen primordia at stages 6-7 (Chen *et al.*, 2016) before its production declines during floral bud elongation.

In the commercial production of cucumbers, it is necessary to select proper chemical agents that can be used in establishing male flowers. Sex differentiation is usually regulated by the use of steroids like silver nitrate ($AgNO_3$), silver thiosulfate (STS), and gibberellic acid (GA_3) (Yadav *et al.*, 2020). As an important phytohormone, ethylene has a significant effect on all aspects of plant growth, including flowering, fruiting, ripening, withering, and detachment (Seesangboon *et al.*, 2018). Ethylene, however, is not washed down on plants directly as it has low water solubility. Rather, silver nitrate and silver thiosulfate are applied as ethylene inhibitors to retain gynoecious lines through developing male flowers (Hallidri, 2004). Silver nitrate was demonstrated in converting female flowers to male flowers in gynoecious cucumber plants (Beyer, 1976) and caused bisexual flowers in monoecious melons (Li *et al.*, 2011). The silver thiosulfate and gibberellic acid are most commonly used in the manipulation of sex expression in cucumbers.

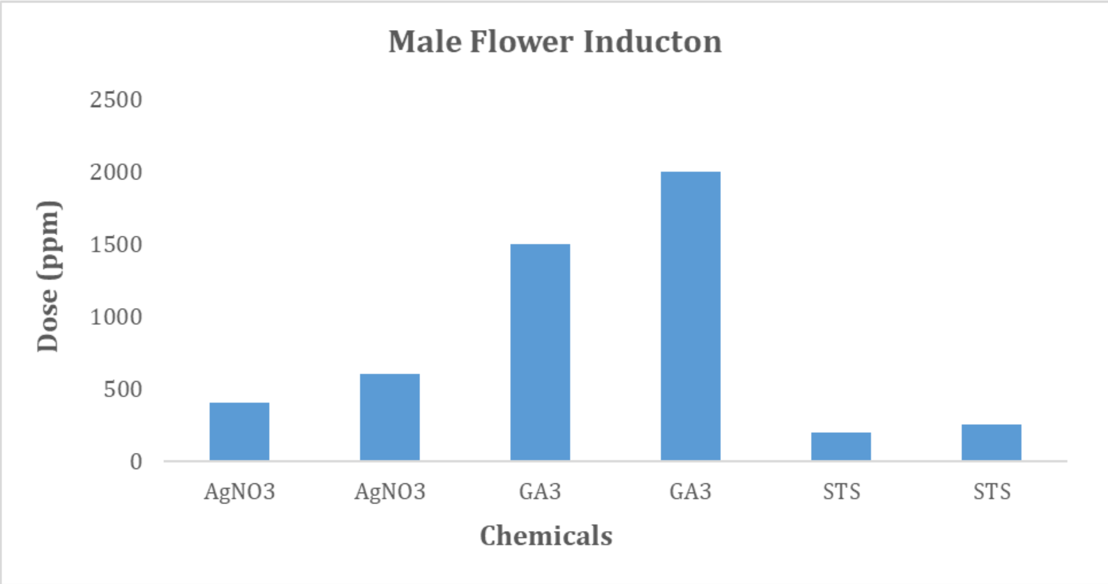

Figure 2. Different chemical treatments and their applied doses (ppm) used for male flower induction in cucumber (*Cucumis sativus* L.).

Figure 2 shows the highest male flower induction in the gynoecious cucumber breeding line with the use of these chemicals, $AgNO_3$, GA_3, and STS, at various concentrations, without depressing cucumber growth. $AgNO_3$ results in the maximum number of male flowers between 400 and 600 ppm, while GA_3 produces maximum male flowers at 1500 and 2000 ppm. Silver thiosulfate results in the peak of male flower induction in cucumber at 200 and 250 ppm.

The flowering patterns make most types of cucumbers favor gynoecious varieties. Gynoecious cucumbers that give yield without fertilization, as well as increased yield. Also, the system to facilitate the induction of male flowers to be more efficient and environmentally sustainable will also reduce the utilised input to produce AB sold. The purpose of the study is to further fill in the current gap of knowledge and present practical advice to cucumber growers and horticulturists, explaining how to induce male flowers in gynoecious cucumber plants. The results of this review might be useful for supporting greater fruit and crop production or more economical, sustainable cucumber production.

Effect of Silver Nitrate

A straightforward, expedient method of inducing male flowers is to apply anti-ethylene agents. Both types of anti-ethylene compounds contribute to the regulation of the flowering process through the inhibition of perception by using silver thiosulfate (STS) (Veen, 1983) or silver nitrate ($AgNO_3$) (Kumar *et al.*, 2009), as well as the biosynthetic stage inhibitor, aminoethoxy vinyl glycine (AVG). Adverse effects due to ethylene are managed by the utilization of ethylene inhibitors. Use of $AgNO_3$ solution is considered a critical component in identifying reproductive characters of cucumbers by altering the female-male flower ratio in monoecious plants or gynoecious plants (Stankovic & Prodanovic, 2000). The gynoecious plants acquire higher seed yield per fruit due to successful pollination of their female flowers by staminate flowers induced to pollinate female flowers of other varieties of gynoecious nature by $AgNO_3$ (Koyama, 2008). $AgNO_3$ solution enhances androgenic response of the gynoecious-type cucumbers (Amirian *et al.*, 2020) and agronomic response of stamen growth in female flowers of Coccinia grandis (Ghadge *et al.*, 2014). $AgNO_3$ usage also causes hermaphrodite flowering and seed production of the dioecious species of Cucurbicaceae, including the kakrol (Momordica dioica Roxb.) and pointed gourd (Trichosanthes dioica Roxb.) (Q. Li *et al.*, 2022). Flowers with an ethylene connection result in the frequent pollination of the plant and proper pollination through the generation of pollen (Hassan & Miyajima, 2019). $AgNO_3$ treats plants of melon species, shifting male and bisexual flowers below the main stem (Ye *et al.,* 2020).

Dai *et al.* 2022) examined how silver nitrate induced the stamen in the female inbred lines. The Silver Nitrate solution is the one that has been used in reproducing hermaphrodite flowers in gynoecious inbred lines. The number of true flowers between 2 to 5 was regarded as seedlings. The silver nitrate concentrations were at 150, 300, 450, and 600 mg/L. The plants at the four-leaf stage and silver nitrate being sprayed at 450 mg /L gave the maximum pollen in the hermaphrodite flowers.

Gosai *et al.* (2020) examined both theoretical and practical implications of different plant growth regulators (PGRs) applied to regulate cucumber growth and development. In their study, altering the plant structure and growth through foliar application of PGRs has a significant impact on a wide range of physiological and/or developmental processes that include vegetative growth, sex expression, yield, and components of production of cucumbers. Auxins, together with gibberellins, induced secondary growth, and on the other hand, ethylene inhibited secondary development. At the concentration of 300 ppm-400 ppm, ethylene led to a higher level of femaleness, besides promoting fruit smoothness at 200 ppm-300 ppm. Besides, 400 ppm of silver nitrate ($AgNO_3$) was also observed to enhance the formation of male flowers in cucumbers. Because of the significant effect of PGRs on vegetative growth and fruit quality, exogenous application of plant growth regulators has become a mandatory exercise among Nepali cucumber farmers.

Hassan and Miyajima (2019) conducted research in which Silver nitrate ($AgNO_3$) was applied to treat vines in a range of doses (50 to 2,000 mg/L) with hazardous effects. Hermaphrodite flowers could only be produced using 800 and 1000 mg/L of $AgNO_3$. Hermaphrodite flowers passed through a number of morphological modifications. Among them, tri-furcated encouraged the development of three anthers and the fusion of the most masculine flower stigmas into a single structure. Flowers treated with 100 to 1000 mg/L of $AgNO_3$ produced fruits that were parthenocarpic. In every way, parthenocarpic fruits performed worse than typical open-pollinated fruits.

Sanwal *et al.* (2011) investigated the potential for sexual crossover between two genetically female plants, focusing on the effects of silver nitrate ($AgNO_3$) on sex modification in cucurbits. Their research involved spraying $AgNO_3$ on 30-day-old female plants, which led to the development of hermaphroditic flowers. The maximum male flowers appeared when 500 mg/L AgNO3 was used on female plants. The development of hermaphrodite flowers started after 17-21 days of spraying with the $AgNO_3$ (depending on the doses), and it took 8-17 days. The hermaphroditic flowers thus induced had a viability of pollen similar to that of the natural male flowers. This method allowed the obtaining of one plant with better genotype traits of females.

Karakaya and PADEM (2011) conducted the experiment in order to determine the impacts of silver nitrate ($AgNO_3$) on the flowering outputs of cucumber. Silver nitrate application was also done by spraying 250, 500, 750, and 1000 ppm of silver nitrate to the plants' growth tips. A random parcel trial pattern was used in the investigation, and 4 replications of 5 plants were used. Based on the results of this study, $AgNO_3$ increased the number of male flowers through the induction of male flowers. The quantity of male flowers always increased, according to the seasons (during summer or winter).

Kumar and Wehner (2012) performed an experiment in which a factorial, randomized block design was used to check the rate of natural outcrossing by applying the growth regulators. To ensure pollen availability, silver nitrate was applied to induce male flower formation, while ethrel was used to promote gynoecy. Isolation blocks have gynoecious plants that were not treated. The rise in outcrossing rate at the node of treated plants was quite effective, according to the results. Growth inhibitors and plot size, however, had a big impact. High outcrossing rates (54%) were seen in treated plants compared to untreated plants (30%). For maximal outcrossing, families should be treated with growth regulators before being intercrossed in an isolation block.

Moon *et al.* (2010) performed research to compare the impact of silver nitrate in long and short-day durations. When female plants grown under long-day conditions were treated with silver nitrate during the vegetative stage and then moved to short-day conditions, the difference in flowering time between sex-reversed male flowers and normal female flowers was only 1 to 3 days. Female seeds cultivation produced 141 kg/10 Acre as compared to the normal seed output of 96 kg/10 Acre.

Susaj and Susaj (2010) studied the models for controlling cucumber sex expression from the perspective of breeding and seed production based on the interplay of the plant's hormonal and genetic systems. Ethylene, auxins, and other substances encourage femaleness, whereas gibberellic acid and other chemicals encourage maleness. $AgNO_3$ concentration and the number of treatments affect floral sex expression and the induction of male blooms. At the two to three true leaves stage, the first treatment was administered using $AgNO_3$ concentrations of 100, 200, 300, 400, and 500 ppm. The highest number of staminate flowers was observed in plants treated with 400–500 ppm $AgNO_3$ two or three times within seven days.

Dennis Thomas (2004) conducted an experiment in which filtered, sterilized silver nitrate was incorporated into Murashige and Skoog medium, along with 5 ml of 6-benzylaminopurine (BA). After applying silver nitrate to the culture media, female plants developed male, female, and mixed inflorescences. The highest induction of male inflorescences, reaching 22.4%, was observed at a silver nitrate concentration of 2500 g. In addition to male and female flowers, bisexual blooms were also present in plants treated with silver nitrate.

Stankovic and Prodanovic (2000) investigated sex expression in two inbred cucumber lines by applying different concentrations of silver nitrate (0.01%, 0.02%, 0.03%, and 0.04%). The study aimed to determine the most effective concentration for sex conversion. As the concentration of $AgNO_3$ increased, more male flowers appeared in the gynoecious line, while the number of female flowers decreased compared to the control. However, silver nitrate did not influence male flower development in monoecious cucumber lines. The number of male flowers also varied depending on the planting season.

Effect of Gibberellic Acid

Cucumber development shows male preferences when gibberellins function as tetracyclic diterpenoid phytohormones. Gibberellins (GA) production levels in andromonoecious cucumber plants exceed those of gynoecious and monoecious cucumber varieties according to research by Hemphill *et al*. (1972). According to Pike and Peterson (1969), external applications of GA_3 promote male flower production in monoecious cucumbers and stimulate male floral development in gynoecious plants. The GA signaling pathway is crucial for stamen and anther development in hermaphroditic plants such as Arabidopsis and rice (Oryza sativa) (Plackett *et al.,* 2011; Song *et al.,* 2013). In this pathway, GA binds to GID1 receptors, enabling interactions between DELLA proteins, which act as repressors of GA signaling. This interaction triggers the rapid degradation of DELLA proteins via the ubiquitin-proteasome pathway, thereby removing their inhibitory effects on GA activity and allowing the plant to continue its growth and development (Harberd *et al.,* 2009; Plackett *et al.,* 2014). GAMYB, a key regulator within the GA signaling pathway, is an important downstream gene of DELLA proteins (Achard *et al.,* 2004; Fleet & Sun, 2005). By breaking down DELLA proteins, GA activates GAMYB transcription, promoting flowering and facilitating anther development (Achard *et al.,* 2004).

Chowdhury *et al.* (2023) executed a field experiment on cucumbers throughout the spring-summer months (March to June) of 2022 and 2023, utilizing six different gibberellic acid concentrations. The research evaluated GA_3 at five different concentrations spanning from 100 ppm to 500 ppm, with a control treatment. Three replicated blocks formed the basis of the experimental design. A concentration of 400 ppm GA_3 yielded the most male flowers, yet the 200 ppm GA_3 treatment resulted in the fewest male flowers (22.91 flowers). The highest yield achievements emerged when crops received a 200 ppm GA_3 treatment.

Golabadi *et al*. (2018) conducted research in which the most significant changes in cucumber sex expression were caused by gibberellic acid. According to reports, gibberellic acid can increase the quantity of male flowers. It is generally known that exogenous gibberellin makes cucumbers more androecium. Silver nitrate and silver thiosulfate were shown to be effective male blooming inducers in gynoecious cucumbers. In gynoecious cucumbers, silver thiosulphate produced more male flowers than GA_3. The formation of male cucumber flowers was positively affected by silver thiosulfate.

Chen *et al.* (2003) conducted an experiment in which a single foliar spray of gibberellic acid (GA_3) at doses of 125, 250, 500, or 1,000 mg/L was applied on cucumber plants to induce male flowers in around 170 days. If GA_3 concentrations rose, more plants produced male flowers, and there were, on average, more blossoms per plant. Compared to untreated plants, GA_3-treated plants produce more blooms.

Effect of Silver Thiosulfate

Silver thiosulfate (STS) functions as an ethylene perception blocker, which modifies the flowering process. STS treatment of gynoecious bitter gourd plants results in hermaphrodite flower production in female flowers' staminal tissue, providing better results compared to using $AgNO_3$ or GA_3 at lower concentrations (Mishra *et al.,* 2013). An application of Silver thiosulfate (STS) through foliage helps female hemp plants develop reproductive male structures (Lubell & Brand, 2018). Scientific studies utilizing RNA-Seq analysis between untreated female Cannabis sativa plants and STS-treated plants detected multiple differentially expressed genes (DEGs), which contain transcription elements alongside genes that depict male organ advancement, together with phytohormone pathway elements and characteristics that impact masculine characteristics (Adal *et al.,* 2021). Spring flowers experience undesirable changes, including premature aging, when exposed to ethylene gas. Inhibiting ethylene response mechanisms proves effective for maintaining the freshness of ethylene-sensitive flowers (Serek *et al.,* 2015). The ethylene receptors in petals become blocked by STS, so petals preserve their fresh appearance instead of wilting (Veen, 1983). STS treatment enhances both flower production and duration in cassava while delivering optimal results within shoot apex tissues according to Hyde *et al.* (2020). The simultaneous application of STS with a prolonged light cycle successfully produces potatoes with male-fertile flowers (Kumar *et al.*, 2006).

Minnu *et al.* (2022) discovered the morphologically new gynoecious cucurbit lines at Kerala Agricultural University in India. Silver thiosulfate was used to alter the gynoecious line's sex expression. The application of 200 STS (silver thiosulphate), which is an artificial male flower booster, was applied after the emergence of the first true leaves on gynoecious plants. The gynoecious trait remained stable in progenies cultivated through tissue culture.

Lubell and Brand (2018) studied the effects of silver thiosulfate (STS) on hemp to increase the male flowers. The different concentrations of chemicals were applied as a foliar spray on the four strains of female hemp for the production of male flowers. The most effective results were observed at a 3mm concentration of STS on the strains. Partial conversion of the male flowers occurred at a 0.3 mm concentration of STS. As the research revealed, male production of flower production is extremely easy to attain through STS application.

According to Saha *et al.* (2018), gynoecy is the production of only female flowers on all flowering nodes, which can boost yields and reduce the cost of producing hybrids. Inhibition of ethylene perception using silver thiosulfate succeeded in the production of Hermaphrodite flower buds in gynoecious lines of cucurbits. Nevertheless, in India, molecular breeding in bitter gourd is yet to mature since the molecular resources in India especially lack functional markers for specific traits such as gynoecy, etc. Findings showed that silver thiosulfate was effective in inducing the presence of male flowers more in gynoecious lines of cucurbits.

Airina *et al.* (2013) conducted an experiment titled "Heterosis breeding exploiting gynoecy in cucumber (*Cucumis sativus* L.)." was conducted. In this experiment, a foliar spray of silver thiosulfate at a dosage of 200 ppm was used to successfully induce male flowers in the gynoecious line. The artificially created male flowers were uniform and typical monoecious cultivars with good pollen productivity. As a result, these cucumber lines may be developed for testing under various agroclimatic settings to utilize the vigor of cucumber lines for commercial purposes.

Law *et al.* (2002) researched how Silver thiosulfate ($Ag(S_2O_3)_2^{-3}$) was applied exogenously, which promotes stamen formation in female plants of the cucumber. $Ag(S_2O_3)_2^{-3}$ stimulates stamen growth in asexual plants to the same degree. Even said, female plants that have ($Ag(S_2O_3)_2^{-3}$) may have better stamen growth. As a result, silver ions alone are responsible for the impact seen in female plants.

Effect of GA_3, $AgNO_3$, and $Ag(S_2O_3)_2^{-3}$

Among plant growth hormones, gibberellic acid (GA) and ethylene have the most significant impact on sex expression in cucumbers (Perl-Treves, 2004). Gibberellic acid, known for its role in inhibiting ethylene production, has been found to promote male flower formation (Jutamanee *et al.*, 1994). The stamen serves as the primary site for bioactive GA, playing a vital role in male flower development (Gupta & Chakrabarty, 2013). Studies have established that applying GA externally increases the production of male flowers or delays female flower formation in cucumbers (Peterson & Anhder, 1960). Khan and Chaudhry (2006) reported that applying 400 ppm GA_3 not only encouraged early flowering but also increased the number of both male and female flowers in *Cucumis sativus* L. and *Momordica charantia* L. Additionally, repeated applications of GA_3, which acts as an ethylene synthesis inhibitor, prevented the continuous female phase in self-pollinating female cucumber lines (Gupta & Chakrabarty, 2013). Silver nitrate (AgNO3) and silver thiosulfate (STS) are the other chemical agents, besides plant hormones, that are capable of inducing the male flower production in gynoecious cucumbers (Karakaya & PADEM, 2011). It is a successful readjustment of the gynoecious lines by the outside use of GA_3 (Peterson & Anhder, 1960), silver nitrate (Beyer, 1976), and silver (Den Nijs & Visser, 1980). In two gynoecious breeding lines and two largely female breeding lines, Kalloo and Franken (1978) established that $AgNO_3$ was more effective in the induction of male flowers than GA_3. On the same note, $AgNO_3$ also turned out to positively influence male flower production of cucumbers as reported by Karakaya and PADEM (2011). There is, however limited study on the interaction effects of hormones and chemical treatments on morphological characters and production of male flowers in various growth stages using different frequencies of application rates. The effect of various chemicals on the induction of male flowers to seed production by altering sex expression in parthenocarpic gynoecious cucumber lines was researched by Dhall *et al.* (2022). To experiment with different concentrations of GA_3, silver nitrate ($AgNO_3$), and silver thiosulfate (STS), their experiment was conducted on gynoecious cucumber lines. The analysis of variance indicated that there was a significant influence of the chemical treatments on the factors of male flower induction and the rates of mortality in the plants. Silver thiosulfate was observed to be the most effective chemical in the induction of male flowers since it gave the highest number of male flowers per plant when used at 250 ppm and sprayed weekly, three times at the third to fourth leaf stage.

Sapkota *et al.* (2020) experimented to examine the effects of plant growth regulators on cucumber growth, flowering, and yield. They carried out a study of nine treatments that included control (no applied spray), silver nitrate 250 ppm, ethephon 250 ppm, GA_3 300 ppm, NAA 50 ppm, silver nitrate 500 ppm, ethephon 250 ppm, GA_3 500 ppm, and NAA 100 ppm, all with three replicates each. The findings were that plant growth regulators had a significant influence on growth, flowering, and fruit output in the plants. Plants of the GA_3 300 ppm treatment were found to be the tallest, whereas the shortest plants were found after the NAA 100 ppm treatment. Control treatment yielded the greatest quantity of male flowers, and ethephon 250 ppm enhanced the maximum quantity of female flowers. The GA_3 500 ppm led to the longest of the fruits; on the other hand, the ethephon 250 ppm produced the shortest. Under the GA_3 300 ppm treatment, the number of fruits per plant was high, and the one that had the lowest number of fruits was the control. GA_3 300 ppm also resulted in the greatest fruit yield (109.7 t/ha) and the control in the lowest one (40.53 t/ha). The present results indicate that the recommended concentration of 300 ppm GA_3 stimulates plant growth, flowering, and fruit production more than that of NAA and other proportions of GA_3.

Flajšman *et al.* (2021) experimented to discover how the various exogenous chemicals, i.e., colloidal silver, gibberellic acid, and silver thiosulfate (STS), influence sex expressions in cucumber. Several concentrations were applied to plants with different treatments. As per the results, foliar spray of Silver thiosulfate on the cucumber plant was better than that of $GA_{3,}$ and heavy trimming could not result in enhancing male flower development. The colloidal silver had also been successful in producing the male flowers in the gynoecious plants, as it yielded more male flowers per plant. Results verified the induction of male flowers' viability and fertility.

Verma *et al.* (2018) examined the impact of chemicals (gibberellic acid, silver nitrate, and silver thiosulfate) in altering sex expression in gynoecious cucurbits. This was done using three chemicals, namely: Gibberellic acid at 500, 1000, and 1500 ppm, Silver Nitrate at 250, 500, and 750 ppm, and Silver Thiosulphate at 250, 500, and 750 ppm, which were all sprayed with each other at the 2-3 true leaves stage at five-day intervals until the 10-15 leaves stage. Gibberellic acid (GA_3), silver nitrate ($AgNO_3$), and silver thiosulphate ($Ag(S_2O_3)_2^{-3}$) had more male flowers in gynoecious cucumber cultivars than the control, which had no male flowers. The greater weight of silver thiosulfate did not exhibit harmful effects as that of silver nitrate when used in higher concentrations.

Naik *et al.* (2018) have studied gynoecious vines of the family Cucurbitaceae. Application of gibberellic acid (GA_3) and silver nitrate ($AgNO_3$) at different concentrations through a foliar spray of male and hermaphrodite flowers at the pre-flowering stage is found. GA_3 induced more than 50 percent of the male flowers on the female plant at 1500 ppm, and $AgNO_3$ induced the same at 500 ppm. $AgNO_3$ at its 750ppm produced successfully 36.6 percent male flowers and 33.9 percent hermaphrodite on the same plant. The hermaphrodite bloom had a bigger pollen size (103.57, 103.57, 126.70) compared with the normal one size (94.94, 94.94, 111.12) of the male flower.

Using an experiment, Zhang *et al.* (2017) examined how the Cucurbitaceae family reacts to treatments of gibberellin, ethephon, and silver nitrate regarding sex expression. It was observed that the application of gibberellins (GA_3) and ethephon decreased the percentage of female flowers, caused the time of the first female flowering to be late, and favored the production of male flowers. Conversely, silver nitrate had a more powerful impact on flower development in terms of male status. Both silver nitrate and ethephon treatment produced male bloom in gynoecious plants, but silver nitrate was the most effective in inducing male flowers as compared to ethephon and GA_3.

Golabadi *et al.* (2017) studied how various chemical studies influence the production of cucumber fruit and sex expression. Through the study, it was observed that the induction of male flowers with silver thiosulphate ($Ag(S_2O_3)_2^{-3}$) and silver nitrate ($AgNO_3$) works at different concentrations. Silver thiosulfate was sprayed twice on the damaged crops at 200 and 500 ppm, whereas silver nitrate was sprayed at 100, 200, and 300 ppm. Application of treatments at various growth steps (5, 10, and 15-leaf stages) and single or multiple sprays was done. The variance analysis has revealed that both the chemical used and its frequency have shown a significant effect in inducing male flowers. Silver ions, especially at high concentrations, were relevant in boosting the production of male flowers. Aspects of flower sex exposure (as well as days to initiate male flowers and male flower numbers) were more perturbed by these treatments than vegetative growth and yield-related traits. The experiment pointed out that silver ions can be successfully used to manipulate sex expression and promote flowering of males in female cucumber lines.

Mishra *et al.* (2013) studied gynoecious sex expression in cucumbers that play a very important role in hybrid breeding. The paper examined the possibility of chemical sex manipulation of this (usually a monoecious) plant. The effects of silver nitrate (SN), gibberellic acid (GA_3), and silver thiosulfate (STS) on inducing male flowers in gynoecious cucumber varieties were assessed by the researchers. The conclusions indicated that the STS at 3 mm and 6 mm was effective in developing male flowers, whereas the GA_3 at 2.9 mm and 4.3 mm mainly concentrated on vegetative growth. Silver nitrate 1.2 and 1.5 mm did not make any difference. The most male flowers developed on plants that were treated with 6 mm STS, and the proportions of plants displaying changed sex-expression were 57.63 percent. Stamens were present in male flowers, which started 7 to 15 days after treatment. Silver nitrate was found to be effective in the induction of male flowers in a gynoecious cucumber line when applied at a thickness of 6mm.

Nagar *et al.* (2014) investigated how the concentration of silver nitrate (SN) and silver thiosulphate (STS), the amount of sprays, and the mode of application can influence the induction of staminate flowers in gynoecious cucumber. In the study, it was discovered that the ratio between $AgNO_3$ (400 ppm) and STS (2 mM) was the most practical. Optimum response was obtained under 2 mM of STS twice (once at 2-3 true leaves and second after seven days), followed by two times 400 ppm SN applications as a foliar spray. This treatment made the most staminate flowers (151), the most staminate nodes (20), and the earliest onset of staminate flowers with a maximum of 25.17 nodes per plant.

Ding XiaoTao *et al.* (2013) also performed research on the impact of different amounts of gibberellin (GA_3) solution as well as silver nitrate solution to incite the growth of male flowers in gynoecious cucumber plants. The results proved that the use of these chemicals was effective in causing the flowering of male flowers and, furthermore, was most effective with an increased amount of those chemicals. In the meantime, when these chemicals were at very high doses, the cucumber plants were greatly damaged. As compared to the plants treated by silver nitrate solution studies, the plants treated by GA_3 yield fewer male flowers. The outcome of the experiment showed that the most effective amounts of silver nitrate solution and GA_3 solution in treatment were 250 and 500 mg/kg, respectively.

Yongan *et al.* (2002) have studied the role of ethephon, GA_3, and $AgNO_3$ in regulating sex expression in pumpkin (Cucurbita pepo L.). It was established in the research that 1000 mg/L gibberellin and 200 or 300 mg/L silver nitrate stimulated androecy to augment male flower commencement, but delayed the female flowers. Conversely, ethephon (2-chloroethyl phosphoric acid) at 50 mg/L embellished gynoecy since it stimulated female flower production compared to that of males, which were suppressed. The foliar sprays were carried out at the cotyledon stage, and the various varieties of the pumpkin responded similarly to the sprays. Nonetheless, ethephon in the amount of 100 mg/L damaged or killed the plant, whereas a higher dose of (1000 mg/L) gibberellin caused uncontrolled growth.

Chaudhary *et al.* (2001) studied how to preserve gynoecious cucumber (*Cucumis sativus* L.) lines by facilitation of staminate flowering using chemical induction to preserve the lines by genetic selfing. The experiment was carried out to test the three chemicals, namely silver nitrate ($AgNO_3$), gibberellic acid (GA_3), and silver thiosulfate ($Ag(S_2O_3)_2^{-3}$), to induce male flower formation in gynoecious lines. 300 and 400 ppm of silver nitrate to act as the initial lateral treatment produced the most male flowers. The experiment also established that the silver nitrate treatment done twice was the maximum way of getting staminate flowers to confirm the use of silver nitrate in keeping gynoecious lines.

Table 1. Comparative Analysis of Male Flower Induction Chemicals.

Parameter	Silver Nitrate	Gibberellic Acid	Silver Thiosulfate
Optimal Concentration	400-600 ppm	1500-2000 ppm	200-250 mg/L
Success Rate	60-70%	40-60%	70-80%
Phytotoxicity Risk	Moderate to High	Low	Low
Application Timing	2-3 true leaf stage	2-4 leaf stage	2-3 true leaf stage
Response Time	17-21 days	15-25 days	7-15 days
Economic Feasibility	High	Moderate	Low
Environmental Sensitivity	High	Moderate	Low
Mechanism	Ethylene inhibition	Gibberellin pathway	Ethylene receptor blocking

Table 1 compares silver nitrate, gibberellic acid, and silver thiosulfate for male flower induction in cucumber, highlighting their key quantitative differences. Silver nitrate is effective at 400-600 ppm with a 60-70% success rate but shows moderate to high phytotoxicity and environmental sensitivity. Gibberellic acid requires higher doses (1500-2000 ppm), achieves 40-60% success, and has lower phytotoxicity. Silver thiosulfate, applied at 200-250 mg L^{-1}, provides the highest success rate (70-80%) and the fastest response (7-15 days) with low toxicity, though its economic feasibility is lower.

Analysis of literature reveals distinct performance profiles for each chemical treatment. Silver thiosulfate consistently demonstrates the highest effectiveness with 70-80% success rates in male flower induction, followed by silver nitrate with 60-70% success rates, while gibberellic acid shows 40-60% effectiveness depending on genotype and environmental conditions. Significant variations exist across studies regarding optimal concentrations. For $AgNO_3$, reported effective concentrations range from 250 ppm (Ding XiaoTao *et al.,* 2013) to 600 ppm (multiple studies), indicating strong genotype-environment interactions. Similarly, GA_3 effectiveness varies from 400 ppm (Chowdhury *et al.,* 2023) to 2000 ppm across different studies, suggesting the need for variety-specific protocols.

Phytotoxicity represents a major constraint for silver nitrate applications, with plant damage observed above 1000 mg/L (Hassan & Miyajima, 2019). Economic factors limit silver thiosulfate adoption despite its superior effectiveness due to higher chemical costs. Environmental factors, including temperature, photoperiod, and seasonal variations, significantly affect treatment outcomes but remain poorly quantified across studies.

Treatment response varies significantly with genetic background, as demonstrated by who showed that monoecious lines exhibit no response to $AgNO_3$, while gynoecious lines respond proportionally to concentration increases. Seasonal effects noted by Karakaya & PADEM (2011) indicate higher success rates during optimal growing conditions, suggesting the need for seasonally adjusted protocols.

CONCLUSION

Cucumber is an important vegetable crop with high economic and biological value. It is of five sex expressions, i.e., monoecious, gynoecious, hermaphrodite, andro-monoecious, and androecious. A gynoecious cucumber is a parthenocarpic plant and hence develops only female flowers and yields produce to a great extent without cross-pollination. An important mechanism in cucumber is parthenocarpy. To keep such parthenocarpic lines, different chemicals are applied to induce male blossoms. These researched chemicals are silver nitrate, gibberellic acid, and silver thiosulfate. Parthenocarpic gynoecious cucumbers are treated with different mixtures of plant compounds to different levels of transformation, allowing the formation of male flowers. Artificially produced male flowers make it possible to conserve the genetic constitution of the parthenocarpic cucumber, subsequently being used in the production of inbred lines, newest varieties, and crosses. Maximum production of male flowers on the gynoecious lines was recorded at concentration levels of 400 and 600 ppm of silver nitrate and 1500 and 2000 ppm of gibberellic acid, whereas Silver thiosulfate induced favorably at 200 ppm. These flower-inducing chemicals in males are mandatory for sustaining parthenocarpic lines of cucumbers. The suggested concentrations may help the breeders to maintain and improve parthenocarpic gynoecious lines.

Future Prospects

Future research should prioritize advanced transcriptomic and proteomic studies to elucidate complete molecular pathways involved in chemically-induced sex reversal, enabling more targeted interventions with reduced side effects. Investigation of novel compounds with improved efficacy-to-toxicity ratios represents a promising direction, particularly bio-based ethylene inhibitors or selective hormone receptor modulators that could offer sustainable alternatives to current silver-based treatments. Development of cultivar-specific treatment protocols based on genetic markers or physiological indicators could optimize success rates while minimizing chemical inputs. Additionally, comprehensive environmental impact assessments of silver compounds used in breeding programs are essential for sustainable production practices, alongside research into automated application systems and large-scale treatment protocols for commercial adoption.

The integration of chemical male flower induction with emerging biotechnological approaches, including gene editing and molecular markers, represents the next frontier in cucumber breeding. Success in this field will require interdisciplinary collaboration between plant physiologists, molecular biologists, and breeding specialists to develop more efficient, sustainable, and economically viable methods for maintaining parthenocarpic gynoecious cucumber lines in modern agricultural systems.

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AUTHOR'S CONTRIBUTIONS

EA and NH were responsible for the write-up, and MT led the manuscript writing. SA reviewed the paper, and NP contributed to the reference setting. RA and AL collected data for the paper write-up.

CONFLICT OF INTEREST

All authors declare no conflict of interest

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