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Vishwas Rajeshkumar Acharya
Department of Genetics and Plant Breeding, B.A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Sushil Kumar
Department of Agricultural Biotechnology, Anand Agricultural University, Anand, Gujarat, India

Zeal Acharya
Ph.D., Department of Genetics and Plant Breeding, AAU, Anand, Gujarat, India

Dimple Gor
Ph.D., Department of Plant Molecular Biology and Biotechnology, AAU, Anand, Gujarat, India

Nisha Patel
Department of Genetics and Plant Breeding, College of Agriculture, Vaso, AAU, Anand, Gujarat, India

Akarsh Parihar
Department of Genetics and Plant Breeding, Anand Agricultural University, Anand, Gujarat, India

Corresponding Author:
Vishwas Rajeshkumar Acharya
Department of Genetics and Plant Breeding, B.A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Modification of the sex expression and maintenance of gynoecious parthenocarpic line in cucumber (*Cucumis sativus* L.)

Vishwas Rajeshkumar Acharya, Sushil Kumar, Zeal Acharya, Dimple Gor, Nisha Patel and Akarsh Parihar

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Abstract
Cucumber (*Cucumis sativus* L.), a cross-pollinated yet self-compatible crop, exhibits diverse floral morphologies, including male, female, and hermaphrodite flowers, typically borne solitarily. Sex expression in cucumber is profoundly influenced by growth regulators, which can significantly alter flower morphology. Gynoecious parthenocarpic cucumbers, ideal for off-season cultivation hance naturally lack of male flowers, necessitating the application of growth hormones to induce male flower production for the efficient maintenance of these lines. A study conducted during the *Kharif* 2020 aimed to manipulate the sex expression in the gynoecious parthenocarpic cucumber variety 'Pusa Seedless 6' using a Completely Randomized Design (CRD) with three replications. Treatments included varying concentrations of gibberellic acid (500, 1000, 1500 ppm), silver nitrate (250, 500, 750 ppm), and silver thiosulphate (250, 500, 750 ppm), along with a control (water spray). These chemicals were applied at the two-to-four-leaf stages at a rate of 10 ml per plant. The results revealed significant differences in sex expression parameters, including the days to the appearance of the first male flower, the node position of the first male flower, and the total number of male flowers per plant. Pollen viability assessments confirmed a 100% germination rate in induced male flowers, whereas no male flowers were observed in the control group. Among the treatments, silver thiosulphate ($Ag_2S_2O_3$) at 500 ppm proved to be the most effective treatment without obstructing the plant health, inducing an average of 43.44 male flowers per plant, with the first male flower appearing at the 10.67 node in 43.56 days, demonstrating the potential of this treatment for maintaining gynoecious parthenocarpic cucumber lines efficiently.

Keywords: Sex manipulation, gynoecious, parthenocarpy, growth regulators

Introduction
Vegetables are recognized as an essential food for nutritional security. At present, the global value of fruit and vegetable production exceeds that of all food grains combined, owing to their nutritional and economical importance. Among all the vegetable crops, cucumber (*Cucumis sativus* L.) is one of the important and popular cucurbitaceous vegetable crops grown throughout the tropical and subtropical world. It belongs to family cucurbitaceae having a somatic chromosome number 2n = 2x = 14 with genome size of 243.5 Mb (Huang *et al*., 2009) [4] as first genome sequenced vegetable crop.

Cucumber exhibits a fascinating range of floral morphology. It can have all three types of flowers i.e., male, female and hermaphrodite flower and they generally bear solitary. Gynoecious cucumber is distinguished by its production of predominantly female flowers, a trait advantageous for increasing fruit yield. The genetic control of gynoecy is primarily governed by the Female (F) locus (Yamasaki *et al.*, 2000; Saito *et al.*, 2007) [10, 7]. However, this locus influences sex expression through hormonal and environmental mechanisms, with ethylene playing a central role in promoting female flower development. Conversely, an ethylene action inhibitor shift sex expression from female to male, illustrating the delicate balance of hormonal influences on sex determination (Ando & Sakai, 2002; Yamasaki & Manabe, 2011) [1, 9]. These hormonal interactions indicate that strategically manipulating ethylene levels in cucumber breeding can optimize sex expression, thereby maintenance of gynoecious parthenocarpic inbreds can effectively achieved.

Materials and Methods

A study was carried out during *Kharif* 2020 to modify the sex expression from female to male in order to facilitate the maintenance and multiplication of gynoecious parthenocarpic plants, using the variety Pusa Seedless 6. The materials were evaluated using a Completely Randomized Design (CRD) with three repetitions. Ten plants were selected randomly and subjected to 10 different treatments. The treatments comprised of three levels of each three chemicals *viz.*, gibberellic acid (500, 1000 and 1500 ppm), silver nitrate (250, 500 and 750 ppm) and silver thiosulphate (250, 500 and 750 ppm) along with control water spray. Application of these chemical was performed as per the treatments (Table 1) at two to four true leaf stage of plant (20-25 days after sowing) and subsequent application at seven-day interval. The solutions of chemical were prepared with deionized water and about 10 ml solution per plant was applied with micro sprayer. The observations *viz.*, days to appearance of first male flower, node number on which first male flower appear and total male flowers per plant in each treatment were recorded.

Table 1: List of the treatments to modify the sex expression

Sr. No.	Treatments	Details
1	T_1	Gibberellic acid (500 ppm)
2	T_2	Gibberellic acid (1000 ppm)
3	T_3	Gibberellic acid (1500 ppm)
4	T_4	Silver nitrate (250 ppm)
5	T_5	Silver nitrate (500 ppm)
6	T_6	Silver nitrate (750 ppm)
7	T_7	Silver thiosulphate (250 ppm)
8	T_8	Silver thiosulphate (500 ppm)
9	T_9	Silver thiosulphate (750 ppm)
10	T_{10}	Water spray (Control)

Chemical Preparation

The gibberellic acid (GA) solutions of 500, 1000, and 1500 ppm were prepared by dissolving 500, 1000, and 1500 mg of GA salt, respectively, in distilled water and adjusting the final volume to one liter. Similarly, silver nitrate solutions of 250, 500, and 750 ppm were prepared by dissolving the respective quantities of silver nitrate salt in distilled water, with the final volume made up to one liter. The stock solution of each of 4mM silver nitrate and sodium thiosulphate ($Na_2S_2O_3.5H_2O$) was prepared by dissolving 680 mg silver nitrate in one litre and 1000 mg of sodium thiosulphate separately in one liter of distilled water. For safer use, each bottle was kept in dark and stored in refrigerator till final use. The solution of silver thiosulphate was prepared by slowly pouring the calculated volume of silver nitrate into sodium thiosulphate, stirring rapidly as the solutions were mixed as needed on the day of experiment. To prepare 500 ppm solution of silver thiosulphate from 4mM stock solution of silver nitrate and sodium thiosulphate, 125 ml of each stock solution was mixed and the final volume was brought to one liter. Similarly, using the normality equation, silver thiosulphate in concentration of 250 ppm and 750 ppm were prepared accordingly.

Characters studied

The days to first male flower initiation was recorded on randomly selected 10 plants from each plot. The character was recorded by computing the days required for appearing first male flower in selected plants of the plot from the date of spraying. The node was counted from ground level up to which first male flower appears on randomly selected plants individually to record node of first male flower appeared. The total number of male flowers produced by the selected plants in the plot was recorded by counting all male flowers up to the 30th node.

Pollen germination test for gynoecious variety

Pollen germination tests are essential for assessing pollen viability and reproductive capacity, which are fundamental to the success of plant breeding and hybridization programs. To ensure the fidelity of pollen from induced male flowers, germination tests were carried out using the procedure described below. The pollen from induced male flowers of the gynoecious inbred line Pusa Seedless 6 was used for the pollen germination test. The germination media used during this study consisted of 10% (w/v) agarose, 25% (w/v) sucrose, 0.52 mM KNO_3, 3.06mM $MnSO_4$, 1.66mM H_3BO_3, 0.42mM $MgS0_4{\cdot}7H_20$, and 1.0 µM GA_3 gibberellic acid. The medium was brought to 7.6 pH before adding sucrose and agarose, after which it was liquified by autoclaving and poured into petri plate (35 × 10 mm) covered and stored in a refrigerator until needed. Before use, the plates were removed from the refrigerator and brought to room temperature. Any condensation on the Petri dish lids was removed. The medium was overlaid with a 100 µL of hot 1.5% agarose, which was immediately smoothed with a glass rod and allowed to cool for 30 min. As the agarose overlay cooled, flowers were harvested randomly from the selected the plant the dehisced pollen from these flowers combined into a single sample per replicate, and the sample placed on the surface of the germination medium and allowed to germinate at 80% humidity.

Results and Discussion

The study conducted during *Kharif* 2020 aimed to modify the sex expression of the gynoecious parthenocarpic cucumber variety by inducing male flower. This modification was essential for the maintenance and multiplication of gynoecious lines, which naturally lack male flowers. By promoting the production of male flowers, the study sought to improve hybridization, enable seed set, and enhance the sustainability of off-season cultivation, thereby facilitating controlled breeding programme in cucumber production. The results of study are presented below. The spray of different chemicals concentration *viz.*, gibberellic acid (500, 1000 and 1500 ppm), silver nitrate (250, 500 and 750 ppm) and silver thiosulphate (250, 500 and 750 ppm) along with control of water spray were applied at 2-4 true leaf stage at seven days interval till 10-15 leaf stage to induce male flowers in gynoecious lines and their subsequent effect on sex expression was studied (Table 3). The perusal of results indicated that the treatment differences were found highly significant for days to appearance of male flower, node numbers on which first male flower appear and total male flowers per plant (Table 2). It revealed that the treatments preferred for the current research had extremely varied effects on these traits.

The observations recorded for days to appearance of male flower (Table 3) amongst the different treatments indicated that application of T_4 (250 ppm Silver nitrate) as well as T_6 (750 ppm Silver nitrate) both produced early male flowers in 40.33 days after sowing which was statistically *at par* with T_5 (500 ppm Silver nitrate) as well T_3 (1500 ppm

Gibberellic acid).The data on node number of first male flower indicated that T_8 (500 ppm Silver thiosulphate) produced male flower on lowest node (10.67). This treatment was found *at par* with T_4 (250 ppm Silver nitrate) 10.78 DAS, T_6 (750 ppm Silver nitrate) 11.22 DAS, T_5 (500 ppm Silver nitrate) 11.67 DAS and T_8 (500 ppm Silver thiosulphate) 11.78 DAS. Considering the total number of male flowers per plant across all the treatments (Table 2), the treatment T_8 (Silver thiosulphate $Ag_2S_2O_3$ 500 ppm) with spray of 10 ml per plant at two to four true leaf stage and subsequent application at seven-day interval was found significantly superior to induce the highest number of male flower production (43.44 number of male flowers per plant) without injuring the plant and no male flower was found under control (Water spray). The application of T_8 (500 ppm Silver thiosulphate) produced first male flower on 10.67 node and required 43.56 days to appearance of male flower. In the current experiment, it was found that it would be impossible to maintain the gynoecious line without spraying any chemicals. The male flowers on the gynoecious line did rise when gibberellic acid, silver nitrate, and silver thiosulphate were sprayed on the plants. The present findings are corroborated with the study of Chaudhary *et al.* (2001) [2], Susaj and Susaj (2010) [8] Karakaya and Padem (2011) [5], Golabadi *et al.* (2015) [3], Verma *et al.* (2018) [12] and Singh *et al.* (2022) [11].

Table 2: Analysis of variance for effect of treatments on sex modification related parameters in gynoecious variety Pusa Seedless 6

Source	Degree of freedom	Mean sum of square		
		Days to appearance of male flower	Node number of male flower	Total male Flowers per plant
Treatment	9	572.13**	56.33**	442.07**
Error	20	1.37	0.63	3.47

*, ** Significant at 5 and 1 percent levels, respectively

Table 3: Effect of various chemicals on sex modification parameters in gynoecious cucumber var. Pusa Seedless 6

Sr. No.	Treatments	Details	Days to appearance of male flower	Node numbers of first male flower	Total male flowers per plant
1	T_1	Gibberellic acid (500 ppm)	45.67	15.11	22.33
2	T_2	Gibberellic acid (1000 ppm)	43.89	13.89	26.44
3	T_3	Gibberellic acid (1500 ppm)	42.11	15.44	36.11
4	T_4	Silver nitrate (250 ppm)	40.33	10.78	39.00
5	T_5	Silver nitrate (500 ppm)	41.67	11.67	28.56
6	T_6	Silver nitrate (750 ppm)	40.33	11.22	27.67
7	T_7	Silver thiosulphate (250 ppm)	45.78	12.67	32.67
8	T_8	Silver thiosulphate (500 ppm)	43.56	10.67	43.44
9	T_9	Silver thiosulphate (750 ppm)	45.33	11.78	36.78
10	T_{10}	Water spray (Control)	0.00	0.00	0.00
S. Em. ±			0.68	0.46	1.08
CD at 5%			1.99	1.36	3.17
CV%			3.01	7.03	6.36

*, ** Significant at 5 and 1 percent levels, respectively

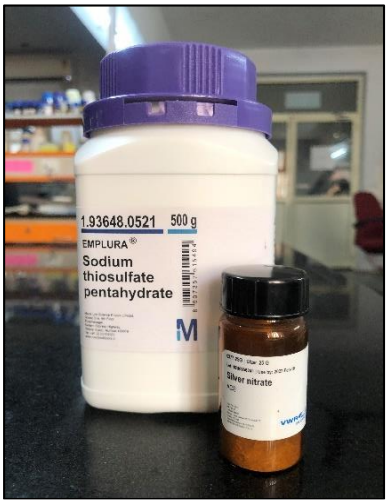

Fig 1: Silver Thiosulfate

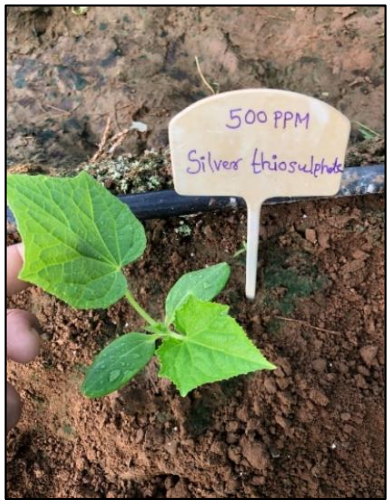

Fig 2: Two true leaf stage

Fig 3: Induce Male Flower

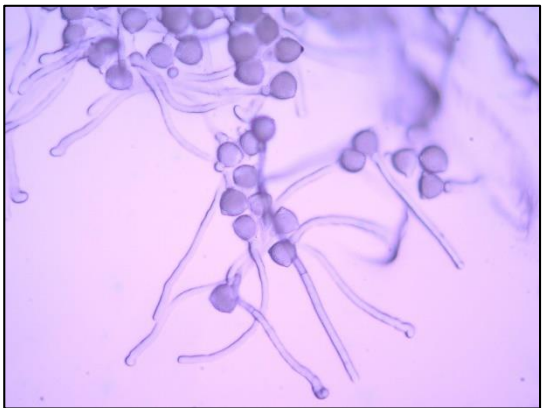

Fig 4: *In vitro* germinated pollen of Pusa Seedless 6

Conclusion

The study successfully demonstrated the modification of sex expression in a gynoecious parthenocarpic cucumber variety by inducing male flowers through the application of gibberellic acid, silver nitrate, and silver thiosulphate. Among the treatments, 500 ppm silver thiosulphate (T_8) proved most effective, producing the highest number of male flowers per plant (43.44) without harming the plant. Early male flower development was achieved with silver nitrate (250–750 ppm), and the lowest node for male flower production was recorded with silver thiosulphate. These findings are essential for the maintenance, hybridization, and controlled breeding of gynoecious cucumber lines, enabling sustainable off-season cultivation.

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