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Maintenance of Gynoecious Lines of Cucumber through Modification of Sex Expression using Gibberellic Acid, Silver Nitrate and Silver Thiosulphate in Cucumber (*Cucumis sativus* L.)

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ABSTRACT

The present experiment was carried out during Kharif (2016) at the Experimental Research Farm, Department of Vegetable Science, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) to gather information on use of chemicals (Gibberellic acid, Silver nitrate and Silver thiosulphate) in modification of sex expression in gynoecious varieties (GYNO-1 and GYNO-2) of cucumber. The experiment was laid out in a RCBD with three replications. Use of chemical in modification of sex expression was assessed through factorial analysis in randomized block design. The treatments consist of three chemicals viz., Gibberellic acid (500, 1000 and 1500 ppm), silver nitrate (250, 500 and 750ppm) and silver thiosulphate (250, 500 and 750ppm) along with control (distilled water) were sprayed at 2-3 true leaf stage at five days interval till 10-15 leaf stage. Amongst the various treatments, chemical silver thiosulphate ($Ag_2S_2O_3$) induced maximum number of mean male flowers followed by silver nitrate ($AgNO_3$) and gibberellic acid (GA_3) whereas no male flower has been recorded in control treatment (Distilled water) in gynoecious cucumber cultivars. Silver thiosulphate in higher concentrations was found less deleterious in comparison to silver nitrate.

Introduction

Cucumber (*Cucumis sativus* L; 2n = 2x = 14), is one of the most economically important members of the family cucurbitaceae. The family cucurbitaceae, consists of 2 subfamilies, cucurbitoideae and zanonioideae, is moderately large, consisting of about 130 genera and 900 species. The latter sub-family is further partitioned into eight tribes of which the Melothrieae includes the genus *Cucumis.* The different species of cucurbitaceae family are distributed primarily in the warmer areas of the world, especially in the tropics and subtropics. They are less frequent in temperate regions because of their frost sensitivity (Xixiang *et al.*, 2015).

Cucumber has three types of flowering habit "gynoecious" which produces only female flowers, "predominantly gynoecious" which bears mainly female flowers along with some male flowers and "monoecious" which produces both male and female flowers somewhat in equal proportions (Mehdi *et al.,* 2012). Gynoecious cucumber is more appropriate for cultivation under protected condition as it produces fruits without fertilization and gives higher yield. Furthermore, these types are affected greatly by environmental factors as well as hormonal levels in the plant system. Growth regulators have tremendous effects on sex expression and flowering in various cucurbits leading to either suppression of male flowers or an increase in the number of female flowers without imposing any deleterious effects on the environment and human health. Exogenous application of plant growth regulators can alter the sex ratio and sequence if applied at the 2-4 leaf stage, which is the critical stage at which the suppression or promotion of either sex is possible (Kumar *et al.,* 2011).

Staminate flower induction is necessary for production of F_1 hybrid in gynoecious cucumber. Gynoecious sex expression has been responsible for exploitation of hybrid vigor in cucumber which has attained a high degree of perfection (Golabadi *et al.,* 2015). The improvement of any crop plant which is ultimate objective of the plant breeder worldwide is not a simple task.

The basic idea behind this study was to develop an appropriate technique for maintaining the gynoecious line under local conditions which is non-existent till present, so that gynoecious line could be used successfully as a parent in hybridization programme of cucumber. Such study aims to develop the basis for any future hybridization programme for improvement and standardization of the local cultivars. So the present study was formulated to evaluate the effect of different plant growth regulators to identify the best treatment (longer male flowering period and high numbers of male flowering) for induction of staminate flower in gynoecious lines.

Materials and Methods

The experimental material for the present investigation was comprised of two cultivars of cucumber viz., two gynoecious lines (GYNO-1 and GYNO-2) maintained at Department of Vegetable Science, UHF, Nauni Solan (H.P.) India. The experiment was carried in Randomized Complete Block Design (RCBD) with 3 replications during Kharif 2016. Row to row and plant to plant spacing of 100 cm × 50 cm was kept in a plot having size 3.0 m × 3.0 m accommodated 18 plants per plot.

The standard cultural practices for raising a healthy crop of cucumber in the mid hills were adopted during the growing period (Anonymous, 2014). Data were recorded on 10 randomly selected competitive plants from each plot/treatment and the average was worked out to record the mean value in each replication. Three chemicals were sprayed at 2-4 true leaf stage at weekly interval for three weeks. Gibberellic acid (GA_3) was sprayed in concentration of 500, 1000 and 1500ppm whereas; silver nitrate ($AgNO_3$) and silver thiosulphate ($Ag_2S_2O_3$) were sprayed in concentration of 250, 500 and 750ppm, respectively. The number of male flowers appeared after sprays were counted to determine the shift in sex expression of gynoecious lines.

Preparation of gibberellic acid solution

The solution of 500ppm of gibberellic acid was prepared by dissolving 250 mg of gibberellic acid salt in distilled water and final volume was made half litre.

Similarly, the solutions of 1000ppm and 1500ppm were prepared by dissolving 500 and 750 mg of gibberellic acid salt, respectively in distilled water and final volume was made half litre in each case.

Preparation of silver nitrate solution

The solution of 250ppm of silver nitrate was prepared by dissolving 125mg of silver nitrate salt in distilled water and final volume was made half litre. Similarly, solutions of 500ppm and 750ppm were prepared by dissolving 250 and 375 mg of silver nitrate salt, respectively in distilled water and final volume was made half litre in each case.

Preparation of silver thiosulphate solution

The solution of silver thiosulphate was prepared as per the method given by Reid *et al.,* (1980). The stock solution of each of 4mM silver nitrate and sodium thiosulphate ($Na_2S_2O_3.5H_2O$) was prepared by dissolving 680mg silver nitrate and 1000mg of sodium thiosulphate separately in one litre of distilled water. For safer use, kept each bottles 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 0.5mM (500ppm) solution of silver thiosulphate from 4mM stock solution of silver nitrate and sodium thiosulphate, 125ml of each stock solution was mixed and the final volume was brought to one litre. Similarly, using the normality equation, silver thiosulphate in concentration of 250ppm and 1000ppm were prepared accordingly.

Time and method of spray

The different chemicals were sprayed during evening hours after the sunset. Single spray of 5ml solution of each chemicals and distilled water was given with an automizer on each plant. Initially, the plants were raised in the polyhouse and 10 plants were included in each treatment for spray with gibberellic acid, silver nitrate, silver thiosulphate and distilled water at 2-4 true leaf stage.

Statistical analysis

The response of different chemicals for the induction of male flowers in gynoecious line were analyzed by two factor analysis in randomized block design (RBD) by using the model as suggested by Panse and Sukhatme (2000). The statistical analysis was carried out for each observed character under the study using MS-Excel and OPSTAT 16.0 software as per the designs of experiments.

Results and Discussion

Sex expression is an important characteristic which determines yield potential of different cucumber varieties. Use of plant growth regulators, which are involved in the maintenance of gynoecious lines leading to modification in the sex form. The spray of different chemicals concentration viz., gibberellic acid (@ 500, 1000 and 1500ppm), silver nitrate (@ 250, 500 and 750ppm) and silver thiosulphate (@ 250, 500 and 750ppm) (Fig. 1 a, b and c) were applied at 2-3 true leaf stage (Fig. 1d) at five days interval till 10-15 leaf stage to induce male flowers in gynoecious lines and their subsequent effect on sex expression was studied (Table 1).

The observations recorded on mean number of male flowers induce through varied concentrations of gibberellic acid showed significant variation (Table 2). The mean comparisons showed that gibberellic acid had significant effect on the number of staminate flower induction whereas no male flower has been noticed in control treatment (Distilled water). Mean number of male flowers induced through gibberellic acid at 1500ppm (42.98) was more and found significantly higher than other two concentrations i.e., at 500ppm

(21.47) and 1000ppm (30.23). Among the cultivars, GYNO-2 produced significantly more number of male flowers (24.67) than GYNO-1 (22.68). Interactions (Concentrations × Varieties) had significant effect on induction of male flower through gibberellic acid. It ranged from 0.00 – 45.10.

Maximum number of male flower induced through GA_3 was recorded in GYNO-1 at 1500 ppm (40.87) and no male flower was found in controlled conditions (Distilled water). Similarly in GYNO-2, mean number of male flower induced through GA_3 at 1500 ppm (45.10) was more and no male flower observed in control (Distilled water) conditions.

These results are in agreement with the reports of Chaudhary *et al.,* (2001), Raymond (2004), Aisha and Chaudhary (2006), Gupta and Chakrabarty (2013), Golabadi *et al.,* (2015), Meena (2015) and Prajapati *et al.,* (2015). These workers had also reported that GA_3 at higher concentrations (1500 ppm) when sprayed at 2-leaf stage induced maximum number of male flowers.

Data pertaining to effect of varied concentrations of silver nitrate on gynoecious cultivars have been presented in table 3. Higher concentrations of silver nitrate adversely affected the overall growth of plants and mean comparisons showed that silver nitrate had significant effect on the number of staminate flower induction whereas no male flower has been noticed in control treatment (Distilled water).

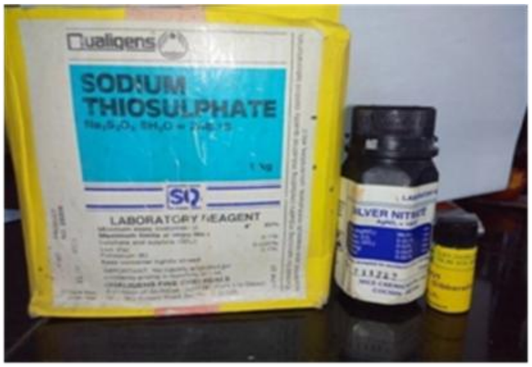
Among the three concentrations of silver nitrate, 250ppm induced more number of male flowers (48.57) and minimum was at 750ppm (31.13). Maximum number of male flower (30.28) was recorded in cultivar GYNO-2 and minimum (28.49) in cultivar GYNO-1. Interactions (Concentrations × Varieties) had non-significant effect on induction of male flower through silver nitrate (Table 3).

The present findings are corroborated with the study of Hirayama and Alonso (2000), Chaudhary *et al.,* (2001), Law *et al.,* (2002), Stankovic and Prodanovic (2002), Hallidri (2004), Karakaya and Padem (2011), Golabadi *et al.,* (2015), Meena (2015) and Prajapati *et al.,* (2015). They also reported that silver nitrate at higher concentrations (500 ppm and above) was phytotoxic and produced burning effect in leaves and exhibited highly retarded growth of plants.

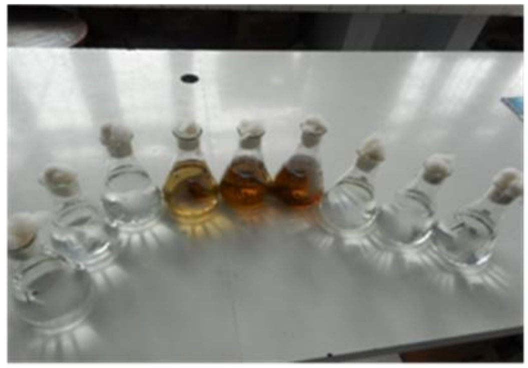
The observations recorded on effect of varied concentrations of silver thiosulphate on gynoecious cultivars of cucumber have been presented in table 4. It is evident from the table that silver thiosulphate spray increased the number of staminate flower whereas no male flower has been recorded in control treatment (Distilled water).

Mean number of male flowers induced through silver thiosulphate at 500ppm (56.00) were more (Fig. 1e) and found significantly higher than other two concentrations of silver thiosulphate applied at 250ppm (39.17) and 750 ppm (44.77).

Among cultivars, GYNO-2 produced significantly more number of male flowers (36.18) than GYNO-1 (33.79). Interactions (Concentrations × Varieties) had significant effect on the induction of male flower through silver thiosulphate. It ranged from 0.00 – 57.60. Maximum number of male flowers induced through $Ag_2S_2O_3$ was recorded in GYNO-1 at 500 ppm (54.40) and no male flower was found in controlled conditions (Distilled water). Similarly in GYNO-2, mean number of male flower induced through $Ag_2S_2O_3$ at 500 ppm (57.60) was more and no male flower observed in control (Distilled water) conditions.



a. Chemical (s) used for spray solution


b. Prepared chemical (s) solution


c. Storage of chemical (s) solution in dark bottles


d. Stage (2 true leaf stage) at which chemical solution was sprayed


e. Induction of male flowers in gynoecious lines after spray with silver thiosulphate at 500ppm

Fig 1 : Preparation and application of chemical solutions for induction of male flowers

Table.1 Analysis of variance for effect of gibberellic acid, silver nitrate and silver thiosulphate on mean number of male flower in gynoecious lines of cucumber

Source / Character	df	Mean sum of squares		
		Gibberellic acid	Silver nitrate	Silver thiosulphate
Replication	2	0.42	4.15	5.40
Factor (Concentrations)	3	1354.80*	1895.25*	2681.27*
Factor (Varieties)	1	24.81*	21.09*	37.78*
Int (Concentrations × Varieties)	3	6.54*	1.64	2.78*
Error	14	2.01	2.66	2.60
Total	23			

*Significance at 5% level of significance

Table.2 Effect of gibberellic acid (GA_3) on mean number of male flower in gynoecious lines of cucumber

Concentrations	Varieties		Mean (Concentrations)
	GYNO-1	GYNO-2	
Distilled Water	0.00	0.00	0.00
500 ppm	21.30	21.63	21.47
1000 ppm	28.53	31.93	30.23
1500 ppm	40.87	45.10	42.98
Mean (Varieties)	22.68	24.67	
$CD_{0.05}$			
Concentrations	0.66		
Varieties	0.47		
Concentrations × Varieties	0.93		

Table.3 Effect of silver nitrate ($AgNO_3$) on mean number of male flower in gynoecious lines of cucumber

Concentrations	Varieties		Mean (Concentrations)
	GYNO-1	GYNO-2	
Distilled Water	0.00	0.00	0.00
250 ppm	47.50	49.63	48.57
500 ppm	36.57	39.13	37.85
750 ppm	29.90	32.37	31.13
Mean (Varieties)	28.49	30.28	
$CD_{0.05}$			
Concentrations:	1.00		
Varieties:	0.71		
Concentrations × Varieties:	NS		

Table.4 Effect of silver thiosulphate ($Ag_2S_2O_3$) on mean number of male flower in gynoecious lines of cucumber

Concentrations	Varieties		Mean (Concentrations)
	GYNO-1	GYNO-2	
Distilled Water	0.00	0.00	0.00
250 ppm	37.43	40.90	39.17
500 ppm	54.40	57.60	56.00
750 ppm	43.33	46.20	44.77
Mean (Varieties)	33.79	36.18	
$CD_{0.05}$			
Concentrations:	1.02		
Varieties:	0.72		
Concentrations × Varieties:	1.44		

Table.5 Analysis of variance for comparison among gibberellic acid, silver nitrate and silver thiosulphate for induction of staminate flower in gynoecious varieties

Chemicals	Mean	Comparison	t_{cal}	t_{tab}
Silver nitrate (C1)	29.55	C1 Vs C2	-23.19	2.01
Silver thiosulphate (C2)	35.09	C1 Vs C3	28.70	2.01
Gibberellic acid (C3)	23.79	C2 Vs C3	55.78	2.01

These results are in line with the views of Chaudhary *et al.,* (2001), Hatwal *et al.,* (2015) Golabadi *et al.,* (2015) and Prajapati *et al.,* (2015). They also reported that silver thiosulphate in higher concentrations (750 ppm) was less deleterious in comparison to silver nitrate.

Amongst the various chemicals, silver thiosulphate (35.09) induced maximum number of mean male flowers followed by silver nitrate (29.55) and gibberellic acid (23.79) in gynoecious cucumber cultivars under study (Table 5).

In comparison to control (Distilled water), where plants had normal growth, the treated/sprayed plants showed slow or retarded growth. Silver nitrate when sprayed at concentrations of 500ppm and 750ppm was phytotoxic and produced burning effect in leaves and plants had retarded growth. Silver thiosulphate in higher concentrations was found less deleterious in comparison to silver nitrate. However, brittleness of stem and leaves was also apparent in silver thiosulphate and gibberellic acid. The silver thiosulphate is widely being used by seed producers for the maintenance of gynoecious cucumber lines over a longer period and it is less phytotoxic compared to silver nitrate.

A comprehensive work considered by Shifriss (1961) has also shown that only some Japanese and Korean races such as Shoigon, Kurume, Seoul Mady, Black Pearl and Chung Yup are source of gynoecism. Further, they reported that sex expression in cucumber is genic and controlled by polygenes. Moreover, it is also highly influenced by the environment. More and Munger (1987) have also further established that stability of sex expression in cucumber to a large extent influenced by temperature and photoperiod.

Spray of gibberellic acid, silver nitrate and silver thiosulphate did increase the number of male flowers on this gynoecious line. Silver nitrate and silver thiosulphate were found to have similar and positive effects on the male expression of cucumber. Ethylene, as one of the growth regulators in plants, has been found to induce feminization. Most of the effects of ethylene can be antagonized by specific ethylene inhibitors. Silver ions (Ag^+) applied as silver nitrate or as silver thiosulphate replace copper ions (Cu^+) which are part of ethylene receptor preventing the receptor from responding to ethylene. Sex expression being a polygenic character as described by Shifriss (1961) suggests that crossing of gynoecious and monoecious line such as K-75 and UHF-CUC-101 with GYNO-1 and GYNO-2 would lead to the improvement of female ratio in the subsequent generations.

From the present studies it is inferred that maintenance of gynoecious line without spraying any chemical under mid hill condition would not be possible but for using them as a male parent, it may be necessary to be spray them with different plant growth regulators which promote induction of staminate flower. Manipulation of sex expression of gynoecious lines would help the breeders and seed producers in development of varieties/hybrids which will combine the fruit fly resistance of land races/local cultivars with gynoecism of exotic female lines. Vishnu Swarup (1991) and Tatligolu (1993) have also reported that silver nitrate to be more phytotoxic as compared to silver thiosulphate and gibberellic acid and thus advocating the use of latter in inducing staminate flowers in all the female lines of cucumber for their maintenance as well as for crossing gynoecious × gynoecious lines to produce all F_1 female hybrids. These all female F_1 hybrids generally grown in the polyhouse to set fruits without pollination *i.e*., parthenocarpically. Hence use of chemicals for induction of male flowers would be required. The technology standardized in the present studies will help the breeder and seed producers in developing cultivars/hybrids of cucumber suited for open cultivation, which were adapted to mid hill environment for production during rainy season.

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