Vol.65: e22200792, 2022
https://doi.org/10.1590/1678-4324-2022200792
ISSN 1678-4324 Online Edition

Article- Biological and Applied Sciences

Standardized Protocol for *In Situ* and *In Vitro* Maintenance of Newly Developed Parthenocarpic Gynoecious Cucumber Inbred

Rajinder Kumar Dhall[1*]
https://orcid.org/0000-0003-3055-9780

Shreedhari Narasimha Hegde[1]
https://orcid.org/0000-0001-7766-4188

Pawan Kumar Malhotra[2]
https://orcid.org/0000-0002-3318-2715

[1]Punjab Agricultural University, College of Horticulture and Forestry, Department of Vegetable Science, Ludhiana, Punjab, India; [2]Punjab Agricultural University, College of Agriculture, School of Agricultural Biotechnology, Ludhiana, Punjab, India

Editor-in-Chief: Alexandre Rasi Aoki
Associate Editor: Adriel Ferreira da Fonseca

Received: 17-Dec-2020; Accepted: 14-Oct-2021.

[*]Correspondence: rajinderkumar@pau.edu; Tel: +91-8283840078 (R.K.D.).

HIGHLIGHTS

- New parthenocarpic gynoecious cucumber line 'PBRK-11' was developed and characterized.
- Application of silver thiosulphate @ 250 ppm induced maximum number of male flowers per plant, minimum plant mortality percentage and induced male flowers for longer duration.
- *In vitro* protocol was standardized to maintain the parthenocarpic gynoecious inbred "PBRK-11".

Abstract: New parthenocarpic gynoecious cucumber line 'PBRK-11' was developed, morphologically characterized and observed that its fruits were dark green, cylindrical, seedless, bitter free, and long (19-22 cm). Besides, different chemicals {GA_3, $AgNO_3$, and $[Ag(S_2O_3)_2^{-3}]$} having different concentrations were used for altering the sex expression of parthenocarpic gynoecious line to induce male flowers for seed production. Analysis of variance showed that chemical applications significantly affect the male flower induction and plant mortality percentage of parthenocarpic gynoecious cucumber inbred. Application of silver thiosulphate @ 250 ppm induced maximum number of male flowers per plant, minimum plant mortality percentage and induced male flowers for longer duration in parthenocarpic gynoecious plants when sprayed at 3-4 leaf stage at weekly interval for three weeks. Besides, *in vitro* protocol was standardized to maintain it. Shoot tip explants cultured on half MS media showed higher regeneration rate of 53% with highest shoot initiation response in 32 days followed by M5 [MS + BAP (1.0 mgL^{-1}) + Kinetin (1.0 mgL^{-1})] media with 48% regeneration. Root induction rate (80%) was high in MS media supplemented with IAA (1.0 mgL^{-1}). The parthenocarpic gynoecious expression was found to be stable in tissue culture regenerated progenies.

Keywords: gynoecious; parthenocarpic; sex modification; micropropagation; *Cucumis sativus* L.

INTRODUCTION

Cucumber (*Cucumis sativus* L.), the fourth most cultivated vegetable grown throughout tropics and subtropics of the world [1,2]. Cucumber has a diverse array of unisexual or bisexual flowering sex types [3]. Though monoecious is the predominant sex form in cucumber, but gynoecious sex form has been exploited for F_1 hybrids production. Gynoecious line as one parent in hybrid breeding has positive impact on yield and earliness [4] but, if gynoecious trait is associated with parthenocarpy, then yield is even higher than the gynoecious hybrids. Parthenocarpy is one such trait in cucumber that is highly preferred by consumers and has a great demand in market. The yield of parthenocarpic gynoecious cucumber varieties are often higher than the monoecious varieties and gynoecious hybrids as there are all female flowers and these female flowers don't need pollination for fruit setting. Moreover, the energy required to produce seeds in conventional seeded cultivars is not needed in parthenocarpic hybrids as these hybrids are seedless and the conserved energy will be utilized to produce more fruits in parthenocarpic gynoecious varieties. However, cultivation of cucumber under protected conditions in India is restricted due to non-availability of suitable parthenocarpic varieties/hybrids from public sector and high cost of the hybrid seeds marketed by the private seed companies [5]. Besides, parthenocarpic gynoecious varieties/hybrids available in the country usually become unstable as gynoecism breaks down at high temperature conditions of protected structures [6]. The Punjab Agricultural University is the pioneer institution in India for developing and commercially exploiting parthenocarpy and gynoecy together in cucumber [7]. Using parthenocarpic gynoecious line PBRK-4, one variety "Punjab Kheera-1" has been developed and released by the institute in the recent past for cultivation only in poly-net house conditions [8] and for commercialization of this variety recently two MOA's has been signed with private seed companies. But, there is also need to develop parthenocarpic gynoecious variety which can be cultivated under open field and low tunnel conditions. In this direction, a new parthenocarpic gynoecious cucumber line "PBRK-11" has been identified from the segregating population of advance breeding line.

Sex expression is an important factor that has a positive effect on yield and that constitutes a major component of cucumber improvement programs. The sex appearance of cucumber is closely connected with its genetics as well as its chemical and environmental conditions [9]. Sex type in cucumber is under the genetic control of three major genes (M, A, and F) [10,11]. Moreover, sex expression is also highly influenced by the environment. The stability of sex expression in cucumber is influenced by temperature and photoperiod to a large extent [12]. In parthenocarpic gynoecious cucumbers, male flower induction is necessary for production of F_1 hybrid seeds and for induction of male flowers (as a pollen source), plant requires an application of growth regulator or other chemical [13,9]. Some researchers have reported the effects of plant growth regulators on the modification of sex expression in cucumber flowers [14,15]. Maintenance of the gynoecious lines has been possible through the exogenous application of gibberellic acid [16], silver nitrate [17], and silver thiosulphate [18]. Among the plant growth hormones, exogenous gibberellic acid (GA_3) had the greatest effects on sex expression in cucumber by increasing the number of male flowers or delaying female flower production as GA_3 inhibit ethylene production [19]. The application of 400 ppm GA_3 led not only to precocious flowering, but also to increased number of pistillate and staminate flowers in cucumber and bitter gourd [20]. In addition, chemicals such as silver nitrate ($AgNO_3$) and silver thiosulphate [$Ag(S_2O_3)_2^{-3}$] appear to be powerful chemical inducers of male flowering in gynoecious cucumbers [9]. The application of $AgNO_3$ induces more male flowers than the GA_3 on two gynoecious and two predominantly female cucumber lines [21]. Although, sex modification by use of chemicals in gynoecy cucumber have been reported but there is need to standardize the dose of different chemicals for parthenocarpic gynoecious cucumber line to induce male flowers and to produce seeds. The present study aims to evaluate the effects of different kinds of chemical compounds at early plant growth stage to identify the best treatment for maximum male expression with minimum plant mortality in parthenocarpic gynoecious cucumber inbred. The best selection could be important with respect to longer male flowering period, high numbers of male flowering, less plant mortality and cost effectiveness.

In segregating breeding population, sex expression in cucumber plants can be confirmed only after appearance of at least 20 nodes and at that stage, if the plant is gynoecious and parthenocarpic it is very difficult to induce male flowers and produce seed of that plant because for male flower induction in gynoecious parthenocarpic plant, plant has to be sprayed at 2-4 leaf stage. Therefore, micro propagation can be used as alternate method for maintenance and multiplication of parthenocarpic gynoecious lines which were identified at later stage of plant growth in the segregating breeding population [22]. A good micropropagation procedure could help the breeders to maintain the seeds of identified parthenocarpic gynoecious plants which are even identified at later stage of plant growth. In cucurbitaceous family, regeneration techniques by *in vitro* methods is demonstrated and the regeneration of plants has been reported from nodal cuttings, excised cotyledons,

leaf explants and anther culture [23,24,25]. Most of the reports are on cotyledonary cultures in cucurbits, which describes indirect plant regeneration from the cotyledons developed from seed explants [26] and somatic embryogenesis from leaf derived calli [27]. Different media compositions have been reported for various set of variable conditions. Hence, the standardization of *in vitro* regeneration protocol for gynoecious parthenocarpic cucumber will be helpful to the breeders to maintain the stable gynoecious parthenocarpic expression in plants. Therefore, the present study was taken up with the objective to establish the *in vitro* protocol and to confirm the stability of parthenocarpic gynoecious inbred lines in the field established progeny.

MATERIAL AND METHODS

The study was carried out at Vegetable Research Farm, Department of Vegetable Science and Plant Tissue Culture Laboratory, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana during 2017-2019. Morphological characterization of the newly identified parthenocarpic gynoecious line, PBRK-11, was done based on DUS test guidelines in PPVFRA [28].

Three chemicals were sprayed on parthenocarpic gynoecious inbred “PBRK-11” at 3-4 true leaf stage at weekly interval for three weeks. Gibberellic acid (GA_3) was sprayed in concentration of 400, 500, 600, 700, 800, 900 and 1000 ppm whereas; silver nitrate ($AgNO_3$) and silver thiosulphate [$Ag(S_2O_3)_2^{-3}$] were sprayed in concentration of 200, 250, 300, 350, 400, 450 and 500 ppm, respectively. The number of male flowers appeared after sprays were counted to determine the shift in sex expression of parthenocarpic gynoecious line. The mortality percentage of plants at seedling stage was also studied after application of each chemical. Inbreds of parthenocarpic gynoecious line were developed through sib mating of female flowers using pollen from the induced male flowers. Population generated from the sib mating between parthenocarpic gynoecious plants and male sibs were raised in pro-trays. The transplanting was done on raised beds in the poly-net house on 7th February, 2019. The experiment was laid out in a Randomized Block Design (RBD) with three replications. Each entry consisted of ten plants per replication. The standard package of practices recommended for the crop was followed to raise a healthy crop [7].

To maintain the parthenocarpic gynoecious inbred under lab conditions during offseason, standardization of *in vitro* protocol was done. Untreated (without GA_3, $AgNO_3$ and $Ag(S_2O_3)_2^{-3}$ sprays), vigorous, pest and disease free parthenocarpic gynoecious plants of “PBRK-11” grown in the polyhouse have been selected as stock plants and nodal cuttings were collected from the plants in morning hours. After removing immature leaf, the explants ranging from 2.5-3 cm in length were prepared and cleaned in running tap water. The cleaned explants were washed repetitively in double distilled water. To reduce the fungal contamination, the explants were treated with mild detergent (Teepol) and Bavistin solution (0.1%) for 15 minutes and then rinsed with sterile distilled water for 3-5 times under aseptic conditions. The explants were surface sterilized with 0.1% (w/v) $HgCl_2$ solution for 5 minutes. The sterilized explants were washed 4-5 times with autoclaved distilled water immediately to eliminate the traces of $HgCl_2$. The explants were cultured *in vitro* on half MS (Murashige and Skoog) and MS medium [29] supplemented with various concentration of BAP, Kinetin and IAA for multiple shoot induction and shoot elongation (Table 1) The elongated shoots were excised and transferred to root induction media (M9 or M10 Table 1). MS media fortified with IAA and IBA were used for root induction. In all the treatments, pH of the medium was adjusted to 5.8, solidified with 0.8% agar and autoclaved at 1.05 kg cm^{-2} for 20 minutes at 121ºC. The cultures were incubated at 25 ± 2ºC with air conditioners and 16/8h photoperiod. Data on regeneration percentage, days to shoot initiation, number of shoots per explants and percent rooting were recorded. DMRT (Duncan"s Multiple Range Test) at $p < 0.05$ used for comparing the means. Rooted plants were carefully taken out from the cultured bottles and washed with tap water to remove the media adhering to the roots. These were hardened by using moist cotton (with half MS solution). After three to four days these hardened plants were transferred to the pots having coco-peat and soil (1:1) to acclimatize to green house conditions.

Table 1. Composition of different media used during *in vitro* studies

Designated No.	MS Media Used	Growth Regulator in mgL^{-1}
M1	Half MS	free
M2	Half MS	0.5 BAP and 0.5 Kinetin
M3	Half MS	1.0 BAP and 1.0 Kinetin
M4	MS	0.5 BAP and 0.5 Kinetin
M5	MS	1.0 BAP and 1.0 Kinetin
M6	MS	1.0 BAP, 1.0 Kinetin,1.0 IAA
M7	MS	1.5 BAP and 1.5 Kinetin
M8	MS	2.0 BAP and 2.0 Kinetin
M9	MS	1.0 IAA
M10	MS	1.0 IBA

RESULTS

Characterization and evaluation of parthenocarpic gynoecious line

Parthenocarpic gynoecious cucumber inbred was morphologically characterized using minimal descriptors and DUS test guidelines (Table 2). Plants of parthenocarpic gynoecious cucumber indred “PBRK-11” were vigorous, bearing 1-2 fruits per node. Its fruits were dark green, cylindrical shaped, dark green, seedless, bitter free, large sized (200-260 g), long (19-22 cm) and require peeling (Figure 1). Intermediate skin lusture was exhibited by the inbred. The shape of fruit at peduncle end was found to be flat whereas it was round at blossom end. Fruit surface of parthenocarpic gynoecious inbred was characterized with numerous deep triangular tubercles, which were non-conspicuous in nature. First fruit picking is possible after 50-55 days after sowing. Its average yield per plant is 2.7- 3.1 kg.

Table 2. Qualitative and qualitative characters of sib mated parthenocarpic gynoecious inbred “PBRK-11”.

Qualitative characters		Quantitative characters	Range	Mean	CV
Plant growth habit	Indeterminate	Days to first female flower	24.4-27.6	25.5	8.7
Stem pubescence	Present	Node at which first female flower appears	2.0-3.0	2.5	6.4
Fruit shape in longitudinal section	Cylindrical	Days to first fruit harvest	50.8-55.4	51.4	8.3
Fruit shape at peduncle end	Flat	Days to last fruit harvest	120-125	122.0	9.8
Fruit shape at blossom end	Round	Number of harvests	6.0-8.2	7.0	6.5
Fruit colour	Dark green	Vine length (cm)	230-300	260	12.9
Fruit skin lusture	Intermediate	Fruit length (cm)	19.0-22.0	19.8	8.2
Fruit ribs	Absent	Fruit diameter (mm)	40.4-46.4	43.3	10.5
Pulp texture	Crispy	Fruit weight (g)	200-260	245	9.6
Fruit sutures	Absent	Number of fruits per plant	12.1-15.2	12.5	14.5
Fruit creasing	Absent	Yield per plant (kg)	2.7-3.1	2.8	12.8
Fruit vestiture	Hairy				
Fruit colour at ripening stage	Yellow				

Figure 1. New parthenocarpic gynoecious inbred “PBRK-11”

Effect of chemicals on male flower induction in parthenocarpic gynoecious lines

Sex expression is an important characteristic which determines yield potential of different cucumber varieties. The observations recorded on mean number of male flowers induce through varied concentrations of different chemicals showed significant variation (Table 3). Among different gibberellic acid (GA_3) concentrations, the maximum number of male flowers per plant was induced by application of GA_3 @ 1000ppm (48.2) and it was found to be significantly higher than other GA_3 concentrations. Besides, application of GA_3 @ 1000ppm also resulted in significantly higher plant mortality (8.8%) (Table 3).

Table 3. Effect of gibberellic acid, silver nitrate and silver thiosulphate on induction of male flowers and plant mortality percentage of parthenocarpic gynoecious plants.

Gibberellic acid			**Silver nitrate**			**Silver thiosulphate**		
Concentration	Number of male flowers/ plant	Plant mortality (%)	Concentration	Number of male flowers/plant	Plant mortality (%)	Concentration	Number of male flowers/ plant	Plant mortality (%)
400 ppm	25.6	1.2	200 ppm	35.8	2.0	200 ppm	70.8	0.0
500 ppm	32.5	1.8	250 ppm	46.9	4.1	250 ppm	99.5	0.0
600 ppm	36.9	3.1	300 ppm	52.4	6.5	300 ppm	75.2	1.6
700 ppm	38.4	5.6	350 ppm	42.8	7.6	350 ppm	68.0	2.8
800 ppm	39.2	7.2	400 ppm	36.4	12.5	400 ppm	55.6	3.9
900 ppm	45.6	8.1	450 ppm	38.5	14.0	450 ppm	45.9	7.2
1000 ppm	48.2	8.8	500 ppm	35.5	15.4	500 ppm	40.5	7.9
CD*	1.21	0.52		2.01	1.25		4.51	0.75
CV**	8.51	6.42		9.74	6.50		7.74	7.05

* Critical difference ; ** Coefficient of variation

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 and more plant mortality percentage. Among the seven concentrations of silver nitrate, 300 ppm induced more number of male flowers (52.4), whereas, minimum male flowers were produced with 500 ppm (35.5). However, silver nitrate @ 300 ppm also results plant mortality of 6.5% (Table 3). The highest plant mortality (15.4%) in 500 ppm may be due to toxicity of silver nitrate at higher concentration.

The observations recorded on effect of varied concentrations of silver thiosulphate on parthenocarpic gynoecious inbred of cucumber have shown that the silver thiosulphate spray increased the number of

staminate flower to maximum extent as compared to GA_3 and silver nitrate. Mean number of male flowers induced through silver thiosulphate at 250 ppm (99.5) were more and found significantly higher than other concentrations of silver thiosulphate (Table 3). Besides, there was no plant mortality by spraying silver thiosulphate @ 200 and 250 ppm but there was more male flower induction with application of 250 ppm and it is significantly higher than 200 ppm and rest of treatments of silver thiosulphate.

Amongst the various chemicals, silver thiosulphate @ 250 ppm induced maximum number of male flowers (99.5) followed by silver nitrate@ 300 ppm (52.4) and gibberellic acid @ 1000 ppm (48.2) in parthenocarpic gynoecious cucumber inbred under study (Table 3). Among all the chemicals used in the study, it was observed that maximum plant mortality (15.4%) was observed with the application of silver nitrate at higher concentration (500 ppm). No doubt, there is almost comparable plant mortality in both GA_3 and silver thiosulphate treatments but the number of male flowers induces is significantly less in GA_3 treatment in comparison to silver thiosulphate.

Micropropagation of parthenocarpic gynoecious line

Among the 8 treatments (Table 1) experimented with shoot culture, M1 (Basal Half MS) medium found to be effective in the shoot regeneration of nodal cuttings in gynoecious cucumber plant (Table 4). Shoot regeneration about 53% was found on basal half MS medium without any addition of growth regulator. Use of growth regulator like BAP, Kinetin and IAA were also responsible for the induction of shoots. M5 medium (MS medium supplemented with BAP 1mgL^{-1} and Kinetin 1mgL^{-1}) also found to produce higher shoot multiplication about 48%. Callus formation observed when more kinetin and BAP were added and decreased regeneration percentage from 18-14 observed in M7 and M8 media. M5 and M1 media were at par showing minimum days 30 and 32 respectively for shoot formation (Table 4). M3 and M8 media requires maximum number of days for the development of shoots. With the increased amount of growth hormones like BAP and Kinetin from 1mg L^{-1} in half MS medium and 2 mg L^{-1} in MS medium take maximum days i.e. 40 and 30 for shoot induction and development (Table 4). It was found that M1 and M5 media produces maximum shoots about 4 and 3 respectively indicating they are at par. With the increased concentration of BAP and kinetin up to 2 mg L^{-1} will decreases the shoot number, however at 1.5 mg L^{-1} BAP and Kinetin in MS medium i.e. M7 showed average of 2 shoots per explant.

Table 4. Effect of different media on percent shoot regeneration, days for shoot initiation and shoots per explant in cucumber.

Treatment (Media)	Regeneration %	Days for shoot initiation	Shoots per explant
M1	53^{a}	32^{de}	4^{a}
M2	28^{cd}	34^{cd}	1^{c}
M3	32^{b}	38^{ab}	1^{c}
M4	24^{de}	36^{b}	2^{b}
M5	48^{a}	30^{e}	3^{ab}
M6	29^{bc}	36^{b}	1^{c}
M7	18^{df}	35^{bc}	2^{b}
M8	14^{ef}	40^{a}	1^{c}

MS medium supplemented with IAA and IBA showed good rooting in gynoecious cucumber plants. Shoots were transferred to the different rooting media for the induction of roots. It was found that MS media having IAA (1mg L^{-1}) was found to be better compared with the MS media supplemented with IBA (1mg L^{-1}). Rooting percentage was found to be 80% in IAA media where as IBA media showed 60% rooting (Figure 2).

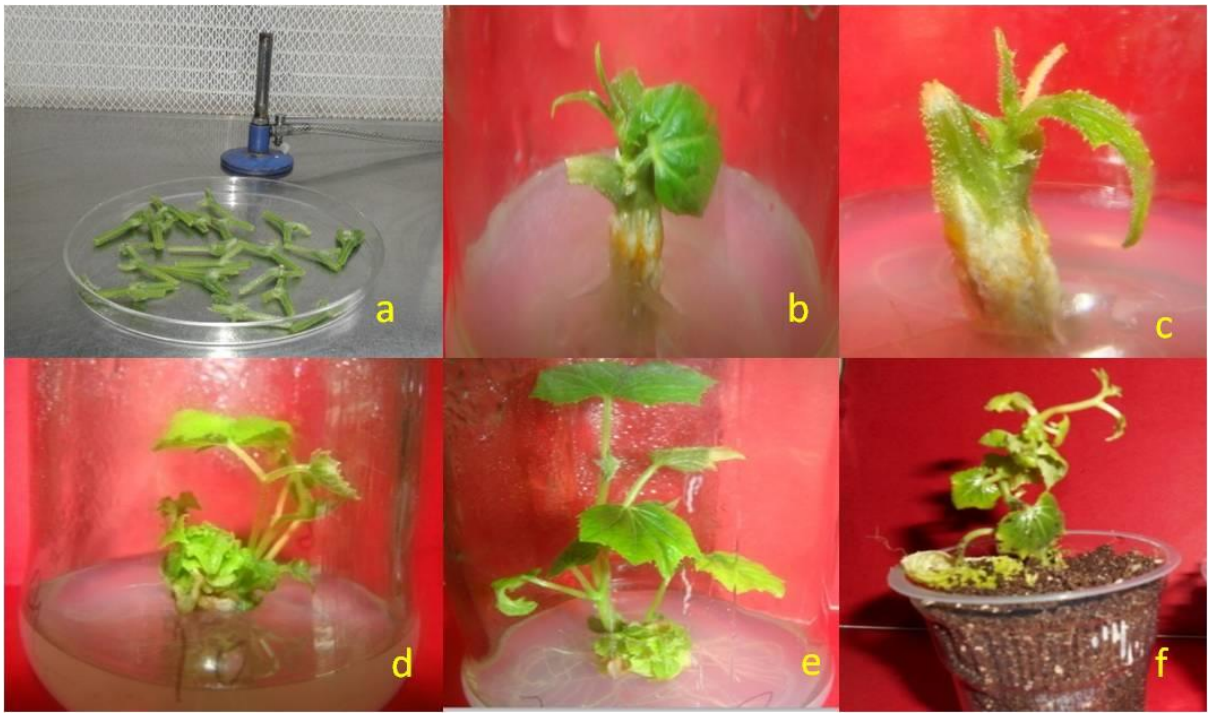

Figure 2. Micropropagation (a-f) of parthenocarpic gynoecious cucumber inbred "PBRK-11" (a) Explants (b) Shoot initiation on half MS media (c) Shoot initiation on MS media supplemented with BAP and kinetin (MS+1.0 mg L^{-1} BAP + 1.0 mg L^{-1} Kinetin) (d) Multiple shoot initiation (e) *In vitro* rooting (f) Planting out and hardening of tissue culture plants

DISCUSSION

Characterization and evaluation of parthenocarpic gynoecious line

The parthenocarpic gynoecious line of cucumber has fruits that needs peeling. Since all the inbreds developed through sib mating were gynoecious and parthenocarpic, it is understood that the parthenocarpic gynoecious line identified at PAU is stable and the gene is of homozygous nature. In India [30,31], stable gynoecious lines in bitter gourd also reported. The fruit size observed was higher than that of earlier reported parthenocarpic gynoecious lines from India. The range of biometric characters observed for the gynoecious inbred was also higher than the previous report (fruit weight 200-260 g, fruit length 19-22 cm). Hence the gynoecious inbred, PBRK-11, can be directly released as variety and it holds enormous potential for future breeding programme for improving fruit character and yield in cucumber.

Effect of chemicals on male flower induction in parthenocarpic gynoecious lines

All the chemical applications in the study had resulted in induction of male flowers but their number varies. Besides, there is variation in plant mortality percentage with application of different doses of chemicals. Another finding of the study was that the number of male flowers increased with increase in doses of GA_3 but application of $AgNO_3$, $[Ag(S_2O_3)_2^{-3}]$ resulted increase in number of male flowers at low doses but at higher doses there was decrease in number of male flowers. These results of GA_3 are in agreement with the reports of [32,20,33,34], as these workers reported that GA_3 at higher concentrations (1500 ppm) induced maximum number of male flowers when sprayed at 2-leaf stage. A new aspect of this finding is that, $AgNO_3$ and $[Ag(S_2O_3)_2^{-3}]$ application had more male flower induction effects than did GA_3. It may be due to effect of silver ions (Ag+) applied as silver nitrate ($AgNO_3$) or as silver thiosulfate $[Ag(S_2O_3)_2^{-3}]$ which replace copper ions (Cu+) which are part of the ethylene receptor preventing the receptor from responding to ethylene [36]. $AgNO_3$ produced more male flowers than GA_3 treatment in cucumber and summer squash, respectively [18,36], a finding that is similar to our results. Similar to these findings [21], different doses of $AgNO_3$ (50, 200, and 500 mg L^{-1}) led to greater effects on male flower production than those of different doses (100, 500, and 1500 mg L^{-1}) of GA_3. On the other hand, it is more expensive to use GA_3 than Ag ions, especially for maintenance of parthenocarpic gynoecious inbred. 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 and more plant mortality percentage. There is maximum plant mortality with higher dose application of $AgNO_3$ which may be due to phytotoxic effect of $AgNO_3$ at higher dose. The higher dose of $AgNO_3$ application also produced burning effect in leaves of surviving plants and exhibited

highly retarded growth of these plants. These types of effected plants take more time to recover and bear male flowers comparatively late. Similar results were reported [9,37-40,33-34]. Among all the treatments of different chemicals, the application of silver thiosulphate @ 250 ppm induced maximum number of male flowers per plant, minimum plant mortality percentage and induced male flowers for longer duration in parthenocarpic gynoecious plants when sprayed at 3-4 leaf stage at weekly interval for three weeks. The technology standardized in the present study will help the breeder and seed producers in maintaining parthenocarpic gynoecious inbred of cucumber.

Micropropagation of parthenocarpic gynoecious line

It was observed that external fortification of growth regulator along with endogenous plant regulator was responsible for *in vitro* morphogenesis. In this study, half MS medium showed good response for shoot initiation without any external supplement of growth regulator. Half MS media showed good response for shoot initiation [41]. MS medium supplemented with 0.1 mg L^{-1} BAP resulted in higher number of multiple shoots [42]. The growth and development of *in vitro* plants are controlled by using growth regulators including auxins, cytokinins, and auxin-cytokinin interactions [43]. Best response for shoot initiation from nodal cuttings was observed on the media containing 1.0 mg L^{-1} BAP with 1.0 mg L^{-1} Kinetin, which took 30 days for shoot initiation. Development of shoots in cucumber was observed by the use of growth regulator BAP and Kinetin [44]. Highest percentage of shoot initiation (62) with an average of eight shoots per explant was reported from shoot tip explants of cucumber when cultured on MS medium supplemented with BA alone [45]. The effects of BA and NAA on shoot proliferation in cucumber studied [46]. Upto 72% of rooting with MS media having IAA (1mg L^{-1}) reported [47]. The rooting can be induced by using IBA and BA [48]. Similar results were also reported in other cucurbits like bitter gourd and ridge gourd [49,50]. This study revealed that higher concentration of both BAP and kinetin had less effect on *in vitro* propagation thus provide a way for standardization of protocol for stabilizing gynoecious sex expression.

CONCLUSION

The new parthenocarpic gynoecious cucumber line ‘PBRK-11’ is amenable to *in-situ* and *in vitro* maintenance. The parthenocarpic gynoecious expression is stable and sex reversal can be achieved application of all the chemicals {GA_3, $AgNO_3$, $[Ag(S_2O_3)_2^{-3}]$} used in the study but application of silver thiosulphate @ 250 ppm induced maximum number of male flowers per plant, minimum plant mortality percentage and induced male flowers for longer duration in parthenocarpic gynoecious plants when sprayed at 3-4 leaf stage at weekly interval for three weeks. Although the nodal cuttings gave maximum shoot regeneration on half MS medium without supplemented with any growth regulator. But the addition of cytokinins (BAP and Kinetin) gave early shoot initiation response in parthenocarpic gynoecious inbred line. Addition of auxin (IAA) gave maximum root initiation response under *In vitro* conditions. An effective protocol for micropropagation of parthenocarpic gynoecious inbred was standardized which will help in maintaining the gynoecious parthenocarpic cucumber plant identified at the later stage of plant growth from the segregating population.

Funding: This research received no external funding
Acknowledgements: The research work was carried out with the support of "Punjab Agricultural University, Ludhiana".
Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES

1. Plader W, Burza W and Malepszy S, Cucumber, Transgenic Crops IV. Biotechnology in Agriculture and Forestry, 2007 Jan;59:181-99.
2. Innark P, Khanobdeeh CH, Samipaks S, Jantasurivarat CH. Evaluation of genetic diversity in cucumber (*Cucumis sativus* L.) germplasm using agro-economic traits and microsatellite markers. Sci. Hortic. 2013 Oct; 162: 278-84.
3. Nam YW, Lee JR, Song KH, Lee MK, Robbins MD, Chung SM, Staub JE, Zhang HB. Construction of two BAC libraries from cucumber (*Cucumis sativus* L.) and identification of clones linked to yield component quantitative trait loci. Theor and Appl Genet. 2005 Apr ;111(1): 150-61.
4. Rao GP, Behera TK, Munshi AD, Dev B. Estimation of genetic components of variation and heterosis studies in bitter gourd for horticultural traits. Indian J. Hort. 2017 Jun;74(2): 227-32.
5. Kumar S, Kumar R, Kumar D, Gautam N, Dogra RK, Mehta DK, et al. Parthenocarpic gynoecious parental lines of cucumber introduced from Netherlands for developing high-yielding, quality hybrids. J. Crop Improv. 2016 Apr; 30(3): 352-69.

6. Cantliffe DJ. Alteration of sex expression in cucumber due to changes in temperature, light intensity, and photoperiod. J. Am. Soc. Hortic. Sci. 1981;106:133-6.
7. Dhall RK, Singh D. Punjab Kheera-1: A cucumber variety for poly-net house cultivation. Progressive Farming 2018;54(9):17-8.
8. Dhall RK. Punjab Kheera-1: A new variety of parthenocarpic cucumber for poly net house cultivation. Veg. Sci. 2019; 46(1-2):135-8.
9. Karakaya D, Padem H. The effects of silver nitrate applications on the flower quantity of cucumbers (*Cucumis sativus* L.). Not. Bot. Hort. Agrobot. Cluj., 2011 May; 39(1): 139-43.
10. Trebitsh T, Staub JE, Neill SD. Identification of a 1-Aminocyclopropane-1-carboxylic acid synthase gene linked to the female (F) Locus that enhances female sex expression in cucumber. Am. Soc. Plant. Biol., 1997 Mar;113(3): 987-95.
11. Wang YH, Joobeur T, Dean RA, Staub JE. Cucurbits. In: Kole, C. (ed.). Genome mapping and molecular breeding in plants, 2007; (5): 315-29 pp.
12. More TA, Seshadri VS. Maintenance of gynoecious muskmelon with silver thiosulphate. Veg. Sci. 1987;14:138-42.
13. Wang YH, Behera TK, Kole CH. Genetics, Genomics and Breeding of Cucurbits. CRC Press 2011.
14. Rafeekher M, Nair SA, Sorte PN, Hatwar GP and Chandan PM, Effect of growth regulators on growth and yield of summer cucumber. J. Soils Crops 2002;12(1):108-10.
15. Bano HA, Khokhar KM. Sex expression and level of phytohormones in monoecious cucumbers as affected by plant growth regulators. Sarhad J. Agric. 2009; 25(2):173-8.
16. Peterson CE, Anhder LD, Induction of staminate flower in gynoecious cucumber with GA_3. Sci. 1960;131(3414):1673-4.
17. Beyer E. Silver ion: A potent anti ethylene agent in cucumber and tomato. Hort. Sci.1976; 11(3): 195-6.
18. Den Nijs APM, Visser DL. Induction of male flowering in gynoecious cucumbers (*Cucumis sativus* L.) by silver ions. Euphytica 1980 Jun;29(2): 273-80.
19. Perl-Treves R. Male to female conversion along the cucumber shoot: approaches to studying sex genes and floral development in *Cucumis sativus*. In: Ainsworth, C.C. (ed.), Sex determination in plants, Bios Scientific Publishers Ltd, Oxford, 1999:189-216.
20. Aisha S, Chaudhary NY. GA_3 improves flower yield in some cucurbits treated with lead and mercury. African J. Biotechnol. 2006;5(2):149-53.
21. Kalloo G, Franken S. Chemical induction of staminate flowers in four determinate gynoecious lines of pickling cucumber. Gartenbauwiessenschaft 1978;43(6): 280-2.
22. Mohiuddin AKM, Abdullah ZC, Chowdhury MKU, Napis S. Enhancement of adventitious shoot regeneration in *Cucumis sativus* L. using $AgNO_3$. J.Plant Biotechnol. 2005 Oct;15(1): 15-23.
23. Naseem A, Mohammad A. *In vitro* mass propagation of (*Cucumis sativus* L.) from nodal segments. Turk J. Bot 2005 Feb;29(3): 237-40.
24. Stipp LCL, Mendes BMJ, Piedade SMDS, Rodriguez APM. *In vitro* morphogenesis of *Cucumis melo var*. inodorus. Plant Cell Tiss. Org. Cult. 2001;65(1): 81-9.
25. Kumar HGA, Murthy HN, Paek KY. Embryogenesis and plant regeneration from anther cultures of *Cucumis sativus* L. Sci. Hortic. 2003 May;98(3): 213–22.
26. Ugandhar T, Venkateshwarrlu M, Gousia B, Srilatha T, Jaganmohan RK. *In vitro* plant regeneration of Cucumber (*Cucumis sativum* L.) from cotyledon and hypocotyl explants. Sci. Res. Rep. 2011 Nov;1(3): 164-9.
27. Usman M, Hussain Z, Fatima B. Somatic embryogenesis and shoot regeneration induced in cucumber leaves. Pak. J. Bot 2011;43(2): 1283-93.
28. PPVFR, Protection of Plant Varieties & Farmers' Right Authority, India. http://www.plantauthority.gov.in/pdf/GField%20pea.pdf, 2007:SG/11/2007
29. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum 1962 ;15(3):473-87.
30. Ram D, Kumar S, Banerjee MK, Kalloo G. Occurrence, identification and preliminary characterization of gynoecism in bitter gourd (*Momordica charantia* L.). Indian J. Agr. Sc.i 2002;72: 348-9.
31. Behera TK, Dey SS, Sirohi PS. DBGy-201 and DBGy-202: two gynoecious lines in bitter gourd (*Momordica charantia* L.) isolated from indigenous source. Indian J. Genet. 2006; 66(1): 61-2.
32. Chaudhary BN, Piluek K, Taychasinpitak T, Sagwansupyakorn C, Development and maintenance of gynoecious lines of cucumber (*Cucumis sativus* L.). Kasetsart J. Nat. Sci. 2001 Jul-Sept;35(3): 242-50.
33. Golabadi M, Golkar P, Eghtedari AR. Use of chemical and hormonal agents for changing sex expression of cucumber for breeding programs. Biharean Biologist 2018 Jun;12(1): 27-32.
34. Prajapati S, Jamkar T, Singh O P, Raypuriya N, Mandloi R, Jain PK. Plant growth regulators in vegetable production: An overview. Plant Arch. 2015;15: 619-26.
35. Abeles FB, Morgan PW, Saltveit JRME. Ethylene in plant biology. Academic Press, New York, 1992;302p.
36. Yongan CH, Bingkui Z, Enhui Z, Zunlian Z. Control of sex expression in summer squash (*Cucurbita pepo* L.). Cucurbit Genetics Cooperative Report 2002;25: 51-3.
37. Hirayama T, Alonso JM. Metal ions are involved in ethylene perception and signal transduction. Plant Cell Physiol. 2000;41(5): 548-55.
38. Law TF, Hardenack SL, Grant SR. Silver enhance stamen development in female white Campion (*Silene latifolia* [Caryophyllaceae]). Am. J. Bot. 2002;89(6): 1014-20.

39. Stankovic L, Prodanovic S. Silver nitrate effects on sex expression in cucumber. Acta Hortic. 2002;579: 203-206.
40. Hallidri M. Effect of silver nitrate on induction of staminate flowers in gynoecious cucumber line (*Cucumis sativus* L.). Acta Hortic.2004; 637: 149-54.
41. Ajay B, Pradeepkumar T, Varun RC. *In Vitro* regeneration of parthenocarpic cucumber (*Cucumis sativus* L.). Int. J. Curr. Microbiol. App. Sci. 2017Jul;6(7): 1711-20.
42. Sangeetha P, Venkatachalam P. Induction of multiple shoots from shoot tip explants of cucumber (*Cucumis sativus* L.). Plant Cell Biotech. Molec. Biol. 2011;12: 1-4.
43. Gaspar T, Kevers C, Penel C, Greppin H, Reid DM, Thorpe TA. Plant hormones and plant growth regulators in plant tissue culture. In Vitro Cell Dev. Biol. Plant 1996 Oct;32(4): 272-89.
44. Vasudevan A, Selvaraj N, Kumar PS, Ganapathi A. Multiple shoot induction from shoot tip explants of Cucumber (*Cucumis sativus* L.). Cucurbit Genet. Coop. Rep. 2001;24: 8-12.
45. Vasudevan A, Selvaraj N, Ganapathi A, Kasthurirengan S, Anbazhagan VR, Manickavasagam M, Choi CW. Leucine and spermidine enhance shoot differentiation in cucumber (*Cucumis sativus* L.). In Vitro Cell Dev. Biol. Plant 2008;44(4): 300-6.
46. Jafar M, Nuray S. *In vitro* clonal propagation of *Cucumis sativus* L. by shoot tip culture. J. Biol. Sci. 2007;7(4): 653-7.
47. Venkateshwaralu M, Direct multiple shoot proliferation of muskmelon (*Cucumis melo* L.) from shoot tip explants. Int. J. Pharma Biol. Plant 2012;48:125-8.
48. Selvaraj N, Vasudevan A, Manickavasagam M, Kasthurirengan S, Ganapathi A. High frequency shoot regeneration from cotyledon explants of cucumber via organogenesis. Sci. Hortic. 2007 Mar;112(1): 2-8.
49. Thiruvengadam M, Praveen N, Chung IM. In vitro regeneration from internodal explants of bitter melon (*Momordica charantia* L.) via indirect organogenesis. African J. Biotech. 2012;11(33): 8218-24.
50. Pradeep kumar T, Sujatha R, Krishnaprasad BT, Johnkutty I. New source of male sterility in ridge gourd (*Luffa acutangula* (L.) Roxb.) and its maintenance through in vitro culture. Cucurbit Genet. Coop. Rep. 2007;30: 60-3.

© 2022 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY NC) license (https://creativecommons.org/licenses/by-nc/4.0/).