Current Journal of Applied Science and Technology

39(4): 17-24, 2020; Article no.CJAST.55013
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

In-vitro Flowering and *in-vivo* Sex Expression of Micropropagated Parthenocarpic Gynoecious Cucumber

Ajay Bhardwaj[1*], T. Pradeepkumar[2] and C. Varun Roch[2]

[1]Department of Horticulture (Vegetable and Floriculture), Bihar Agricultural University, Sabour-813210, Bhagalpur, Bihar, India.
[2]Department of Vegetable Science, College of Horticulture, Kerala Agricultural University, Thrissur-680656, Kerala, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author AB performed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author TP managed the analyses of the study. Author VRC managed and helped in performing the experiment. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2020/v39i430526
Editor(s):
(1) Dr. Aydin Unay, University of Aydin Adnan Menderes, Turkey.
Reviewers:
(1) Washa B. Washa, University of Dar es Salaam, Tanzania.
(2) Qi Cheng, Chinese Academy of Agricultural Sciences, China.
(3) Paul Kweku Tandoh, Kwame Nkrumah University of Science and Technology, Ghana.
Complete Peer review History: http://www.sdiarticle4.com/review-history/55013

Original Research Article

Received 06 January 2020
Accepted 11 March 2020
Published 24 March 2020

ABSTRACT

A micropropagation protocol for parthenocarpic gynoecious cucumber reduces the burden of producing the seeds for each generation and their maintenance *in-vivo*. Thus an experiment was conducted in order to regenerate the plants *in-vitro* to check their performance after micropropagation. The micropropagation resulted in maximum shoot initiation (100%) from seedling excised cotyledonary explants with half strength MS medium supplemented with 0.5 mg/l IAA and 2 mg/l BAP along with half strength MS medium supplemented with 0.25 mg/l IAA for rooting and from stem nodal explants with Full MS + 1.5 mg/l IAA + 2 mg/l BAP media whereas half strength MS media without any hormones resulted in rooting and in both cases there were *in-vitro* flowers and change in their sex expression while grown in *in-vivo* conditions. On an average 61.11 and 48.15 percent survival was recorded from the plants regenerated through cotyledonary explants

**Corresponding author: E-mail: bhardwaj.ajay.phd@gmail.com;*

and stem nodal explants respectively. Out of five survived plants from regenerated parthenocarpic genotype CS 131 three showed monoecious sex expression and two exhibited gynoecious (parthenocarpic) sex expression. Mixed response of sex expression was evident in the regenerated parthenocarpic and gynoecious genotypes.

Keywords: Cucumber; parthenocarpy; gynoecy; micropropagation; in-vitro flowering; regeneration.

1. INTRODUCTION

Parthenocarpy along with gynoecious sex expression is an asset for protected cultivation of cucumber. Cultivation of parthenocarpic gynoecious hybrids is gaining attention of the growers as it is a reliable and profitable venture in India. But still, the growers are left with the option of choosing from the private sector hybrids which costs very high (Rs. 4 to 7 per seed) or from very limited public sector hybrids which are yet to be tested at various places. The development of hybrids exhibiting these traits along with various useful yield attributing characters is a tedious and very risky affair because if a generation is missed for inducing male flowers or failed under *in-vitro* regeneration for seed production will result in complete loss of genetic material. Parthenocarpy is influenced by environmental, physiological, and genetic factors. Environments with low temperature and short day length advance parthenocarpy [1]. Similarly, parthenocarpy is also dependent on certain hormones as evidenced by Kim et al. [2,3] and Boonkorkaew et al. [4] that endogenous IAA concentrations in parthenocarpic ovaries or on fruits were higher than in pollinated cucumbers. Other exogenous plant growth-regulating chemicals such as auxin and auxin transport inhibitors, gibberellin, cytokinin, and brassinosteroids also induce parthenocarpy [5,6,7,8]. Moreover, genetically modifying cucumber by introducing the DefH9-iaaM auxin-synthesizing gene can also result in parthenocarpic plants [9]. Direct organogenesis has already been reported for many cucurbits from various explants *viz*., cotyledons, hypocotyls, cotyledonary node, leaf explants and anther culture. Flower formation on *in-vitro* grown plants has been reported for many species using different explant sources to investigate the influence of medium, plant growth regulators and photoperiod on flowering [10]. *In-vitro* flowering in vegetables is important for selective hybridization with pollen from rare accessions, enabling synchronization of flowering, and studying the physiology of flowering [11,12]. *In-vitro* flowering has also been reported for cucumber [13,14]. A good micropropagation protocol for cucumber could be used for reducing the cost (approx. 30%) of hybrid seed production [15] and moreover, to cope up the risk of maintenance in parthenocarpic and gynoecious cucumber due to their innate seedlessness *in-vivo*. Hence, keeping all these lines in mind the present study was undertaken to get *in-vitro* shooting and rooting from stem nodal explants and to know the performance in terms of sex expression of regenerated plants in polyhouse conditions.

2. MATERIALS AND METHODS

The present study was conducted at Biotechnology laboratory, and polyhouse of Department of Olericulture, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur. For conducting the study three sex forms/types of cucumber viz., gynoecious, parthenocarpic and monoecious genotypes respectively were taken. Details of which are given in Table 1. *In-vitro* seed germination, *in-vitro* regeneration (shooting, rooting and callusing) using cotyledonary leaf explants was already observed in the previous study [16]. *In-vitro* regeneration through field nodal explants and sex expression of regenerants in the polyhouse condition were studied. The stem nodal explants were taken for *in-vitro* culture of four cucumber genotypes. The plants of all the four genotypes of cucumber were sprayed with Bavistin @ 1 g/l twice at 24 and 6 hrs before taking the tender stem nodal cuttings. Then these cuttings were wiped with 70 per cent alcohol cotton swabs. These stems were cut 2-3 cm below the node and 1-2 cm above the node. The side leaves were removed and the bottom portion of the nodes was given a slant cut with the help of sterile blade. After that these nodal cuttings were washed for three minutes in double distilled water. The cuttings were then soaked in mild detergent and 0.1 g Bavistin in 100 ml double distilled water for 10 minutes and were again rinsed with double distilled water for five times. These were then sterilized in 50 per cent ethyl alcohol for five minutes and repeatedly washed again in double distilled water for 3-4 times. The nodal cuttings were then surface sterilized with 0.05 per cent Mercuric chloride

($HgCl_2$) for five minutes and rinsed five times in sterile distilled water. The nodal explants were then placed on two different media compositions in the test tubes containing three per cent w/v sucrose. The pH of the media was adjusted to 5.8 ± 0.1 with 1N HCL or 1N NaOH and then solidified with agar and autoclaved at 121ºC at 15 *psi* for 15-20 minutes. Single nodal explants were inoculated in each culture tube and incubated at 25 ± 2ºC under white fluorescent light for 16 hrs light/8 hrs dark period. The sterilized nodal cuttings were then placed on two media compositions (Table 2) namely, first- half strength MS basal medium [17]; second- full strength MS basal medium supplemented with 2 mg/l BAP and 1.5 mg/l IAA; one nodal cutting was cultured per tube containing 15 ml of medium. The data for 15 samples per treatment were recorded for shoot, root and callus initiation along with response (%) for consecutive three weeks and was subjected to calculation of standard error. The regenerated plants were then placed in coco-peat mixture cups in shade for hardening for two to three days having temperature of 26-28ºC in high humidity (>90%) conditions and then were transplanted in polyhouse (modified naturally ventilated polyhouse with the dimensions of 24m length and 16 m width) protected with 60 mesh insect proof net for observing their sex expression. These plants were transplanted in the month of June, 2017 which is the monsoon season in Kerala and have outside average temperature 28 ± 2ºC and relative humidity more than 80%. The data on survival percentage and sex expression was recorded for the live plants available from the initial sample size of 15.

Table 1. Details of genotypes used for tissue culture

Sex form/type	Variety
Gynoecious cucumber	EC 709119 (Gy-14)
Parthenocarpic cucumber	CS 130
Parthenocarpic cucumber	CS 131
Monoecious cucumber hybrid	L-04

Table 2. Details of media composition for stem nodal explants

Media	Composition
A_1	Half MS (Basal Media)
A_2	Full MS + 1.5 mg/l IAA + 2 mg/l BAP

3. RESULTS AND DISCUSSION

Seed germination of two parthenocarpic (CS 130 and CS 131), one gynoecious (EC 709119) and one monoecious (L-04) genotype was observed *in-vitro* with half strength MS [17] basal medium and 100 percent germination was achieved [16]. *In-vitro* germination of cucumber cultivars was also observed by Margaret et al. [18] and Alam et al. [15]. Maximum shoot initiation (100%) from seedling excised cotyledonary explants was obtained with the media composition of half strength MS medium supplemented with 0.5 mg/l IAA and 2 mg/l BAP. The half strength MS medium supplemented with 0.25 mg/l IAA followed by half MS + 0.5 mg/l IAA were found best for rooting and the half MS media accompanying 0.25 mg/l IAA and 2 mg/l BAP for callusing in all the genotypes [16]. Similar type of varied shoot initiation response for different genotypes were also reported by Wehener and Locy [19], Rhonde and William [20], Hooymons et al. [21], Mohiuddin et al. [22] and Ugandhar et al. [23]. *In-vitro* rooting resorting to various auxin and cytokinin concentrations was also achieved by Handley and Chambliss [24], Cade et al. [25], Misra and Bhatnagar [26], Chovelon et al. [27] and Ugandhar et al. [23].

Micro-propagation from stem nodal cuttings is always preferable over cotyledonary explants. Shoot initiation from stem nodal explants was achieved in A_2 (Full MS + 1.5 mg/l IAA + 2 mg/l BAP) media whereas half strength MS media without any hormones resulted in rooting of various parthenocarpic, gynoecious and monoecious cucumber genotypes in the present study (Fig. 1). Monoecious (L-04) and parthenocarpic genotype (CS 130) showed 100 percent response for shoot initiation with A_2 media (Table 3). Monoecious genotype (L-04) took minimum days (7.00±0.58) for shoot initiation followed by parthenocarpic genotype CS 130 (11.00±0.58). On an average 83.34 percent shoot initiation response was achieved and it took 13.00±2.52 days for shoot initiation irrespective of genotypes. Gynoecious (EC 709119) and parthenocarpic genotype (CS 130) showed 100 percent response for root initiation (Table 3). Minimum days (6.50±0.41) for rooting were taken by parthenocarpic genotype (CS 131) followed by monoecious genotype, L-04 (8.00±1.63). Gynoecious genotype was late for showing root initiation response in A_1 media. On an average 83.34 percent root initiation response was achieved and it took 7.86±0.46 days for root initiation irrespective of genotypes. The shoot

and root regeneration from nodal explants were also observed by Custers and Verstappen [28] Sarowar et al. [29] Vasudevan et al. [30], Margaret et al. [18] and Alam et al. [15].

3.1 *In-vitro* Flowering

In-vitro male flowers were obtained in all the media compositions used in the previous study [16]. Male flowers were obtained in gynoecious genotype (EC 709119), parthenocarpic genotype (CS 131) and monoecious genotype (L-04), which is kind of a first report in gynoecious and parthenocarpic genotypes. The *in-vitro* female flower from stem nodal explants was obtained in gynoecious genotype when cultured in A_1 media composition (Fig. 2). The male flowers were extracted from the tubes and pollen fertility test was done with one per cent acetocarmine solution. It was found that the male flowers obtained from gynoecious and parthenocarpic genotypes were partially fertile and from monoecious genotypes were fully fertile (Fig. 2). This might have happened due to high concentration of cytokinin hormone used in the media. It had been earlier reported that flowering of cucumber in tissue culture depends on the type of explant, media composition, type of plant growth regulators and their concentration [31]. Production of the single parthenocarpic cucumber fruits by use of an automated culture system administering compressed air earlier has also been reported Tisserat and Galletta [32]. The air circulation for decreasing the ethylene effects might be one of the reason for sex modification. *In-vitro* male flowering in monoecious cucumber was also reported by various researchers namely Rajasekaran et al. [13], Msikita et al. [14] and Kielkowska and Havey [31].

Fig. 1. Stages of *in vitro* plant regeneration; a: Multiple shoot regenerating; b: Root initiation; c: Regenerated plant; d: Hardened plant

Table 3. Effect of different media for shoot and root initiation from nodal explants for different genotypes

Media	EC 709119		CS 130		CS 131		L-04		Average of all genotypes	
	Days taken for shoot initiation*	Shoot initiation response (%)	Days taken for shoot initiation*	Shoot initiation response (%)	Days taken for shoot initiation*	Shoot initiation response (%)	Days taken for shoot initiation*	Shoot initiation response (%)	Days taken for shoot initiation*	Shoot initiation response (%)
A_2	18.50±2.04	66.67	11.00±0.58	100	15.50±0.41	66.67	7.00±0.58	100.00	13.00±2.52	83.34
	Days taken for root initiation*	Root initiation response (%)	Days taken for root initiation*	Root initiation response (%)	Days taken for root initiation*	Root initiation response (%)	Days taken for root initiation*	Root initiation response (%)	Days taken for root initiation*	Root initiation response (%)
A_1	8.33±1.20	100.00	8.50±0.41	100	6.50±0.41	66.67	8.00±1.63	66.67	7.83±0.46	83.34

'' Data are Mean ± Standard error, n=15; NR-No response*

Table 4. Survival percentage and number of plants showing gynoecious and monoecious sex expression among the plants regenerated from cotyledonary and nodal explants in polyhouse

Variety	No. of surviving plants from cotyledonary explants	Survival percentage from cotyledonary explants (%)	No. of surviving plants from nodal explants	Survival percentage from nodal explants (%)	No. of plants having monoecious sex expression	No. of plants having gynoecious sex expression
EC 709119	6 (10)	60.00	3 (7)	42.86	7	2
CS 130	4 (9)	44.44	3 (6)	50.00	7	0
CS 131	5 (9)	55.56	0 (6)	0.00	3	2
L-04	7 (8)	87.50	7 (8)	87.50	14	0
Total	22 (36)	61.11	13 (27)	48.15	31	4

Value in parenthesis represents the total plants tried for polyhouse cultivation

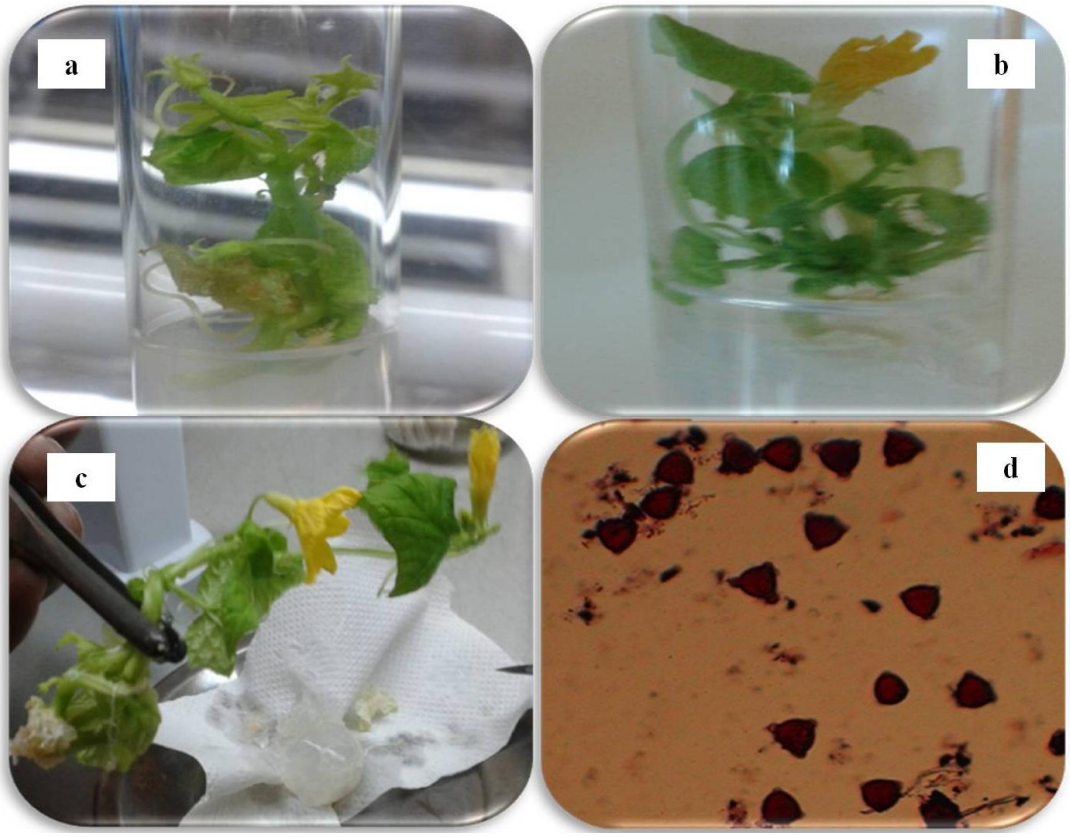

Fig. 2. *In vitro* flowering; a: Female flowers; b: Male flowers; C: Pollen extraction; D: Pollen viability

3.2 Evaluation of Regenerated Plants in the Polyhouse

On an average 61.11 and 48.15 percent survival was recorded from the plants regenerated through cotyledonary explants and stem nodal explants respectively (Table 4). Maximum survival percentage (87.50 %) was achieved in monoecious genotype (L-04) and minimum survival percentage of 44.44 percent was observed in parthenocarpic gynoecious genotype (CS 130) regenerated through cotyledonary explants. The maximum survival of 87.50 percent was recorded in monoecious genotype (L-04) regenerated though stem nodal explants. Parthenocarpic genotype (CS 131) failed to survive in the field condition. Out of all survived plants of gynoecious genotype (EC 709119), seven plants showed monoecious sex expression and two plants exhibited gynoecious sex expression (Table 4). In the parthenocarpic genotype (CS 130) all the survived (seven) plants showed monoecious sex expression. The five survived plants from parthenocarpic genotype CS 131 have shown monoecious sex expression for three plants and gynoecious (parthenocarpic) sex expression for two plants. All the survived plants of the monoecious genotype (L-04) were monoecious in sex expression. On an average out of 35 plants, 31 plants showed monoecious sex expression irrespective of genotypes. Only four plants (two from gynoecious and two from parthenocarpic genotype) showed gynoecious sex expression in the field condition. Variation in survival percentage was also recorded by Vasudevan et al. [30] and Ugandhar et al. [23].

4. CONCLUSION

Mixed response of sex expression in the regenerated parthenocarpic and gynoecious cucumber genotypes (regenerants) was evidenced from the current study which attributes to various growth factors involved in changing the sex expression of the plants. Most probable reason might be the higher concentration of growth hormone used. Hence it can be

concluded that this study unwraps a novel issue which require further scientific insights.

ACKNOWLEDGEMENTS

We thank Kerala Agricultural University, Vellanikkara and UGC, New Delhi for providing the financial assistance for the PhD work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wu Z, Zhang T, Li L, Xu J, Qin X, Zhang T, Cui L, Lou Q, Li J and Chen J. Identification of a Stable Major-effect QTL (Parth 2.1) Controlling Parthenocarpy in Cucumber and Associated Candidate Gene Analysis *via* Whole Genome Re-Sequencing. BMC Plant Biol. 2016;16: 182-195.
2. Kim IS, Okubo H, Fujieda K. Endogenous levels of IAA in relation to parthenocarpy in cucumber (*Cucumis sativus* L.). Sci. Hortic. 1992a;52:1–8.
3. Kim IS, Okubo H, Fujieda K. Genetic and hormonal control of parthenocarpy in cucumber (*Cucumis sativus* L.). J. Fat. Agr. 1992b;36:173–181.
4. Boonkorkaew P, Hikosaka S, Sugiyama N. Effect of pollination on cell division, cell enlargement and endogenous hormones in fruit development in a gynoecious cucumber. Sci. Hortic. 2008;116:1–7.
5. Cantliffe D. Parthenocarpy in the cucumber induced by some plant growth regulating chemicals. Can. J. Plant Sci. 1972;52:781-785.
6. Kim IS, Okubo H, Fujieda K. Studies on parthenocarpy in *Cucumis sativus* L.- (4)-Effects of exogenous growth regulators on induction of parthenocarpy and endogenous hormone levels in cucumber ovaries. J. Kor. Soc. Hortic. Sci. 1994a;35: 187–196.
7. Kim IS, Yoo K, Fujieda K, Okubo H. Studies on parthenocarpy in *Cucumis sativus* L.-(5)-Influence of exogenous plant growth regulators on growth and diffusible IAA level of cucumber ovaries. J. Kor. Soc. Hortic. Sci. 1994b;35:196–200.
8. Fu FQ, Mao WH, Shi K, Zhou YH, Asami T, Yu JQ. A role of brassinosteroids in early fruit development in cucumber. J. Exp. Bot. 2008;59:2299–2308.
9. Yin Z, Malinowski R, Ziolkowska A, Sommer H, Plcader W, Malepszy S. The *DefH9-iaaM*-containing construct efficiently induces parthenocarpy in cucumber. Cell Mol. Biol. Lett. 2006;11:279–290.
10. Bhat MA, Mujib A, Junaid A. *In-vitro* regeneration of *Solanum nigrum* with enchanced solasodine production. Biol. Plant. 2010;54:757–760.
11. Franklin G, Pius PK, Ignacimuthu S. Factors affecting *in-vitro* flowering and fruiting of green pea (*Pisum sativum* L.). Euphytica. 2000;115:65–73
12. Sheeja TE, Mandal AB. *In-vitro* flowering and fruiting in tomato (*Solanum lycopersicum* L.). Asia Pac. J. Mol. Biol. Biotechnol. 2003;11:37–42.
13. Rajasekaran K, Mullins MG, Nair Y. Flower formation *in-vitro* by hypocotyls explants of cucumber (*Cucumis sativus* L.). Ann. Bot. 1983;52:417–420.
14. Msikita W, Skirvin RM, Juvik JA, Splittstoesser WE, Ali N. Regeneration and flowering *in-vitro* of 'Bulpress Hybrid' cucumber cultures from excised seed. Hort. Sci. 1990;25:474–477.
15. Alam MF, Amin R, Uddin ME, Kumar SB, Islam MM. Regeneration of shoot from nodal explants of *Cucumis sativus* considering different hormonal concentration. Int. Res. J. Biological Sci. 2015;4(7): 48-52.
16. Bhardwaj A, Pradeepkumar T, Roch VC. *In-vitro* regeneration of parthenocarpic cucumber (*Cucumis sativus* L.). Int. J. Curr. Microbiol. App. Sci. 2017;6(7):1711-1720.
17. Murashige T, Skoog F. A revised medium for rapid growth bioassays with tobacco tissue culture. Physiol. Plant. 1962;15: 473–497.
18. Margaret S, Maheswari U, Ambethkar, Vasudevan, Sivanandhan, Selvaraj. Direct regeneration of multiple shoots from nodal explants of West Indian Gherkin (*Cucumis anguria* L.). Int. J. Innovative Res. Sci. Engg. Technol. 2014;3(6):13876-13881.
19. Wehener TC, Locy RD. *In-vitro* adventitious shoot and root formation of cultivars and lines of *Cucumis sativus* L. Hort. Sci. 1981;16:759-760.
20. Rhonde LG, William AD. An *in-vitro* technique for the production of multiple shoots in cotyledon explants of cucumber

(*Cucumis sativus* L.). J. Plant Cell Tiss. Org. Cult. 1990;20:177-183.
21. Hooymans CM, Hakkert JC, Jansen J, Custers JBM. Competence for regeneration of cucumber cotyledons is restricted to specific developmental stages. Plant Cell Tiss. Org. Cult. 1994;39:211-217.
22. Mohiuddin AKM, Abdullah ZC, Chowdhury MKU, Napis S. Enhancement of adventitious shoot regeneration in *Cucumis sativus* L. using $AgNO_3$. Plant Tiss. Cult. 2005;15:15-23.
23. 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;1(3):164-169.
24. Handley LW, Chambliss OL. *In-vitro* propagation of *Cucumis sativus* L. Hort. Sci. 1979;14:22-23.
25. Cade MR, Wehner CT, Blazich AF. Somatic embryos derived from cotyledons of cucumber. J. Amer. Soc. Hort. 1990; 115(4):691-696.
26. Misra AK, Bhatnagar SP. Direct shoots regeneration from the leaf explant of cucumber. Phytomorphol. 1995;45:47-55.
27. Chovelon V, Restier V, Giovinazzo N, Dogimont C, Aarrouf J. Histological study of organogenesis in *Cucumis melo* L. after genetic transformation: Why is it difficult to obtain transgenic plants? Plant Cell Rep. 2011;30:2001–2011.
28. Custers JBM, Verstappen ECP. Improvements of *in-vitro* growth of cucumber. Cucurbit Genet. Coop. Rep. 1989;12:20-22.
29. Sarowar S, Oh HY, Hyung NI, Min BW, Harn CH, Yang SK, Ok SH, Shin JS. *In-vitro* micropropagation of a *cucurbita* interspecific hybrid cultivar – a root stock plant. Plant Cell Tiss. Org. Cult. 2003;75: 179–182.
30. Vasudevan A, Selvaraj N, Ganapathi A, Choi CW, Manickavasagam M, Kasthurirengan S. Direct plant regeneration from cucumber embryonal axis. Biol. Plant. 2007;51(3):521-524.
31. Kiełkowska A, Havey MJ. *In-vitro* flowering and production of viable pollen of cucumber. Plant Cell Tiss. Organ Cult. 2012;109:73-82.
32. Tisserat B, Galletta PD. Production of cucumber fruits from the culture of 'Marketmore-76' plantlets. Plant Cell Rep. 1993;13:37-40.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/55013