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Callogenesis, Regeneration of Shoot and Root of Brinjal (*Solanum melongena L.*)

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Abstract: Brinjal is one of the most popular, nutritional vegetable crops. It plays a vital role in the national economy as a cash crop. Biotechnological applications of eggplant (*Solanum melongena* L.) for improvement of quality and quantity of crop. Tissue culture techniques are largely used for the increasing value of this crop in these days. There is a different combination of different hormones used for *in vitro* regeneration of brinjal. The hormones 2, 4- D, IAA, BAP, NAA and kinetin used in various combinations with different ratio. Various explants such as stem and leaves were used for regeneration but stem showed better response. Among the treatments 2, 4-D at 2mg/l showed better callus formation. In case of stem, among the different combinations 2.0 mg/l BAP + 0.5 mg/l NAA showed better callus induction formation and also somewhat regeneration. Cytokyanins such as kinetin at 0.5mg/l with combination of Auxin (IAA) 1.5mg/l and coconut water showed highest percentage of direct shoot regeneration. Explants showed direct shoot regeneration after about 4, 5 weeks of inoculation. Moreover regeneration of roots on two different types of media,one is hormone free MS media and other is supplemented with 0.5mg/l IAA occurred but hormone free MS media was most favorable as it has given rooting after about 2 to 3 weeks after shooting.

Key words: Brinjal · Callogenesis · Coconut water · Kinetin · IAA

INTRODUCTION

Eggplant (*Solanum melongena L.*) belongs to the family *Solanaceae* and genus *Solanum*. In all over the world there are about 25 refined species of a genus *Solanum* that includes the potato, tomato and various eggplant species [1]. *Solanum melongena L*. also called an eggplant and have different names in different societies and civilizations as aubergine or brinjal. It is a commercially important vegetable as well as a cash crop. It is found mostly in moderate and tropical parts of the globe. As compare to other crop plants like tomato, it is rich in vitamins and minerals that increase its total nutritional value [2].

It is vegetable crop grown in summer and covers about 8670 hectors of whole area of the Pakistan and its largest share for sowing area is subcontinent Punjab that shares about 4890 hectors and its production is about 60890 tones [3].Plant tissue culture offers a well organized method for making materials free from pathogen and preservation of germplasm in order to control upon this situation. For the improvement of crops normally helpful tool is tissue culture as in plant breeding the probable value of tissue culturing has been extensively predicted. Principle of totipotncy is responsible for regeneration of commercially important plants via tissue culture [4]. Tissue culture improved the quantity, yield and quality of crops through regeneration at high frequency and genetic engineering technologies. After culturing, explants produced callus on appropriate media. A lot of research programs have been taken out to examine factors that affect and enhance plant regeneration. After explants formation from seeds both callus induction from explants and regeneration require a suitable concentrations of plant growth hormones supplemented with cultured media. Tissue culture analysis data and protocols for embryo formation, organogenesis and protoplast culturing have been well recognized [5-7].

Plant regeneration from tissue culture is a vital component of biotechnology and it is necessary for change in genetic makeup of plants for making it diseased free [8]. A proficient and reproducible regeneration

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system *in vitro* is considered as a vital part of successful transformation. *In vitro* regeneration of eggplant from different explants through organogenesis, there is anumeral reports available by [9-11] and for somatic embryogenesis reports available by [12]. Regeneration of shoot from segments of hypocotyls of eggplant has been documented in the presence of growth hormone IAA [9]. Shortly after this importance of tissue culture was noticed as it was a significant technique which was used for the increase yield of genotypes [13]. Formation of callus and ability of plants to regenerate have been iberated or studied in eggplant from different explants such as root tip, leaf segments and shoot tip explants [14].

MATERIALS AND METHODS

Seeds of brinjal variety NS-797 were taken from Horticulture Department of National Agriculture Research Center, Faisalabad, Pakistan.

Sterilization: All materials for tissue culture lab experiment such as scissors, forceps, glasswares (petri dishes, flasks, media jars and test tubes) washed with running tap water and after wrapping in aluminum foil or newspaper, autoclaved at pressure 15 to 20 atm and high temperature 120°C to remove contaminants and made them aseptic.Culture medium was used in the experiment called MS media [15].

Stock Solutions: To make solutions, measured quantity of MS salts (reagents) taken in beaker from stock A, B and C such as 1900mg of KNO_3 and 1650mg and so on. Similarly that of stock D, E and F in milligrams, was taken and distill water was added to the mark of 1000ml to make 1 liter of medium. After that it was become final volume of these stocks as shown in Table. So, MS media containing different stocks was obtained. Already prepared MS media in this experiment used contain measured amounts of stocks and other ingredients. Stocks of growth regulators such as IAA, NAA, BAP and kinetin were also prepared in 1M sodium hydroxide by dissolving 0.5mg in 50ml distilled water. Weight by volume stocks were prepared in 1/1 ratio.

Surface Sterilization and Germination of Seeds: Seeds were washed with running tap water for 2 - 3 minutes in petridish. Floating seeds were considered to be empty and they were discarded. Later on, the nutritional seeds were surface sterilized with 20% (w/v) sodium hypochlorate for 3 - 4 minutes inside Laminar flow and finally washed four to five times with sterile distilled water. The seeds were then kept on a sterilized petri dish containing sterile filter paper to remove excess of water droplets. The surface sterilized seeds were then inoculated into test tube containing agar solidified basic MS medium with sucrose for supporting seed germination and seedling development. 1 - 2 seeds were inoculated in each test tube.

Explants Sterilization: Seeds were used as source of explant for tissue culture experiments. Seeds were excised by inoculating them on wet filter paper. Embryos swelled up and then were excised and after surface sterilization these sterilized seeds were inoculated into test tube containing agar solidified MS medium [15], with sucrose for supporting seed germination and seedling development. 1 - 2 seeds were inoculated in each test tube.

Callus Induction Medium: Before shifting to callus induction medium seedlings were broken down into small pieces, each piece acting like as an explant. For callus induction three different concentration of 2, 4-D (2, 3 and 5mg/l) were used for brinjal variety NS-797. Also the effect of kinetin and coconut milk was tested for embryogenic callus induction with combination of IAA (Table 1). Callus Induction frequency was calculated by the following formula:

Callus induction frequency = Number of embryogenic calli produced/Total Number of uncontaminated explants inoculated X 100

Maintenance Media: After formation of two types of calli (embryogenic calli and non-embryogenic calli) in callus induction medium, they were transferred to maintenance medium. Only embryogenic calli were used for further analysis as for maintainenance and shifted to medium supplemented with BAP 2mg/l and 0.5 mg/l of NAA and another combination of hormones kinetin + IAA.

Regeneration Media: Before shifting to regeneration medium, calli were broken down into small pieces, each piece acting like an embryo, so that hormones can reach each cell of the proliferated callus. Later on, calli were transferred from maintenance medium to regeneration medium supplemented with different concentrations of growth hormones such as BAP,kinetin, IAA and NAA. Initially regeneration was tested on medium containing different concentrations of IAA (0, 0.3mg/l and 0.5mg/l) and kinetin (0, 1mg/l, 1.5mg/l) + coconut milk (Table 2,3).

Table 1: Various CIM (Callus induction media) containing different concentrations of hormones used for NS-797 cultivar of brinjal.

CIM1	CIM2	CIM3	CIM4	CIM5
2 mg/L 2,4-D	3 mg/l 2, 4-D	2 mg/l BAP, 0.5mg/l NAA	0.5 mg/l IAA, 1.5Kinetin+ coconut water	5 mg/l 2,4-D

Table 2: Various regeneration media on different concentrations of Indole Acetic Acidand Kinetin and coconut milk:

	Kinetin		
IAA	0 mg/L	1.0 mg/L	1.5 mg/L
0 mg/L	RM1	RM2	RM3
0.3 mg/L	RM4	RM5	RM6
0.5 mg/L	RM7	RM8	RM9

Table 3: Composition of regeneration media containing various concentrations of hormones (kinetin and Indole Acetic Acid)and coconut milk

Type of Regeneration media	Composition of regeneration media
RM_1	0 mg/l IAA + 0 mg/l Kinetin + MS media
RM_2	0 mg/l IAA + 1 mg/l Kinetin + MS media
RM_3	0 mg/l IAA + 1.5 mg/l Kinetin + MS media
RM_4	0.3 mg/l IAA + 0 mg/L Kinetin + MS media
RM_5	0.3 mg/l IAA + 1 mg/L Kinetin + MS media
RM_6	0.3 mg/l IAA + 1.5 mg/L Kinetin + MS media
RM_7	0.5 mg/l IAA + 0 mg/l Kinetin + MS media
RM_8	0.5 mg/l IAA + 1 mg/l Kinetin + MS media
RM_9	0.5 mg/l IAA + 1.5 mg/l Kinetin + MS media

Table 4:Regeneration media on different concentrations of NAAand BAP.

	Benzyl Amino Purine	
NAA	0 mg/l	2 mg/l
0 mg/L	RM10	RM11
0.3 mg/L	RM12	RM13
0.5 mg/L	RM14	RM15

Table 5: Composition of regeneration media containing various Conc. of hormones NAA and BAP

Type of Regeneration media	Composition of regeneration media
RM_{10}	0 mg/l NAA + 0 mg/l BAP + MS media
RM_{11}	0 mg/l NAA + 2 mg/l BAP + MS media
RM_{12}	0.3 mg/l NAA + 0 mg/l BAP + MS media
RM_{13}	0.3 mg/l NAA+ 2 mg/l BAP+ MS media
RM_{14}	0.5 mg/l NAA + 0 mg/l BAP + MS media
RM_{15}	0.5 mg/l NAA + 2 mg/l BAP+ MS media

Regeneration was also experimented with different concentrations of NAA (0, 0.3mg/l and 0.5mg/l) in combination with different concentrations of BAP (0mg/l, 2mg/l) as shown in (Table 4,5). Finally the best concentrations of growth hormones in combination were tested and selected. Cultures were kept at 27°C, with 12 hours light cycle in every 24 hours.

Shoot Regeneration Media: After callus formation, the same regeneration media that was applicable for callus induction also used for shoot formation containing 0.5mg/l IAA and 1.5mg/l kinetin + coconut milk.

Root Regeneration Media: For root formation, hormone free MS media and MS media supplemented with 0.5mg/l IAA were used.

RESULTS AND DISCUSSION

Brinjal (*Solanum melongena* L.) is one of the most popular, delicious and nourishing vegetable. Major loss of this crop plant occurs throughout the world due to abiotic stress that is responsible for decreasing its production. About 55 to 60% loss of brinjal occurred by biotic factor as harmful pest (*Leucinodes orbonalis* Guen) was reported and it has become major risk of diseases for cultivation of brinjal [16]. Biotechnological applications of eggplant for improvement of quality and quantity have been used as important tools for solving biotic and abiotic stress problems of breeding of vegetable [7]. A lot of research programs have been carried out to examine factors that affect and enhance plant regeneration and control upon stress. Plant regeneration from tissue culture is a vital component of biotechnology and it is necessary for change in genetic makeup of plants for making it diseased free and stress free. For regeneration of plants, after explants formation from seeds, callus induction from explants and then regeneration require suitable concentration of plant growth hormones supplemented with cultured media [17]. Cytokinins usually increase cell division and induce shoot formation and auxiliary shoot proliferation and auxins promote root formation. High cytokinin to auxin ratio enhances shoots proliferation while high auxin to cytokinin ratio results in root formation [18]. Explants cultured on MS media supplemented with different combinations and concentrations of BAP (0, 0.5, 2.0 mg/l) and NAA (0, 0.5 and 1.5 mg/l), 2, 4-D (2, 3 and 5mg/l), IAA (0, 0.5 and 1.5) and kinetin (0, 0.5) as given in (Table 6,7, 10) and (Figure 6,7) etc.

Seed Germination of Brinjal: The seeds of brinjal were allowed to germinate on basic MS medium. The germination was initiated after duration of 10 days of

Table 6: Percentage of callus induction of brinjal on different calii induction media (CIM) containing various combination of hormones.

Callus induction media	%age of callus formation	%age of embryogenic callus formation	%age of Non embryogenic callus Formation
CIM1=2mg/l 2,4D	40	20	80
CIM2=3mg/l 2,4D	65	30	70
CIM3=0.5mg/l IAA+1.5mg/l kinetin + cocout milk	80	80	20
CIM4=NAA0.5mg/l+ BAP 2mg/l	50	60	40
CIM5=5mg/l 2,4D	33	15	85

Table 7: Analysis of Variance (Anova) of brinjal *in-vitro* callus induction on five different callus induction media (CIM)

Analysis of Variance (Anova) Table					
	Df	S.S	M.S	F-value	P-value
Between	4	6118.200	1529.550	81.359	0.0000
Within	15	282.000	18.800		
Total	19	6400.200			

Coefficient of Variation = 8.45%

Fig. 1: Seed germination and formation of brinjal seedling from seeds after 2-3 weeks of inoculation on basic MS media.

inoculation by placing them periodically in dark at 27°C, for 3-4 days and in light for 5-6days. Seeds were germinated and 90% of seeds produced seedlings after about 15 days of inoculation on basic MS medium. Size of seedling formation was 3-4cm long. Germination and seedling formation from seed is shown in (Figure 1).

Callus Induction of Brinjal: Seedling was cut into small pieces with help of scissor and each piece operated like explant. Various explants were used for this purpose and among these explants; stem was comparatively more responsive for callus induction than other explants such as leaf and root. Growth hormone such as 2, 4-D was functional in order to optimize callus induction for undifferentiated splitting up of cells from the explants used. NS-797 cultivar of brinjal showed good comeback and calli started to appear from shoot explants after just

Fig. 2: Callus induction from explants of brinjal on MS media supplemented with IAA (0.5mg/l), Kinetin (1.5mg/l) and 25% coconut milk after 20 days of inoculation.

5 to 6 days of inoculation on CIM1 (2mg/l 2, 4-D+MS media). Over 40% of explants showing callus induction on CIM1 (2mg/l 2, 4-D+MS media).

Percentage of calli induction was increased upto 65.08 % in CIM2 (3mg/l 2, 4-D+MS media) and it has produced non embryogenic calli within 22 days of inoculation of explants. When we increased concentration of same hormone 2, 4-D up to 5mg/l frequency of callus induction again decreased much and only 33.1% of the explants formed calli. Hormone (2, 4-D) used for induction of callus in brinjal and 3mg/l of 2, 4-D was best for callus induction from stem explants in about 15 days. 2, 4-D was the hormone of choice for callus induction in wheat by [19], who reported 2, 4-D was the major ingredient in callus induction.

Similarly another combination of hormones such as 2mg/l BAP (Benzyl amino purine) and 0.5mg/l NAA (Naphthalene acetic acid) on MS media formed 50% of calli induction from shoot explants after about 25 days of inoculation. Similar type of result as calli formation by inoculation of explant in BAP and NAA was also documented by [20], who had been performed experiment by taking same conc. of these hormones 2mg/l BAP and 0.5mg/l NAA in MS media.At 1.5mg/l of kinetin and 0.5mg/l Indole acetic acid in coconut milk with Murasiage and skoog media about 80% of explants produced calli

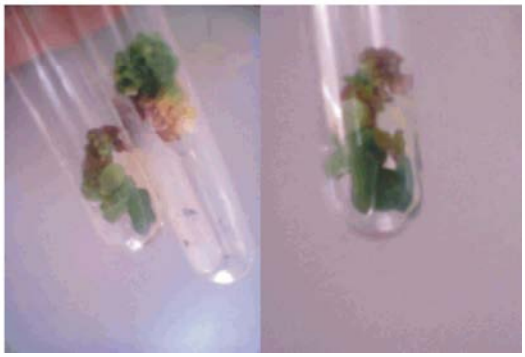

Fig. 3: Shoot induction of Brinjal after 25days of inoculation on MS media supplemented with 0.5mg/l NAA and 2mg/l BAP.

rapidly. After 2 weeks, calli induction appeared and after three weeks clear appearance of calli observed. Callus induction is shown in (Figure 2).

Regeneration: After callus induction in various CIM media, the appearance of white color of calli in maintenance medium occurred, that started to turn light green after about 4 to 5 weeks of inoculation in CIM3 media which indicated that they are now be able of regeneration in regenerating medium. Two to three growth hormones were responsible for regeneration i.e. Indole Acetic Acid (IAA), Benzyl Amino Purine (BAP) and Kinetin.

Regeneration of Brinjal Shoot on NAA and BAP: It was resulted that at 0 mg/l concentration of NAA there observed no regeneration and no plantlets were produced. Calli changed to brownish after 2 to 3 weeks and at last died. Similarly in absence of another hormone BAP there were no plants produced.NS-797 has given no results at 0.5mg/l of NAA.At 0.5mg/l of BAP with combination of NAA (1.5mg/l), about 10.5% of the calli produced small shoot lets (Table 8). Green spotting was observed after few days. Majority of these calli produced greenish color. Regeneration frequency was increased with the increase of BAP concentration. Finally at 2.0 mg/l of BAP with combination of 0.5mg/l NAA, about 59.5% of the calli produced plantlets and days required for this were 15days.Calli produced greenish color. Regeneration of shoot is shown in (Figure 3).

$$\text{Percentage of regeneration frequency} = \frac{\text{Number of shoots regenerated from calli}}{\text{Total Number of callus inoculated}}$$

Table 8: Percentage of Brinjal shoot regeneration on various (RM) regeneration media containing different conc. of hormones NAA and BAP.

Variety of brinjal	conc. of NAA + BAP(mg/l)	% age of regeneration
NS-797	R1= 0 + 0.5	0
	R2= 0.5+2.0	59.5
	R3= 1.5+ 0.5	10

Table 9: Percentage of shoot regeneration on different regeneration media containing 25% coconut milk and various concentrations of kinetin + IAA.

Concentration of kinetin + IAA in(mg/l)		% age of regeneration
NS-797	R4=0.5 + 0	0
	R5=1.2 + 0.5	15.5
	R6=1.5+0.5	70

Table 10: Analysis of variance (ANOVA) of brinjal *in-vitro* shoot regeneration on different regeneration media (RM).

Analysis of Variance (Anova) Table					
	Df	S.S	M.S	F-value	P-value
Between	5	18152.333	3630.467	382.54	0.0000
Within	18	171.000	9.500		
Total	23	18323.333			

Coefficient of Variation = 10.75%

[20], described that the explants cultured on MS medium without hormones did not produce any callus while calli induced in medium supplemented with BAP and NAA.The combined effect of explants and different combinations of BAP (Benzyl Amino Purine) and NAA (Naphthalene Acetic Acid) on callus induction.

Regeneration of Brinjal Shoot on Kinetin, Iaa and Coconut Milk: For further improvement of regeneration of brinjal, different concentrations of Kinetin and IAA (Indole Acetic Acid) in a balanced ratio were used for experiment with the addition of coconut milk in combination Table 9). It was found that kinetin could not give regeneration alone but could give regeneration with additional hormone such as IAA. When the concentration of kinetin was set up as about 1.5mg/l and that of IAA as 0.5mg/l containing coconut milk 25% then it was found that calli changed their color into greenish and at last gave the regeneration of shoot within test tube. It was established as a new protocol for the regeneration of shoot. The result was found after about 26 days of inoculation and regeneration frequency was increased upto70%. Similar case was reported by [19], who documented that by the addition of another hormone such as NAA with kinetin in MS medium, regeneration frequency increased in comparison to explants shifted from medium containing individually 2, 4-D or kinetin.

Fig. 4: Regeneration of Brinjal shoot after 40 days of inoculation on MS media containing IAA (0.5mg/l), Kinetin (1.5mg/l) and 25% coconut milk.

Fig. 5: Root regeneration from shoots of brinjal after 40 days of inoculation on hormone free MS medium.

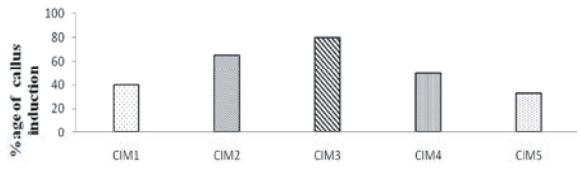

Fig. 6: Calli induction of brinjal on different callus induction media (CIM) containing various conc. of growth hormones (2, 4-D, BAP+ NAA and IAA+ Kinetin).

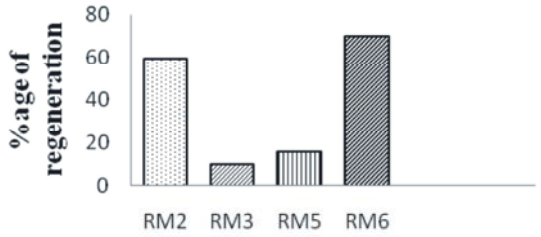

Fig. 7: Regeneration of Brinjal shoot on different regeneration media (RM) containing hormones (BAP+NAA, IAA + Kinetin) and coconut water

According to [14], when Kinetin was supplemented into the MS medium, the size of callus was decreased. Similar case appeared here and callus size in comparison to calli produced by 2.0 mg/l BAP + 0.5 mg/l NAA decreased and most of the energy consumed by shoot induction.Similar findings of using IAA and kinetin for regeneration described in *S. melongena* by several authors [5, 21], who described about protocols of tissue culture for *de novo* organ formation and protocols for somatic embryogenesis. Regeneration of shoot shown in (Figure 4).

Regeneration of Roots of Brinjal (*Solanum Melongenal*): Roots were regenerated finally either diretly from callus or shoots. Roots were regenerated within 1-2 weeks after the inoculation of regenerated shoots on media (0.5 IAA + MS medium) as well as hormone free MS media. Mostly the shoots regenerated roots on basic MS media and a few on Media (0.5 IAA + MS medium). Most of the shoots developed roots by days 10 to 13. Higher number of roots was induced on MS 0 or hormone free media. Similar type of results for formationof root in plant growth hormone-free medium have been described in *S. melongena* by[19, 22]. Regeneration of root shown in (Figure 5).

CONCLUSIONS

It is concluded that 2, 4-D was the hormone of choice and favorable for both embryogenic and non embryogenic callus induction.Eggplant has given maximum regeneration frequency of shoot when Kinetin and IAA (Indole Acetic Acid) were used in combination with coconut milk.NAA (Naphthalene Acetic Acid) and BAP (Benzyl Amino Purine) were found to be better for embryogenic calli induction and shoot regeneration.

REFERENCES

1. Samuels, J., 2009. The Solanaceae: novel crops with high potential. Organic Grower, 9: 32-34.
2. Kalloo, G., 1993. Eggplant (Solanum melongena L). In: Kalloo, G. (Ed.), Genetic Improvement of Vegetable 573 Crops. Pergamon Press, Oxford, pp: 587-604.
3. Anonymous. 2007. Agriculture Statistics of Pakistan 2006-2007. Govt. of Pakistan, Ministry of Food, Agriculture and Livestock, Islamabad.
4. Krikorian, A.D. and D.L. Berquam, 1969. Plant cell and Tissue culture. The Role of Haberlandt. Bot., 35: 59-88.

5. Collonier, C., I. Fock, V. Kashyap, G.L. Rotino, M.C. Daunay, Y. Lian, I.K. Mariska, M.V. Rajam, A. Servaes, G. Ducreux and D. Sihachakr, 2001. Indirect Shoot Organogenesis Of Eggplant (Solanum Melongena L.). Plant Cell Tiss.and Org. Cult, 65: 91-107.
6. Kashyap, V., S.V. Kumar, C. Collonnier, F. Fusari, R. Haicour, G.L. Rotino, D. Sihachakr and M.V. Rajam. 2003. Biotechnology of eggplant. Scientia Horticultura, 97: 1-25.
7. Khatun, F., M.B. Meah and K.M. Nasiruddin. 2006. Regeneration of eggplant through anther culture. Pakistan Journal of Biological Sciences. 9: 48-53.
8. Khizar, H.B., N. Kausar, U. Rashid, K. Hussain, K. Nawaz and E.H. Siddiqi, 2013. Effects of Biotic Stresses on Egg Plant (Solanum melongena L.). World Applied Sciences Journal, 26(3): 302-311.
9. Kamat, M.G. and R.NA, 1978. Vegetative multiplication of eggplant (*Solanum melongena* L.) using tissue culture technique. Plant Sci. Lett., 13: 57-65.
10. Sharma, P. and M.V. Rajam, 1995.Genotype, explant and position effects on organogenesis and embryogenesis in eggplant (*Solanum melongena* L) J. Exp. Bot., 46: 135-141.
11. Magioli, C., A.P.M. Rocha, D.E. De Oliveira and E. Mansur, 1998. Efficient shoot organogenesis of eggplant (*Solanummelongen*aL.) induced by thidiazuron. Plant Cell Rep., 17: 661-663.
12. Yadav, R., P. Arora, D. Kumar, D. Katyal, N. Dilbaghi and N. Chaudhury. 2009. High frequency direct plant regeneration from leaf, internode and root segments of Eastern Cottonwood, pp: 76-89.
13. Brown, D.C.W. and T.A. Thorpe, 2011. Crop improvement through tissue culture. W. J. Microbio and Biotech, 11: 409-415.
14. Jahan, M.A.A. and S. Hadiuzzama, 1996. Callus induction and plant regeneration from different explants of *Solanum nigrum* L. seedlings. Plant Tiss Cult, 6(1): 57-62.
15. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiology, Lancaster. 15: 473-497.
16. Krishnaiah, K., 1980. Assessment of crop losses due to pests and diseases. In: Govindu HC (ed), Uni. of Agri. Sci. Tech. Ser. 33, Bangalore, pp: 259-267.
17. Fari, M., I. Nagy, M. Csanyi, J. Mitkyo and A. Rasfalvy, 1995. Agrobacterium mediated genetic transformation and plant regeneration via organogenesis and somatic embryogenesis from cotyledon leaves in eggplant (Solanum melongena L. cv. 'Kecskemeti lila'). Plant Cell Reports, 15: 82-86.
18. Larue, C.D., 1936. The growth of plant embryos in culture. Bull. Torrey Bot., 63: 365-382.
19. Sarker, R.. H.S. Yesmin and M.I. Hoque, 2006. Multiple Shoot Formation in Eggplant (*Solanum melongena* L.). Plant Tissue Cult. & Biotech, 16(1): 53-61.
20. Jayasree, T., V. Paban, M. Ramesh, A.V. Rao and K.J.M. Reddy, 2001. Somatic embryogenesis from leaf cultures of potato. Plant Cell Tiss. Org. Cult., 64(1): 13-17.
21. Kashyap, V., S.V. Kumar, C. Collonnier, F. Fusari, R. Haicour, G.L. Rotino, D.Sihachakr and M.V. Rajam, 2012. Biotechnology of eggplant. Scientia Horticulturae, 1846: 1-25.
22. Taha, R.M. and M. Tijan, 2002. An *in vitro* production and field transfer protocol for *Solanum melongena* plants. South African J. Bot., 68: 447-450.