International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **4(7),** 48-52, July **(2015)** *Int. Res. J. Biological Sci.*

Regeneration of Shoot from Nodal explants of *Cucumis sativus* considering different Hormonal concentration

Firoz Alam M., Ruhul Amin, Ekhlas Uddin M., Sudhangshu kumar Biswas and Monirul Islam M.
Department of Biotechnology and Genetic Engineering, Islamic university, Kushtia, BANGLADESH

Available online at: www.isca.in, www.isca.me
Received 31st May 2015, revised 27th June 2015, accepted 8th July 2015

Abstract

Cucumber (Cucumis sativus), one of the most economically important vegetable (cucurbit) crops, is eaten raw or cooked and is popularly used as salads in south Asia. Considering the nutrition value of Cucumis sativus, the present investigation was undertaken to develop a rapid and efficient in vitro multiplication and regeneration system of this species using in vitro nodal explants. Addition of cytokinin is essential to induce shoot formation from the explants. Of the two cytokinin tested, BAP was more effective than kinetin at concentration of 1.5mg/l yielded best response (87%) on shoot formation. Kinetin at 1.5mg/l showed the highest shoot regeneration frequency (53%). When combination of auxin with IAA (0.5 mg/l) + BAP (1.0 -5.0 mg/l) and IAA (0.5 mg/l) + KN (1.0-5.0 mg/l) for shoots development, greater frequency (70%) were produced at IAA (0.5 mg/l) + BAP (3.0 mg/l) whereas IAA + KN were greater frequency (67%) at 3.0 mg/l KN. For root induction, four concentration of NAA were used. The maximum frequency of root formation (83%) was achieved within 3 week when isolated in vitro raised shoots were cultured on MS medium containing 0.5mg/l NAA.

Keywords: Cucumber, *In vitro,* regeneration, explants, cytokinin, kinetin, MS medium.

Introduction

Cucumber (*Cucumis sativus* L.), is one of the most important vegetable crop, originated from South Asia. It is a popular crop in Bangladesh, cultivated primarily for its fruits for slicing and pickling, juice extraction and for the preparation of traditional local medicines. Cucumber is an excellent source of potassium and fiber with moderate Vitamins A and C, folic acid, phosphorous, and magnesium[1]. This fruits contains water (95%), protein (1%) and carbohydrates (4%). Its seeds and pulp have been used from long days. It has strong antioxidant potential that exhibited chilling and soothing effect to irritated skin caused by sun or cutaneous eruption[2].

Currently, the plants (edible or non-edible) with strong antioxidant potential have concerned great attention to the protection against acne vulgaris related with oxidative stress in living systems[3,4]. The plant parts used in cosmetic preparations for its various therapeutic properties like antioxidant, antiseptic, anti-inflammatory, anti-keratolytic and antibacterial action[5,6]. Its alcoholic pulp extract is used to prepare an ointment for external applications that refresh, smooth the dry skin and can also be used for throat related illness[7]. The seeds of *Cucumis sativus* L. are diuretic, tonic refrigerant[8] and the extractable alcoholic odorous is used in some posy perfumes[9].

The most important vegetables positions among the ten, *Cucumis sativus* L. produced in the nationwide, but it is subjected to many pathogens and pests[10]. Direct organogenesis has been reported for many cucurbits from various explants viz., cotyledons[11] hypocotyls[12] cotyledonary node[13] leaf explants[11,14,15] and anther culture[16]. A good micro-propagation protocol for cucumber could be used for reducing the cost (30%) of hybrid seed production.

Material and Methods

Source of seeds: The seeds of Cucumber (*Cucumis sativus*) were collected from horticulture center, Kushtia, Bangladesh.

Sterilization of Seeds: Mature and healthy seeds were separated carefully and cleaned with tap water. The cleaned seeds were washed repetitively in double distilled water. Now the procedure of sterilization was done under aseptic conditions. To reduce the fungal contamination, Ethyl alcohol (70%) were treated for 30 seconds and then washed with sterile distilled water for 3-5 times. The seeds were surface sterilized with 0.1% (w/v) $HgCl_2$ solution for four minutes. The sterilized seeds were washed 4-5 times in distilled water immediately to eliminate all traces of $HgCl_2$ and left to air dry.

Explants Source: The sterilized seeds were aseptically inoculated on MS medium[17] containing in culture tubes for germination. The culture tubes were incubated in dark at 26ºC for germination and then shifted to culture room providing cool-white-fluorescent light (18/6h photoperiod) and allowed to grow. The cultures were checked daily to note the response. Nodal explants (10-12 mm) were excised from 3-4 weeks old *in vitro* raised seedlings which serves as an explants.

Shoot development and proliferation: The nodal explants were placed vertically with the proximal region facing up in

each culture tube of MS medium with different concentrations of BAP (0.5-3.0 mg/l), Kinetin (0.5-3.0 mg/l), combination of IAA (0.5 mg/l) + BAP (1.0 -5.0 mg/l) and IAA (0.5 mg/l) + KN (1.0-5.0 mg/l). The culture medium was prepared with 3% (w/v) sucrose and 0.8% agar. The pH was adjusted (5.8) after the addition of growth hormones. The nutrient media was autoclaved at 1.05 kg cm^{-2} for 20 minutes at 121°C. The cultures chamber were maintained at 25 ±2° C with white fluorescent light for light and dark (16/8h) periods. The number of shoots produced after 3weeks was counted.

Root Induction: Isolated single shoots (5 centimeters) were inoculated in MS medium with NAA (0.1-1.0 mg/l) for rooting. The cultures were maintained as described above.

Results and Discussion

The plant regenerated from nodal segment is considered to be one of the most promising ways for multiplying a selected variety true to its type. The nodal segment with preexisting meristem is better for propagation since its easy manipulation, high propagation rate and retains of clonal fidelity[18-20].

Different species of Cucurbitaceae have difference in micropropagation. The difference might have arisen due to many other factors like- genotypes, medium composition, physical growth factors like- light, temperature, moisture etc[21]. Khalafalla *et al* reported that MS medium proved to be the best medium for multiple shoots induction[22]. Addition of growth stimulating hormones (cytokinin) was essential to multiple shoot formation. Faria and Illg (1995)[23] have shown that shoots formation capability depends on concentrations of the growth hormones. BAP and KIN are broadly used for shoot multiplication[24].

For the establishment of *in vitro* culture, surface sterilization of explants was mandatory for the medium used in these techniques are also suitable for microbial growth. $HgCl_2$ was optimized for the surface sterilization because the chlorine gas released from $HgCl_2$ was very penetrating that it destroyed the microorganisms present in most tissue of the explants.

In this attempt of surface sterilization with 0.1% ($HgCl_2$) for treatment duration of 1-2 min. showed 100% of contamination. Maximum cultured explants showed fungal contaminations within 3 to 4 days for incubation. About 82-80% contamination free cultures were obtained when the explants treated for 4-5 min with 0.1% $HgCl_2$ (figure-1). In this case, most of the explants were showed green and healthy growth and formation of auxiliary shoots. But 100% of the explants died when treated with $HgCl_2$ for 10 minutes.

Effects of different concentrations of BAP, KN, IAA + BAP and IAA + KN on shoot proliferation from nodal explants of *Cucumis sativus:* Shoot development was evaluated on MS medium with different types and concentrations of cytokinins such as BAP and KN. Shoot development was observed on both the hormones. The frequency of shoot induction was counted from 0-87% and 0- 53% respectively on BAP and KN with MS medium. After 3-4 weeks large number of shoots was produced (figure-2a to 2c).

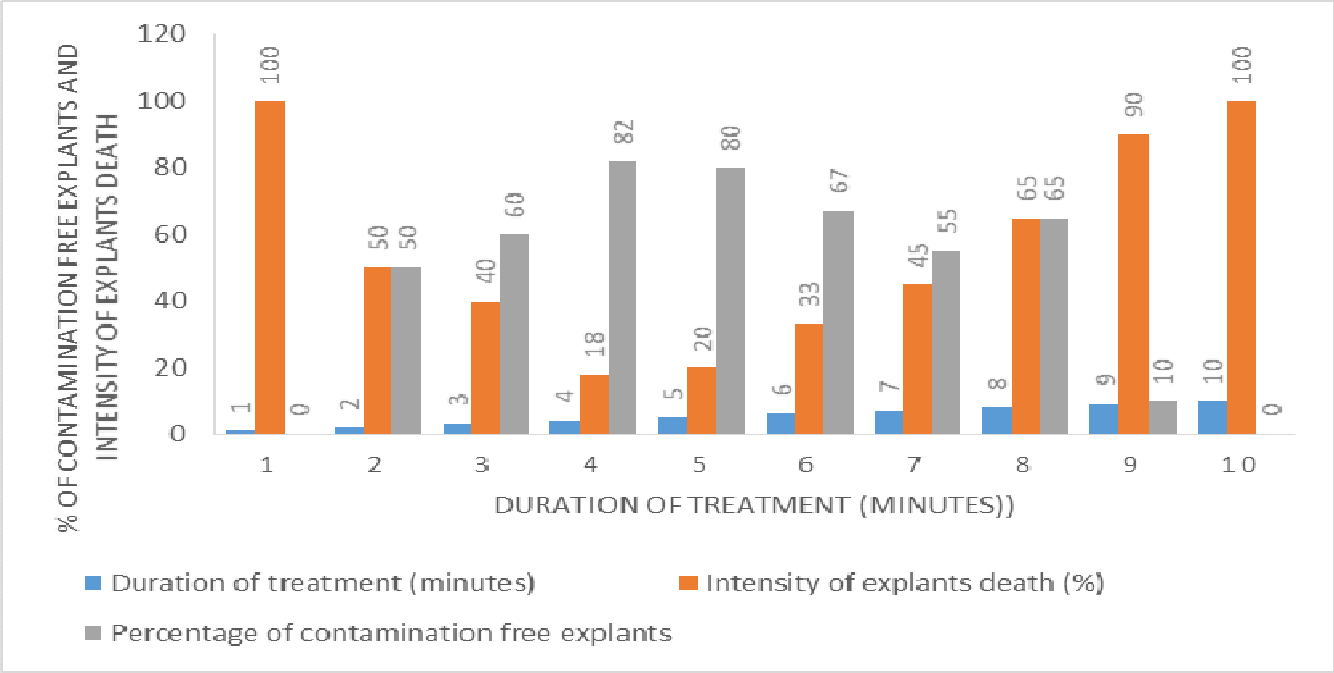

Figure-1
Contamination free explants and intensity of explants death after treatment of $HgCl_2$

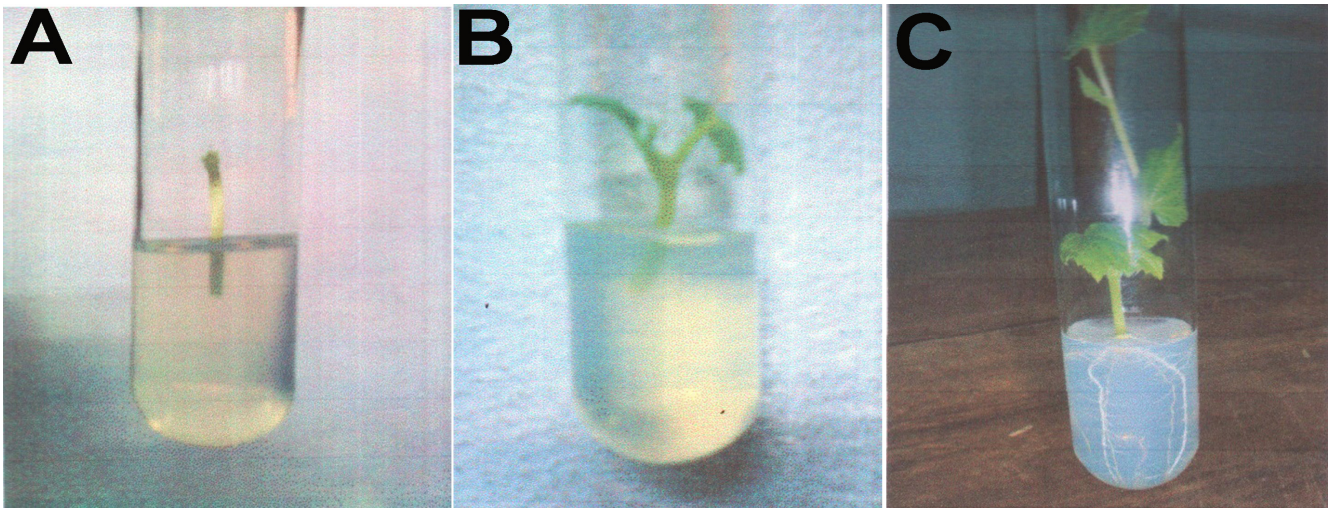

Figure-2
(a) Inoculated explant **(b) Shoot Regeneration** **(c) Multiple Root from Regenerated Shoot**

Among the six concentrations of BAP at 1.5mg/l yielded the best response on shoot proliferation which accounted to 87% while kinetin (1.5mg/l) showed the highest 53% of shoot regenerated (table-1). When the concentration was increased (0.5-1.5mg/l) the percentage of explants show proliferation and number of shoots per culture increased gradually. Further increased in concentration to 2.0-3.0mg/l did not improve any of the parameter but reduce the proliferation of shoots and at the highest level (3.0mg/l) the cultured explants failed to proliferate any shoot. The length of shoot ranges from 0-2.2 cm calculated in both the cytokinins. Shoots development on MS medium with 1.0mg/l of BAP was calculated 2.2 cm height (3 weeks). Although MS medium with 1.0mg/l of KN reached 1.9 cm height in the same period.

Table-1
Effects of different concentrations of BAP and KN in MS medium for Shoot multiplication and proliferation

Hormonal concentrations (mg/l)		Shoot proliferation (%)	Mean shoot length (cm)
BAP	0.5	47	2.1
	1.0	60	2.2
	1.5	87	1.9
	2.0	63	1.6
	2.5	40	1.1
	3.0	0	0.0
KN	0.5	20	1.0
	1.0	30	1.9
	1.5	53	1.6
	2.0	43	1.5
	2.5	20	1.1
	3.0	0	0.0

The study showed that most of the concentration of BAP was cooperatively more effective than KN for shoot induction which has also been reported previously[15,25]. Similarly Hoque *et al.* have also observed that the highest frequency of shoots formation on BAP compared to KN[26].

In combination of auxin with IAA (0.5 mg/l) + BAP (1.0 -5.0 mg/l) and IAA (0.5 mg/l) + KN (1.0-5.0 mg/l) (table-2), the highest frequency (70%) were produced at BAP (3.0 mg/l) + IAA (0.5 mg/l). IAA + KN were not as much of responsive compared to IAA + BAP in inducing shoots. With IAA (0.5 mg/l) + KN (3.0 mg/l) was more responsive for shoots formation with high frequency (67%). As the concentration of BAP and KN was increased in combination with IAA from 4-5 mg/l the rate of shoots formation were significantly reduced.

Table-2
Effects of different concentrations of IAA + BAP and IAA + KN in MS medium for Shoot multiplication and proliferation

Hormonal concentrations (mg/l)		Shoot proliferation (%)	Mean shoot length (cm)
IAA + BAP	0.5+1.0	60.0	1.5
	0.5+2.0	63.0	1.9
	0.5+3.0	70.0	2.3
	0.5+4.0	67.0	2.1
	0.5+5.0	53.0	1.8
IAA + KN	0.5+1.0	50.0	1.6
	0.5+2.0	60.0	2.1
	0.5+3.0	67.0	2.2
	0.5+4.0	53.0	1.7
	0.5+5.0	47.0	1.5

This outcome is similar to that of T Ugandhar *et al.*[8]. BAP alone or in combination with other cytokinins showed essential for shoot formation[27]. Earlier reports also recorded that the formation of multiple shoots in cucurbits in *in vitro* by using different explants with cytokinins alone with (BAP/KN)[28] or combination of cytokinins (BAP/KN) with auxins (NAA, IAA and IBA). The combination of auxin with cytokinin is essential for shoot induction has been described in culture of *Cicer ariutum* leaf[29] of cytokinin BAP proved most effective for shoot induction than KN.

Through this experiment it was realized that BAP was the most effective cytokinin and the most preferred concentration of BAP was 1.5mg/l. At this concentration KN produces remarkable lower number of shoot. This is why BAP is used for further experiment.

Development of Roots from regenerated Shoots: The regenerated shoots were cultured on 1/2MS medium with NAA for healthy root development. Four concentrations of NAA were used to induce root in *C. sativus.* The highest number of root formation (83%) and root length (2.2 cm) was achieved within 3 weeks on 1/2MS medium containing 0.5mg/l NAA (table-3). Similarly Ahmad and Anis (2005) have found that 1/2MS medium supplemented with NAA showed better frequency with mean root length (3.86cm)[30]. Jabeen *et al.* also observed that frequency of rooting with NAA was more compared to IAA and IBA in *Solanum nigrum.* The lowest number of root per explants was calculated on medium containing 1.0mg/l of NAA[31].

Table-3
Effects of different concentrations of NAA for root induction

Hormonal concentrations (mg/l)	% of root induction	Number of roots/shoot	Mean root length (cm)
0.1	53	1.12	1.8
0.5	83	2.46	2.2
0.7	63	2.21	2.1
1.0	0	0	0

Conclusion

In recent years, many investigations have been performed in establishing reliable regeneration protocol for important vegetable crops primarily because of both primary and essential steps for facilitating gene introduction and crop improvement. The present experiment showed that an effective protocol for micropropagation of *Cucumis sativus* depends on various key factors like choice of explants, surface sterilization, growth regulators and their combination at different concentrations. This simple practice is reliable and may be useful for propagation of *Cucumis sativus* in a shorter period. This protocol can reduce the costs of high yield seed production and also be subjected for further studies.

References

1. Mahmoud O. and Arash M., Rapid micropropagation of Cucumis sativus var. Dastgerdi (Iranian cultivar) by Node Culture Technique, *British Biotechnology Journal* **4(6),** 733-739 **(2014)**

2. Budhiraja H, Gupta R.K. and Nand P., Formulation and characterization of *Cucumis sativus* extract in the treatment of acne, *World Journal of Pharmacy and Pharmaceutical Sciences*, **3(12)**, 1043-1057 **(2014)**

3. Kähkönen M.P, Hopia A.I, Vuorela H.J, Rauha J.P, Pihlaja K, Kujala T.S. and Heinonen M., Antioxidant activity of plant extracts containing phenolic compounds, *J Agric Food Chem,* **47(10)**, 3954-3962 **(1999)**

4. Krishnaiah D, Sarbatly R. and Nithyanandam R., A review of the antioxidant potential of medicinal plant species. *Food Bioprod Process*, **89(3)**, 217-233 **(2011)**

5. Cowan M.M., Plant products as antimicrobial agents, *Clin Microbiol Rev*, **12(4)**, 564-582 **(1999)**

6. Kumar G.S, Jayaveera K.N, Kumar C.K, Sanjay U.P, Swamy B.M. and Kumar D.V., Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria, *Trop. j. pharm. res*, **6(2)**, 717-723 **(2007)**

7. Mahajan A, Gill N.S. and Arora R., *Cucumis anguria:* A medicinally important plant of cucurbitaceae family, *International Journal of Recent Advances in Pharmaceutical Research*, **4(3)**, 21-27 **(2014)**

8. Ugandhar T, Venkateshwarrlu M, Begum G, Srilatha T. and Jaganmohanreddy K., *In Vitro* plant regeneration of Cucumber (*Cucumis sativum* (*L.*) from cotyledon and hypocotyl explants, Science Research Reporter, **1(3),** 164-169 **(2011)**

9. Pandey B.P., *Economic Botany,* New Delhi: S. Chand and Co. Ltd, 76-77 **(2000)**

10. Lebeda A, Widrlechner M.P, Staub J, Ezura H, Zalapa J. and Křístková E. Cucurbits (Cucurbitaceae; *Cucumis* spp., *Cucurbita*spp., *Citrullus*spp.), Chapter 8. In: Singh R [ed.], *Genetic Resources, Chromosome Engineering and Crop Improvement Series*, vol. 3 – *Vegetable Crops*, CRC Press, Boca Raton, FL, U.S.A, 271–376 **(2007)**

11. Misra A.K. and Bhatnagar S.P. Direct shoot regeneration from the leaf explants of cucumber (*Cucumis sativus L.*), *Phytomorphology*, **45**, 47-55 **(1995)**

12. Mohiuddin A.K.M, Chowdhury M.K.U, Zaliha C. Abdullah and Suhaimi N., Influence of silver nitrate (ethylene inhibitor) on cucumber in vitro shoot regeneration, *Plant Cell, Tissue and Organ Culture*, **51**, 75-78 **(1997)**

13. Ambetkar A, Maheswari U.C, Margaret S, Sivanandhan G. and Selvaraj N., Micropropagation of *Trichosanthes anguina L.* via cotyledonery node, *International Journal*

of Applied Bioresearch, **4**, 6-11 **(2012)**

14. Stipp L.C.L, Mendes B.M.J, Piedade S.M.D.S. and Rodriguez A.P.M., *In vitro* morphogenesis of *Cucumis melo* var. inodorus, *Plant Cell Tissue and Organ Culture,* **65**, 81-89 **(2001)**

15. Kathal R, Bhatnagar S.P. and Bhojwani S.S., Regeneration of plants from leaf explant of *Cucumis melo* cv. Pusasharbati, *Plant Cell Reports,* **7**, 449-451 **(1988)**

16. Kumar H.G.A, Murthy H.N. and Paek K.Y. Embryogenesis and plant regeneration from anther cultures of *Cucumis sativus* L., *Scientia Hort,* **98**, 213-222 **(2003)**

17. Murashige T. and Skoog F., A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures, *Physiologia Plantarum*, **15**, 473-497 **(1962)**

18. Kates M., Factors affecting proliferation of shoot in mulberry auxillary bud culture, *J. Seric. Sci. Jpn*, **62**, 152-161 **(1993)**

19. Pattnaik S.K. and Cland P.K., Rapid clonal propagation of three mulberries, *Morus cathayana* Hemsl., *M. Ihou* Koiz. And *M. serrata* Roxb., through *in vitro* culture of apical shoot buds and nodal explants from nature trees, *Plant Cell Rep,* **16**, 503-508 **(1997)**

20. Chita V.D.S. and Padmaja G., Clonal Propagation of mulberry (*Morus indica* L. cultivar M-5) through *in vitro* culture of nodal explants, *Sci. Hot,* **80**, 289-298 **(1999)**

21. Pierik R.L.M., *In vitro* culture of higher plants. *Martinus Nijhoff Publ,* Dordrecht, Boston, Lancaster, 364-370 **(1975)**

22. Khalafalla M.M., Elgaali E.I. and Ahmed M.M., *In vitro* multiple shoot regeneration from nodal explants of *vernonia amygdalina.* An important medicinal plant, *African Crop Science Conference Proceedings,* **8**, 747-752 **(2007)**

23. Faria R.T. and Illg R.D., Micropropagation of *Zingiber spectabile* Griff, *Sci. Hoticult*, **62**, 135-137 **(1995)**

24. Padmapriya, H., Karthikeyan, A.V.P., Jahir Hussain, G., Karthi, C. and Velayutham, P., An efficient protocol for *in vitro* propagation of *Solanum nigrum* L. from nodal explants, *Journal of Agricultural Technology,* **7(4),** 1063-1073 **(2011)**

25. Venkateshwarlu. M., Direct multiple shoots proliferation of muskmelon (*Cucumismelo* (l.) from shoot tip explants, *International Journal of Pharma and Bio Sciences,* **3(2)**, 645-652 **(2012)**

26. Hoque M.D and Mansfield J.W., Effect of genotype and explant age on callus induction and subsequent plant regeneration from root-derived callus of Indica rice genotypes, *Plant Cell Tiss. Organ Cult.,* **78**, 217- 223 **(2004)**

27. Chaturvedi R. and Bhatnagar S.P., High frequency shoot regeneration from cotyledon explants of water melon cv. Sugar baby, *In vitro Cellular and Developmental Biology-Plant*, **37**, 255-258, **(2001)**

28. Mahzabin F, Parvez S. and Alam M.F., Micropropagation of *Cucurbita maxima* Duch. through shoot tip culture, *Journal of Biosciences*, **16**, 59-65 **(2008)**

29. Arokiaswamy S, Varghese G. and Ignacimuthu S., High frequency regeneration of chick pea (*Cicer arietium* L.) Plantlets from leaf callus, *Phytomorphology,* **50**, 297-302 **(2000)**

30. Ahmad N. and Anis M., *In Vitro* Mass Propagation of *Cucumis sativus* L. from Nodal Segments, *Turk J Bot,* **29**, 237-240 **(2005)**

31. Jabeen, F.T.Z, Venugopal, R.B, Kiran, G, Kaviraj, C.P. and Rao, S., Plant regeneration and in vitro flowering from leaf and nodal explants of *Solanum nigrum* (L). - An important medicinal plant, *Plant Cell Biotechnology and Molecular Biology*, **6(1and2)**, 17-22 (2005)