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An Efficient Callus Induction and Plant Regeneration of *Solanum nigrum* (L.) - An Important Antiulcer Medicinal Plant

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Summary

An efficient protocol devised for rapid callus induction and plantlet regeneration from young leaves, internodal explants of *Solanum nigrum* was described. For *in vitro* callus induction auxins such as 2, 4-D, IAA and NAA in combination with cytokinin BAP were used. High frequency of green compact callus was obtained in leaf explants cultured on MS medium supplemented with 3.0 mg/l NAA+0.5 mg/l BAP. The present study also describes successful plant regeneration from *in vitro* derived callus of young leaves. BAP or Kn alone or in combination with NAA and IAA was used for regeneration of plantlets from callus culture. High frequency and maximum number of multiple shoots were induced on MS medium supplemented with 3.0 mg/l BAP + 0.5 mg/l NAA. All the *in vitro* raised shoots with a length of 3-5 cm were transferred to rooting medium supplemented with different concentrations of IBA (0.25 – 1.0 mg/l). The best rooting response was observed on 0.5mg/l IBA. The well rooted plantlets were transferred to polybags containing soil + vermiculite in 1: 1 ratio for hardening. Finally the hardened plantlets were transferred to field conditions for maximum survivability.

Key Words: Callus induction, Plant regeneration, Leaf, Inter node, 2, 4-D, NAA, IAA, BAP, *Solanm nigrum* (L.)

Abbreviations: 2, 4-D- 2, 4-Dichloro phenoxy acetic acid; BA- 6-benzyladenine; Kn- kinetin; NAA- α-naphthalene acetic acid; IAA- indole-3-acetic acid

Introduction

Solanacae family comprises a number of plants widely known for the presence of variety of natural products of medicinal significance mainly steroidal lactones, glycosides, alkaloids and flavanoids. *Solanum nigrum* L. (Black night shade) a member of the solanacae, has a wide range of medicinal values. The herb is antiseptic, antidysentric and antidiuretic used in the treatment of cardiac, skin disease, psoriasis, herpivirus and inflammation of kidney. The fruits and leaves have been traditionally used against various nerve disorders [19]. It has very important gastric ulcerogenic activities [2] Berries are bitter, pungent and are useful in heart diseases, piles, dysentery [11]. *Solanum nigrum* presently grown as a homestead plant, it is often cultivated in homestead gardens as pot plants. The plant has been considered ethonobotanically important due to its use in traditional and health care system for curing severe ulcers, gastritis and stomachache. Most prominent medicinal properties are the presence of alkaloids, solamargin and solasonine which yield solasodine as glycone has great demand in pharmaceutical industries. Solasodine has embryogenic, teratonic as well as antifungal and antiviral activities [10]. Recent studies describe solasodine production from *in vitro* grown callus cultures of *Solanum nigrum* [28]. Although callus has proved better for the synthesis of alkaloids in several cases [4].

Considering the high economical and pharmacological importance of secondary metabolites, industries are deeply interested in utilizing plant tissue culture technology for large scale production of these substances [15]. Hence present investigation was undertaken to study callus induction, multiple shoot regeneration using young leaf, internodal explants of *Solanum nigrum.*

Materials and methods

Collection of Plant material

Young leaf and inter nodal explants of *Solanum rigrum* (L.) were collected from two month old seed germinated field grown plants growing in biotechnology garden, S.V.University, Tirupati A.P. India.

Surface sterilization

Explants were washed thoroughly under running tap water to remove the traces of dust etc. followed by treatment with 10% teepol/tween-20 for 5 minutes. Then the explants were sterilized in 70% ethanol for a minute, and finally with 0.01% $Hgcl_2$ for 1-2 minutes and washed 3-4 times with sterile double distilled water.

Culture medium

Young leaf and inter nodal explants (1-2 cms) were inoculated on MS medium [16] containing 3% sucrose and gelled with 0.8% agar supplemented with various concentration of auxins such as 2,4 –D, IAA and NAA in combination with cytokinin BAP. The pH of the medium was adjusted to 5.8

before gelling with agar and autoclaved for 20 minutes at 121^{0}C for 15 lbs pressure.

Sub culturing

The cultures were maintained by regular subculture at 4 week intervals on fresh MS medium.

Culture conditions

The growth room conditions maintained for *in vitro* cultures were 26 ± 2^{0}C and 60-70% relative humidity, light intensity was 3000 lux with a photoperiod of 18 hrs day light and 6 hrs dark. Each experiment was conducted at least thrice with 20 replicates per treatment.

Results

Young leaf and internodal explants of *Solanum nigrum* were cultured on MS medium supplemented with different concentrations of auxins 2, 4-D, IAA and NAA in combination with cytokinin BAP. Callus tissue was initiated from both leaf and inter nodal explants after two weeks of inoculation.

Influence of auxin: cytokinin on callus induction

Effect of 2, 4-D alone or in combination with BAP on callus induction

Callus induction was observed on MS medium supplemented with different concentrations of 2, 4-D alone or in combination with BA. Callus was initiated along the cut portions after 12-15 days of inoculation, initially leaf foldings and bulging of internodes were observed. Depending on the concentration and combination of harmones used a wide range of variation in frequency of callus formation and nature of callus was observed. At lower concentration of 2, 4-D light brown callus, where as at higher concentrations dark brown callus was formed. After supplementing BAP to the culture medium colour complexity of callus turns from brown to light green. From leaf explants light green, fragile callus was formed on 2, 4-D (1.0 mg/L) and BAP (0.5 mg/L) supplemented medium (Table-1).

Effect of IAA, NAA in combination with BAP on callus induction

Callus initiation was also observed on MS Medium supplemented with different auxins such as IAA, NAA in combinations with BAP. In BAP and IAA supplemented MS medium after two weeks of inoculation callus was initiated from young leaf and internodal explants, well profused callus was obtained after 4 weeks. In the combination of IAA (3.0 mg/L) and BA (0.5 mg/L), profuse, green organogenic callus was obtained from leaf explants (Table-1; Figure-1). In combination of BA and NAA callus was initiated from both the explants. Well profused, dark green organogenic callus was induced on MS medium supplemented with 3.0 mg/L NAA and 0.5 mg/L BA using leaf explants. In internodal explants, green organogenic callus was obtained on 3.0 mg/L NAA and 0.5 mg/L BA. In lower concentrations of NAA (0.5 – 1.0 mg/L) and BA (0.5 mg/L) less amount of callus was formed in comparison to higher concentrations (Table-1). Among the different combination of auxin: cytokinin tested, BA and NAA proved to be better in terms of inducing high frequency of greenish compact callus.

Table-1: Effect of different concentrations of auxins such as IAA, NAA, 2, 4-D singly or in combination with cytokinin BA on induction of callus from young leaf and internodal explants of field grown *Solanum nigrum* plants.

MS+					
Plant Growth Regulators (mg/L)		Type of explant			
		Leaf		Internode	
2, 4-D		Intensity of callus formation	Nature of callus	Intensity of callus formation	Nature of callus
0.5		-	No callus formed	-	No callus formed
1.0		+ +	Brown loose, fragile	+	Light brown, fragile
1.5		+ +	Cremish brown, fragile	+ +	Dark brown, fragile
2.0		+ +	Dark brown, fragile	+ +	Dark brown, fragile
2,4-D	BA				
0.5	0.5	+	Light brownish green, fragile	+ +	Whitish green, fragile
1.0	0.5	+ +	Light green, fragile	+ +	Light green, fragile
2.0	0.5	+ +	Light brown, compact	+ +	Light brown, compact
IAA	BA				
0.5	0.5	+	Light brown, fragile	+ +	Cremish , fragile
1.0	0.5	+ + +	Light brownish white, fragile	+ +	Yellowish green, fragile
2.0	0.5	+ + +	Cremishgreen, compact, organogenic	+ + +	Light green, nodular
3.0	0.5	+ + +	Light green, compact, organogenic	+ + +	Green, organogenic
NAA	BA				
0.5	0.5	+ +	Light brownish, fragile	+	Cremish brown, fragile
1.0	0.5	+ +	Light yellowish green, compact	+ +	Light brownish green, fragile
2.0	0.5	+ + +	Light green, compact	+ +	Green, nodular
3.0	0.5	+ + +	Dark green, compact organogenic	+ + +	Green, organogenic

Intensity of callus: +, low; ++, moderate; +++, high.

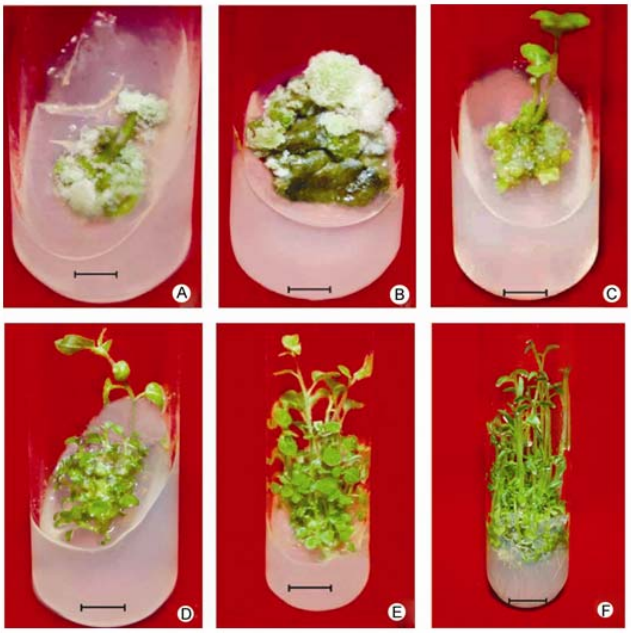

Figure-1: Callus formation from internodal and leaf explants.
A) MS + NAA (2.0 mg/L) + BAP (0.5 mg/L) (bar 1 cm = 0.8 cm); B) MS + NAA (3.0 mg/L) + BAP (0.5 mg/L) (bar 1 cm = 0.9 cm) Indirect shoot regeneration from leaf derived organogenic callus. Initiation of multiple shoots from callus; C) MS + BA (1.0 mg/L) + Kn (0.5 mg/L) + NAA (0.1 mg/L) (bar 1 cm = 0.9 cm); D) MS + BA (2.0 mg/L) + IAA (0.5 mg/L) (bar 1 cm = 0.8 cm) Elongation of multiple shoots; E) MS + BA (3.0 mg/L) + IAA (0.5 mg/L) (bar 1 cm = 1.1 cm); F) MS + BA (3.0 mg/L) + NAA (0.5 mg/L) (bar 1 cm = 1.0 cm)

Plant regeneration from callus

Effect of BAP on indirect shoot regeneration

Well profused callus derived from young leaf explants was sub-cultured on fresh MS medium supplemented with different concentrations of BA alone or in combination with auxins IAA/NAA. After two weeks of subculture, shoot buds were emerged on leaf derived callus surface. In BAP supplemented MS medium at lower concentration of BAP (0.5 mg/L) the frequency of shoot initiation was low (50%), further increase in BAP concentrations (> 0.5 mg/L) enhances the frequency of shoot initiation. High frequency and maximum number (4.2 ± 0.18) of multiple shoots were induced on BA (3.0 mg/L) supplemented medium(Table-2).

Table-2: Effect of different cytokinins (BA / Kn) alone or in combination with IAA / NAA on multiple shoot regeneration from *in vitro* grown callus of *Solanum nigrum*

MS+

Plant Growth Regulator (mg/l)				Regeneration frequency (%)	Mean no. of shoots per callus	Mean shoot length (cm)
BA	Kn	IAA	NAA			
0.5	-	-	-	50	1.4 ± 0.21	3.6± 0.21
1.0	-	-	-	60	3.0 ± 0.28	3.2 ±0.17
2.0	-	-	-	65	3.6 ±0.41	2.8 ±0.18
3.0	-	-	-	70	4.2 ±0.18	2.0 ±0.18
1.0	0.5	0.1	-	60	3.5±0.50	6.4±0.35
2.0	0.5	0.1	-	75	4.0±0.18	5.2±0.34
3.0	0.5	0.1	-	70	5.2±0.34	4.0±0.18
1.0	-	0.5	-	70	6.2 ±0.19	5.4 ±0.34
2.0	-	0.5	-	82	7.6 ±0.35	3.6 ±0.21
3.0	-	0.5	-	90	10.4 ±0.21	3.0 ±0.28
1.0	0.5	-	0.1	70	6.0±0.28	6.0±0.17
2.0	0.5	-	0.1	80	7.4±0.34	5.0±0.28
3.0	0.5	-	0.1	72	8.5±0.50	3.6±0.35
1.0	-	-	0.5	80	9 ±0.26	5.6 ±0.53
2.0	-	-	0.5	92	11.5 ±0.50	4.2 ±0.34
3.0	-	-	0.5	95	17.8 ±0.18	3.8 ±0.17

Observation: After 4 weeks, values are mean ± S.E. of 20 independent determinants

Effect of BAP and NAA/IAA combination on indirect shoot regeneration

The presence of cytokinin along with auxin is necessary for indirect adventitious shoot induction. Maximum number of shoots (17.8 ±0.18) was induced on MS medium supplemented with 3.0 mg/L BAP and 0.5 mg/L NAA, but maximum shoot length was observed on 1.0 mg/1 BAP and 0.5 mg/L NAA (Table-2; Figure-1). In the present study MS medium supplemented with NAA in combination with cytokinin has shown to promote shoot bud differentiation. In IAA and BA supplemented MS medium maximum number of shoots (10.4 ± 0.21) was obtained on 3.0 mg/L BAP and 0.5 mg/L IAA. When two cytokinins such as BA (1.0-3.0 mg/L) and Kn (0.5 mg/L) in combination with the NAA/ IAA (0.1 mg/L) supplemented, the explants showed reduced frequencies (60-80%), less mean number of shoots (3.5-8.5). But maximum mean shoot length (4.0-6.4 cms) was observed (Table-2).

In vitro rooting

Well developed shoots with a length of (3-5cm) were excised and transferred to MS medium supplemented with different concentrations of auxins such as NAA, IBA (0.25 – 1.0mg/l). In IBA supplemented MS medium the number of roots, root length were high compare to NAA supplemented medium. High frequency and maximum number of roots were induced on MS medium supplemented with 0.5mg/l IBA (Table-3;Figure-2).

Table-3: Effect of auxins IBA, NAA on root induction from *in vitro* regenerated shoots of *Solanum nigrum*

Plant Growth Regulator (mg/l)	Frequency of root initiation (%)	Mean no. of roots	Mean shoot length (cm)
IBA			
0.25	80	11.0 ± 0.28	3.2 ± 0.18
0.50	95	18.5 ± 0.17	4.5 ± 0.31
0.75	90	15.4 ± 0.22	3.6 ± 0.35
1.00	76	12.8 ± 0.31	2.8 ± 0.17
NAA			
0.25	80	14.5 ± 0.34	3.0 ± 0.28
0.50	90	16.8 ± 0.17	4.2 ± 0.18
0.75	75	12.4 ± 0.22	2.8 ± 0.17
1.00	65	10.6 ± 0.35	1.6 ± 0.35

Results are mean ± S.E of 20 replicates

Figure-2: *In vitro* rooting and hardening of *Solanum nigrum* (L.)
A) Initiation of *in vitro* roots on MS medium containing 0.5 mg/l IBA (bar 1cm = 0.72)
B) Plantlet showing elongated root system
C) Hardening of plantlet in poly bag containing soil and vermiculite in 1:1 ratio
D) Plantlet in field conditions.

Acclimatization and hardening

Well rooted plantlets were separated from the culture tubes, washed and transferred to polybags containing soil + vermiculite in 1:1 ratio for hardening. Finally the hardened plantlets were transferred to field conditions for maximum survivability (Figure-2).

Discussion

Callus cultures represent clumps of unorganized parenchymatous tissues formed by vigorous proliferation by

the mitotic cell division from the small explants in culture showing no polarity. Callus initiated from the cut portions of the explant, where cells at the cut ends undergo mitosis, which leads to callus formation. It may be due to wound reaction or effect of exogenous growth regulator. The texture of callus varied according to the nature of cytokinin and also on auxin: cytokinin ratio [14].

In 2, 4-D alone supplemented MS medium explants shows brown colour callus formation. Brown colour of the callus showed sensitivity of plant tissues to 2, 4-D. This is in agreement with the findings in *Ipomea aquatica* [17]. The colour is being mainly influenced by the location of phenolic secondary metabolites in cells. If the accumulation of the phenolics in the cytoplasm, it undergoes oxidation and polymerization and oxidized products appear brown [12]. 2, 4-D in combination with BAP supplemented medium light green callus was formed. 2, 4-D along with BA was noted to be a potent hormonal combination for stimulating callus induction. Similar results were reported in *Biophytum sensitivum* [13] using leaf explants, where 2, 4-D in combination with BA induced green compact callus.

Callus initiation was also observed on MS medium supplemented with different auxin such as IAA, NAA in combination with BAP. Where green nodular to organogenic callus was formed. Similar callusing response was noted in *Holostemma adakoidein* [14], *Justicia gendarussa* [1] and *Erythrina variegate* [25]. Lower concentration of NAA and BA induced less amount of callus formation compare to higher concentration. Similar observations were reported in *Asperagus* [23], *Biophytum sensitivum* [13]. Among the different combination of auxin: cytokinin tested, BA and NAA proved to be better interms of inducing high frequency of greenish compact callus formation.

Plant propagation through callus required the induction of organogenic callus. The source of explant origin and its physiological state are critical factors for organogenic callus induction [7]. Callus induction is a prerequisite for adventitious improvement including induction of somaclonal variations and embryoids. In BAP alone supplemented MS medium high frequency of shoot initiation was observed. These results are similar to the findings reported in *Vigna radiate* [27] and *Piper longum* [4]. The presence of cytokinin along with auxin is necessary for indirect shoot induction as noted by Skoog and Miller [26]. In the present study addition of auxins such as IAA/NAA to the culture medium in combination with cytokinin, BA enhances the frequency of shoot initiation and shoot number. These results are in agreement with those in *Rawolfia tetraphylla* [6] and *Withania somnifera* [9], where NAA in combination with cytokinin has shown to promote shoot bud differentiation. Enhanced multiple shoot induction by addition of IAA was also reported in *Solanum trilobatum* [3]. Among the auxins tried (IBA, NAA and IAA) for *in vitro* rooting, the best rooting response was observed on IBA (0.5 mg/l) supplemented medium. Similar *in vitro* rooting response was reported in *Anisochilus carnosus* [8], *Coleus blumei* [21] and *Quisqualis indica* [20].

In the present study BA in combination with NAA exhibited better morphogenesis. These results are in line with previous reports in *Asteracantha longifolia* [18].

Conclusion

Vegetative plant parts especially leaves are desirable explants for *in vitro* improvement because of regeneration from these explants would preserve the genetic homozygosity of the parent genotype. Callus culture system offer many advantages as a model system for several biological investigations. Hence in the present investigation a standardized protocol has been devised for *in vitro* callus induction and regeneration of *Solanum nigrum* from young leaf explants

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References

[1] Agastian P., Lincy Williams and Ignacimuthu S. 2006. *In vitro* propagation of *Justicia gendarussa* Burm. A medicinal plant. Indian Journal of Biotechnology. 5: 246-248.

[2] Akhtar M.S and Munir M. 1989. Evaluation of gastric ulcerogenic effects of *Solanum nigrum, Brassica olevacae* and *Ocimum basillicum* in rats. J.Ethanopharmacology. 27:163-176.

[3] Arokiasamy D.I., Muthunkumar B., Natarajan E. and Johu Britto S. 2002. Plant regeneration from node and inter nodal explants of *Solanum trilobatum* (L.). J. Plant Tiss. Cult., 12(2): 93-97.

[4] Bhat S.R., Chandel, K.P.S. and Malik. 1995. Plant cultivated piper species. Plant Cell Rep., 14: 395- 402.

[5] Datta A. and Srivastava P.S. 1997. Variation in vinblastin production by *Catharanthus roseus* during *in vivo* and *in vitro* differentiation. Phytochemistry. 46:135-137.

[6] Ghosh K.C. and Banerjee N. 2003. Influence of plant growth regulators in vitro propagation of *Rauwolfia tetraphylla* L. *Phytomorphology*. 53 11-19.

[7] Harms C.T., Baktir, I. and Oertil, J.J. 1983. Plant Cell Tiss. Org. Cult., 2:93-102.

[8] Jayachandran R. 2004. *In vitro* culture root formation in *Anisochilus carnosus*. Journal of Swamy Bot. Club. 21:27-30.

[9] Kannan P., Ebenzer G., Dayanadan P., Abraham G.C. and Ignacimuthu S. 2005. Large-scale production of *Withania somnifera* (L.) Dunal using *in vitro* techniques. Phytomorphology. 55: 259-266.

[10] Kim Y.C., Che-Quing Ming, Gunatilaka A.A. and Kingston D.G. 1996. Bio active steroidal alkaloids from *Solanum umbelliferum.* Journal of Natural Products. 59(3):283-285.

[11] Kritikar K.R. and Basu B.S. 1987. Indian Medicinal Plants. 3:1784-1781

[12] Lukas A.M., Christoper D.G., Rebecca A.S. and Virgnia W. 2000. AN9-a petunia glutathione s-transferase required for anthocyanin sequestration, is a flavonoid binding protein. Plant Physiol., 123: 1561-1570.

[13] Shivanna M.B., Vasantha kumari M.M. and Mangala C. 2009. Regeneration of *Biophytum sensitivum* (Linn.) DC. through organogenesis and somatic embryogenesis. Indian Journal of Biotechnology. 8:127-131.

[14] Martin K. 2002. Rapid propagation of *Holostemma ada-kudien* Schult. A rare medicinal plant through axillary bud multiplication and indirect organogenesis. Plant Cell Rep., 21:112-117

[15] Misawa M. 1994. Plant Tissue Culture: an alternative for production of useful metabolites. FAO, Agriculture Services Bull., Rome.87 pp.

[16] Murashige T. and Skoog F. 1962. A revised medium for rapid growth and bioassays with *Tobacco* tissue culture. Physiol. Plant., 15:473-497.

[17] Nagendra Prasad K., Siva Prasad M., Shivamurthy G.R. and Aradhya S.M. 2006. Callus induction from *Ipomoea aquatica* leaf and its antioxidant activity. Indian Journal of Biotechnology. 5:107-111.

[18] Panigrahi J., Behere M., Maharana S. and Mishra R.R. 2007. Bimolecular changes during *in vitro* organogenesis of *Asteracantha longifolia* (L) nees – A medicinal herb. Indian J. Exp. Biol., 45:911-919.

[19] Perez G.R.M., Perez L.A., Garcia D.L.M. and Sosa M.H. 1998. Neuropharmacological activity of *Solanum nigrum* (L.) fruit. J. Ethanopharmacology. 62:43-48.

[20] Poornima D. and Shivamurthy G.R. 1998. Root formation in *Quisqualis indica* (L.). J Swamy Bot. Club. 22:37-38.

[21] Rani G., Talwar D., Nagpal A. and Virk G.S. 2006. Micropropagation of *Coleus blumei* from nodal segments and shoot tips. Biol. Plant., 50(4): 496-500.

[22] Rao S. and Padmaja 1996. Genotypic and explants differences in the establishment of callus and shoot regeneration in chickpea *Cicer arietinum* L. in role of *biotechnology in pulse crops,* edited by Irfan A Khan and S.A. Farook (Ukaaz Publications, India), 159-170.

[23] Sanghamitra Nayak., Sumitra Sen., Nayak S. and Sen S. 1998. Regeneration of *Asparagus robustus* Hort. Journal of Herbs Spices and Medicinal plants. 5(4): 43-50.

[24] Saritha K.V., Prakash E., Swamy P.M. and Naidu C.V. 2003. Indirect shoot regeneration from leaf explants of *Spillanthus acmella* Murr. J. Plant Biol., 30(1): 31-36.

[25] Shasthree T., Madhavi S. and Mallaiah B. 2009. Regeneration of plantlets of *Erythrina variegate* L. by organogenesis. Research Journal of Biotech., 4(3): 30-37.

[26] Skoog F. and Miller C.O. 1957. Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*. Symp. Soc. Exp. Biol., 11: 118- 131.

[27] Srinatha Rao., Prabhavathi Patil and Kaviraj C.P. 2005. Callus induction and organogenesis from various explants in *Vigna radiate* (L.). Indian Journal of Biotechnology. 4:556-560.

[28] Yoganath R., Bhakyaraj R., Chanthuru A., Parvathi S. and Palanivel S. 2009. Comparative analysis of Salosodine from *in vitro* and *in vivo* cultures of *Solanum nigrum.* Kathmandu University. J. Sci. Eng. Tech., 5:99-103.