Tissue Culture Study of Eclipta alba for Induction of Multiple Shoots on Nodal Explants.

Ritika Kumari
Research Scholar (M.Sc., Ph.D. in Botany),

University Department of Botany, B.R. Ambedkar Bihar University,
Muzaffarpur, Bihar 842001, (India)

ABSTRACT:

In the present study single nodal explant of *Eclipta alba* was inoculated in Murashige and Skoog (MS) medium supplemented with different concentrations of N_6-Benzyl amino purine and kinetin alone or with α-naphthalene acetic acid. It was noted that MS basal medium supplemented with different concentrations of BAP or KN alone induced shoot buds on the nodal explants of *Eclipta alba*, but when the nodal explants were inoculated in BAP+ 0.25 mg/l NAA, the percentage response for shoot bud initiation was 94.38, the number of shoot buds per explant 11.32 and length of the shoot buds after 3 weeks was 6.12 cm. Similarly, explants were inoculated in MS+1.5 mg/l KN+ 0.25 mg/l NAA; the percentage of response for shoot bud initiation was 96.42. The number of shoot buds 12.72 and the length of the shoot buds per explant was 6.74 cm. When nodal explants were inoculated in MS+ 1.0 mg/l BAP alone the highest percentage of response was 88.25, the number of shoot bud per explant 9.56, and mean length of the roots 5.45. Similarly, nodal explants were also inoculated in MS + 1.5 mg/l KN alone, the highest percentage of response was 90.24, the number of shoot buds 10.15, and the mean shoot length 4-84 cm respectively. Well grown plantlets were used for rooting *in vitro*. Plantlet was inoculated in MS + different concentrations of IBA and IAA separately. Maximum 88.74 percentage of response for root initiation was observed in MS + 1.5 ppm IBA; Here the mean number of roots per plantlet was 7.54 and mean length 4.25. The highest percentage 77.82 for root induction was noted in the plantlets when inoculated in MS + 1.5 ppm IAA. Here the mean root number was 6.38 and mean length 3.82 cm. Therefore, MS + 1.5 mg/l KN + 0.5 mg/l NAA was best culture condition for shoot bud initiation, while MS + 1.5 ppm IBA was the best medium for root initiation in the plantlets raised *in vitro*.

KEY WORDS:

Tissue culture, *Eclipta alba*, Shoot bud, Nodal explants, Inoculation, Rooting medium

INTRODUCTION:

Eclipta alba (L.) Hassk, of the family *Asteraceae* is an important medicinal herb, used by the common rural people for the treatment of different diseases. This herb grows near the bank of drains, or other moist places, even in dry condition can be recognized among the other herbs due to its white flowers and luster green rough leaves. Rural people use the extracts of the plant to cure their wounds, new cuts, and liver ailments as suggested by the Vaidya. This juice is also rubbed on the scalp for hair growth. For its medicinal values, different workers have provided experimental

evidence such as Thyagrajan (1991) as an antifungal agent, Franca *et al*; (1995) reported that it acts as an antihepatotoxic agent. Ananthi *et al*; (2003) reported antihypoglycemic activity of the plant. Venkatasan and Ravi (2004) reported that it has antifungal activity. Datta *et al*; (2009) reported that extracts promote hair growth. Sunita *et al*; (2010) the extracts have antibacterial activity. Chaudhary *et al*; (2011) reported the anticancer activity of the plants. The main active principles of *E. alba* are Wedelolactone and dimethyl wedelolactone (Franca *et al*; 1995).

Micropropagation of medicinal plants has been reported by different workers. Some of them are being mentioned here. Arora and Bhojwani (1989) reported micropropagation of *Saussurea lappa* an endangered medicinal plant. Thyagarajan and Kumar (1991) reported its application. Bhuyan *et al*; (1997) reported micropropagation of *Murraya koenigii* an important medicinal plants. Venkatasan and Ravi (2004) in *Eclipta alba*; Jain *et al*; (2003) in *Rauwolfia serpentina*; Gawde and Paratkar (2004); in *Eclipta alba*; Neeti and Kothari (2005) in *E. alba*; Bhaskaran and Jayabalan in *E. alba*; Devnath *et al*; (2006); isolated bioactive compounds from micropropagated plantlets; Khale Kuzzaman *et al*; (2008) in *Adhatoda vasica*; Baber *et al*; (2009) in *Crataeva nurvala*; Biswas *et al*; (2009) in *Boerhaavia diffusa*; Karthikeyan *et al*; (2009) in *Centella asiatica*; Ling *et al*; (2009) in *Mirabilis jalapa*. Hussain *et al*; (2010) in *Mimosa pudica*; Sharma *et al*; (2013) in *E. alba*. Sharma *et al*; (2014) in *E. alba*, Yesmin *et al*; (2015); Bhoyer *et al*; (2015) in *Withania somnifera*.

With the incredible increase in the requirement for natural herbal drugs due to their negligible side effects, the demand for medicinal plants as raw materials has also increased many folds. Due to this there is brutal harvesting of such plants from their natural habitat. Further the urbanization and industrialization have also reduced the habitat of these valuable plants. The best alternative to supply the raw materials as per demand as well as to conserve these medicinal plants in their natural population is the *in vitro* micropropagation. Through this technique, a large number of plants may be produced within a limited period. *Eclipta alba* growing in natural habitat is also facing threats due to aforesaid reasons. Keeping these ideas in mind this work was done to produce micro-shoots from the internodal segment of *E. alba*.

MATERIALS AND METHODS:

Eclipta alba growing on the campus of the University Department of Botany, was located and identified with the help of the flora and my teacher. The healthy plants were then collected from the natural habitat. Both the lower and upper parts of the plant were dissected out. Similarly, leaves were pruned. The stem was first washed in the running tap water for 40 minutes. It was also treated with Tween 20, and again rinsed with tap water for 15 min. The stem was cut into small pieces with a sharp blade so that each piece had at least one node. Further treatment was done in the aseptic condition of the laminar airflow cabinet. All the segments prepared above were treated with 50% Ethyl alcohol for 70 sec, followed by treatment with 0.1% $HgCl_2$ for 3-4 minutes. The nodal segments were taken out and rinsed with pre-sterilized distilled water for 3-4 times so that

even the trace of chemical was washed away completely.

Preparation of Culture medium:

The required amount of all the ingredient of Murashige and Skoog (1962) basal medium were taken from the stock solutions prepared earlier. To the above 3% sucrose and different concentrations of plant growth regulators here, BAP, KN alone, and with NAA were added. The pH was adjusted to 5.8, before adding 0.8% agar in molested condition. 20 ml of each medium was dispensed in 125 cc culture flasks. The flasks were plugged with cotton plugs, covered with the muslin cloth. All plugs were wrapped with aluminum foil to avoid wetting during autoclaving. All culture medium containing flasks were autoclaved at 121^0C for 15-20 minutes. Flasks were taken out allowed to cool at room temperature and stored at low temperature for 3 days. Then they were used for inoculation. Flasks showing contamination were discarded after autoclaving. Inoculation was done in the aseptic condition of the laminar airflow cabinet. All the precautions were taken during inoculation to avoid any contamination. Inoculated culture flasks were incubated in the culture room, where the temperature was maintained at 26 ± 1^0C, humidity at 66-68%, and light, supplied by white fluorescent tube light (Philips) with 3000 lux. The photoperiod was 16 hrs. Cultures were watched on an alternate day; infected cultures were taken out and discarded after autoclaving. Observations were made for, percentage of response for shoot bud initiation, time taken for shoot bud initiation, number and length of the shoot buds, etc. All the experiments were done in triplicate and at a time 20 culture were used for one concentration of plant growth regulators. Data collected after taking the mean were presented in table-1. Well grown plantlets were used for rooting in the separate medium. Here also observation sere made for the different parameters as mentioned above.

RESULTS AND DISCUSSION:

Data related to shoot buds initiation, their growths, etc. were presented in table-1. From the table, it may be noted that MS+ six (0.25, 0.5, 1.0, 1.5, 2.0, 2.5 ppm) different concentrations were inoculated with single nodal explants. Here the highest percentage of response 88.25, the maximum number of shoot buds 9.56, and the maximum lengths of shoot buds per node was 5.45 cm respectively. This was followed by 82.56 percentages of response 7.28 number of shoot buds and 4.28 cm length of the shoot buds per explants, respectively. Similarly, explants were inoculated in MS + six different concentrations of KN. Here the highest 90.24 percentage of response, 8.1 number of shoot buds and 4.84 cm l,engths of shoot buds at each node were observed in MS + 1.5 mg/l KN. This was followed by explants inoculated in MS + 1.0 mg/l KN, where the percentage response for shoot bud initiation was 83.42, the number of shoot buds 5.66, and length of shoots per node 4.12.

Nodal explants were also inoculated in MS + BAP 1.0 mg/l + 0.1 to 2.0 mg/l NAA. Here the highest percentage of response 94.38, the maximum number of shoot buds 11.32, and the length of the shoot buds per node 6.12 cm were observed. This was followed by the nodal explants inoculated in MS+ 1.0 mg/l BAP + 0.50 mg/l NAA. Here the percentage of response was 88.52, the number of shoot buds 7.60 and the length of

the shoot buds at each node 7.60, and mean length 5.18 cm respectively. Similarly, nodal explants were also inoculated in MS+ 1.5 mg/l KN + 5 different concentrations of NAA. Here the highest percentage of response 96.42, the highest number of shoot buds 12.72, and the length of the shoot buds per node were 6.72 in MS + 1.5 mg/l KN + 0.25 mg/l NAA respectively. This was followed by the nodal explants inoculated in MS + 1.5 mg/l KN + 0.5 mg/l NAA, where the percentage of response was 92.16, the number of shoot buds 10.64, and the length of the shoot buds at each node 5.22 cm. Here MS medium without growth regulators was used as control. Here the percentage of response was 32.42, number of shoot buds 2.16, and length of the shoot buds per node 2.64 cm respectively.

Rooting of the *in vitro* raised plantlets:

For the induction of roots in the *in vitro* raised plantlets, MS medium was supplemented with six (0.25, 0.5, 1.0 1.5, and 2.0 ppm) of IBA and IAA separately. Data obtained with respect to the percentage of response, number root per plants and root length per shoot were presented in table-2. From the table, it was noted that the highest percentage of response 88.74 for rooting, the maximum number of roots 7.54, and lengths of roots per explant 4.25 cm were found in MS + 1.5 ppm/l IBA. This was followed by the percentage of response 72.32, the number of shoots 5.42 and length 3.80 cm in MS + 2.0 ppm/l IBA respectively. The highest percentage of response 77.82, the maximum number of roots. 6.38 and maximum length of 3.82 cm of roots per explants were obtained in MS + 1.50 ppm/l IAA. This was followed by the maximum percentage of response 66.54, the maximum number of shoots 4.26, and root length 3.25 in MS + 2.0 mg/l IAA respectively. MS medium without growth regulators acted as control where the percentage of response for rooting was 26.54, the number of roots 2.34, and root length 2.25 respectively.

DISCUSSION:

From the table, it was observed that Internodal explants responded in MS + BAP or KN at different concentrations but among the cytokinin KN alone or along with NAA was more suitable in comparison to BAP on the similar concentrations on combinations. When BAP was used, 1.0 mg/l concentration was significantly higher than other concentrations of BAP. Similarly, when MS + KN alone was used 1.5 mg/l was better than that of the rest concentrations. Siddique and Anis (2008); Saha *et al*; (2016); and Thockom and Maitra (2017) have obtained similar results. Therefore, the present findings an in agreement with the above findings. Sharma *et al*; (2013) also reported that the synergistic effects of cytokinin auxins are better than that of the cytokinin alone. Similar reports have also been given by Gawde and Paratkar (2004); in the case of *Eclipta alba*, Bansali and Kumar *et al*; (2018) are of the opinion that for micropropagation either BAP or Kinetin may be used but when used with auxins either IAA or IBA, it gives better results. Therefore, present findings corroborate with the above findings.

In the present work, maximum rooting was found when the plantlets were inoculated in MS + 1.5 ppm IBA. Here the root induction was in 88.74% of the plantlets inoculated. The maximum number of roots 7.54 and a root length of 4.25 cm was noted. Similar results in different medicinal plants

including *Eclipta alba* have also been reported by Bhashkaran and Jayabalan (2005); Rooting on medium containing IBA was also observed by several workers indifferent medicinal plants species. Such as Komalavalli and Rao (2000) in *Gymnema sylvestre*, Sivanesan and Jeog (2007) in *Pentanema indicum* Ling, Shahin-Uz Zaman *et al*; (2009) in *Azadirachta indica*.

CONCLUSION:

In the present study, multiple shoots from nodal explants in MS medium supplemented with six different concentrations of BAP and KN were used separately. The concentrations of BAP and Kinetin at which maximum results were obtained were supplemented with found different concentrations of IBA and IAA. It was noted that MS + 1.0 mg/l BAP + 0.25 mg/l NAA gave the best results while MS + 1.5 mg/l KN + 0.25 mg/l IBA was found more superior in all respects. This protocol may be exploited at a commercial scale so that the desired amount of *Eclipta alba* plants may be supplied as raw materials to the different pharmaceutical companies.

ACKNOWLEDGEMENT:

The author is grateful to the Head, Department of Botany, B.R.A. Bihar University for providing library and laboratory facilities during this research work.

TABLE-1

Showing impact of plant growth regulators on % response, number of shoot buds, length of shoot buds on nodal explants of *Eclipta alba* in MS medium after three weeks.

Plant Growth Regulator (ppm)			Impact *in vitro*		
BAP	KN	NAA	% response for shoot bud initiation	No. of shoot buds per explant	Shoot length (cm)
0.0	0.0	0.0	32.42	2.16	2.64
0.25	--	--	54.28	3.62	3.66
0.50	--	--	72.46	6.45	4.54
1.0	--	--	88.25	9.56	5.45
1.5	--	--	82.56	7.28	4.28
2.0	--	--	68.32	5.72	3.72
2.5	--	--	56.18	4.24	2.84
--	0.25	--	58.74	4.36	3.18
--	0.50	--	77.65	4.88	3.72
--	1.0	--	83.42	5.66	4.12
--	1.5	--	90.24	10.15	4.84
--	2.0	--	74.36	6.74	3.52
--	2.5	--	62.15	5.12	2.45
1.0	--	0.10	89.75	9.88	4.34
1.0	--	0.25	94.38	11.32	6.12
1.0	--	0.50	88.52	9.54	5.80
1.0	--	0.75	84.65	7.60	5.18
1.0	--	1.0	81.24	7.15	4.24
--	1.5	0.10	91.72	8.38	4.88
--	1.5	0.25	96.42	12.72	6.74
--	1.5	0.50	92.16	10.64	5.22
--	1.5	0.75	86.30	7.58	4.56
--	1.5	1.0	72.52	7.46	3.85

BAP = 6, Benzyl Amino Purine
KN = Kinetin
NAA = Naphthalene Acetic Acid

TABLE-2

Impact of auxins supplemented in MS medium on root initiation, root numbers, root length on the *in vitro* raised plantlets of *Eclipta alba*.

Plant Growth Regulator (ppm)		Impact on root initiation		
IBA	IAA	% response for root initiation	No. of root per shoot	Root length/shoot
0.0		26.54	2.34	2.25
0.25		48.36	4.28	2.88
0.5		62.22	5.62	3.72
1.0		76.45	5.88	3.88
1.5		88.74	7.54	4.25
2.0		72.32	5.42	3.80
	0.25	41.65	3.76	2.58
	0.5	56.72	4.82	2.86
	1.0	68.34	5.16	3.45
	1.5	77.82	6.38	3.82
	2.0	66.54	4.26	3.25
IBA = Indole Butyric Acid IAA = Indole Acetic Acid				

REFERENCES:

Ananthi J., Prakasan A. and Pugalendi K.V. (2003):
Antihyperglycemic activity of *Eclipta alba* leaf on alloxan- induced diabetic rats.
Yale J. Biol. Med. 76: 97-102.

Arora R., Bhojwani S.S. (1989):
In vitro propagation and low temperature storage of *Saussurea lappa* C.B. Clark, an endangered medicinal plant.
Plant Cell Rep. 8: 44-47.

Babar S.B., Walia N. and Kaur A. (2009):
Large scale *in vitro* multiplication of *Crataeva nurvala* Meth.
Mol. Biol. 547: 61-70.

Bhansali Shikha and Ashwani Kumar (2018):
In vitro studies on callus induction of *Eclipta alba* (L.) Hassk: An important medicinal plant of traditional medicine.
J. Biol. Sci. Opin. 6(3): 66-71.

Bhaskaran P. and N. Jayabalan (2005):
An efficient micropropagation system for *Eclipta alba* a valuable medicinal plants.
In vitro Cell and Devel. Biol. Plant. 41: 532-539.

Bhoyar, P.I., M.P. Suryavanshi & V. Krishnasamy (2015):
Callus induction and establishment of suspension culture from *Withania somnifera*: The Indian Zinseng.
The Bioscan 10(4): 1567-1571.

Bhuyan, A.K., Pattanik S., Chand, P.K. (1997):
Micropropagation of curry leaf tree (*Murraya koenigii* (L.)) by axillary proliferation using intact seedling.
Plant Cell Rep. 16: 779-782.

Biswas, A., Bari, M.A., Roy M. and Bhadra S.K. (2009):
Clonal propagation through nodal explants culture of *Boerhaavia diffusa* L. A rare medicinal plant.
Plant Tissue Cult & Biotech 19(1): 53-59.

C. Raghvendran, D. Kamalanathan and D. Natrajan (2014):

A rapid propagation of nodal explants of *Eclipta alba* (L.) a multipurpose medicinal herb.
Research in Biotechnology 5(2): 6-12.

Chandran, Hema, M. Meena, Barupal T., and Kanika Sharma (2020):
Plant tissue culture as a perpetual source for production of industrially important bioactive compound.
Biotechnology Report 26: 1-9

Chaudhary, H., Dhuma V., Singh J., Kamboj S.S. and S. Seshadari (2011):
Evaluation of hydro alcoholic extract of *E. alba* for its anticancer potential, an *in vitro* study.
J. Ethnopharmacol. 136: 363-367.

Chen, C.C., Chen S.J., Sagere A.P. and Tsay H.S. (2001):
Adventitious shoot regeneration from stem internode explants of *Adenophora triphylla* (Thumb) A. (*Campanulaceae*) an important medicinal herbs.
Bot. Bull. Aead. Sin . 42: 1-7.

Datta R., Singh A.T., Mukherjee A., Bhatt B., Ramesh B. and A.C. Burman (2009):
Eclipta alba extract with potential for hair growth promoting activity.
J. Ethnopharmacol. 124: 450-456.

Devnath M., Malik C.P. and Besen P.S. (2006):
Micropropagation tool for production of high quality plant based medicine.
Curr. Pharm. Biotechnol. 7(1): 33-49.

Franca, S.C., BErtoni B.W., Pereis AMS (1995):
Antihepatotoxic agent in micro propagated plantlets of *Eclipta alba*.
Plant Cell Tissue Organ Cult. 108(3).: 455-464

Gawde, A.J., Paratkar G.T. (2004):
Micropropagation of *Eclipta alba* Hassk: an approach to shorten the protocol.
Indian J. Biotechnol. 3: 128-132.

Hussain, M.K., and Anis M. (2006):
Rapid *in vitro* propagation of *E. alba* (L.) through high frequency axillary shoot proliferation.
Aeta Physiologial Plantarum 28(4): 325-330.

Hussan, A.K.M.S., Sultana Ra., Jahan M.A.A. and Khatun R. (2010):
In vitro mass propagation of *Mimosa pudica* using shoot tip and nodal explant.
Bangladesh J. Sci. Ind. Res. 45(2): 95-100

Jain, V., Singh, D., Saraf S and Saraf. S. (2003):
In vitro micropropagation of *Rauwolfia serpentina* through multiple shoot generation.
Ancient Science of Life 23(1): 44-49.

Karthikeyan, K., Chander C. and Kulothumgan (2009):
Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Centella asiatica*.
Indian J. Biotechnology 8: 232-235.

Khalekuzzaman, M., Rahman M.S., Rashid M.H. and Hossain M.S. (2008):
High frequency *in vitro* propagation of *Adhatoda vasica* Nees through shoot tip and nodal explants culture.
J. Bio. Sci. 16: 35-39.

Komalvalli, N. and Rao M.V. (2000):
In vitro micropropagation of *Gymnema sylvestre* A multipurpose medicinal plant.
Plant Cell Tiss. Organ Cult. 61(2): 97-105

Ling A.P.K., Tang K.Y., Gansau J.A., Husein S. (2009):
Induction and maintenance of callus from leaf explants of *Mirabilis jalapa* L.
Medicinal and Aromatic Plant Sci. Biotech. 3(1): 42-47.

Murashige T. and Skoog F. (1962):
A revised medium for rapid growth and bioassay with tobacco tissue cultures.
Physiol. Plant. 15: 473-497.

Neeti, D. and S.L. Kothari (2005):
Micropropagation of *Eclipta alba* (L.) Hassk: An important medicinal plant.
In vitro Cellular and Develop. Biol. Plant 41: 658-661.

Prakash P., D. Sharumathy, Sweta Sunki, D.Nand Gopal & G. Narendra Kumar (2015):
Micropropagation of *Eclipta alba* using humic acid as media component.
Plant Archieves Vol. 15(1): 181-185.

Saha, S. and Roy S., Sengupta C. and Ghosh P.D. (2014):
Micropropagation and analysis genetic stability in regenerated plantlets of *Ocimum canum*.
Ind. J. Pl. Physiol. 19: 179-183.

Sen, M.K., S. Nasrin, S. Rahman, Jamal A.H.M. (2014):

In vitro callus induction and plantlet regeneration of *Achyranthes aspera* L., a high value medicinal plant. *Asian Pac. J. Trop. Biomed*. 4(1): 40-46.

Sharan, A.K., Dubey S.R., Kumar R. Chandra V., Singh B.P., Kumar G., Kumari S. (2014):
A novel protocol for propagation of *Eclipta alba* (L.) Hassk.
Int. J. Pure Appl. Bioscience 2:137-141.

Sharma A., Bhansali S., and Kumar A. (2013):
In vitro callus induction and shoot regeneration in *Eclipta alba* (L) Hassk.
Int. J. of Life Sci. and Pharma Res. 3(2): 43-46.

Siddique, I. and Anis M. (2008):
An improved plant regeneration system and ex vitro acclimatization of *Ocimum basilicum* L.
Aeta Physiol. Pl. 30: 493-499.

Sivasankari S., A. Lavanya and R. Karthic (2015):
Establishment of *in vitro* aseptic culture of *Eclipta alba* (L.) Hassk-a traditional hair growth promoting plant.
Int. J. of Adv. Res. in Biol. Sciences 2(10): 1-6.

Sunita, D., Kataria S.K., Sastry K.V. and S.V.S. Rana (2010):
Phytochemical screening of methanolic extract and antibacterial activity of active principles of Hepatoprotective Herb, *Eclipta alba*.
Ethanol. Leaflets 14: 248-258.

Thirupathy, S., Sisubalan, N., & Ghouse B.M. (2014):
Callus induction from wild medicinal plant. *Tephrosia hookekiana* W & ARN.
Int. J. of Recent Scientific Research. 5(6): 1027-1030.

Thyagarajan R.K. and Krishna Kumar N.R. (1991):
Antifungal activity of some compound isolated from *Eclipta alba* Hassk, and *Wedelia trilobata* L.
Geobios 26: 91-94.

Venkatasan S. and R. Ravi (2004):
Antifungal activity of *Eclipta alba*.
Ind. J. Pharm. Sci. 66: 97-98.

Yesmin, S.A., Hashem and M.S. Islam (2015):
Micropropagation of an important medicinal herb. *Eclipta alba* (L.)
Univ. J. Biol. Sci. 4(1): 61-69.