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MICROPROPAGATION OF *ECLIPTA ALBA* USING HUMIC ACID AS MEDIA COMPONENT

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Abstract

The potential of humic acid as a growth supplement in micropropagation of *Eclipta alba* is reported in the present investigation. *Eclipta alba* explants were grown in MS media with BAP and Kinetin as growth factors and it has been observed that BAP was more effective for *in vitro* regeneration of the explants. On the other hand, when humic acid was supplemented along with the growth factors, there is an increase in the shoot proliferation within a reduced period of time. The optimum concentration of humic acid required was found to be 300 mg/100 ml for the effective growth of explants. This study therefore supports the use of humic acid as a suitable growth supplement in micropropagation studies.

Key words : Media component, micropropagation, progeny plants, tissue culture, *Eclipta alba*.

Introduction

The contemporary world of research has seen an enormous interest in the *in vitro* regeneration of plants especially those which are medicinally and economically important. This is because of the remarkable increase in the demand for natural herbal drugs as they have fewer side effects. Also, the depletion of natural resources due to urbanisation and industrialisation made their survival vulnerable that has made plant researchers opt for a technique, which could bring about large scale multiplication of plants using a simple, rapid and genetically stable method (Sharma *et al.*, 2013).

Micropropagation offers a feasible method of swiftly multiplying the plant stock material producing large number of progeny plants using plant tissue culture techniques. As this technique uses explants as the raw material, minimal quantity of the plant stock material is required hence making this a much sought after procedure.

Eclipta alba is one such medicinally important herb that posses various functions. This is used as dyeing agent for blackening of hair and also prevents premature greying. It is well known as an anti-aging agent in Ayurvedic medicine. It is also known to display anti hepatotoxic activity (Wagner *et al*., 1986; Franca *et al*., 1995), antimicrobial activity, antipyretic, antioxidant, immunomodulatory and nootropic properties (Ragavendran *et al*., 2014).

Humic acid, an organic compound which is a product of biotic or abiotic degradation of dead plant and animal material helps to makes the soil fertile and productive, supply nutrients to plants, increases water retention capacity and promotes seed germination (Salman *et al*., 2005). In this context, the present study was carried out to utilize humic acid along with the required growth factors for micropropagation of *Eclipta alba* to understand its influence on the growth of the explants thereby assessing the potential of humic acid as a nutritional supplement for micropropagation studies.

Materials and Methods

Collection and treatment

Eclipta alba plants were collected from the campus of Sathyabama University, Chennai (Tamil Nadu), India. Shoot tips were dissected out and the nodal segments were initially washed with Tween 20 and kept under running tap water for 15 min. Further sterilization procedures were done in a laminar airflow cabinet, which consisted of 50% alcohol treatment for 70 sec followed by 0.1N $HgCl_2$ for 4 min. Two washings of sterile distilled water were given after each of the above mentioned steps.

Humic acid production

Humic acid is extracted as per the standard

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procedures given in International Humic Substances Society (IHSS). Crude Humic acid is used for the experiment. 10 gm of coal (leonardite) sample was weighed, ground and then passed through a sieve to get the fine particles. This was then treated with 100 ml 4% potassium hydroxide and mixed thoroughly and ensured that it is completely dissolved. Water soluble salt of humic acid thus formed is filtered through a Whatman filter paper No.42 to separate it from insoluble residues. The pH is adjusted to 2 using conc HCl. The humic acid will be precipitated at the bottom of the flask and is used for further experiments.

Medium and culture conditions

The explants were placed on sterile blots and were inoculated in 25 × 150 mm glass culture tubes containing MS medium (Murashige and Skoog, 1962) with 3% sucrose and supplemented with various concentrations of 6 - benzyladenine purine (BAP) and kinetin (KIN) to identify the optimum concentration for the shoot formation. Further set of experiments were carried out to grow the explants using the optimal concentrations of BAP and KIN along with humic acid (pH of the medium adjusted to 5.7) to study the effect of humic acid on shoot initiation. The medium was solidified with 0.7% agar and sterilized by autoclaving at 121°C and 15 lb pressure. The cultures were incubated at 25±2°C, 16 hr photoperiod was provided using cool fluorescent tubes with 3000 lux light intensity. After 14 days the response of the explants in terms of shoot formation and number of leaves grown was observed.

Results and Discussion

Two sets of experiments were carried out separately where in *Eclipta alba* explants were used for micropropagation using BAP and Kinetin as growth factors supplemented in MS media. The explants were grown using BAP in different concentration (0.5 to 2.5 mg/L) for a period of 14 days to identify the optimal amount required for the growth. It was observed that a concentration of 1.5 mg/L was found to be ideal for maximum shoot proliferation with maximum number of leaves (table 1). As the concentration increased beyond 1.5 mg/L, there is a significant decrease in shoot proliferation.

Similarly, Kinetin was also used for the growth of the explants in different concentrations (0.5 to 2.5 mg/L) and the results were provided in table 2. It was observed that maximum proliferation was noticed at 1.0 mg/L Kinetin.

In the present study, it was noticed that BAP supplemented MS media was found to be favourable for explants growth compared to that of Kinetin supplemented MS media. Growth response was noticed within 14 days with significant shoot proliferation (80%) while the shoot proliferation was only 50% with Kinetin thereby suggesting that BAP is more suitable for the growth of *Eclipta alba* explants. Another study carried out by Baskaran and Jayabalan (2005) also demonstrated that BAP was more effective in bringing about the growth of the explants compared to that of Kinetin when *Eclipta alba* explants were grown in MS medium supplemented with BAP and Kinetin. These results corroborate with the results obtained by Indra and Upendra (2000) and Thiruvengadam and Jayabalan (2000) where in explants of Indian strawberry and *Dalbergia* respectively also showed significant shoot growth with BAP as the growth factor. Moreover, higher concentrations of the cytokinins resulted in the reduction of shoot growth which is further supported by studies conducted by Hu and Wang (1983) and Baskaran and Jayabalan (2005).

Further, study was conducted by supplementing the media along with the growth factors BAP and Kinetin with different concentrations of humic acid to understand its effect on the growth of explants and its suitability as a growth supplement in micropropagation techniques.

Two sets of experiments were carried out separately by supplementing MS media along with optimized concentration of BAP and Kinetin along with different concentrations of humic acid (0.1 - 0.5 mg/100 ml) and the results are provided in tables 3 and 4.

Interesting results were obtained where it was observed that humic acid was found to influence the shoot proliferation and time of response positively (Fig 1). The explants in MS media with BAP and humic acid were found to show shoot proliferation (90%) similar to that of BAP without humic acid but this response was observed within 11 days unlike the former case where in the growth response was observed after 14 days. The optimal concentration of humic acid was identified to be 300 mg/100ml. This result clearly indicates that humic acid is showing a positive influence in the growth of the explants.

In other case, where MS media is supplemented with kinetin and different concentrations of humic acid, a remarkable increase was noticed in the shoot proliferation rate 80% (from 50%, media with Kinetin without humic acid) within 8 days thereby pointing the profound influence of humic acid on the growth of the explants and time period. These results are supported by the results obtained by Goenadi and Sudharama (1995) which also stated that shoot/root initiation was significantly fastened in the

Table 1 : Standardization of BAP for *Eclipta alba.*

S. no.	BAP (mg/l)	Number of explants inoculated	Percentage of shoot proliferation (%)	Average no. of shoots	Average no. of leaves	Shoot proliferation response (days)
1	0.5	10	40	1.0±0.2	2.0±0.3	14
2	1.0	10	50	1.6±0.3	7.6±0.4	14
3	**1.5**	**10**	**80**	**4.0±0.3**	**16±0.2**	**14**
4	2.0	10	60	2.0±0.2	1.3±0.3	14
5	2.5	10	30	1.0±0.2	1.6±0.2	14

Results represent mean ± SD of three replicated experiments.

Table 2 : Standardization of kinetin for *Eclipta alba.*

S. no	Kinetin (mg/l)	Number of explants inoculated	Percentage of shoot proliferation (%)	Average no. of shoots	Average no. of leaves	Shoot proliferation response (days)
1	0.5	10	30	2±0.5	2.6 ±0.4	10
2	**1.0**	**10**	**50**	**3±0.2**	**5.3±0.2**	**10**
3	1.5	10	30	2±0.4	4.6±0.3	10
4	2.0	10	20	2±0.3	2.6±0.2	10
5	2.5	10	20	1±0.5	2.8±0.4	10

Results represent mean ± SD of three replicated experiments.

Table 3 : Standardization of BAP and humic acid for *Eclipta alba.*

S. no.	BAP (1.5 mg/l) + humic acid (Concentration %)	Number of explants inoculated	Percentage of shoot proliferation (%)	Average no. of shoots	Average no. of leaves	Shoot proliferation response (days)
1	HA - 0.1	10	60	3.0±0.4	14±0.4	11
2	HA - 0.2	10	80	5.0±0.3	17±0.5	11
3	**HA - 0.3**	**10**	**90**	**6.3±0.2**	**22±0.3**	**11**
4	HA - 0.4	10	70	2.6±0.3	5.3±0.5	11
5	HA - 0.5	10	60	3.3±0.2	9.3±0.2	11

Results represent mean ± SD of three replicated experiments.

Table 4 : Standardization of kinetin and humic acid for *Eclipta alba.*

S. no.	Kinetin (mg/l) + Humic acid (%)	Number of explants inoculated	Percentage of shoot proliferation (%)	Average no. of shoots	Average no. of leaves	Shoot proliferation response (days)
1	HA - 0.1	10	70	2.3±0.5	8.0±0.4	8
2	**HA - 0.2**	**10**	**80**	**2.6±0.2**	**9.0±0.3**	**8**
3	HA - 0.3	10	70	2.6±0.2	7.3±0.3	8
4	HA - 0.4	10	70	2.6±0.3	7.6±0.5	8
5	HA - 0.5	10	60	2.0±0.4	6.0±0.2	8

Results represent mean ± SD of three replicated experiments.

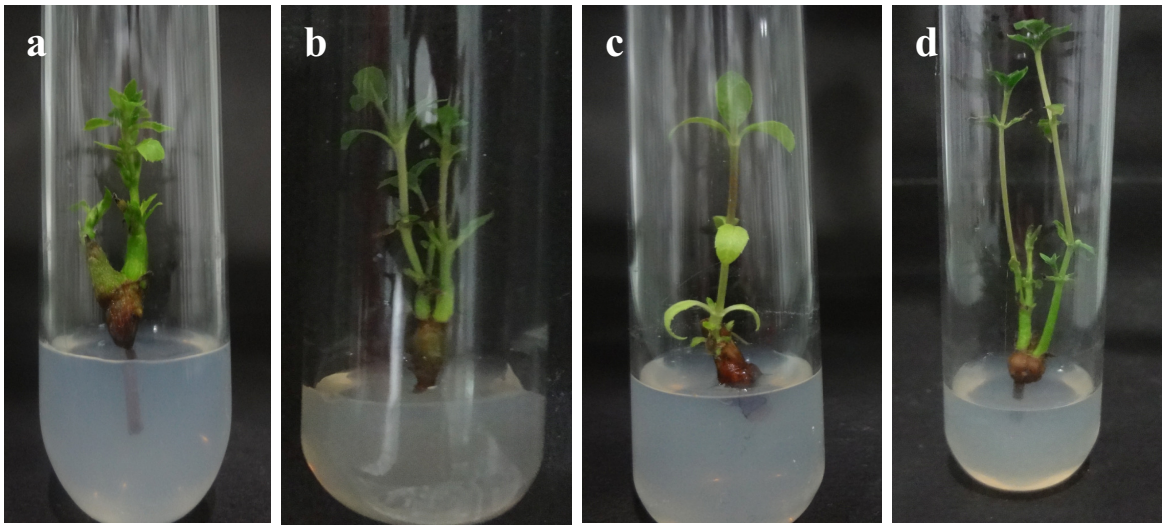

Fig. 1 : Shoot germination in *Eclipta alba* **a)** only BAP, **b)** BAP with 0.3% humic acid, **c)** only Kinetin, **d)** Kinetin with 0.3% humic acid.

presence of humic acid for the explants of *Gnetum gnemon*, *Elletaria cardamomum* and *Pogostemon cablin*. Similarly, another study carried out also used humic acid in the media along with nicotinamide and the results obtained were favourable by significantly increasing all morphological criteria of growth (El-Bassiouny *et al.*, 2014). The potential of humic acid has been further studied by Prakash *et al.* (2010), where in humic acid was extracted using chemical and biological methods using alkaline solution (KOH or NaOH) and *Trichoderma viride* respectively from lignite. The chemically produced humic acid was successfully used to grow *Spirulina plantensis,* and germination of seeds of *Raphanus sativus* (Prakash *et al.*, 2014a, b). Studies were also conducted on the growth of *Pleurotus florida* (Prakash *et al.*, 2011) was studied in terms of the biomass produced, growth rate of *Stevia rebaudiana* (Prakash *et al.*, 2012) and *Morus alba* (Prakash *et al.*, 2013) using humic acid in the form of potassium humate. Further, studies were carried out to optimize the media for the production of humic acid using RSM methodology and study its effect on the growth parameters of *Vigna mungo* (Prakash *et al.*, 2014). The potential of humic acid as seaweed liquid fertilizer on the growth of *Arachis hypogaea* was evaluated (Prakash *et al.*, 2014) and it was noticed that there is significant improvement on the growth, biochemical and yield characteristics of groundnut in presence of humic acid. In view of the potential benefits offered by humic acid, this study reports the possible utilization of humic acid in micropropagation studies as a supplement for growth along with other required growth factors in efficient in vitro regeneration of plants through tissue culture as a means for crop improvement.

Conclusion

In vitro regeneration of commercially and medicinally important plants for their products is the need of the day. The present study reports the utilization of humic acid as a growth supplement for the *in vitro* regeneration of *Eclipta alba* explants. The results showed that the initiation period of the shoots has significantly shortened in presence of humic acid along with the growth factors BAP and Kinetin. The optimum concentration of humic acid required for the increased shoot proliferation has been observed to be 300mg/100ml. This study clearly indicates that humic acid not only influences the agricultural field but also can be effectively used in micropropagation techniques.

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