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RESEARCH ARTICLE

Diversity in callus organization in Eclipta alba Hassak

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Abstract

Callus development has been regarded as a pre-requisite condition for effective micro propagation. *Eclipta alba* a wild medicinal plant seems to follow this principle quite readily and effectively. This plant can be effectively propagated on defined culture media (MS, supplemented with NAA (1mg/L) and BAP (2 mg/L) . During present study an attempt has been made to evaluate the response of different plant part of *Eclipta alba* in micro-propagation. Attempt has also been made to report the type of callus formed during propagation. It has been noticed that callus formation in sub-cultured plant is more rapid, less time consuming (20 days) and exhibits diversity in structural organisation. The type of callus formed has been found to be deep black and tightly packed, light black spreading and diffused, transparent white, transparent white mixed with green, and transparent grey. Growing seed after 30 days of incubation, induced unorganized mass of cells of two different colours. These were transparent grey (Large) and transparent white (Minute attached to the media) in colour. The sub-cultured plant exhibited profusely growing transparent and white calluses. The node ex-plant developed a bulbous callus which was rooty and shooty to a limited extent. When leaf ex-plant was used it yielded calluses of different kind such as green, white minute grey and minute nodular callus.

Introduction

Plant cells have central characteristic for cell differentiation. Plant parts in a culture media generate unorganized cell masses, such as callus or tumors. There is various recognized mechanism known for induction of callus as a result of which various kinds of callus is induced. For example, callus with no apparent organ regeneration typically are called friable or compact callus. Other callus stimulates some degrees of organ regeneration are called rooty, shooty, or embryonic callus, depending on the organs they generate (Zimmerman, 1993; Frank *et al*., 2000)).Therefore, the term callus includes cells with various degrees of differentiation. Review of literature on fate of callus during regeneration suggests that two plant hormones, auxin and cytokinin, determines the state of differentiation and dedifferentiation (Skoog and Miller, 1957) of the regenerating callus. The literature further reveals that the regenerating callus have wide use in both basic research and industrial applications (George and Sherrington, 1984; Bourgaud *et al.,* 2001). However, despite its extensive use, our knowledge of the molecular mechanisms underlying callus formation has been limited until recently. Besides this our knowledge about diversity in callus organization is also limited. During present study an attempt has been made to report the diverse kind of calluses induced during propagation of Eclipta alba. The description given below is the outcome of such an attempt.

MATERIAL AND METHODS

2.1. Source of ex-plant

The experimental plant was collected the university campus. Various parts of the plant such as tip of the stem, node of the stem, were used as the source of ex-plant.

Sterilization

The plant material was first washed in running tap water for 2 hours and then in sterilized distilled water mixed with Tween 20. Washing in Tween 20 was performed with vigorous shaking to remove surface adhered contaminants. The plant sample was washed with sterilized distilled water mixed with Bavistin (01%) and was further washed three times in sterilized distilled water before it was transferred to laminar flow chamber for transfer to the culture media. The plant sample was further surface sterilized with 0.1 %(w/v) mercuric chloride ($HgCl_2$) for 2min followed by thorough rinsing in autoclaved distilled water for at least 7 to 8 times under strict aseptic condition. To avoid bacterial contamination the plant sample was treated with Mox solution (500 mg Mox dissolved in 100 ml of sterilized distilled water). The sterilized ex-plant used was of the size of 0.5 to 1cm containing single node. The part of the leaf was also used as source of ex-plant.

2.2. Culture medium and condition

MS medium was used for in-vitro culture of ex-plants (Murashige and Skoog, 1962). This medium was fortified with 3% sucrose, and 0.8% agar-agar (used to solidify the medium). The pH of the medium was adjusted to 5.8 by adding 1N NaOH / 1N HCl and then autoclaved at121 C for 20 minutes. α –Naphthalene acetic acid- NAA (1 mg/L) and 6-Benzylaminopurine - BAP (2 mg/L) were used to observe its effect on callus development shoot initiation and root initiation. The cultures were maintained at 25 ± 1°C under 16 h photo-period provided by white fluorescent tubes.

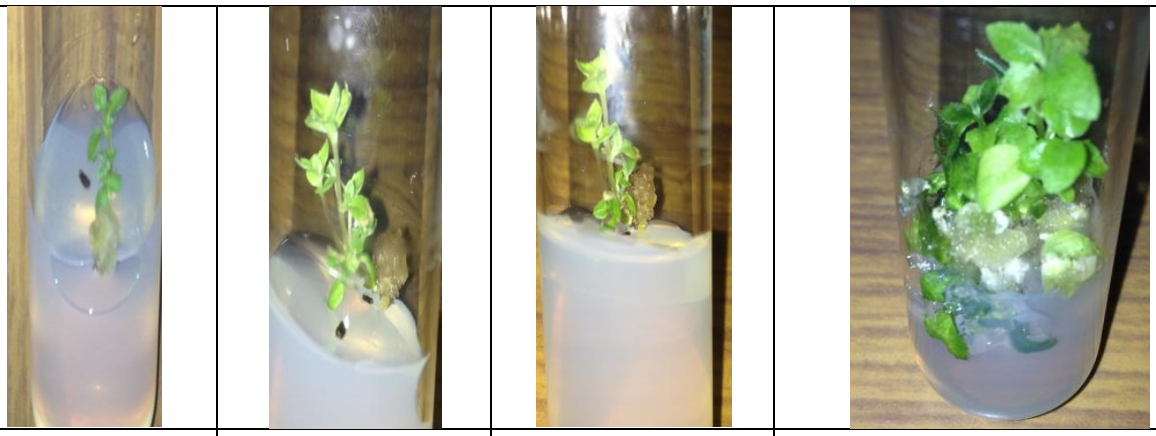
3. Results and Discussion

Review of literature on micro propagation of *E. alba* suggest(Baskaran and Jayabalan, 2005: Borthakur *et al*.,2000;Devendra *et al*.,2011;Dhaka and Kothari,2005;Gawde and Paratkar, 2004; Hussain and Anis, 2006;Sayeed Hassan and Farhana, 2008;Sharma *et al*.,2013)) that this plant can be effectively propagated on MS medium. There is report of its propagation from every part of the plant but there is scanty report about the kind of callus development in this plant. Hence, propagation studies were carried with the sole intention to monitor the kind of callus development in the plant.

3.1 Calluses from Seed

The seed was directly transferred to the culture media where it started growing after 7 days of inoculation. Along with normal growth of the plant like shoot and leaf, unorganized tissues appeared to grow from the basal region of the plant after 20 days of incubation. It appeared that this mass of tissue originated from the seed and remained attached to the main plant but also it appeared that lower most outgrowth remained in close contact to the culture media. It was felt that the lower most calluses are drawing nutrition both from the main plant and also directly from the culture media. . On close examination of the emerged growth (Callus) three different structure of different size could be noticed

1. Large sized growth 1-1.5 mm, very light grey, having smooth surface. This smooth surface turned irregular with lapse of time (Fig –B, Fig-C)
2. One very small sized growth identical to that described above.
3. One very small sized growth but white in colour.
4. Profusely growing transparent and white callus on the surface of the culture media.



A	B	C	D

Fig. 1:

A. Germinating plant from seed after 7 days.

B. Multi shooting in the geminating plant also showing unorganized mass of cells after 25 days.

C. Unorganized mass of cells showing irregular outer surface after 30 days

D. Sub cultured plant showing transparent and white calli.

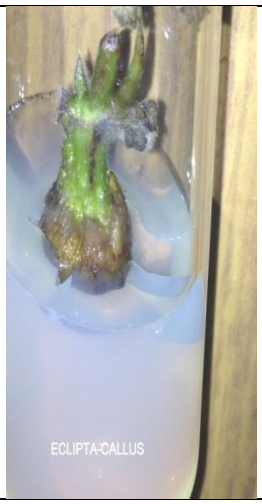
An attempt to further grow this different kind of Callus failed in the same composition of the media. Hence these calluses were recognized as frail type. Attempt to grow these calluses by changing the concentration of the growth hormone is in progress. In yet another experiment keeping all the parameters similar to that described above, nodal segment of the plant was transferred to the culture media. The fate of these explants has been described.

3.2 Calluses from Node

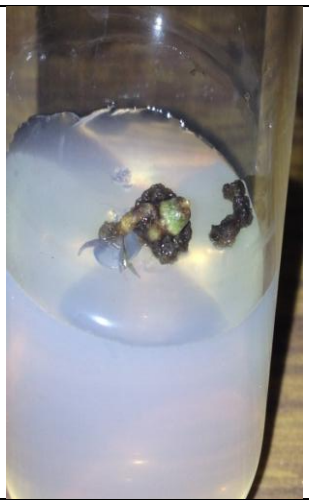
Node of the plant *Eclipta alba* ,01 cm in length and healthy in appearance was inoculated on the MS media supplemented with α –Naphthalene acetic acid- NAA (1 mg/L) and 6-Benzylaminopurine - BAP (2 mg/L).After 10 days of incubation a bulbous mass of tissue was observed ,sunken in the media. Initially the colour of this mass was green but with lapse of time (25days) its colour turned black (Fig.2 A and Fig B).The upper portion of the bulbous callus, however, remained green but without showing any kind of proliferation. After 30 days of growth of this callus, it was removed from the growing tube, and transferred to a freshly prepared culture media as an attempt to do subculture. The callus was gently removed from the tube washed with sterilized distilled water cut into small pieces and again surface sterilized with 0.1 %(w/v) mercuric chloride ($HgCl_2$) for 2 min followed by thorough rinsing in autoclaved distilled water for at least 7 to 8 times under strict aseptic condition. This bit of callus was then transferred to the culture media having the same composition which was used for induction of callus.

After 25 days of incubation of sub cultured callus a patch of greenish mass of tissue was observed which was considered as beginning of embryonic shooting. Even after 40 days of incubation the growth of the shoot remained arrested (Fig.2 C).

A. Bulbous Callus emerging from lower portion of the node.



B. Enlarged view of A.



C Bulbous callus showing rooting and shooting (embryogenic).

Fig.2

In yet another set of experiment the nodal segment after 25 days of incubation, yielded a greenish white lump of tissue which exhibits the diversity of the callus development in this medicinally important plant. Further sub-culture of this callus is underway (Fig 2D).

D Greenish white callus emerging from Node.

Fig.2

Considering rapid method of callus development through seed as well as node, an attempt was made to test the efficacy of leaf segment as ex-plant for micro propagation. It is the cellular organization of the leaf (thin and expanded) which makes them prone to microbial exposure as well as environmental stress. Keeping this in mind, fresh leaf was collected, suitably washed, and surface sterilized and inoculated on MS media containing α – Naphthalene acetic acid- NAA (1 mg/L) and 6-Benzylaminopurine - BAP (2 mg/L).

3.3. Calluses from Leaf as ex-plant

After 25 days of transfer of leaf onto the media, greenish white unorganized mass of tissue began to grow from the petiole region of the leaf ex-plant. After 30 days of incubation a new shoot emerged from this callus (Fig 3A).Thus the callus from the leaf ex-plant exhibited functional shooty characteristic. In yet another tube callus development was noticed as pin head sized callus of white and grey colour (Fig 3B).

	
A. Greenish transparent callus from leaf, also showing new shoot.	B. Pin head size callus white and grey emerging from leaf
Fig 3.	

To observe the effect of plant part on the induction of callus fresh stem tip was used as ex-plant. To evaluate the effect of a particular plant part on growth, two parts of the tip were used.

1. Tip with old plant having a young bud.
2. Tip from young plant.

3.4. Stem tip as ex-plant

After 15 days of incubation the bud was observed to get transformed into a mature flower having a normal shooting (Fig 4A). The new stem tip, however, culminated into a bulbous callus which proliferated into multi-shooting (Fig 4B).

	
A. Old stem tip	B. New stem tip
Fig 4.	

The practice of sub culturing which is very common step followed during micro propagation was also adopted to mark the development of calluses in the sub cultured ex-plant.

3.5. Induction of callus during sub culture:

Callus development from the nodal segment yielded a satisfactory and challenging opportunity and hence, a part of this callus was transferred to MS media supplemented with α –Naphthalene acetic acid- NAA (1 mg/L) and 6-

Benzylaminopurine - BAP (2 mg/L).This resulted into a profuse multi-shooting of the callus after 20 days of transfer(Fig 5). In a quick succession freshly growing plant was removed cut into bits and transferred to MS media for further growth and differentiation.

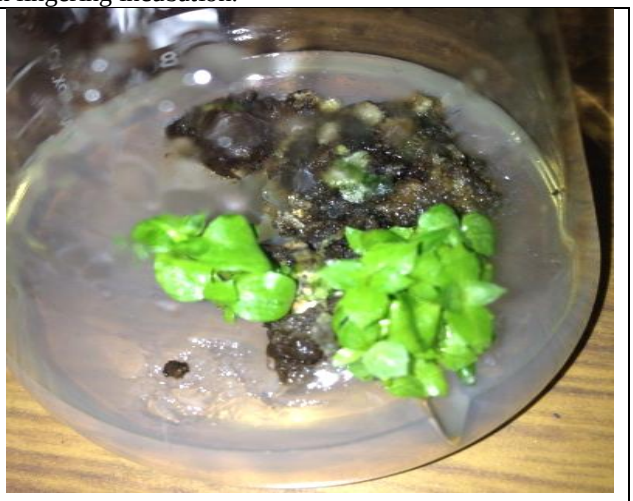
Fig 5.The Host plant which was used to induce callus during sub culture

Each transferred ex-plant after 20 days of incubation developed into irregular mass of tissue. This had different colours and different structural organization. The mass of callus increased its dimension with lapse of time (Fig 6).The callus thus formed were of different colours and different texture such as:

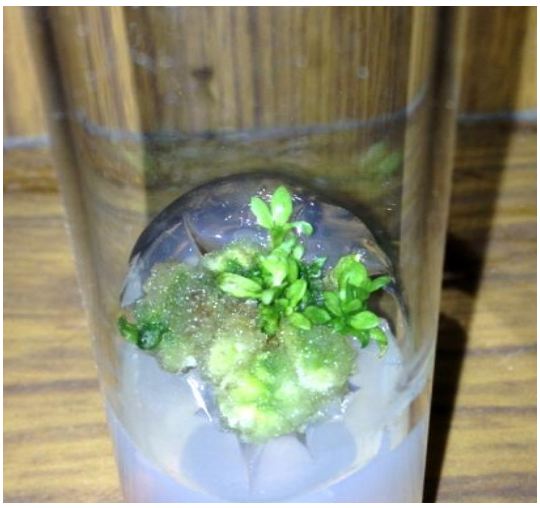
1. Deep black and tightly packed (Fig 6 A).
2. Light black, spreading and diffused (Fig 6 B).
3. Transparent white (Fig 6 C).
4. Transparent white mixed with green colour (Fig 6 C).
5. Transparent grey (Fig 6 D).Chalky white(Fig 6 E)

The entire calluses developed in this attempt were found to be functional and shooting. Large number of plantlets was noticed to emerge .The number of which increased with lingering incubation.

A Black callus showing multi-shooting.



B. Black callus showing multi –shooting in a flask.



C. White and Greenish callus showing multi shooting.

D White and grey callus showing multi-shooting.

Fig 6.

E. White chalk coloured callus with multi-shooting

Fig 6

Plants develop callus or other tumors after exposure to various harsh growth conditions (Freytag *et al*.,1988). This is obviously a big commitment for plants since they have to give up their fully established body plans and start a new developmental program once again. Callus organization in *Eclipta alba* exhibits diversity to the extent that various kind of calluses have been noticed during present study. Callus has been widely used in both basic research and industrial applications (George and Sherrington, 1984; Bourgaud *et al*., 2001). Studying callus has numerous important implications in other areas of biology as it addresses questions of, for example, how multi cellular organisms perceive and transduce endogenous and environmental signals and how they induce or maintain cell differentiation/dedifferentiation (Hooker and Nabors, 1977). In *Eclipta alba* different part of the ex-plant induces different kind of calluses. The time of induction of callus is greatly reduced when ex-plant is sub cultured. A combination of NAA and BAP in uniform concentration has been instrumental in induction of calluses in each ex-plant. Various growth hormones are known to have such inducing effect (Saunders and Shin, 1986; Saunders and Tsai,1999) in wide variety of plants. This consists of auxin and cytokinin (Ikeuchi *et al*., 2013), BAP or KIN in combination with NAA or 2, 4-D (G.rel, 1997; Gurel *et al*., 2001). Mikami T and Kinoshita T (1988) reported various kinds of calluses from various parts of rice ex-plant and to this finding genotypic effect were postulated. This may hold true during present study as well, because different plant parts were used for micro-propagation of *Eclipta alba*. The ex-plants were also procured from various sources. In wild varieties (the source of ex-plant during present study) difference in chromosome number is common. This has an impact over gene dose resulting into diversity in callus organization this may hold true during present study. The stress conditions results into induction of calluses. This seems to be common during present study as ex-plant has been obtained from various sources; they had to face stress conditions (cutting, treatment with alkali, treatment with mercuric chloride, pesticide treatment).

Any one of these factors is sufficient to provide a moment of stress condition resulting into induction of callus of diverse shape, size and colour.

CONCLUSION

During present study diversity in callus organisation has been noticed in *Eclipta alba*. The calluses are both regenerating and friable kinds. The calluses are also of diverse colour and shape which makes this test system suitable to unravel the mystery of various kinds of callus development in different test system. It has been postulated that calluses are the products of stress conditions. Different kinds of calluses are formed also due to different chromosome number in the wild host plant. During present study different conditions were created such as various parts of the host plant was used as ex plant, various chemical treatments were given to the ex plant prior to inoculation. Since a definite molecular mechanism is involved in callus formation a little modification in the protocol can result into different kind of stress to the host plant. These aspects have to be evaluated by adopting scientific principle to draw a conclusion in which *Eclipta alba* can be a pioneer test system.

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