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HIGH FREQUENCY CALLUS INDUCTION FROM SHOOT BASE EXPLANTS OF *ALOE VERA* (L.) BURM.F AN IMPORTANT MEDICINAL PLANT

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ABSTRACT
The present study aimed to evaluate the callus induction from the shoot base derived calli of *Aloe vera* was described. The shoot base explants were excised from *in vivo* plantlets cultured on Murashige and Skoog (MS) medium supplemented with 2, 4-dichloro phenoxy acetic acid 2, 4-D/IAA (1.0 - 3.0 mg/L). After one week the initiated cell debris was transferred on MS medium fortified with IAA (2.5 mg/L). The sub cultured cell debris gave rise to luxuriantly growing calli after two weeks. These calli were sub cultured on MS medium supplemented with various concentrations of IAA (2.0 - 30 mg/L) alone and with NAA (1.0 - 5.0 mg/L) for efficiency callus induction. The efficient callus was observed on full strength MS medium supplemented with 2, 4-D (2.5 mg/L) along with NAA (2.0 mg/L) in three weeks of time.

KEYWORDS: *Aloe vera*; Murashige and Skoog medium; Callogenesis; Shoot buds.

INTRODUCTION
Aloe vera has been used externally to treat various skin conditions such as cuts, burns and eczema. It is alleged that sap from *Aloe vera* eases pain and reduces inflammation. It has antiseptic and antibiotic properties which make it highly valuable in treating cuts and abrasions. It has also been commonly used to treat first and second degree burns, as well as sunburns and poison oak, poison ivy and poison sumac infections and eczema. It can also be used as a hair styling gel and works especially well for curly or fuzzy hair. It is also used for making makeup, moisturizers, soaps, sunscreens, shampoos and lotions. *Aloe vera* gel is useful for dry skin conditions, especially eczema around the eyes and sensitive facial skin. Its juice may help some people with ulcerative colitis, an inflammatory bowel disease. Aloe has been marketed as a remedy for coughs, wounds, ulcers, gastritis, Diabetes, Cancer, headaches, arthritis, immune-system deficiencies, and many other conditions when taken internally. However, the general internal use is as a laxative. The lower leaf of the plant is used for medicinal purpose. If the lower leaf is sliced open, the gel obtained can be applied on the affected area of the skin. Aloe (*Aloe vera*) is an important and traditional medicinal plant belonging to the family Liliaceae. It is indigenous to Africa and Mediterranean countries. It is reported to grow wild in the islands of Cyprus, Malta, Sicily, Canary cape, Cape Verde and arid tracts of India. This is a hardy perennial tropical plant that can be cultivated in drought prone areas and is one of the crops whose potential is yet to be exploited, despite being identified as 'a new plant resource with the most promising pospects in the world'. In India, it is scattered in the wild, along the coast of southern India.

Plant callus is a mass of undifferentiated cells derived from plant tissue (explants) for use in biological research and biotechnology. In plant biology, callus cells are those cells that cover a plant wound. To induce callus formation, plant tissues are surface sterilized and then plated onto *in vitro* tissue culture medium. Plant hormones, such as auxins cytokinins, and gibberellins are supplemented into the medium to initiate callus formation or somatic embryogenesis. Callus tissue initiation has been described for a number of plant taxonomic divisions.

Callus cells are not necessarily genetically homogeneous because a callus is often made from structural tissue, not individual cells. Nevertheless, callus cells are often considered similar enough for standard scientific analysis to be performed as if on a single subject. For example, an experiment may have half a callus undergo a treatment as the experimental group, while the other half undergoes a similar but non-active treatment as the control group.

The morphogenic response of a tissue culture depends on the endogenous growth substances present at the time of

culture. The callus formation from wounds is due to the effect of endogenous growth hormones was reported for the first time by Sinnot (1960). It is also dependent on the concentration of growth substances such as auxins alone or auxin-cytokinins present in the medium for inducing the callus. Callus is made of an amorphous aggregate of loose, parenchyma cells which proliferate from the mother cells (explant). When an explant is wounded a callus is usually formed at the cut ends of the explant. Gautheret (1939) and Nobocourt (1939) first time reported the plant tissue culture of callus, involving explants of cambial tissues isolated from carrot root and carrot respectively. The general growth characteristic of a callus involves a complete relationship between the plant material used to induce callus and the composition of the medium and the culture conditions (light, temperature, etc) during the incubation period (Murashige, 1974). All the plant tissues containing living cells may be induced to form dedifferentiated mass of cells, callus, but tissues may vary in terms of their lag period before an active growth is induced, mostly all the multicelluler plants are potential source for ignition of callus (Yeoman and Macleod 1977).

The application of plant tissue culture method is crop improvement depends upon the induction of viable callus cultures and capable maintenance *in vitro* (Vasil *et.al.*, 1979). Establishment of callus cultures from the explants can be divided in to three stages induction of cell division, continued proliferation and structural and physiological dedifferentiation.

Some calli are heavily liquefied and hard in texture where as other break easily in to small fragments and readily separated are termed as "Friable Callus" Generally in tissue cultures two types of calli are recognized.

They are:

1. Embryogenic callus which is smooth, compact and cream or yellowish in colour and consist of iso-diametric cells.
2. Non – Embryogenic callus that is generally rough crystalline yellow to brown in colour.

The nature of the callus tissue its texture, compactness, friable and coloration also depends on the genotype, age of the explant and even the season. The callus growth within a plant species is also dependent on various factors such as the original position of the explant within the plant and the growth conditions.

The exogenous supply of growth regulators is frequently necessary in calogenesis. This necessity refers to the type, concentration, and relation auxin/cytokinin, genotype of the donor plant and the endogenous content of hormones. According to George and Sherrington (1984), the combination of auxins and cytokinins promote cellular differentiation and also organogenesis. Among the growth regulators used in callus induction, 2,4-D (2,4- dichlorophenoxy acetic acid), NAA (1-naphthaleneacetic acid), BAP (6-benzylaminopurine) and TDZ (thidiazuron) are the most important.

Callus formation take place under the influence of exogenously supplied growth regulators present in the nutrient medium. The type of growth regulator requirement and its concentration in the medium depends strongly on the genotype and endogenous hormone content of an explant. These requirements can be put into three categories.

1. Auxin alone (Especially in monocotyledons).
2. Cytokinin alone.
3. Both Auxin and Cytokinin.

Callus is somewhat an abnormal tissue which has the potentially to produce normal roots or shoots and embryoids, which develop into complete plant lets depending on the level of phyto-hormones i.e. Auxin and Cytokinins present in the nutrient medium. Induction of callus from an explant plays a great role in tissue culture technology for production and multiplication of somaclonal variants with agronomically important characters isolation of pharmaceutically and therapeutically important chemicals from medicinally important plants using cell suspension cultures and also for conservation of the species. In view of all these, an attempt has been made in the present investigations to establish the effective protocol by using various growth regulators in inducing the callus from different explants in *A.vera* medicinal and commercial plant.

Aloe vera is the oldest medicinal plant ever known and the most applied medicinal plant worldwide. Extracts of *Aloe vera* is a proven skin healer. *Aloe vera* help to soothe skin injuries affected by burning, skin irritations, cuts and insect bites and its bactericidal properties relieve itching and skin swellings. It is known to help slow down the appearance of wrinkles and actively repair the damaged skin cells that cause the visible signs of aging. Aloe is a powerful detoxifier, antiseptic and tonic for the nervous system. It also has immune-boosting and anti-viral properties. Research has proven that adding *Aloe vera* to one's diet improves digestion. As a general health tonic. *Aloe vera* is a useful source of vitamins. *Aloe vera* Gel contains a large range of vitamins - even vitamin B12, Vitamin A, contains B-Group vitamins, Vitamin C, Vitamin E and folic acid. *Aloe vera* Gel contains important ingredients including 19 of the 20 amino acids needed by the human body and seven of the eight essential ones that just cannot be made.

Some of the most important pharmacological activities of *Aloe vera* are antiseptic (Capasso *et al.*, 1998), anti-tumor (Winter *et al.*, 1981), anti-inflammatory (Yagi *et al.*, 1998), wound and burn healing effect (Heggers *et al.*, 1993), anti diabetic (Rajasekaran *et al.*, 2006) and as an adjunct to current AIDS therapy (Mc Daniel *et al.*, 1990). *Aloe vera* propagates vegetatively in its natural

state. However, propagation rate is very slow because a single plant can produce only three to four lateral shoots in a year. Moreover, the production of *Aloe* leaves is insufficient to meet the industry demand in India (Aggarwal and Barna 2004) and the production of cosmetics, foods and pharmaceuticals containing *A. vera* has experienced a slow increase due to limited availability of raw material with high quality (Campestrini *et. al.*, 2006). Therefore, there is a need to develop suitable, an alternative method for traditional propagation of *A. vera.*

The present research focuses on the influence of 2, 4-dichloro phenoxy acetic acid 2, 4-D/IAA and 2, 4-D (1.0- 3.0 mg/L) in combination with NAA (1.0- 3.0 mg/L) on Callus induction of *A. vera*.

METHODOLOGY

Shoot base of 2.0-3.0 cm were collected from offshoot-derived elite individual of the superior genotype of *Aloe vera* on the basis of higher yield of leaf biomass. The explants first were washed thoroughly in running tap water for 15 minutes. After that they were again washed with liquid detergent and Tween -20 for 10 minutes with gentle shaking. After washing with detergent explants were again washed with running tap water to remove any traces of detergent for 15 minute and kept in 1% w/v solution of Bavistin for one hour. After that explants was shifted to the 1% v/v solution of savlon for 1-2 minutes. After these treatments shoot tip were taken inside the laminar hood for further sterilization. Here 2-3 sterile water washings are given. After these washings, explants were taken out and dipped in 70% ethyl alcohol for 30 seconds and then dip into alcohol for 20 second, explants were surface sterilized with freshly prepared 0.1% w/v aqueous solution of mercuric chloride for 5 minutes. After mercuric chloride treatment, explants were thoroughly washed for 4-5 times with sterile water to remove any traces of mercuric chloride. Medium was autoclaved at 121^{o}C for 20 minutes.

For callus induction the Shoot base from 7-week-old axenic 8-week old seedlings were excised, these explants were inoculated to MS medium supplemented with various concentrations of (1.0- 3.0 mg / L) of auxins such as 2,4–D/IAA and 2, 4-D (1.0- 3.0 mg/L) in combination with NAA (1.0- 3.0 mg/L) on Callus induction shoot base explant growth regulators were used as auxine alone in culture media. All media were adjusted to pH 5.8 before addition of 0.8% agar agar and autoclaved at 121^{o}C and 103 K pa for 20 minutes cultures in 25 x 150 mm cultures tubes.

Data Analysis

At least 12 replicates were maintained for each treatment and data was recorded after 4 weeks of cultures. Each experiment was repeated at least twice with similar results and data presented are of one representative experiment. All the data were statically analyzed.

RESULTS

Callus induction ability of different explants such as hypocotyls, cotyledon and leaf was investigated by using varying concentrations of different auxins individually. Callus proliferation was initiated at the cut surfaces of the explants studied and later it covered the entire surface. Both color and texture of the callus also varied with growth regulators supplemented. The results are presented in (Table-1) and shown in (Fig.-I). The explants *viz.* Leaf base cultured on MS medium supplemented with different concentrations (1.0-3.0 mg/L) of auxin such as 2,4-D/ IAA and 2, 4-D (1.0- 3.0 mg/L) in combination with NAA (1.0- 3.0 mg/L) on Callus induction individually exhibited initiation of callus after 15 days of incubation while it took 12-15 days in hypocotyls.

Leaf base explants

For callus induction Leaf base explants (0.6-0.8 cm^2) from 6 weeks old axenic seedling were excised and inoculated to MS medium supplemented with various concentrations of 2,4-D /IAA(1.0 - 3.0 mg/L) NAA and 2,4-D (1.0 - 3.0 mg/L). Callus proliferation was initiated at the cut surface of the leaf base explants. The results are presented in (Table-1) and shown in (Fig.-I).

Effect of 2, 4-D

On 2,4-D supplemented medium early induction was observed in all concentration of 2,4-D. High amount of callus was induced at (2.0 mg/L). Different callusing response was recorded in leaf base expands at all the concentrations of 2,4-D. Morphology of callus was found to be varied at different levels of 2,4-D. White friable callus was found at (2.5 and 3.0mg/L) concentration. Low amount of callus was induced at high concentrations of 2,4-D. Highest percentage (80%) of response was observed at (2.0 mg/L). (Fig-1 a,b)

Effect of IAA

On IAA supplemented medium moderate amount of callus was observed at (2.0 and 3.0 mg/L) IAA. Low amount of callus was observed at (1.0, 2.5 and 3.0 mg/L) IAA. Morphology of callus was also found to be varied at different levels of IAA. White compact callus was induced at (1.0mg/L) white friable callus obtained at (1.5 and 2.0 mg/L) IAA. Green nodular callus was observed at (2.5, and 3.0 mg/L) IAA. 68% callusing response was recorded in Leaf base explants at (2.0 mg/L) concentration of IAA. (Fig.-I-d).

Effect of 2, 4-D with NAA (1.0- 3.0 mg/L)

Effect of 2, 4-D (1.0- 3.0 mg/L) in combination with NAA (1.0- 3.0 mg/L) on Callus induction ability of leaf base explants is shown in. High percentage (86%) of response was observed at (2.0 mg/L) 2, 4-D and NAA. Responding callus was also varied at different levels of 2, 4-D + NAA. Whereas moderate amount of callus was observed at (1.0 and 1.5 mg/L) 2, 4-D +NAA. High amount of callus was induced at (2.0 and 2.5 mg/L) 2, 4-

D +NAA. A Green friable callus was induced at (2.5 and 3.0 mg/L) 2, 4-D +NAA. (Fig.-I-e).

DISCUSSION

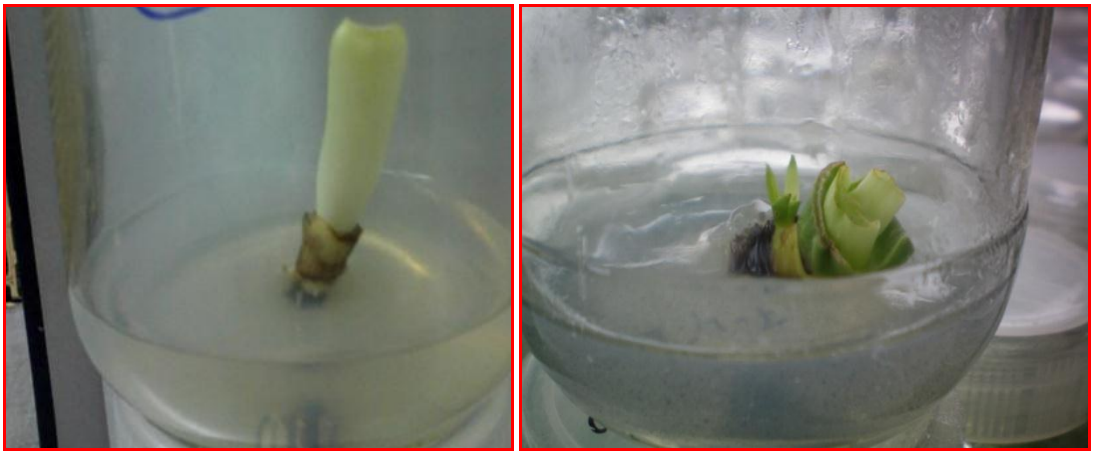
In the present investigation, *In vitro* callus were directly initiated from Leaf base explants of *Aloe vera* cultured on MS media devoid of plant growth regulators (2,4-D/IAA) and 2, 4-D +NAA). At this high concentration of 2, 4-D and NAA the morphogenetic response was very low in leaf base explants. This difference in callusing ability suggests the presence of different levels of endogenous hormones in the tissue. The auxin such as 2, 4-D +NAA, IAA and 2,4-D alone suppressed shoot bud formation in leaf base explants studied but promoted callusing. The MS basal medium was the most effective for callusing of leaf base explants. The explants cultured on MS basal medium supplemented with different auxins of 2, 4-D, NAA and IAA showed varied response for callusing. Among the three types of auxins 2, 4-D and IAA found to be very effective (Singh *et al*., 2009).

Morphogenetic response of Shoot base explants of *A. vera* on MS medium with different concentrations of 2, 4-D D, IAA and NAA+2, 4-D

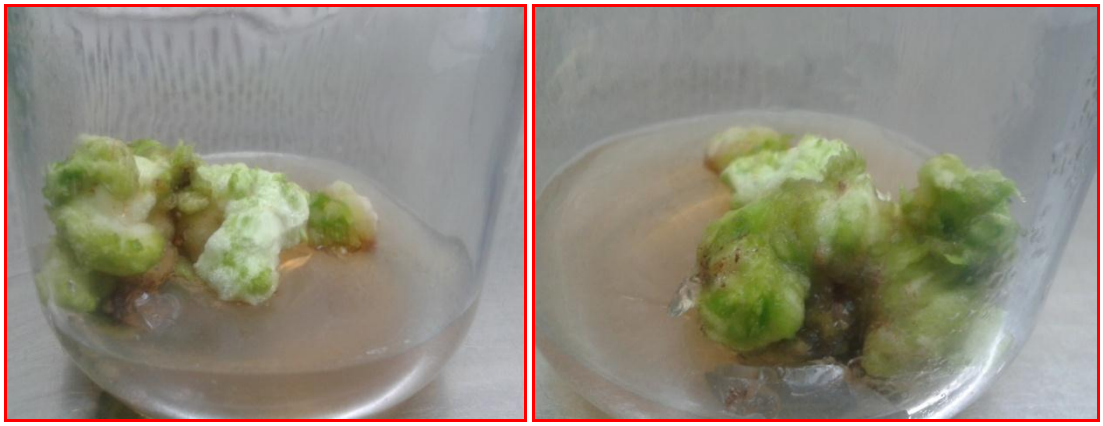
Hormone cone (mg/L)	% of cultures responding	Morphology	Callusing response
2.4 – D			
1.0	53	Green friable	++
1.5	60	Green friable	+++
2.0	80	Green friable	+++
2.5	70	White friable	++
3.0	58	White friable	+
IAA			
1.0	58	White compact	+
1.5	60	White friable	++
2.0	68	White friable	+++
2.5	50	Green nodular	+
3.0	45	Green nodular	+
NAA+2.4 – D			
1.0+1.0	80	Green friable*	++
1.5+1.5	82	Green friable	+++
2.0+2.0	86	Green friable with Small shoot Buds	+++
2.5+2.5	78	Green friable	+++
3.0+3.0	70	Green friable	++

Relative amount of Callus formation: - - = No, + = low, ++ = moderate, +++ = high
*** Embryogenic callus**

However in the present investigation calli were initiated from leaf base explants of *Aloe vera* after four weeks of culture on MS medium fortified with different auxins 2, 4-D, NAA and IAA (1.0-5.0 mg/L). About 80% callusing response with green friable callus was observed in leaf explants cultured on MS medium supplemented with NAA (3.0 mg/L). Variations in the callus forming ability of different explant types, has been reported in many plants. Different explants (stem, root, leaf and shoot apex) were placed in the medium to compare their growth responses.



a **b**



c d

e

Fig.-I: High Frequency Callus formation of *A. vera* (L.) from shoot base explants
a): *In vitro* seedling after 30 days of culture; b): Explants producing small green shoot buds on 2,4-D (1.0 mg/L); c):White callus induced on IAA (2.5mg/L) d): White friable Callus with green sectors was formed on MS+IAA (2.0mg/L) e): Small Green shoot buds developed on MS+2,4-D (2.5 mg/L)+ NAA (2.0 mg/L).

Among the auxins tested, NAA induced the high yield of callus followed by IAA and 2,4-D, similarly, Omar (1988) observed the same findings with NAA in *Rhazya stricta* a medicinal plant. Where Skoog and Miller (1957) recorded the higher callogenesis with auxins and cytokinins both together act synergistically to promote either cell division or expansion depending upon other factors within the cell which reacts with these hormones (Settler field 1963).

Among auxins, 2,4-D exhibited better response in terms of callus induction as compared to NAA supplemented in the MS medium. The callus so produced was whitish green in colour and soft in texture. Presence of 2, 4-D has been shown to be essential for callus formation in *Capsicum annum* (Gupta *et.al.,* 1990). NAA played an important role in callus formation in *Withania somnifera* (Kannan *et. al.,* 2005).

In vitro techniques using micropropagation and tissue culture offer a great possibility to overcome this problem. Micropropagation using stem and lateral shoot pieces of *Aloe vera* has already been proved successful (Natali *et al.,* 1990; Roy and Sarkar 1991; Mayer and Staden 1991; Aggarwal and Barna 2004). However, source of explants, their sterilization procedure, media composition, culture conditions, phenolic browning of explants and media discoloration greatly affect shoot regeneration from different genotypes of the same species. *Aloe vera* exudes lot of phenolic substances into the culture media which could decrease the survival of explants (Roy and Sarkar 1991). Concentration of phenolic compounds may vary in different genotypes of the same species (Glynn *et al.,* 2004), and also those were grown under different climatic conditions (Kjaer *et al.,* 2001).

The auxin 2,4-D has been determined as a potent callus inducing phytohormone in studies with many plant species. *Capsicum* (Gunay and Rao 1978). *Cucumis sativus* (Rajasekharan *et.al.,* 1983). Where as in the present investigation 2,4-D induced less amount of callus proliferation compared to all other auxins used in all the explants studied. (Praveen *et.al.,* 2003) have studied the callusing ability of different explants in *Strychnos prolatorum* on various growth substances Viz: IAA,

NAA and 2,4-D. They observed that the maximum callus growth on MS medium containing 2,4-D in contrast to our present findings.

Callus produced from different explants showed variability in texture, form and coloration. This difference is dependent upon the responses of plant tissues to various growth promoting substances. Thus successful callus induction dependents upon various factors such as composition of the nutrient medium hormonal balance besides the type, age and genotype of the explant (Narayana swamy, 1977).

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