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RESEARCH ARTICLE

SUCCESSFUL EXPLANT RESPONSE OF *Aloe barbadensis* THROUGH MICROPROPAGATION FOR THE RAPID REGENERATION OF PLANTS.

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Key words:-
Aloe vera, Tissue culture, Phytochemicals, Antioxidant property, Organic fertilizer.

Abstract

There is considerable use of *A. barbadensis* in folk medicine in the southernmost United States, and some cosmetics and patent medicines generally found on the market are prepared from the gel in the leaves and from the juice. There is a lack of production of Aloe leaf to meet the industry demand and so it is necessary to undertake large scale cultivation of Aloe. Large scale propagation can be standardized through Tissue culture where the yield is more and highly free from diseases, pests. Poor natural propagation by means of axillary shoots and the presence of male sterility are the two major barriers in rapid propagation of *A. barbadensis.* This plant is highly nutritive which contains the phytochemicals and antioxidants used for therapeutic purposes. In this study, the production and development of the medicinal plant was done through tissue culture and the phytochemical compounds and antioxidants for therapeutic use will be identified after its hardening. Phytochemical studies and the antioxidant activity tests from the hardened leaves prove the medicinal property in Aloe vera and the Aloe vera gel obtained can be used as an organic fertilizer for the enrichment of plants. This implies that the tissue culture method serves as the best time consuming method for obtaining Aloe vera as a multi-purposeful plant.

Introduction:-

Aloe barbadensis is one of the richest sources of health for human beings coming from nature. It has been grown as an ornamental plant widely. Products of the plant are used in the treatment of various ailments. *Aloe vera* is a unique plant which is a rich source of many chemical compounds and plays an important role in the international market (Dwivedi N.K *et al.*, 2014).

Active Ingredients:-

Fresh aloe juice/gel from the inner leaf parenchyma contains 96% water, polysaccharides (mucilage) consisting mainly of D-glucose and D-mannose, tannins, steroids, enzymes, plant hormones, amino acids, vitamins, minerals, and a small amount of barbaloin. The dried latex from the superficial pericycle cells contains at least 28% hydroxyanthracene, calculated as anhydrous barbaloin, which is a mixture of aloinAand aloin B, resin and saponins.The enzymes in aloe are destroyed at temperatures above 70° C. Fresh leaves and carefully made extracts

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therefore have the greatest effect, while heated, powdered dry extracts have much weaker or even negative effects. The ingredients in Aloe can be grouped into the following categories: Vitamins, Minerals, Sugars, Enzymes, Lignins, Amino Acids, Anthraquinones, Saponins, Fatty Acids, Salicylic Acid (Eufrocinio C. Marfori and Aubrey B. Malasa, 2005).

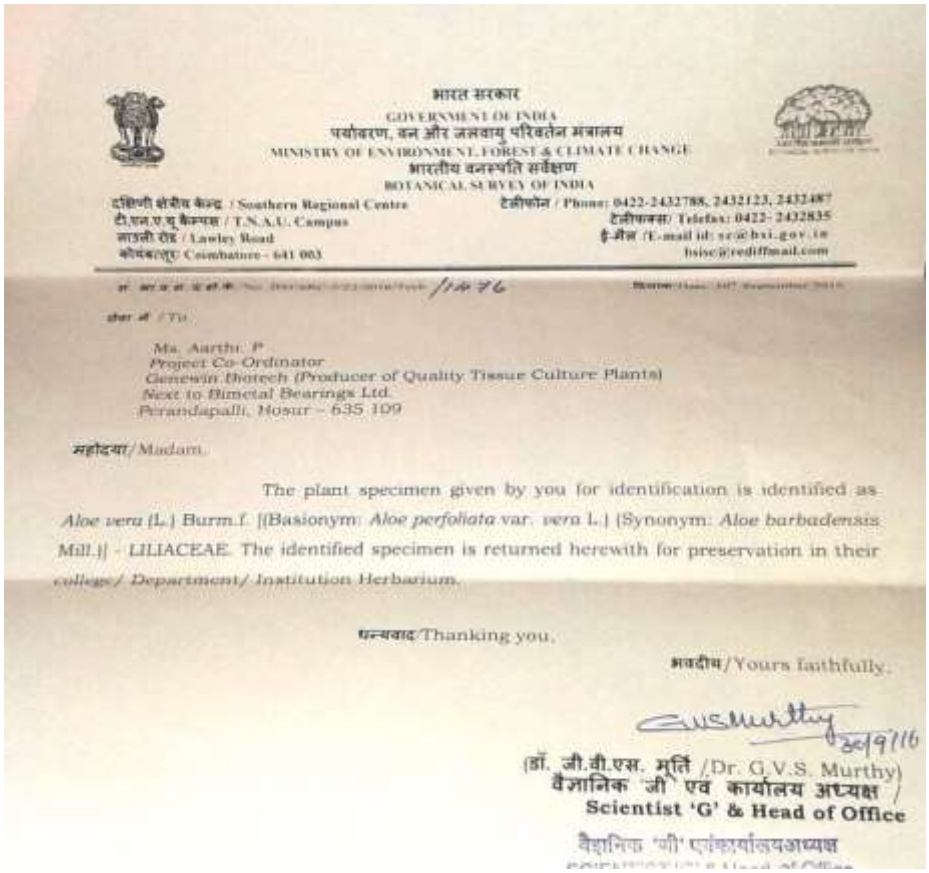
Key Actions:-

It heals wounds, is emollient and stimulates secretions of bile. *Aloe* is laxative. Gels of different strengths, oil extracts, leaf concentrates, and powders are available. South African *Aloe* gives quality gel. The processed gel is used indifferent preparations such as Drugs, Cosmetics, Soft drinks, Food Preparations, Moisturizers, Healthcare products (Baksha R *et al*., 2005).

Materials and methods:-

Collection of explants

The explants for the research studies were identified as Aloe barbadensis by Ministry of Environment, Forest and Climate Change, Botanival Survey of India. After the authorized identification, they were maintained in the Green house at Genewin Biotech, Hosur for further use in the Research study.



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/To

Ms. Aarthi. P
Project Co-Ordinator
Genewin Biotech (Producer of Quality Tissue Culture Plants)
Next to Bimetal Bearings Ltd.
Perandapalli, Hosur – 635 109

महोदया/Madam,

The plant specimen given by you for identification is identified as *Aloe vera* (L.) Burm.f. [(Basionym: *Aloe perfoliata* var. *vera* L.) (Synonym: *Aloe barbadensis* Mill.)] - LILIACEAE. The identified specimen is returned herewith for preservation in their college/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

30/9/16

(डॉ. जी.वी.एस. मूर्ति /Dr. G.V.S. Murthy)
वैज्ञानिक 'जी' एवं कार्यालय अध्यक्ष /
Scientist 'G' & Head of Office
वैज्ञानिक 'जी' एवंकार्यालयअध्यक्ष

Fig 1:- Identification Of Species.

Preparation Of Explants :-

The explants were collected based on the maturity of the leaves from the healthy *Aloe vera* plant. The New emerging leaves were selected as explants. The collected explants were brought to the production laboratory and washed thoroughly in running tap water for 10 min in order to eliminate the muddy dust particles from the explants (Baksha R *et al*., 2005).

Fig 2:- Explant Preparation

Explants Sterilization:-
The new bud explants were soaked in antifungal and antibacterial solution, carbendazim (0.1%) and streptocycline (0.1%) for 15 minutes. The sterilization is followed by wiping of leaves very carefully using 70% ethanol with sterilized cotton. Finally, explants were treated using detergent, Polysorbate 20 for 20 minutes. The explants were washed with sterile water three times to ensure the complete removal of foam.

The explants then ready for layer by layer removal for the initiation of shoot regeneration. The collected explants were soaked in 10% Sodium hypochlorite for 40 min and the first layer is removed. After the removal of first layer, the explants were again soaked in 10% NaOCl for 40 min and the second layer was removed.
The sterilization is further carried out inside laminar air flow chamber using surface sterilant and 2 sets of experiments were performed.

SET 1: Mercuric chloride (0.1%) as surface sterilant for 3, 5, 7, 9 min. The explant were removed from the mercuric chloride solution and washed with sterile water thrice to eliminate the toxic effects of Mercuric chloride.
SET 2: Sodium hypochlorite (NaOCl) as surface sterilant at various concentrations such as 10%, 20% and 30% for 20 min.
Bud break was recorded in each trial in 3- 4 weeks (Diwakar Aggarwal).

Mortality Rate:-
Mortality rate, or death rate, is a measure of the number of deaths (in general, or due to a specific cause) in a particular population, scaled to the size of that population, per unit of time.
The Mortality rate was calculated by

% Mortality = Explants contaminated / Total no of Explants X 100

Fig 3:- Explant Selection

Explants Initiation:-
Initiation stage gives rise to regeneration of new shoots from the selected explants in 15 days. The growth was frequently monitored every week and recorded. The surface sterilized explants were inoculated in following MS basal media treatments + Sucrose 3% with various growth regulator concentrations.
AVIM 1: 6BAP – 0.5 mg/l + NAA – 0.1 mg/l

AVIM 2: 6BAP – 1 mg/l + NAA – 0.1 mg/l
AVIM 3: 6BAP – 1.5 mg/l + NAA – 0.1 mg/l
AVIM 4: 6BAP – 2 mg/l + NAA – 0.1 mg/l
AVIM 5: 6BAP – 2.5 mg/l + NAA – 0.1 mg/l

Before inoculation, the outer scaly leaves like thorns were removed aseptically for the better performance of the explants. The explants were placed in the prepared media; the mean parameters were calculated. The inoculated jars were incubated (Hans B. Juneby).

Fig 4:- Explant Initiation

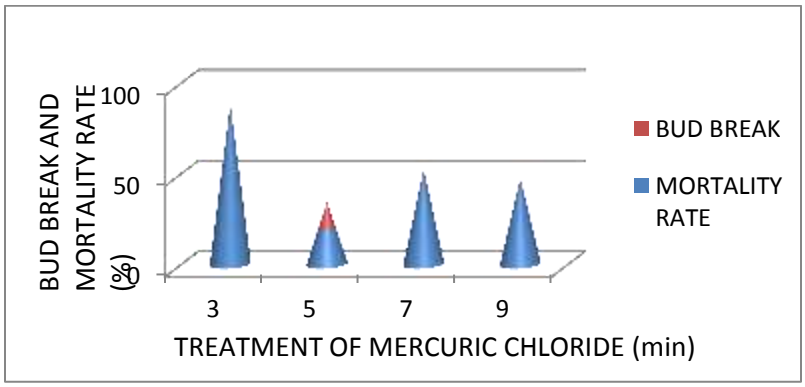
Culture Conditions:-
The explants are subjected under light intensity for 10-12 h in the growth room. Photoperiod provided by cool white fluorescent lamps of 1500-3000 lux, temperature of about 25 ± 2 °C and humidity of 35 - 40%. The observation will be recorded after 4-5 weeks.

Results and discussion:-

Effect of surface sterilization:-

Treatment Of Mercuric Chloride (%)	No Of Explants Taken	Mortality Rate (%)	No Of Explants Not Responded (Nos)	Bud Break (%)
0.1% $Hgcl_2$ – 3 Min	20	86±2.3	12	2±1.2
0.1% $Hgcl_2$ – 5 Min		20±3.5	66	14±0.3
0.1% $Hgcl_2$ – 7 Min		51±3.3	48	1±3.2
0.1% $Hgcl_2$ – 9 Min		47±4.1	53	0

Table-1: Effects of surface sterilization



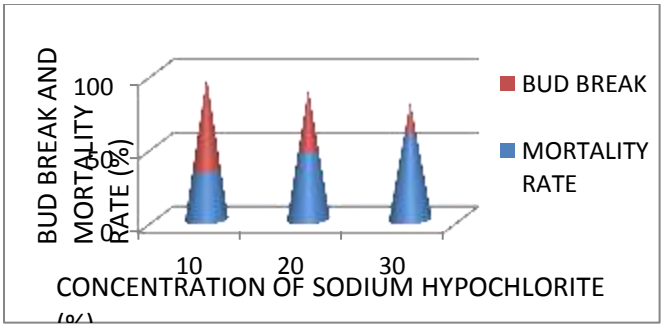
Graph 1:- Mortality rate with Mercuric chloride

Fig 5:- Surface Sterilization

Majority of the explants survived after treatment when they were exposed to mercuric chloride for about 5 min. Treatment with 3 min were found with more mortality of the explants whereas treatment for more than 5 min found to have mortality but comparatively less but the explants were non-responsive (Zarreen badar *et al.*, 2013).

TREATMENT OF SODIUM HYPOCHLORITE (%)	NO OF EXPLANTS TAKEN	MORTALITY RATE (%)	NO OF EXPLANTS NOT RESPONDED	BUD BREAK (%)
10	20	34±3.2	3	63±5.4
20		41±2.7	17	42±2.8
30		59±1.4	20	21±1.5

Table-2: Mortality rate of shoots



Graph 2:- Bud break and Mortality rate

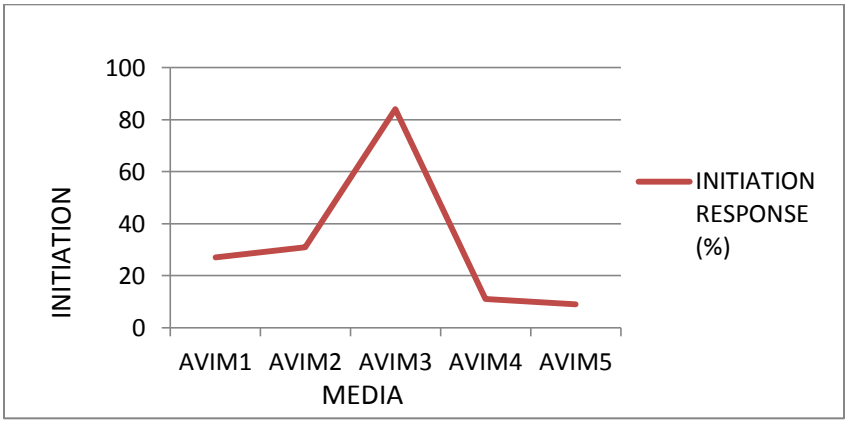
Majority of the explants survived after treatment when they were exposed to Sodium hypochlorite at 10% and the success rate was found as 63%. Other concentrations of Sodium hypochlorite showed success rate of bud break of about 42% and 21% and less percentage of explants also were non responsive (Bradley, 1992).

Initiation for shoot regeneration:-

TRIAL MEDIA	NO.OF EXPLANTS TAKEN	NO.OF EXPLANTS RAISED SHOOTS	MEAN HEIGHT OF SHOOTS (cm)	MEAN % INITIATION RESPONSE
AVIM1	20	9.3	0.8	27
AVIM2		10	0.92	31
AVIM3		16	2.1	84
AVIM4		5.1	0.77	11
AVIM5		4.2	0.51	9

Table 3:- Initiation for shoot regeneration.

Key: AVIM – Aloe vera Initiation Media.



Graph 3:- Initial response.

Fig 6:- Initial Shoot Response

Initiation of nodal response was found as 84% which trailed with the combination of 6BAP – 1.5 mg/l + 0.1mg/l with mean height of 2.1 cm. other media trials with 6BAP of about 0.5,1,2 mg/l were found with lesser shoot regeneration and less shoot height and further transferred to multiplication stage (Zarreen badar *et al.*, 2013).

Conclusion:-

This study is evident that the Aloe vera can be rapidly produced through Tissue culture. It is identified that the commercial propagation could not meet the high demands of medicinal Aloe vera, plant tissue culture proved to be a promising technology in the production of novel improved plant species with higher Quality and Quantity. Regeneration from the explants using various growth regulators were trialed and the best regeneration response was recorded which will be further transferred for the multiplication.

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References:-

1. Dwivedi, N.K., Indiradevi, A., Asha, K.I., Asokan Nair, R. and Suma A. (2014): A protocol for micropropagation of *Aloe vera* L. (Indian Aloe) – a miracle plant. Res. Biotechnol., 5(1): 1-5.
2. Eufrocinio C. Marfori and Aubrey B. Malasa. (2005): Tissue Culture for the Rapid Clonal Propagation of *Aloe barbadensis* Miller. Philipp. Agric. Sci., 88(2).

3. Baksha, R., Miskat Ara Akhter Jahan, Rahima Khatun and John Liton Munshi. (2005): Micropropagation of *Aloe barbadensis* Mill. Through *In vitro* Culture of Shoot tip Explants. Plant Tissue Cult. Biotechnol., 15(2): 121-126.
4. Diwakar Aggarwal, Tissue culture of a medicinal plant - A*loe vera* L.
5. Hans B. Juneby. Aloe barbadensis a legendary medicinal plant. Department of Medicinal Chemistry, Uppsala University, Sweden.
6. Zarreen Badar, Saifullah Khan, Saifullah, Kashif Ali, Syed Ghulam Musharraf and Muhammad Iqbal Choudhary. (2013): I*n vitro* and biotransformational studies of A*loe barbadensis* mill. Pak. J. Bot. Sci., 46(2): 679-685.
7. Bradley, P.R., (1992): British Herbal Compendium, British Herbal Medicine Association: Bournemouth, UK.