International Journal of Research in Advent Technology, Vol.6, No.8, August 2018
E-ISSN: 2321-9637
Available online at www.ijrat.org

Improved *Invitro* Rapid Shoot Amplification and Root Induction in *Aloe Barbadensis* for the Successful Acclimatization

[1]Aarthi Pugazhendhi, [2]D. Sathish Sekar
Research Scholar, Periyar University, Salem, Tamil Nadu, India
Associate Professor, Department of Biotechnology, PEE GEE College of Arts & Science, Dharmapuri, Tamil Nadu
Corresponding Author: Aarthi Pugazhendhi

Abstract- A medicinal perennial herb, Aloe vera belonging to the Liliaceae family is being used for several purposes as the herb possesses medicinal and therapeutic values. Their need could not be met by their natural propagation for which this study aimed at developing an efficient protocol for the shoot proliferation and root induction using the explants which responded well into breaking of buds after several treatments followed by the survival of plants in the soil. In this study, this succulent plant Aloe vera after the Initiation of buds, the responded cultures were assessed for the mass multiplication. The potential of the media in developing the shoots were identified and tabulated along with the challenges faced after Initiation and during proliferation. Rooting was induced in Aloe vera which produced lengthy and hairy like structured roots with ½ MS media. The rooted plants were subjected to acclimatization in the green house for the exposure of sunlight and humidity. The hardened plants were found to be morphologically similar to the mother plants.

Index Terms-Aloe vera, Tissue culture, Initiation, Multiplication, Root induction, Acclimatization, Survival.

1. INTRODUCTION

Aloe vera (Aloe barbadensis) has eminence in medicinal, pharmaceutical and cosmetic applications. few popfor its marvelous medicinal properties. This plant is one of the richest natural sources of health for mammals including human beings. It is a succulent plant species from the genus *Aloe*. It grows liberally in tropical climates and has been used for centuries as a medicinal plant. Actual name of Aloe Vera is Aloe Barbadensis Miller belonging to the Liliaceae family, with 360 species (Shail Bala Sanghi, 2015). Its exudates are transparent, slippery mucilage coming out of leaf. This mucilage was applied to inflamed skin and during the 20th century, it was helpful for radiation burns.

Aloe vera has 75 potentially active constituents, vitamins, enzymes, minerals, sugar, lignin, saponin, salicylic acid and amino acids (Richard 2005). Many properties associated with Aloe species are contributed by inner gel of the leaves that are anti-diabetic, anti-inflammatory, peptic ulcers, antitumor, anticancer Properties, activity effects on the Immune System, adverse reactions, Laxative effects, wound healing, antiseptic, vitamins, minerals, enzymes, amino acids, stress, sugars. (Gajendra Mahor and Sharique A Ali, 2016).

These remarkable qualities have influenced industrial and commercial production of *Aloe vera* and Aloe vera value added products throughout the world. The main constraint in this production chain is to obtain healthy primary culture for a sustainable supply of the plant materials and through organic as it is being consumed as food. The common practice for the reproduction of *Aloe* is conventional vegetative propagation.However, this practice should be avoided because it can also allow the propagation of diseases frequently present in the mother plants, because it happens during the conventional vegetative propagation of other plant species such banana, potatoes, strawberry, and sugarcane. This problem can be minimized using in vitro propagation of Aloe vera (Crocomo and Oliveira, 1995).

Need for organic Aloe vera juice has increased predominantly in recent times due to its properties in healing. Organic farms are more protected monitored farms by self and contract farming by pharmaceutical and food industries. Every industry struggles to get quality planting material without any disease. Also the quantity required for

farming is also high, which is 10000 plants per acre. The numbers are really high which is hard to produce in conventional methods of propagation. Generally, Aloe vera is a slow growing plant and it yields a maximum of 1 leaf per week when the optimum conditions are provided. In this conditions, As Aloe vera is succulent variety propagation becomes challenging as invitro cuts release sticky mucilage inviting bacterial contaminations and high chances of endogenous bacteria development. Present study is more specific to advanced techniques in hardening section of Aloe vera and rapid shoot multiplication. This method of propagation enables the grower society to obtain the clonal plantlets with standard size and disease free plantlets on time.

2. MATERIAL AND METHODS

PLANT MATERIAL - MOTHER SOURCE:

Aloe vera plants were obtained from the field collection Rajasthan, identified by TNAU-botanist and further maintained in the Green house in Genewin Biotech, Hosur.

SURFACE DISINFECTION TREATMENTS:

Apical buds explants, each ≈1 cm^3, were dissected from young lateral shoots of mother plants. The explants were primarily treated with 0.1% antifungal and antibacterial agents for 20 minutes and taken for surface sterilization *invitro*. Treatment was carried out with 4% sodium hypochlorite for 40 minutes. Some layers of base were trimmed and removed followed by surface sterilization with 4% Sodium hypochlorite for 15 minutes. Explants were then placed on semisolidified Murashige and Skoog medium (Murashige and Skoog, 1962) with 30 $g \cdot L^{-1}$ sucrose, and 2 $mg \cdot L^{-1}$6-benzylaminopurine (6-BAP); the pH was adjusted to 5.8 and agar 5.2 $gm \cdot L^{-1}$. In this treatment, 80 buds were individually inoculated and incubated in the growth room at 25 °C ± 2 under light (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) with a photoperiod of 16/8 h. The effect of the disinfection treatments was evaluated after 30 days in the culture medium taking into account the rates of explant contamination, necrotic buds, non responsive and successive bud break (SBB).

Fig 1 (a)

Fig 1(b)

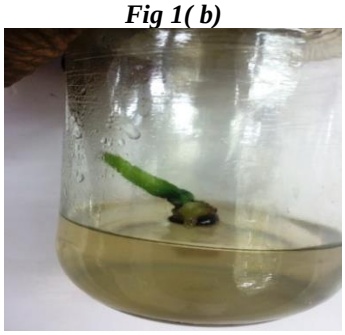

Fig 1 (c)

Fig 1 (a)- Explant selected; 1(b)- prepared explants for surface sterilization. Left side- trimmed explants underwent first treatment with sodium hypochlorite. Right side- Explant subjected for second treatment with sodium hypochlorite with further trimming. 1 (c) – explants Bud break

3. SHOOT PROLIFERATION

After 30 d of explants initiation, the best green apical shoot explants bearing axillary buds (GAS) were selected for further transfer. The shoots emerged from the explant after initiation were further transferred to multiplication stage containing cytokinnins for rapid propagation and allowed for 30 days of incubation.

TROUBLE SHOOTING WITH THE CYTOKININS:

- During Multiplication, the cytokinnin hormone selected were Kinetin and BAP for the higher multiplication ratio efficiency.
- During the 3rd week observation, callus formation was observed which lowered the multiplication ratio.

TABLE 1: TRIALS ON PGR CONCENTRATION FOR MULTIPLICATION

Trials	GROWTH REGULATORS
T1	Kin+ 6BAP – 1 + 1mg/l
T2	Kin+ 6BAP – 1 + 2 mg/l
T3	Kin+ 6BAP – 1 + 3 mg/l
T4	Kin+ 6BAP – 0.5 + 0.5 mg/l
T5	Kin+ 6BAP – 1 + 1mg/l
T6	Kin + 6 BAP– 2 + 1 mg/l
T7	Kin + 6 BAP – 3 + 1 mg/l

ROOTING STAGE:

Shoots proliferated were transferred to a rooting medium for the formation of roots. This stage is crucial in order to induce the establishment of fully developed plantlets. Auxins are class of growth regulators mainly concerned with inducing cell division. Role of this group in tissue culture are involved with elongation of stem, internodes, apical dominance, abscission and rooting. Different factors favoring root initiation have been trialed. The explants after Multiplication had passed on to next stage rooting for knowing the ability of the plants to induce roots after the shoot proliferation. It has been initialized with the media and various growth regulator combinations.

TABLE 2: MEDIUM TRIALS ON ROOTING WITH DIFFERENT GROWTH REGULATORS IN COMBINATIONS

MEDIA NAME	MEDIA + GROWTH REGULATOR	CONCENTRATION (mg/l)
RM 1	½ MS + IBA	0.5
RM 2		1
RM 3		1.5
RM 4		2
RM 5	Full MS + IBA	0.5
RM 6		1
RM 7		1.5
RM 8		2

IBA with ½ MS and full MS media were trialed out for the thick and lengthy induction of roots *invitro* for easy adaptation in the soil during hardening.

HARDENING:

Hardening refers to the process of acclimatizing the plants from invivo temperatures to the exvivo. The hardening of invitro raised plantlets is the final successful stage where the better survival and healthy establishment of plants can be witnessed. Direct transfer of tissue culture raised plant from the lab to the field is not possible due to high rate of indulged environment with a very high humidity, varied light and temperature condition and also concerned about the protection of plants from the attack of microbial and other agents.

Transfer of plantlets to soil is the most critical step in after micropropagation. Acclimatization stage involves the maintenance of the plantlets under highly protected conditions in in vitro i.e. high humidity, low irradiance, low CO2 levels and high sugar content.

PRIMARY HARDENING:

The rooted plants were sent for hardening in the Green house at Genewin Biotech for acclimatization of plants to the environment. In primary hardening, the plants were kept and maintained in the tunnel for a period of 45 days in order to avoid over exposure to sunlight which leads to mortality.

SECONDARY HARDENING:

After primary hardening, the plants were transferred to the secondary hardening stage wherein the plants were removed out of tunnels and given 50% sun light inside the green house for its acclimatization to the environment. This stage includes another 45 days.

4. RESULTS AND DISCUSSION

As a medicinal plant, Aloe exhibited its importance now-a-days in treating several diseases and the demand for it shall be achieved through tissue culture by mass propagation and the result of the same was tabulated.

TABLE 3: RESPONSE OF EXPLANTS ON SURFACE STERILIZATION AND BUD BREAK

No of inoculated buds	Contaminated buds	Necrotic buds	Non responsive	Bud Break
80	3	18	7	52
Rate of Subject	2.4%	14.4%	5.6%	41.6

TABLE 4: EFFECT OF PGRS IN MULTIPLICATION OF ALOE VERA

Trials	GROWTH REGULATORS	STAGE	OBSERVATION
T1	Kin+ 6BAP – 1 + 1mg/l	1st Subculturing	Emergence of single shoot
T2	Kin+ 6BAP – 1 + 2 mg/l		Multiplication with 2-3 shoots
T3	Kin+ 6BAP – 1 + 3 mg/l		High multiplication with healthy shoots
T4	Kin+ 6BAP – 0.5 + 0.5 mg/l		Idle explants with tiny shoots emerging
T5	Kin+ 6BAP – 1 + 1mg/l		Less multiplication
T6	Kin + 6 BAP– 2 + 1 mg/l		Tall shoots with multiplication
T7	Kin + 6 BAP – 3 + 1 mg/l		Tall shoots with roots

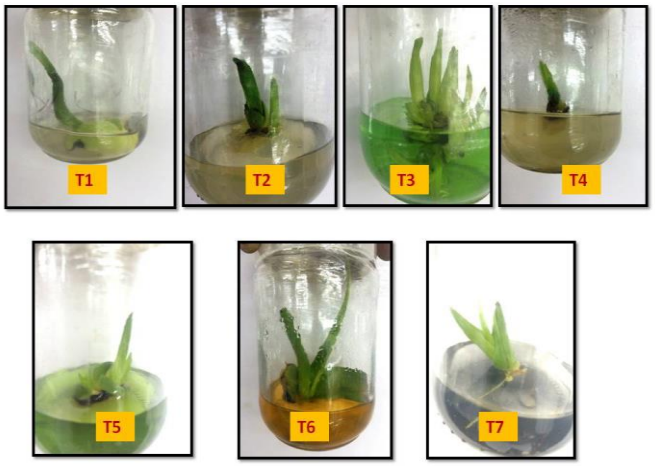

Fig 2: Growth responses of various treatments such as T1-T7 for cytokinins and auxins as per the observations tabulated.

ELONGATION AND ROOT INDUCTION:

Developed *Aloe* plantlets were transferred to culture medium without 6-BAP to induce elongation and rooting for a 30-d period.

TABLE 5: EFFECT OF ROOTING EFFICACY IN ALOE VERA BY VARIOUS COMBINATIONS OF PGRS

TRIAL MEDIA	MEAN ROOT LENGTH (cm)	MEAN ROOT NUMBERS (cm)	RESPONSE %
RM1	5.6 ± 0.11	7	96 ± 0.15
RM2	5.72 ± 0.15	5	68 ± 0.25
RM3	3.6 ± 0.11	5	54 ± 0.32
RM4	2.4 ±0.07	6	56 ± 0.32
RM5	2.1 ±0.04	4	42 ±0.39
RM6	2.1 ±0.04	3	30 ± 0.41
RM7	2.22 ±0.043	3	24 ± 0.41
RM8	2.24 ±0.043	4	16 ± 0.43

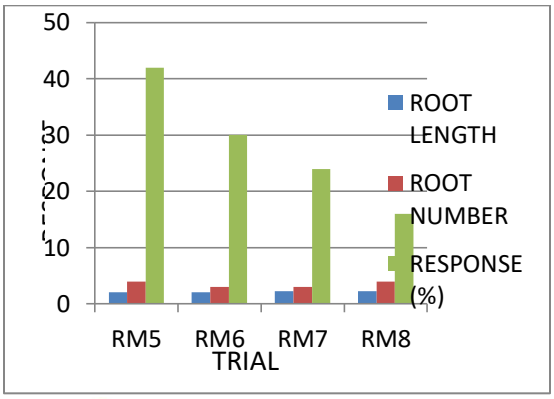

Fig 3 (a) Rooting efficacy in RM8

Fig 3(b) Rooting efficacy in RM5

HARDENING AND ACCLIMATIZATION:

HARDENING:

During secondary hardening, the media contained the following trails to know the survival rate and mortality rate of the produced plants.

Fig 4: PLANT OUT OF ROOTING MEDIA

H1 - VAM
H2 – Coir pith
H3 – Red soil: Sand: Coir pith
H4 – Red soil

Varying the media varied the mortality rate as well. It was found that VAM as the media for the plants found to show less mortality rate than the other media. Mortality rate was showed as 1.6% with the VAM followed by 3% with the coir pith

TABLE 6: SURVIVAL RATE OF ACCLIMATIZED ALOE

MEDIA TRIALS	**MEAN HEIGHT OF PLANT (cm)**	MORTALITY **(%)**	SURVIVAL RATE (%)
H1	8.3 ± 1.2	1.6	98.4
H2	5 ± 0.9	3	97
H3	4.4 ± 0.7	3.1	96.9
H4	4.1 ± 0.7	3.5	96.5

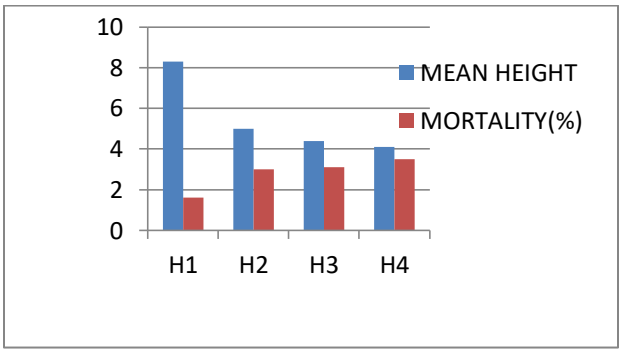

Fig 5: WELL HARDENED ALOE PLANT

INFERENCE OF TISSUE CULTURE:

At the end of the tissue culture process, rapidly multiplied plants through micropropagation are genetically similar. A single explant of Aloe vera found to produce several thousand plants within a short period of time independent of climate and time. The hardened plants are true to the type. These acclimatized plants are healthy and free from the pathogens. This technique of producing the plants can be carried out throughout the year independent of seasons.

ACKNOWLEDGEMENT:

The authors record their sincere thanks to Dr. V. Palani, Managing Director, Founder and Managing Director of Genewin Biotech, Hosur and Mrs. Manasa Satheesh for providing Technical support and necessary laboratory facilities to carry out this work successfully in the DBT certified laboratory.

REFERENCES

[1] O.J.Crocomo, and E.T. Oliveira. " Biotecnologia de plantas como um programa de produção vegetal". Biofábrica Produção Industrial de Plantas '*In Vitro*' ed GeraldL.T.S. (UFSCar, Araras), pages 18–21, 1995.

[2] Gajendra Mahor , and Sharique A Ali. "Recent update on the medicinal properties and use of Aloe vera in the treatment of various ailments", Bioscience Biotechnology Research Communications, **9**(2): pages 273-288, 2016.

[3] T. Murashige,and F. Skoog. " A revised medium for rapid growth and bioassays with tobacco tissue cultures". Physiologia Plantarum, Copenhagen, **15**: pages 473–497, 1962.

[4] L.W.Richard. "Aloe vera gel: Update for dentistry". Pharmacology Today, pages 6-9, 2005.

[5] Shail Bala Sanghi, "Aloe vera: A Medicinal Herb", Internationournal of Research – Granthaalayah, **3**(11): pages 32-34.

[6] O. O. Agarry, M. T. Olaleye, and C. O. Bello-Michael. "Comparative antimicrobial activities of *Aloe vera* gel and leaf," African Journal of Biotechnology, **4(12)**: pages 1413-1414, 2005.

[7] B. N. Meyer, N. R. Ferrign, J. E. Putnan, L. B. Jacobsca, D. E. Michols, and J. L. Molaughlin, Brine Shrimp. "A Convenient General Bioassay for Active Plant Constituents", 1982

[8] Razdan. @A wider survey of existing literature suggests that BA is the most reliable and useful cytokinin". Research in Biotechnology, **5**(1), 1992.

[9] Giuseppe Cristiano, Bernardo Murillo-Amador, and Barbara De Lucia. "Propagation Techniques and Agronomic Requirements for the Cultivation of Barbados Aloe (*Aloe vera* (L.) Burm. F.)—A Review", Frontiers in Plant Science, 7, 1410, 2016

[10] Enio Tiago de Oliveira1 and Otto Jesu Crocomo. "Large-scale Micropropagation of *Aloe vera"*, Horticultural Science, **44(**6): pages 1675 – 1678, 2009.