Journal of
Plant Science & Research

Open Science Publications

Volume 7, Issue 2 - 2020

www.opensciencepublications.com

Development of Efficient Micropropagation Technique for Conservation of *Adansonia digitata* A Rare Medicinal Plant

Research Article

Kumari Anjali*

Plant biotechnology laboratory, University Department of Botany, India

***Corresponding author:** Kumari Anjali, Plant biotechnology laboratory, University Department of Botany, B.R.A.Bihar University, Muzaffarpur, Bihar, India; E-mail: k.anjali.bot@gmail.com

Article Information: **Submission:** 10/09/2020; **Accepted:** 20/10/2020; **Published:** 24/10/2020

Abstract

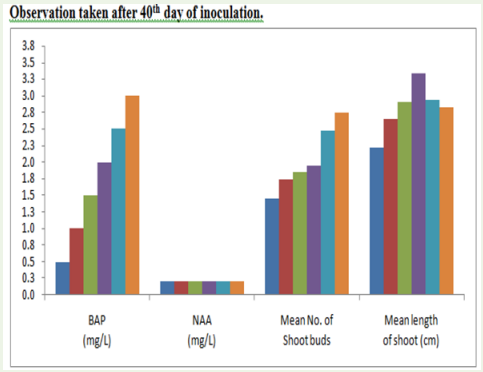
Micropropagation technique is being utilized for the production of large scale planting materials through tissue culture. *Adansonia digitata* commonly known as Baobab, is dioecious plant and therefore, general seeds formation depends upon the plantation of both the plant in the same garden. In Turki, at Muzaffarpur district, Bihar a single plant of *Adansonia digitata* is growing and even the oldest person of the area are not sure about its exact age. The plant is in such a condition that any time it can be uprooted and the germplasm of this tree shall be eroded. In the present research its micro propagation has been carried out. Here MS (Murashige and skoog 1962) basal medium was supplemented with various concentration of BAP either alone or two different concentration of NAA. Kinetin at various concentration either alone or in combination with NAA was used. It was supplemented with 3% sucrose and gelled with 0.8% agar. The nodal explants were prepared for inoculation through general method of preparation. It was inoculated in the culture medium and was incubated in the cultural room maintained at 26 °C and 300 lux light through fluorescent tube. After 20 days it was observed that highest response with respect to initiation of axillary bud was at 3.0 mg/l + antioxidants + 0.2 mg/l NAA. The percent response at this condition was 92 while the mean number of shoots was 2.60 and mean length of shoots was 2.12 cm after 40th day of inoculation. Well grown tissue cultured raised plantlets were used for rooting *in vitro*. Half strength MS basal medium supplemented with 4.0 mg/l NAA promoted maximum rooting where the mean number of root per plantlets was 2.0 among 56 % of the explants after 28 days of incubation.

Keywords: Micropropagation; Conservation; Nodal explants; Axillary bud

Introduction

The plant *Adansonia digitata* commonly known as African Baobab is a dioecious plant. The tree trunk has widest diameter than any other tree. It belongs to the family Bombacaceae. The base of the plant is broad and rapidly narrowing upward. The flower are deciduous, white and stalked. The male and female flowers are on separate plant. It is named "Tree of life" because almost every part of it can be used for healing purposes. According to the United Nations (2005), the fruit pulp of *Adansonia digitata* is traditionally used for the treatment of fever, diarrhoea, dysentery, haemoptysis and small pox in human. Leaves are also used to treat disease of urinary tract and eyes. The bark of this plant is used for the treatment of malaria and other fevers [1]. Several Ayurvedic medicines are prepared from the raw materials obtained from the medicinal plants. As these plants are growing as a wild species, they are harvested from their natural habitat. Brutal harvesting of these medicinal plants for other land use have forced them to become extinct or on the verge of extinction [2]. Micropropagation through tissue culture have been found the best alternative for the conservation of such medicinal plant. In *in vitro* culture of *A.digitata* different basal medium were used for the growth of different stages of explant. The different concentration of Cytokinin like BAP & Kinetin were used for this purpose. It was reported that the increased concentration of BAP may suppress the growth of some stages of explant in *in vitro* culture of *A.digitata* [3]. The germination of seed under natural condition and plant formation is time taken

ISSN: 2349-2805



Graph 1: Graph representing initiation of axillary shoot buds on nodal expalnt of *Adansonia digitata*.

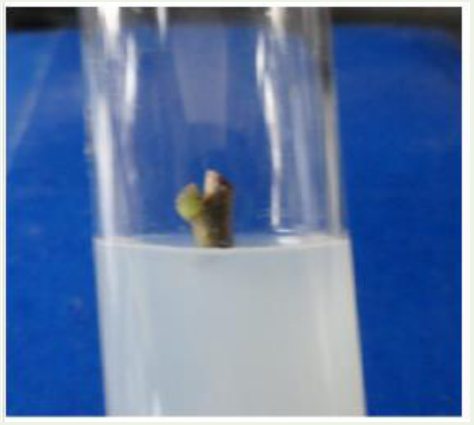


Figure 1: Showing Initiation of bud on nodal explant of *A. digitata*.

Figure 2: Showing Initiation of bud on nodal explant of *A. digitata*. on 20 day old culture.

and tough therefore, protocol for *in vitro* micropropagation through seed germination was carried out by several workers [4]. In the present work, protocol shall be developed that may be utilized for the production of large number of planting materials which shall be true to its genotype. Thus, micropropagation of this rare medicinal plant which is alone in Bihar can be conserved. There is a need of large scale multiplication and popularization of its cultivation. Also, awareness about its medicinal value among common people is to be needed [5]. In all these work along with BAP the use of different cytokinin were used in few work either alone or in combination. In the present research work experiments have done to produce shoot bud proliferation through micropropagation of *Adansonia digitata* from nodal explants as the nodal explants contains pre- existing meristematic buds.

Citation: Kumari A. Development of Efficient Micropropagation Technique for Conservation of *Adansonia digitata A Rare Medicinal Plant.* J Plant Sci Res. 2020;7(2): 197

Figure 3: Showing Initiation of bud on nodal explant of *A. digitata*. of 30 days old Culture.

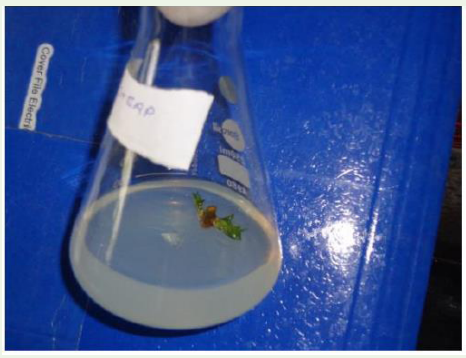


Figure 4: Showing Initiation of bud on nodal explant of *A. digitata*. of 40 days old Culture.

Figure 5: Showing *in vitro* single shoot regeneration after 50 days old culture.

Materials and Methods

Adansonia digitata L. is present in Turki at muzaffapur, district of Bihar. The plant is very very old, when we collected branches and cultured it, there was excessive exudation of phenolics and inspite of the addition of antioxidants the explants become black always. To avoid the problem in the present work young plantlets has been procured from nursery at Baroda and planted in the pot. From this, we use explants for micropropagation.

MS (Murashige and skoog, 1962) basal medium supplemented with (3% W/V) Sucrose and gelled with (0.8% w/v) agar was used (Hi Media Mumbai) [6]. The medium was supplemented with various concentration of (0.5-3.8 mg / l) BAP either alone or with two different concentration of (0.1-0.2 mg/l) NAA. Kinetin at various concentrations either alone or with NAA was used. Preparation of culture media were done as the methodology of tissue culture experiments.

20 ml of above medium was taken in culture tube and 35 ml of medium was taken in culture jar plugged with cotton plugs. All tubes

Citation: Kumari A. Development of Efficient Micropropagation Technique for Conservation of *Adansonia digitata A Rare Medicinal Plant.* J Plant Sci Res. 2020;7(2): 197

Figure 6: Showing *in vitro* regenerated 60 days plantlets with root.

and jars were wrapped with aluminium foil. The pH was adjusted at 5-8 and autoclaved at 15lb pressure and at 121 °C for 20 minutes. The tubes and jar were taken out and allowed to cool at the room temperature. Some of the tubes were kept tilted to make slant and were allowed to cool at the room temperature. These medium containing tubes and jar were stored at low temperature. Tubes showing any contamination were discarded.

The nodal explants were prepared by cutting into small pieces (8-10mm). All these explants were washed in running tap water in a flask whose mouth was closed with thin muslin clothes for half an hour. It is also treated with 0.1% $Hgcl_2$ for 1-2 minutes, and rinses 3-4 times with distilled water to remove even the traces of chemicals from the surface. In the first culture the medium became brown and the explants were infested with fungal mycelium.

To get rid from the above problem of exudation of phenolics, the medium was supplemented with antioxidants taken as 50 mg/l to 100 mg/l (Citric acid + Ascorbic acid) each. The antioxidant (100 mg/l citric acid + 100 mg/l Ascorbic acid) completely inhibits exudation and oxidation of phenolics. The explants were also treated with 0.1% Bavistin solution (fungicides) so that any fungal contaminants may be killed. All explants were further washed with sterile distilled water to remove even the traces of the chemicals from the surface. Inoculation was done in the Laminar flow chamber and inoculated tubes and jars were incubated in the culture room at 26±1 °C with light intensity of 3000 Lux generated by white fluorescent tubes.

Observation was made on an alternate day and cultured tube showing any contamination and phenolics were discarded. The multiplication of shoot bud was obtained in the same medium after subculture. The plantlets were rooted in half strength MS basal medium + sucrose + 0.8% agar and various concentration of IBA (0.5-5.0) mg/l and NAA (0.5-5.0) mg/l either alone or in combination. Half MS Basal medium supplemented with 4.0 mg/l NAA promoted maximum rooting, where the mean number of roots per plantlets was 2.0 among 56% of the explants after 28 days of inoculation. Well grown plant with vigorous root were taken out. Root were washed to remove culture medium and were transferred in pot containing sterile soil, sand and compost in 1:1:1 ratio. The hardening was done in artificial moist chamber made with the help of poly bag.

Result

In our present work, we had started our experiment taking MS medium along with the BAP of different concentration starting from (0.5 mg/l-3.8 mg/l) and NAA from (0.1-0.2) mg/l. The MS basal medium was found to be suitable for *in vitro* regeneration of *A.digitata* [7]. The culture tubes and flask inoculated with the nodal explants were incubated in the culture room with controlled temperature and light. From the review of literature, it was cleared that preparation of culture media for a particular explant is a completely trial and error method, with different concentration of cytokinin and Auxin with basal medium in either alone or in different combination. A particular combination of basal medium along with cytokinin is only suitable for a particular explant and may be a growth inhibitor for other explant [8,9]. After 3 week to 4 week of primary culture, explants showed growth response on different culture medium when supplemented with different concentration of BAP (0.3-3.8) mg/l and NAA (0.1-0.2) mg/l and antioxidant (Ascorbic acid + citric acid) 100 mg/l each after 28 days of inoculation. From the graph, we saw that when the concentration of BAP (0.5) m/l and NAA (0.2) mg/l was taken, the mean number of shoot buds is 1.46 and mean length of shoots (in cm) was 2.22, while when the concentration of BAP is increased from (0.5) mg/l to (2.5) mg/l and concentration of NAA (0.2) mg/l was taken, the mean no. of shoot buds were also increased. It was clear that, at the concentration of BAP (3.0 mg/l) + (0.2) mg/l NAA +antioxidant, the percent response at the condition was 92, while the mean number of shoots bud was 2.60 and mean length of the shoots was 2.82 cm after 40^{th} days of inoculation. At lowest concentration of BAP i.e (0.5) mg/l minimum percentage of response, lowest mean numbers of shoot bud and length were noted. The combination of BAP increasing from (2.0) mg/l and NAA (0.2) mg/l was most effective in inducing shoot buds from nodal explant of *A.digitata*. After taking several efforts at different concentration of BAP (0.3 -3.8) mg/l the best concentration was standardized. i.e BAP (3.0) mg/l +(0.2) mg/l NAA and (Ascorbic acid + citric acid) 100 mg/l each (Graph 1).

Rooting

Rooting in well grown plantlets raised through tissue culture was initiated in ½ MS basal medium supplemented with different concentration of IBA (0.5-5.0 mg/l) and NAA (0.5-5.0) mg/l either alone or in combination. The best result of maximum induction was

Citation: Kumari A. Development of Efficient Micropropagation Technique for Conservation of *Adansonia digitata A Rare Medicinal Plant.* J Plant Sci Res. 2020;7(2): 197

½ MS + (4.0 mg/l) NAA, where the mean number of roots was 2.0 among 56% of the explant. In ½ MS medium IBA (0.5-5.0 mg/l) alone or in combination with NAA (0.5-5.0 mg/l) the response was less than the above.

Discussion

Micropropagation technique is being exploited for large scale production of plantlets which are mostly true to the genotype of its mother stock. This technique is also being used in case of tree species [10]. *In vitro* micropropagation of *A.digitata* had been done by taking explants from seed germination, cotyledonary nodes, two nodes segment, terminal apex and even more [11,12]. In some experiment of multiple shoot bud formation from *A.digitata* embryo were also used as explant in two different basal medium MS medium and WPM (Woody Plant Medium) [13].

In the Present work the initiation of axillary shoot buds on nodal explant were obtain with MS medium supplemented with BAP (0.5-3.0) mg/l and NAA (0.1 -0.2) mg/l either alone in combination, Antioxidants were also used for removal of exudation of phenolics [14]. The different concentration of BAP and NAA used proved successful for microprogation of *A.digitata* through nodal explants. Nodal segment as explants and cytokinin are one of the most important hormones for shoot proliferation [15]. From the survey of literature it is apparent that BAP is the most reliable and effective cytokinin for shoot bud induction.

Acknowledgement

Author is highly obliged to the Head of the Department providing the research facilities in the University Department of Botany. I am also very much thankful to my mentor Dr. L.N.Shukla for their cooperation,and Department of science and technology from where we got financial support to carry out my research in University Department of Botany.

References

1. Wickens GE (2008) The baobabs: pachycauls of Africa, Madagascar and Australia.
2. Assogbadjo AE, Kyndt T, Chadare FJ, Sinsin B, Gheysen G, et al. (2009) Genetic fingerprinting using AFLP cannot distinguish traditionally classified baobab morphotypes. Agrof Syst 15: 157-165.
3. Ishii K, Kambou S (2007) *In vitro* culture of an African multipurpose tree species: *Adansonia digitata*. Propagation of Ornamental plants 7: 62-67.
4. Singh S, Rai S, Khan S (2010) *In vitro* seed germination of *Adansonia digitata* L.: An endangered medicinal tree. Nanobiotech Univer 1: 107-112.
5. Assogbadjo AE, Kakai GR, Edon S, Kyndt T, Sinsin B (2011) Natural variation in fruit characteristics, seed germination and seedling growth of *A.digitata* L. in Benin. New Forest 41: 113-125.
6. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15: 473-497.
7. Kumari A, Kumari R, Shukla LN (2015) Development of technique for efficient *in vitro* propagation of Jatropha curcas L.from nodal explant. Indian J Appl Res 5: 40-43.
8. Jagannathan V (1988) Present status of plant tissue culture in India; Proceedings of the National seminar on plant tissue culture. (New Delhi: ICAR publication) pp. 1-10.
9. Boxus Ph (1974) The production of strawberry plant by *in vitro* micropropagation. J Hort Sci 49: 209-210.
10. Johri, Sanjay M (2008) Can tissue culture conserve Kalpvriksha? Merinews article.
11. Roll E, Brunonia F, Bruni R (2014) An optimized method for *in vitro* propagation of Africanbaobab (*Adansonia digitata* L.) using two-node segments. Plant Biosystems 150: 750-756.
12. N'Doye AL, Sambe MAN, Sy MO (2012) Propagation of African Baobab (*Adansonia Digitata* L., Bombacoideae, Malvaceae) Germplasm through *in vitro* cloning. Adv Env Biol 6: 2749-2757.
13. Fasola TR, Okerenkporo MT (2019) Micro propagation of Baobab and economic plant. J Am Sci 15: 48-53.
14. Kumari A, Ranjana, Shukla LN (2011) Reduction of phenolics and endophytic fungus in the nodal and intermodal explants of Jatropha curcas L. Indian J Sci Res 2: 45-49.
15. Bhojwani SS (1980) *In vitro* propagation of garlic by shoot proliferation. Scientia Horticulturae 13: 47-52.

Citation: Kumari A. Development of Efficient Micropropagation Technique for Conservation of *Adansonia digitata A Rare Medicinal Plant.* J Plant Sci Res. 2020;7(2): 197