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# MICROPROPAGATION OF *Bauhinia acuminata* L.

**Md. Shamim Akhter* , Dr. S.M. Mahbubur Rahman, Md. Hafizur Rahman**

Biotechnology and Genetic Engineering Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh.

*Corresponding author's e-mail: shamim11_akhter@yahoo.com

**Abstract**

Seeds of *Bauhinia acuminata* L. were used as an experimental material for micropropagation. The effect of growth regulators concentrations on morphogenetic development was studied. Seeds were germinated on MS medium supplemented with $GA_3$ (0.10, 1.0 and 2.0 $mgL^{-1}$) and without $GA_3$. Satisfactory germination was observed at media with 1.0 $mgL^{-1}$ $GA_3$. Subsequent propagation from plantlet was performed on MS medium supplemented with various concentrations of BA and NAA or IBA. For Shoot proliferation, combinations of BA and NAA (0.20+0.10, 0.30+0.10, 0.4+0.1, 0.50+0.10 and 1.0+0.10 $mgL^{-1}$) were used. Reasonable shoot formation was observed at 0.50 $mgL^{-1}$ BA + 0.10 $mgL^{-1}$ NAA. For rooting IBA (0.20, 0.60 and 0.80$mgL^{1}$) and NAA (0.20, 0.60 and 0.80$mgL^{-1}$) were used. The highest numbers of roots were observed at NAA 0.60 $mgL^{-1}$.

**Keywords**: Bauhinia, micropropagation, $GA_3$, BA,NAA, IBA.

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## Introduction:

The term *Bauhinia* is derived from the new Latin word 'Bauhin'. The synonym of Bauhinia is 'dwarf white orchid tree'. This is a genus of more than 200 species of flowering plants in the subfamily Caesalpinioideae of the large flowering plant family Fabaceae, with a pan tropical distribution. The genus was named after the Bauhin brothers, Swiss-French botanists: Jean *Bauhin* (1541–1612) and and Gaspard *Bauhin* (1560–1624)[1]. Many species are widely planted in the tropics as ornamental, particularly in northern India, Vietnam and southeastern China.

Many species, cultivars, and varieties are available. *Bauhinia acuminata* L. is seedless and would not present such a litter problem. It is also the most spectacular and most wanted *Bauhinia* spp., bearing six-inch, orchid-like flowers of rich reddish or rose purple during the winter but is very tender to freezing temperatures. *Bauhinia variegata*, most popular, produces in winter and spring most nearly orchid-like blossoms of purplish casts or pure white in cultivar `Candida'.

*Bauhinia purpurea*, most variable, produces narrow-petaled, red-purple to blue-purple flowers in late fall and early winter while leaves are on the trees. *Bauhinia monandra* produces pink, single-stamened flowers all summer. *Bauhinia acuminata* also blooms all summer but with white flowers[2].

It is a cultivated shrub of 3 m tall; young stems, petioles and inflorescence axes with sparse curled pubescence. Stipules lance-linear, 5-12 mm long, acuminate, curled puberulent, caducous; largest colleter swollen, divergent, 1.5-2.1 mm long. Leaves with petioles 1.5-4 cm long; blades ovate, broadly ovate or suborbicular, 5.4-11.3 (20) x 3.7-11.3 cm, divided about 1/3 their length, membranous, glabrous adaxially, densely puberulent abaxially, base cordate to rounded, apex of lobes acute. Inflorescences axillary racemes, 2.5-5.8 cm long; peduncles negligible; bracts and bracteoles lance-linear, 3-9 mm long, puberulent, especially on margins, caducous. Flowers with pedicel 6-12 mm long; hypanthium 5-9 mm long; calyx limb spathaceous, 28-37 mm long, with few scattered hairs abaxially, apex of 5 spidery lobes 1.7-4.1 mm long; petals not clawed, elliptic to oblanceolate, 39-49 (60) x 20-25 (30) mm, glabrous, white; fertile stamens 10, filaments strigose at base; ovary stipitate, strigose on sutures, stigma peltate, bilobed. Legumes linear, 7.5-15 x 1.7-1.8 cm, glabrous, brown; seeds suborbicular, ca. 10 mm in diameter" [3], [4], [5].

*Bauhinia* is grown in the best in full sun or high, shifting pine shade and it thrives in any well-drained soil but in alkaline soils and micronutrient deficiency can show interveinal chlorosis (yellowing) on the leaves. The wood tends to be weak and sprouts are often seen growing from the base of the tree creating an unkempt appearance. Besides this the fallen leaves messy because they are large and decompose slowly. Chewing insects and borers may present and this can create problem for Bauhinia. Because of over-watering because it may tend to turn the foliage yellow[6], [7].

They are excellent on raised beds ,sunny banks or rock gardens,where they thumble and spread over rocks or retaining structures. In nature, they inhabit rocky, open locations, where they have to be tough and drought tolerant. They are very much at home in roof and balcony gardens, in low maintenance gravel gardens, as edging along paths and driveways and, of course, in beds and borders. They can be planted in containers, seasonal window boxes, or shrub gardens. *Bauhinia* should be planted where they will receive at least 4-5 hours of full sun each day. They thrive in fertile, fast draining, and slightly alkaline (pH 6.75) soil[8], [9]. Testa of unstratified seeds contains high concentrations of ABA. It has been found that seeds with intact integument germinate more slowly than those without integument[10]. Therefore, the effect of ABA could be reduced in unstratified seeds by removing the integument. The ABA level in the seed can be reduced by both high and low temperatures[11].

Seeds of *B. acuminata* possess an undeveloped rudimentary embryo that requires an extended period of high temperature for breaking the dormancy. This situation can be overcome by treating the dormant seeds with $GA_3$ and cytokinin[12] and the action of cytokinin are both antagonistic to the inhibitory effect of ABA and complementary to the action of $GA_3$. Therefore, the regulation of seed dormancy by temperature for germination, applied growth regulators at different concentrations and testa removal were investigated through the experiment. In sexual reproduction, seeds will not truly

reproduce their parents and a substantial amount of variation will occur among the seedlings. The *in vivo* clonal propagation of plant is often difficult, expensive and even unsuccessful. Alternative means of Tissue culture methods offer a vegetative propagation and the identity e.g. disease resistance, tolerance to environmental stress, flowering times and yields etc. of mother plants can be maintained through this method. Clonal propagation through tissue culture popularly known as micro propagation can be achieved in a short time and space and it is possible to produce plants in a large scale starting from a single individual. Thus, micropropagation is the only commercially viable approach[13]. Leaves of Begonia and some other ornamentals produce shoots on a large scale through micropropagation [14]. However, it is difficult to produce root from shoot tip for developing a plantlet can be hardened in soil. Therefore, the study was undertaken considering the following objectives: i) To test shooting efficiency of *B. acuminata* in MS media. ii) To find out the suitable auxin and determine the optimum concentration of auxin for effective rooting in semisolid medium. iii) To observe the rooting efficiency/ morphological development of root of *Bauhinia* in semisolid medium. iv)To develop a suitable protocol for formation of root in semisolid medium that would be effective for the sustainable hardening of *Bauhinia* in soil.

## Related Research

Orchids were the first plants to be commercially propagated through tissue culture [15]. The first experiments in orchid tissue culture were carried out in the1940s in Knudson's laboratory at Cornell University. More than a decade later, Georges Morel at the Central Station for Plant Physiology in France reported that excised *Cymbidium* shoot tips could be induced to form multiple plantlets when cultured on seed germination media supplemented with phytohormones. Shortly after this report, Donald Wimber at Brookhaven National Laboratory developed a successful method of using tissue culture to commercial propagate *Cymbidium. Phalaenopsis* proved more difficult to tissue culture propagate. During the 1980s, many different researchers throughout the world developed tissue culture protocols for *Phalaenopsis* including Intuwong and Sagawa at the University of Hawaii, Reisinger and others at the University of California, Zimmer and Pieper at the Technical University in Germany[16].

Micropropagation is the use of plant tissue culture techniques to generate high quality, genetically uniform plants. It is the only way to produce virus free clones of infected plants. The first plants to be commercially propagated in this manner were *Cymbidium* orchids, but the method is used for most plants that are commonly propagated vegetatively. Familiar examples include tulips, potatoes, & lilies. Micropropagation is also useful for reproducing copies of plants with ideal (forestry and fruit trees) or unique (introductions of a new rose or orchid hybrid) characteristics for commercial distribution. With a limited amount of materials and laboratory space, millions of plants can be produced. From miniature African Violets to the tallest California redwoods, the variety of plants that can be propagated in vitro is almost unlimited[17].

Cytokinins are often used to stimulate growth and development; Kinetin, BAP, 2iP and BA being in common use. They usually promote shoot differentiation, especially if added together with auxin. In higher concentration (1-10 $mgL^{-1}$) they can induce adventitious shoot formation but root formation is generally inhibited. They promote axillary shoot formation by decreasing apical dominance[18]. Many growth active substances, phytohormones as well as other types of compound have included in the culture medium to manipulate organogenesis *in vitro* [19]. *In vitro* culture is often impossible without regulators whether an auxin and/or a cytokinin have to be added to a nutrient medium. Organogenesis dependents also on the type of explant and genotype of the plant species. A large number of plant species responded in presence of suitable auxin or cytokinin balance in forming shoots and roots[20]. Thin cross section culture system is about seven times more efficient in protocorm like bodies formation than shoot tip culture[21].

For *Dendrobium gouldll cv pinwathana*, phytamax (phytamax + 2.0 $mgL^{-1}$ BAP + 0.5 $mgL^{-1}$ NAA + 2% sucrose +2$gL^{-1}$ peptone) was found to be most efficient in which highest percentage of seed germination occurred with full vigor[22]. It was reported that maximum number of multiple shoots regeneration was observed in *Chrysanthemum* cv. Super Yellow and Light Violet from the MS media supplemented with 0.5 $mgL^{-1}$ IAA+ 0.5$mgL^{-1}$ NAA + 0.8$mgL^{-1}$ Kn + 2.0$mgL^{-1}$ BAP + 10% CW and 0.5$mgL^{-1}$ IAA + 0.8$mgL^{-1}$ Kn + 10%CW combinations[23]. Seed germination of different *Bauhinia* species was also observed in Phytamax medium[24]. Although requirement of seed germination of orchids varies from species to species, it was observed that Phytamax medium was suitable for seed germination of orchids. It was found that seed germination was enhanced when coconut water (CW-20%) was added to the phytamax medium containing 2.0$mgL^{-1}$ BAP and 0.5 $mgL^{-1}$ NAA[25].

The media widely used to initiate growth of tissue on culture media and their several modifications based on changes mostly in macro element incorporations[26]. Higher concentrations of cytokinin (2-10 $mgL^{-1}$) were employed for shoot regeneration. A high level of auxin to cytokinin is root promoting, whereas, the opposite condition i.e., an increased concentration of cytokinin and low auxin (a medium in which the ratio of cytokinin to auxin concentration and its inhibition by the addition of kinetin, has been shown in a particular variety of *Armoracia rusticana* [27]. In tissue culture various methods have been widely employed in clonal propagation of Bauhinia. In this case monopodial Bauhinia are propagated by the seeds and the excision of shoot tip. Success was obtained in many monopodial white dwarf orchid plant like *Bauhinia* [28]. For mass propagation of Bauhinia in commercial purposes millions of plantlets have been produced by tissue culture techniques. For this purposes various explants have been used by several workers namely, shoot tip auxiliary bud leaf base leaf tip dormant bud on flower stalk inflorescence and root tip [29].

Seeds also be cultured for the Micropropagation of Bauhinia. Juvenile leaves were found to be very suitable material for propagation and production of additional plants in *Bauhinia acuminata* . The *Bauhinia* is a kind of plant which has a slow growth. Accelerating the growth of it can be done using plant growth regulators. Very recently this is reported that the importance of coconut water for inducing protocorm like bodies formation from shoot tip explants of *Phalaenopsis* without using plant growth regulators[30]. The most important growth regulator content of coconut water are perhaps the

cytokinins. However, coconut milk inhibited root and leaf production of Bauhinia seedlings. On the other hand, for *Bauhinia monendra* the best seed germination and seedling growth was reported on Knudson C medium[31].

## Materials and Methods

In this experiment, the plant materials (seeds) were collected from mature plants. Seeds of *B. acuminata* were used. At first, seeds were washed under running tap water. Then they were rinsed with sterilized distilled water. Finally, seeds were dipped into absolute alcohol for 30 seconds and then washed with distilled water. Afterwards seed coat of the seeds was opened by forceps which was then inoculated aseptically onto MS medium.

MS basal medium[26] supplemented with different concentrations and combinations of $GA_3$ (0.1, 1.0 and 2.0 $mgL^{-1}$) was prepared as a media for germination. After mixing all stock solutions and growth regulators at appropriate volume, 3% sucrose was added. The pH of the medium was adjusted to 5.7-5.8 and then agar (0.7%) was added and dissolved. The media were dispensed in the 40×150 mm glass bottles in a volume of 20-25 ml. Each treatment consisted of 10 glass bottles. The media were sterilized by autoclaving at 121ºC for 20 minutes.

For shoot proliferation, 4-5 weeks germinated seedlings were taken. And every node was cultured in solidified MS basal medium supplemented along with BA + NAA (0.2+0.1, 0.3+0.1, 0.4+0.1, 0.5+0.1 and 1.0+0.1 $mgL^{-1}$). Culture media were dispensed in the glass bottles and sterilized as mentioned above. About 3-4 weeks period was required for shoot proliferation. For rooting, regenerated shoots were sub-cultured in solidified MS medium supplemented along with IBA or NAA (0.5-2.0 $mgL^{-1}$) and their combinations (0.5-2.0 $mgL^{-1}$) including coconut water (5%). Culture media were dispensed in the glass bottles and sterilized as mentioned above. Ten shoots were inoculated for each of the treatment.

The culture was incubated under 12 hr photoperiod (cool-white fluorescent light) providing light intensity 3000 lux. The temperature and relative humidity were maintained at 26± 2 ºC and 78% respectively. Weekly Visual observation of culture was made and frequency of culture showing plantlet, shoot and root formation and multiplication was recorded. The data pertaining to shooting and rooting per culture were analyzed subjected to standard deviation. Data presented in the tables were analyzed as means ± SD according to Mian and Mian (1984)[32].

## Discussion

Dormancy is caused for the presence of ABA. But it also may happened by the cause of cold temperature ($15^0C/20^0C$). Seeds of *B. acuminata* possess an undeveloped embryo. Dormancy can be overcome by treating the dormant seeds with $GA_3$ and cytokinin [33]. In this study, the germination percentage was 95 which are acceptable; this indicates that $GA_3$ might reduce the effect of ABA. Hundred percent germination was obtained with $GA_3$ treatment in *A. cherimola* which has rudimentary embryo[34].

*B. acuminata* showed remarkable response in the formation of shoots and roots in semisolid culture media. MS semisolid media supplemented with three different concentration and combination of cytokinins & Auxins (BA+NAA) and different concentrations of auxins (IBA, NAA) were used to determine the rooting efficiency of *B. acuminata*. Each of the three auxins showed a ready response to induce shooting and rooting and started to develop shoots and roots more or less within 5-7 days. Data were recorded after 4 weeks. The best results came from the treatment using NAA. Each of three concentrations group of NAA produced highest number of roots per shoot with highest root length.

**Table-1: Effect of $GA_3$ on the germination of *B. acuminata*:**

| Conc. Of growth regulators ($mgL^{-1}$) $GA_3$ | % germination |
|---|---|
| Control | 70 |
| 0.10 | 72.5 |
| 1.0 | 95 |
| 2.0 | 72 |

**Table-2. Effect of different concentration and combination of cytokinin and auxin on shoot formation:**

| Cytokinin+Auxin | Concentration($mgL^{-1}$) | No. of shoots per culture | Avg. Shoot length (cm) |
|---|---|---|---|
| BA+NAA | 0.20+0.10 | 1.33 ± 0.58 | 3.58±0.75 |
| BA+NAA | 0.30+0.10 | 1.63 ± 0.80 | 3.49±0.80 |
| BA+NAA | 0.40+0.10 | 1.80± 0.83 | 2.91±0.45 |
| BA+NAA | 0.50+0.10 | 2.50 ± 0.91 | 3.86±0.80 |
| BA+NAA | 1.0+0.10 | 2.15 ± 0.89 | 3.71± 0.92 |

**Table: 3 Effect of different concentration auxin on root formation:**

| **Auxin** | **Concentration($mgL^{-1}$)** | **No. of Roots per culture** | **Root length (cm)** |
|---|---|---|---|
| IBA | 0.20 | 2.50±0.71 | 2.38±0.99 |
| | 0.60 | 2.70±1.19 | 2.50±0.80 |
| | 0.80 | 2.71±1.22 | 2.43±0.82 |
| NAA | 0.20 | 2.57±1.18 | 2.71±1.28 |
| | 0.60 | 3.44±0.68 | 3.11±0.74 |
| | 0.80 | 2.70±0.78 | 2.60±1.02 |

A number of treatments of cytokinin (BA) ranging from 0.20-1.0 $mgL^{-1}$ (0.20, 0.30, 0.40, 0.50, 1.0 $mgL^{-1}$) with auxin (0.10 $mgL^{-1}$) were employed for shoot proliferation. The highest number of shoots/culture was observed at the concentration of

0.50mgL$^{-1}$ BA with 0.10mgL$^{-1}$ NAA. This was 2.50 ± 0.91 The maximum shoot length (3.86±0.80cm) was found in this concentration (Table-2).

In these treatments, the highest number of shoot formation was found at concentration of 1.0mg/L BA+ 0.05mg/L NAA. Highest shoot length was originated when cultured on a medium containing MS + 0.1 mg/L NAA+0.05 mg/L[35]. The longest shoot originated from nodal segments when cultured on MS medium supplemented with 0.1 mg/L NAA + 1.0 mg/L[35]. but in this experiment the longest shoot from nodal segment cultured on MS medium supplemented with 0.50mgL$^{-1}$ BA with 0.10mgL$^{-1}$ NAA.

Plate-1*: Bauhinia acuminata L*. Plate-2. Germination of *B. acuminata* on MS medium containing $GA_3$ 1.0 mgL$^{-1}$ Plate-3: Shoot formation of *B. acuminata* on MS medium containing BA 0.50 mgL$^{-1}$ + NAA 0.10 mgL$^{-1}$. Plate-4: Root formation of *B. acuminata* on MS medium containing NAA 0.60 mgL$^{-1}$.

In the case of root proliferation three concentrations of IBA viz. 0.20, 0.60 and 0.80 $mg^{-1}$ and NAA viz, 0.20, 0.60 and 0.80 $mg^{-1}$ were employed for root induction. All the treatments produced root with varying root number and length. NAA 0.60 $mg^{-1}$ obtained maximum number of roots 3.44±0.68 but the maximum root length 3.11±0.74 was found in the concentration of 0.60 $mg^{-1}$ NAA. (Table-3). Plate 3 shows the maximum number of root.

In this investigation, it is found that the effect of NAA (3.11±0.74) was greater than IBA (2.50±0.71). The best root growth (3.11±0.74 cm) was found at 0.60mg/L NAA as compared with IBA (0.6mg/L). The highest number of roots/shoot (3.44±0.68) formed at 0.60mg/l NAA was better than IBA. It was reported that a very low concentration of NAA (0.49 µM) produced significantly higher number of roots per shoot[36] and higher concentrations of NAA favored formation of malformed and thick roots[37].

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