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# Callus induction and multiple shoot regeneration in *Ajuga bracteosa* Wall ex. Benth.-An important medicinal plant growing in Kashmir Himalaya

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## Abstract

*Ajuga bracteosa* Wall ex. Benth is an important medicinal plant growing in Kashmir Himalaya. During the present study an efficient and rapid in vitro protocol has been established viz; Callus induction and multiple shoot regeneration. Callus induction has been achieved from different explants viz., leaf, petiole and internodal cuttings. Maximum callus production was obtained when leaf explants were inoculated on Murashige and Skoog (1962) medium supplemented with BAP (benzylaminopurine 5.0 mg/l) after 19 days of inoculation. In the petiole explant callus was induced on MS (Murashige and Skoog ) medium supplemented with BAP (2.0 mg/l) + IAA ( Indole-3-acetic acid 3.0 mg/l) after 51 days of inoculation and from internodal explant callus was induced on MS medium supplemented with BAP (2.0 mg/l) + NAA(α-Naphthalene acetic acid 5.0 mg/l) after 35 days of inoculation. Callus derived from leaf explants differentiated into multiple shoots on MS medium supplemented with different concentrations of auxins (IAA, NAA) and cytokinins (BAP, Kinetin). Maximum multiple shoots regenerated on MS medium supplemented with BAP (5.0 mg/l) after 28 days of inoculation.

**Keywords:** *Ajuga bracteosa*, Callus, multiple shoots, Auxins, Cytokinins.

## Introduction

*Ajuga bracteosa* Wall ex. Benth. of family Lamiaceae is commonly known as 'Bungle' in English and 'Jan-i-adam' in Kashmiri. It is a perennial erect, ascending hairy herb, often prostrate with oblanceolate or sub-spathulate leaves and grows up to 5-50cm tall. It is distributed in subtropical and temperate regions of Kashmir to Bhutan, Pakistan, Afghanistan, China, Malaysia, Western Himalayas, plains of Punjab and upper Gangetic plains of India at an altitude of 1300m .[1, 2] In Pakistan it is found in northern hilly areas, where in local Hindi/Punjabi language it is called kori booti (means bitter herb) owing to its bitter taste.[3] It is found along roadsides, open slopes, and rock cervices.[4] The plant is used for the treatment of gout, rheumatism, palsy and amenorrhoea.[5-9] Locally the leaves help in curing headache, pimples, measles, stomach acidity, burns, boils.[10, 11] It is effectively used against jaundice, hypertension, sore throat and as a blood purifier.[12] Anti-inflammatory and anti-cancerous properties of *Ajuga bracteosa* have been reported.[13] Investigators have also reported anti-malarial activities.[14, 15] It could also be an alternative to Artemisia currently being used as an anti-malarial.[16] In vitro evaluation of antihelmentic efficacy of *Ajuga bracteosa* on Ascaridia galli (a poultry worm) has also been studied.[17]

The limited distribution of this plant coupled with its tough habitat and unrestricted

exploitation of its medicinal properties have made *Ajuga bracteosa* an endangered plant species.[16]The present study focused on standardization of effective *in vitro* protocols of *Ajuga bracteosa* for high rate of callus induction followed by multiple shoot regeneration in a short duration for its mass propagation.

## Materials and Methods

*Ajuga bracteosa* was collected from Jawahar tunnel Jammu and Kashmir and later transplanted at Kashmir University Botanical Garden (KUBG). The specimen was collected and processed for herbarium preparation and latter deposited at Kashmir University Herbarium (KASH) Voucher Specimen Number 1844 (Ref.No.F1/Herbarium-Specimen vouchers) KU/2013). Leaves, petiole and intermodal explants of *Ajuga bracteosa* were collected from plants grown at KUBG. Explants were first washed thoroughly under running tap water for 30 min in order to remove dust, dirt and other unwanted materials. These were then washed with a detergent solution Labolene 1% v/v containing 2-3 drops of surfactant, Tween 20 (1% v/v). This was followed by washing with tap water to remove the detergent and finally washed 2-3 times with double distilled water under laminar air flow hood. Finally the explants were disinfected with 2 % sodium hypochlorite solution (NaOCl) for 10 minutes. After 10 minutes disinfectant solution was decanted and the surface sterilized explants were washed 5-6 times with double distilled water so as to remove any traces of the sterilant. The sterilized plant material was then aseptically inoculated on to the culture medium in the laminar airflow cabinet. MS medium supplemented with different concentrations and combinations of 6-benzylaminopurine (BAP), α-Napthalene acetic acid (NAA), Indole-3-acetic acid (IAA) were used for callus induction. The cultures were maintained at 25± 2°C, light intensity of 3000 lux and a regular photoperiod of 16 hrs.

## Results

### Callus induction

*From leaf explant*

Callus was induced when leaf explants were inoculated on MS medium supplemented with NAA (3 mg/l) , BAP (5 mg/l) and BAP (5 mg/l) + IAA (2 mg/l) in a time period of 20,19 and 30 days respectively. The best results were obtained on MS medium supplemented with BAP at a concentration of 5 mg/l (Fig. 1 Table 1).


**Figure 1:** Callus initiation from leaf explant [A MS+NAA (3 mg/l)], [B MS+BAP (5 mg/l)], [C MS+BAP (5 mg/l) + IAA (2 mg/l)

**Table 1:** Effect of different growth regulators on callus induction from leaf explant of *Ajuga bracteosa* (*30 replicates per treatment)

| MS medium | BAP (mg/l) | NAA (mg/l) | IAA (mg/l) | Mean no. of days for Callus production | % Response |
|---|---|---|---|---|---|
| + | _ | _ | _ | _ | 0 |
| + | 2 | _ | _ | 47 | 40 |
| + | _ | 3 | _ | 20 | 60 |
| **+** | **5** | **_** | **_** | **19** | **100** |
| + | 5 | _ | 2 | 30 | 80 |
| + | _ | 2 | _ | 22 | 30 |

*From petiole explant*

Petiole explants produced callus when they were inoculated on MS medium supplemented with BAP (2 mg/l) + IAA (3 mg/l) .Callus was obtained on petiole explants after 51 days of inoculation. (Fig. 2 Table 2)

*From internodal explant*

Internodal explants produced callus on MS medium supplemented with BAP (2 mg/l) + NAA (5 mg/l) after 35 days of inoculation. (Fig. 3 Table 3)


**Figure 2:** Callus initiation from petiole explant [D MS+BAP (2 mg/l) + IAA (3 mg/l)]


**Figure 3:** Callus initiation from internodal explant [E BAP (2 mg/l) +NAA (5 mg/l)]

**Table 2:** Effect of different growth regulators on callus induction from petiole explant of *Ajuga bracteosa* (*30 replicates per treatment)

| MS medium | BAP (mg/l) | NAA (mg/l) | Mean no. of days for Callus production | % Response |
|---|---|---|---|---|
| + | _ | _ | _ | 0 |
| + | 0.5 | _ | _ | 0 |
| + | 1.0 | 0.5 | 65 | 30 |
| + | 2.0 | **3.0** | **51** | **100** |
| + | 3.0 | 2.0 | 60 | 40 |
| + | 4.0 | 3.0 | _ | 0 |
| + | 5.0 | 1.0 | _ | 0 |

**Table 3:** Effect of different growth regulators on callus induction from internodal explant of *Ajuga bracteosa* (*30 replicates per treatment)

| MS medium | BAP (mg/l) | NAA (mg/l) | Mean no. of days for Callus production | % Response |
|---|---|---|---|---|
| + | _ | _ | _ | 0 |
| + | 0.5 | _ | _ | 0 |
| + | 1.0 | 0.5 | _ | 0 |
| + | 1.5 | 0.5 | _ | 0 |
| + | _ | 2.0 | 35 | 40 |
| + | 2.0 | 3.0 | 38 | 45 |
| + | 2.0 | 5.0 | 35 | 100 |
| + | 3.0 | _ | 37 | 60 |
| + | 4.0 | 3.0 | _ | 0 |
| + | 5.0 | 1.0 | _ | 0 |

**Shoot multiplication**

Multiple Shoots were obtained on MS medium supplemented with auxins and cytokinins individually and in combinations. Cytokinins like BAP were used in concentrations of BAP(3mg/l) and BAP(5 mg/l).Different combinations of BAP with IAA also gave good results BAP(5 mg/l) +IAA(2 mg/l),BAP(2 mg/l) +IAA(3 mg/l), BAP(2 mg/l) +IAA(5 mg/l). Kinetin was used individually and in combination with IAA Kinetin (2 mg/l) +IAA(2 mg/l).

Best results were obtained on MS medium supplemented with BAP (5 mg/l) with an average number of 10.5 shoots, 28 days after inoculation (Fig 4, Table 4).


**Figure 4:** Multiple shoot formation in *Ajuga bracteosa* on [**F** MS+BAP (5 mg/l)], [**G** MS +BAP (5 mg/l) + IAA (2 mg/l)], [ **H** MS+KN (2 mg/l) + IAA (2 mg/l)],[ **I** MS+BAP (3 mg/l).

**Table 4:** Effect of different growth regulators on multiple shoot formation from leaf derived callus of *Ajuga bracteosa*

| **MS medium** | **BAP** mg/l | **NAA** mg/l | **IAA** mg/l | **KN** mg/l | **Average number of shoots±SE** | **Average height of shoots(cm)±SE** | **Number of Days** | **% response** |
|---|---|---|---|---|---|---|---|---|
| + | 2 | _ | 3 | _ | 6.3±0.59 | 3.5±0.5 | 39 | 40 |
| + | 2 | _ | 5 | _ | 4.6±0.56 | 6.5±0.5 | 36 | 30 |
| + | 3 | _ | _ | _ | 8.5±0.68 | 2.5±0.30 | 32 | 60 |
| + | 5 | _ | _ | _ | 10.5±1.54 | 4.8±0.48 | 28 | 100 |
| + | 5 | _ | 2 | _ | 9.5±0.70 | 2.5±0.30 | 30 | 80 |
| + | _ | _ | 2 | 2 | 6.9±0.60 | 2.2±0.2 | 35 | 40 |
| + | _ | _ | _ | 3 | 5.9±0.60 | 1.5±0.22 | 38 | 20 |

## Discussion

During the present study, different plant growth regulators like auxins and cytokinins used either individually or in different combinations produced callus from different explants of *Ajuga bracteosa*. Maximum callus production was obtained from leaf explants inoculated on MS medium containing BAP (5 mg/l). Juvenile leaves responded better as compared to older leaves. The callus produced was compact and green. Callus from leaves after sub culturing produced multiple shoots. Maximum shoot formation was achieved on MS medium supplemented with BAP 5 mg/l. This is in contrast to the results of Srivastav *et al*., 2013 showing maximum callus induction from leaf explants of *Ajuga bracteosa* on MS medium supplemented with BAP (5 mg/l) and IAA (2 mg/l) after 10 days of inoculation.[16] The callus developed multiple shoots on MS medium supplemented with IAA (2 mg/L) + BA (5 mg/L) that elongated within 8 weeks.

## Conclusion

A procedure was developed for callus induction and multiple shoot regeneration in *Ajuga bracteosa* from three different explants viz., leaf, petiole, and internodal cuttings. MS medium supplemented with different plant growth regulators. Among all the explants, leaf explants proved to be more responsive as they produced the maximum amount of callus in less number of days when inoculated on MS medium containing BAP. Multiple shoots were obtained from leaf derived callus on MS medium supplemented with BAP with an average number of 10.5 shoots.

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## Conflict of interest

Declared none.

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