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Research Article

EFFECT OF 6-BENZYL AMINO PURINE HORMONE ON THE SHOOTING GROWTH OF *OCIMUM GRATISSIMUM* L.

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ABSTRACT
BAP (benzyl amino purine) is a cytokine hormone. It causes the shooting effect in plants. We used different concentrations of BAP in MS (Murashige's and Skoog) media, which stimulate shoots regeneration in different explants of *Ocimum gratissimum*. The nodal explants and shoot tips were taken and sterilized using bavistien, tween 20 and mercuric chloride. Then explants were introduced into MS media containing various concentrations of BAP concentrations. 0.5 mg/l BAP showed the best performance of proliferation by inducing shoots in 95 % cultured nodal explants. They produced the highest number (12.0 ± 0.5) of shoots per culture on the medium with 2.3 ± 0.29 cm average length of shoots per culture. On the same medium, shoot tip explants, produced shoots in 75 % of the culture. They produced the highest number (6.9 ± 0.23) of shoots per culture, their average length being 1.8 ± 0.29 cm. This study supports the rapid multiplication of this useful medicinal plant by *in vitro* conditions.
Keywords: *Ocimum gratissimum,* BAP, Nodal explants, Shoot tip explants.

INTRODUCTION

Ocimum gratissimum is propagated by the seed germination and stem cutting. The problem associated with conventional method of propagation is very poor germination rate of seeds (<10 %) and cutting takes 28 days for rooting. It produces around 100 tons of essential oil annually. It is a globally important economic crop because it has a trade value of US $15 million per year. It is also widely used in indigenous system of medicines. *Ocimum gratissimum* is a valuable multipurpose medicinal plant belonging to family Lamiaceae. It is widely distributed in tropical and warm temperature region. It also grows semi-wild in different villages groves. *Ocimum gratissimum* is commonly used in the treatment of upper Respiratory Tract Infections, Diarrhea, Headache, Fever, Ophthalmic, Skin diseases and pneumonia. Extracts of plant contain antimicrobial[1], antifungal[2], antimalarial[3] and antiprotozoal activity. Thymol (32-65 %) and eugenol are the active compounds present in volatile aromatic oil obtained from the leaves[4]. It also contains xanthenes, terpenes[5] and lactones. Many authors' reports that explants from mature field grown plants were better than seedlings explants. Such instances were recorded in *Asclepias curassivica*, *Prosopis juliflora*, *Terminalia belerica*, *Solanum nigrum*[6], *Carica papaya*[7], and *Gemilina arboreal*. Normally, other species like *O. bacilium* shows good response toward plant regeneration in MS media in the presence of BAP combined with auxins as reported by various authors[8]. Although it is conventionally cultivated through seeds, the seedling progeny shows viability due to cross-pollinated nature of the plant. So far there is no earlier report on *in vitro* plant regeneration of this species. It is therefore, necessary to develop efficient and reliable culture technique for *in vitro* regeneration[9] of the plant. We have evaluated the effect of BAP (benzyl amino purine) hormone on the shooting growth on *Ocimum gratissimum.*

MATERIAL AND METHOD

Explants were collected from one and a half year old field. Mature plants were cut into 1.0-2.0 cm nodal segments and used as explants for induction of multiple shoots. Explants were then washed under tap water for 30 minutes. After that it is treated with Tween 20 (5 % solution) for 5 minutes. Now these explants were washed 7-8 times with sterilized distilled water. Later on, the explants were treated with bavistien (5 % solution) for 5 minutes and again washed three times with sterilized distilled water. These explants were transferred into Laminar Air Flow and sterilized with 0.1 % mercuric chloride for less than one min and immediately washed three times with sterilized distilled water. Now put it on 5 % calcium hypochlorite filter paper for drying. Under aseptic conditions, MS media (Murashige's and Skoog media)[10] were prepared containing 3 % (w/v) sucrose. pH of the media was adjusted to 5.8 prior to the addition of agar 0.8 % and autoclaved at 121°C temperature and 15 psi (per square inches) pressure for 15 minutes. Media was supplemented with different concentrations of 6-benzyl amino purine (BAP: 0.25, 0.5, 1.0 and 2.0 mg/l) at 45°C temperature of media. Media was poured into flasks and allowed to be solidifying. Then explants were inoculated in the media in aseptic conditions in Laminar Air Flow. Finally cotton plug was fitted in the mouth of the flask and aluminum foil was applied. Cultures were then incubated at 26 ± 2°C with 16 hours photoperiod by cool white fluorescent tubes and 70-75 % relative humidity.

Table 1: Effect of Different Concentrations of BAP (Benzyl Amino Purine) in MS (Murashige's And Skoog) Medium of *Ocimum gratissimum* for Multiple Shoot Induction from Nodal Explants

Growth regulators (mg/l) BAP	Percent of explants showing response	No. of shoots	Average length of shoots (cm.)
0.25	60.0	10.8 ± 1.6	2.1 ± 6.0
0.50	95.0	12.0 ± 0.5	2.3 ± 0.29
1.00	90.0	11.9 ± 0.5	2.3 ± 0.34
2.00	70.0	6.0 ± 0.2	2.0 ± 0.30

Table 2: Effect of Different Concentrations of BAP (benzyl amino purine) in MS (murashige's and skoog) Medium of *Ocimum gratissimum* for Multiple Shoot Induction from Shoot Tip Explant

Growth regulators (mg/l)BAP	Percent of explants showing response	No. of shoots	Average length of shoots (cm.)
0.25	50.0	4.9±1.6	1.6±0.25
0.50	75.0	6.9±0.23	1.8±0.29
1.00	75.0	6.6±0.5	1.7±0.34
2.00	60.0	3.5±0.15	1.2±0.30

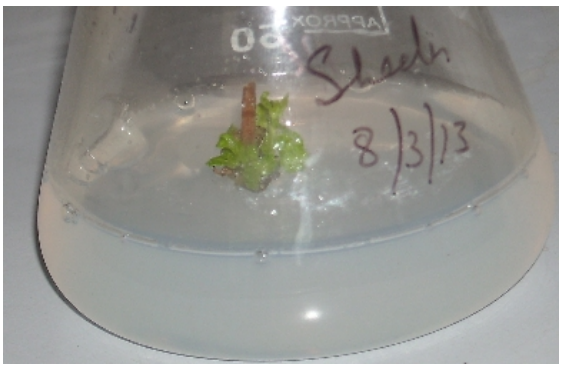

Figure 1: Shoot Regeneration From Nodal Explant

RESULTS AND DISCUSSION

Nodal and shoot tip explants from field-grown mature plants of *Ocimum gratissimum* were cultured on modified MS (half strength of major and full strength of minor salts) medium supplemented with different concentrations of BAP. Initially shoot segments containing nodal zone produced multiple shoot buds on all the cytokine (BAP) supplemented media. The synergistic effect of BAP for direct plant regeneration was also evaluated. For this explants from the *in vitro* grown shoots were also incorporated in this experiment along with explants of the mature field grown plants for proliferation of axillary shoots. The proliferation efficiency of nodal explants from mature plants was significantly higher than that of shoot tips explants when evaluated 45 days after proliferation. As a supplement, 0.5 mg/l BAP showed the best performance of proliferation by inducing shoots in 95 % cultured nodal explants (Table 1). They produced the highest number (12.0 ± 0.5) of shoots per culture on the medium with 2.3 ± 0.29 cm average length of shoots per culture (Table 1). On the same medium, shoot tip explants, produced shoots in 75 % of the culture (Table 2). They produced the highest number (6.9 ± 0.23) of shoots per culture, their average length being 1.8 ± 0.29 cm (Table 2). In the present study, the axillary buds on further multiplication survived well and developed as individual plants. The nature and concentration of cytokine used in this study influenced proliferation of axillary shoots derived from nodal and shoot tips segments of mature plants and *in vitro* raised shoots, respectively. The results of this experiment also indicate that 0.5 mg/l BAP concentration was more effective for multiple shoot induction in node explants (Figure 1) as compared to shoot tip explants.

CONCLUSION

Direct shoot multiplication is preferred for generating true-to-type plants then callus regeneration. This study supports the rapid multiplication of this useful medicinal plant by *in vitro* conditions. This report provides a sample protocol for the micro propagation of *O. gratissimum.* Shoots can be easily derived from node cultures on BAP containing medium. The efficiency of the system could be improved to give rise to more shoot proliferation. This approach offers a means for producing identical plantlets from nodal explants of *O. gratissimum.*

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