



REGULAR ARTICLE

EFFICIENCY OF MEDIA'S FOR PROPAGATION OF MEDICINAL TREE *GARDENIA GUMMIFERA* LINN.F- AN ENDANGERED MEDICINAL PLANT

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SUMMARY

In this work interaction of different media viz B5, MS, LS and whites were tested for shoot proliferation. Proliferation of shoots occurred only when these media are fortified with 2mg l^{-1} BAP and 0.5mg l^{-1} NAA. However, only the callus was formed when growth regulators were avoided. Multiple shoots were obtained from all types of media tested. Nodal segments in MS media shows the better response. Regenerated plantlets were acclimatized and successfully transferred in the field.

Key words: Efficiency of media, *Gardenia gummifera*, Tree, Endangered

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1. Introduction

Gardenia gummifera grows in nature through seeds. However, it's regeneration rate is very poor and plant becomes endangered.¹⁴ It is one of the important tree species of medicinal value belonging to family Rubiaceae. It contain resin, volatile oil, a coloring matter gardenin -A.¹⁰ it can be helpful in treating digestive problems including dyspepsia, diarrhea, an astringent and expectorant for nervous conditions and spasm.¹³

The poor regeneration of *Gardenia gummifera* through seeds and stem cuttings poses difficulties. This poor propagation coupled with over exploitation for pharmaceutical use has depleted the species from it's natural habitat. Therefore, widening the gape between demand and supply and thus putting further pressure on the species. Owing to these factors, the species at the verge of extinction and will extinct soon if proper steps are not taken for its conservation. To our knowledge there have been not found any reports of regeneration of *Gardenia gummifera* Linn.f through tissue culture till date.

The conservation of this plant may be

possible by adopting tissue culture technique. In vitro plant regeneration is an important and essential component of plant biotechnology. The frequency of callus induction and plant regeneration are influenced by many factors such as genotype, type of explant and composition of media.⁶ Nutrient composition is considered to be the major source of variation in plant tissue culture.⁸ In view of the problems of conventional propagation and high demand of planting material, the large scale multiplication of this tree species can only be met efficiently and economically in a short span of time, by in vitro propagation. The aim of the present work was to ascertain the most appropriate basal culture media and growth hormones for in vitro regeneration of *Gardenia gummifera* linn.f.

2. Materials and Methods

Experiments studies were conducted on *Gardenia gummifera* in the Department of Botany at Government Motilal Vigyan Mahavidyalaya Bhopal (M.P). Different types of media's were used such as Murashige

and Skoog¹¹ (1962), Gambrogs medium³ (B₅), Whites medium¹⁵ and Linsmaier medium⁹ during the entire studies. Growth regulators used through out this experimental work were BAP, NAA, IAA, IBA etc. The medium was heated and then dispensed in either test tubes or flasks and autoclaved at a temperature of 121°C and a pressure of 15 lbs psi for 15 minutes. The cultures were kept in a culture room incubator with 16 hour light cycle in every 24 Hour. The temperature was regulated at 25 ± 1°C. The explants of *Gardenia gummifera* were collected from Botanical Garden of Government Motilal Vigyan Mahavidyalaya Bhopal (M.P). Healthy young shoots were used as the starting material. Young leaves and nodal segments were washed separately under running tap water allowed by 5% (V/V) solution of dextron (a neutral liquid detergent) for 5 minutes and surface sterilization using 0.1 % (W/V) mercuric chloride. Leaf segments were sterilized for 7-9 minutes, while nodal segments were treated for 10-12 minutes. Decanting of the mercuric chloride was followed by repeated washes (3 times 5 minutes each) in sterile water. The explants were cut into appropriate sizes 0.4 cm-0.5cm using sterile forceps and scalpel. All the operations and inoculations were carried out under strict

aseptic conditions in laminar airflow cabinet. For the induction of shoot formation, the explants were cultured on different types of media's supplemented with different levels of plant growth regulators.

3. Results and Discussion

In the present study, we first examined the effect of medium on shoot formation to improve the culture conditions. When the nodal explants were inoculated on various types of solid media (with and with out the growth regulators), the explants showed the better growth of shoots on MS medium supplemented with 2mg^l⁻¹BAP and NAA 0.5 mg^l⁻¹NAA than on B₅, WH and LS. The effect of different media with and without the growth regulators on micro propagation of *Gardenia* are presented in table 1.

The callus proliferation from nodal explants occurred with in one month on medium free of growth regulators. No shoot proliferation occurred on any type of media unless growth regulators are not fortified. Callus obtained from different types of media (fig. A, B, C) were transferred and subcultured on the respective media fortified with different concentrations of BAP and NAA. In all types of media the shoot proliferation occurred at 2 mg^l⁻¹ BAP and 0.5 mg^l⁻¹ NAA only.

Fig (A-C): Callus formation without growth regulators

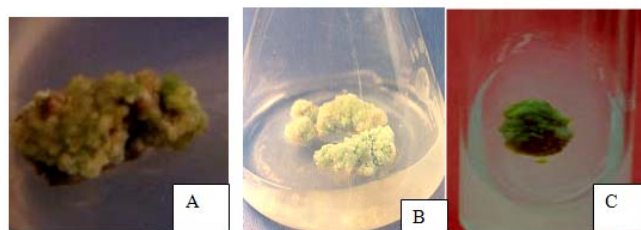
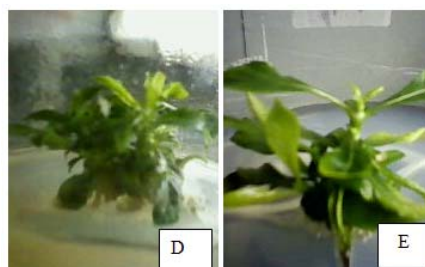


Fig (D-E): Shoot formation When Media (MSABN) was fortified with 2mg^l⁻¹BAP and 0.5mg^l⁻¹NAA



Shoot proliferation from different media's the MSBAN elicited maximum number of shoots (fig D-E) per explant as compared to rest of the media studied. This can be attributed to different nitrate ammonium ratio of medium which is considered to be an important factor for nitrogen uptake and pH regulation during culture.⁴ The mean length of shoots obtained from MSABN media as significantly higher than those on other media. Thus the best results on micro propagation of *Gardenia gummiifera* was obtained on MS medium. It is also observed in *Goedrum*.² The present

findings have also been in agreement with previous reports in tissue culture in *Pterocarpus*.^{7,12}

Rooting experiments were conducted in half-strength of all media's tested. However, MS half strength media was found to be more prominent for root induction when it was fortified with 1mg l^{-1} of IAA (fig. F-G). Roots elongated up to 5-7cm within 10 days. The effectiveness of strength of MS basal medium supplemented with auxins on root induction has been reported in many medicinal plants.^{1,5}

Fig (F-g): Root formation in half-strength (MSABN) Media. (H): Hardening of *Gardenia gummiifera* in potting mixture



The ultimate success of in vitro propagation lies in successful establishment of plants in the soil. The high survival rate in the present study (80-85 %) indicates that this procedure could be easily adapted for large scale propagation. This study will be fruitful to meet the escalating demand of this plant and will be helpful to conservation of this threatened medicinal plant. Each value represents the means \pm SE of 5 replications by using Fischer's T-test and the values are not significantly different at $p \leq 0.01$.

MSA(Murashige and Skoog media), MSABN(Murashige and Skoog media with 2mg l^{-1} BAP and 0.5mg l^{-1} NAA), B5A (Gambrog's media), B5ABN (Gambrog's medium with 2mg l^{-1} BAP and 0.5mg l^{-1} NAA),LSA (Linsmaier and skoog medium),LSABN (Linsmaier and skoog medium with 2mg l^{-1} BAP and 0.5mg l^{-1} NAA),WHA (Whites media),WHABN (whites medium with 2mg l^{-1} BAP and 0.5mg l^{-1} NAA).

Influence of various culture media on shoot formation and growth of *Gardenia gummifera* linn f.

Culture media	No. of shoots explant ⁻¹	Shoot length
MSA MS media w/o growth regulators	Callus formation only	Not Applicable
MSABN 2mg ⁻¹ BAP and 0.5mg ⁻¹ NAA	2.20 ± 0.19	3.80 ± 0.13
B ₅ A Gambrog's media w/o growth regulators	Callus formation only	Not Applicable
B ₅ ABN 2mg ⁻¹ BAP and 0.5mg ⁻¹ NAA	1.28 ± 0.24	2.17 ± 0.17
WHA Whites media w/o growth regulators	Callus formation only	Not Applicable
WHABN 2mg ⁻¹ BAP and 0.5mg ⁻¹ NAA	1.88 ± 0.21	2.84 ± 0.15
LSA Linsmaier and skoog w/o growth regulators	Callus formation only	Not Applicable
LSABN 2mg ⁻¹ BAP and 0.5mg ⁻¹ NAA	1.96 ± 0.23	3.48 ± 0.17

Each value represents the means ± SE of 5 replications by using Fischer's T-test and the values are not significantly different at p ≤ 0.01.

MSA (Murashige and Skoog media), MSABN (Murashige and Skoog media with 2mg⁻¹BAP and 0.5mg⁻¹NAA), B₅A (Gambrog's media), B₅ABN (Gambrog's medium with 2mg⁻¹BAP and 0.5mg⁻¹NAA), LSA (Linsmaier and skoog medium), LSABN (Linsmaier and skoog medium with 2mg⁻¹BAP and 0.5mg⁻¹NAA), WHA (Whites media), WHABN (whites medium with 2mg⁻¹BAP and 0.5mg⁻¹NAA).

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