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In vitro propagation of garlic (*Allium sativum* L.) using clove segment explants

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Abstract

The present investigation was undertaken to study the *in vitro* propagation of garlic to produce disease-free plants and a sufficient number of seed bulbs for the practical cultivation of a new variety. Rapid propagation was performed using clove segments as explants, which were initiated on Murashige and Skoog medium supplemented with four different concentrations of BAP (0.5-2.0 mg/l) in combination with Kn (0.5 mg/l). After 1 week of inoculation, initiation was obtained in the medium supplemented with 2.0 mg/l BAP + 0.5 mg/l Kn. Shoots were regenerated from *Allium sativum* clove explants. The Best Elongation was observed on MS medium supplemented with 1.5 mg/l BAP and 0.5 mg/l Kn. The most efficient rooting response was obtained on MS medium supplemented with 1.5 mg/l IBA.

Keywords: *Allium sativum*, Garlic, *in vitro* propagation, mother plant, Bavistin, MS media, BAP, Kn, IBA

Introduction

Garlic is the second most widely used cultivated *Allium* after onion. It has long been recognized worldwide as a valuable spice for foods and a popular remedy for various ailments and physiological disorders. It is used worldwide to flavor various dishes. *Allium sativum* L., commonly known as garlic, is a member of the onion family (Alliaceae). Garlic has been used throughout the ages for both culinary and medical purposes. Garlic (*Allium sativum*) is an aromatic monocotyledonous bulbous plant and an annual spice belonging to the subfamily Allioideae of the family Alliaceae. It is used worldwide as a condiment and for its medicinal properties (Mawalkar *et al.*, 2018) [4]. Garlic has an unusually high concentration of sulphur-containing compounds. Sulphur compounds, including allicin (thio2-propene-1-sulfinic acid S-allyl ester), were confirmed to be the main active components in the root bulb of the garlic plant. (Mehta *et al.*, 2013) [5] It is used in component in the preparation of drug and pesticide. Garlic is an important culinary as well as medicinal herb due to its ability to improve the taste of food and to its antibiotic, antitumor, and antithrombotic effects in animal cells. Garlic (*Allium sativum* L.) is propagated exclusively vegetatively, a process that has a low coefficient of multiplication and potential for transmission of viral diseases. (Muhammad *et al.*, 1997) Garlic (*Allium sativum*) is an aromatic monocotyledonous bulbous plant and an annual spice belonging to the subfamily Allioideae of the family Alliaceae. It is used worldwide as a condiment and for its medicinal properties. (Mawalkar *et al.*, 2018) [4] Garlic is the world's oldest cultivated crop. It is an approximately 5000-year-old indigenous herb originating in Central Asia (Kazakhstan), South Asia, or southwestern Siberia. Garlic is closely related to the onion, shallot, leek, and chive. It is easy to grow and can be cultivated year-round. It is cultivated in both temperate and tropical climates (Mawalkar *et al.*, 2018) [4]. Allicin has a wide range of biological and pharmacological activities, including anticoagulation, antihypertensive, antimicrobial, antibiotic, antiparasitic, antimycotic, antiviral, antitumoral, antioxidant, anti-aging, antiplatelet, detoxification of heavy metals, fibrinolysis, and hypolipidemic (lipid-lowering) activities. It is an immune enhancer and modulator. (Mehta *et al.* 2013) [5]

Need for propagation of Garlic: The propagation rate of garlic in the field is low; therefore, it takes many years to produce a sufficient number of seed bulbs for the practical cultivation of a new variety.

Many garlic cultivars are susceptible to diseases caused by viruses and fungi and suffer from insect pests. *In vitro* regeneration or micropropagation is the best alternative to overcome these hurdles, and it holds tremendous potential for rapid multiplication and production of high-quality, uniform, and disease-free planting material. (Mawalkar *et al.* 2018)^[4]

Materials and Methods

Source of explant and surface sterilization: Clove segments were collected as explants from mature, healthy cloves from DOGR, Pune, and brought to the tissue culture laboratory of the Department of Plant Biotechnology, College of Agricultural Biotechnology, Loni. These explants were thoroughly washed under running tap water 4-5 times. They were then treated with 0.1% Bavistin for 3 min. In the next step, the samples were washed with Tween 20 for 2-3 min. Surface sterilization was performed with mercuric chloride (HgCl₂) (0.1%) for 30 s under aseptic conditions and finally washed 3-4 times with sterile distilled water to remove traces of HgCl₂ and these sterilized explants were cultured on MS medium with different concentrations of growth regulators.

Initiation stage and culture conditions: Clove segments in the initiation stage were transferred to four different MS media supplemented with BAP (0.5-2.0 mg/l) in

combination with Kn (0.5 mg/l). The inoculated culture bottles were incubated in an incubation room with sterile controlled environmental conditions, such as temperature 25±2 °C, relative humidity (RH) 70%, 16 h photoperiod/day with 2000-3000 lux light intensity under cool white fluorescent tubes (Philips, India).

Shoot elongation: Different hormones, such as BAP and Kn, with multiple combinations, were successfully used for Shoot Elongation. Shoots were transferred to fresh MS medium with different concentrations of BAP and Kn for elongation.

Rooting: The explants were transferred for rooting in MS media supplemented with two different concentrations of IBA.

Results and Discussion

Shoot initiation: Data obtained after 7 days of culture initiation showed that shoots of *Allium sativum* could be established on all tested media, including the medium. The best result was obtained on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l Kn. (Table 1). The shoot length (average shoot length was 2.5 cm) was observed on medium supplemented with 2.0 mg/l BAP + 0.5 mg/l Kn (Table 1, Fig. no. 1).

Table 1: Observation of various combinations of hormone BAP and Kn on shoot initiation of *Allium sativum* after 7 days.

Sr. No.	MS medium+BAP mg/l+Kn mg/l	Number of Bottles Inoculated	Number of Bottles Growth Found	Rate of Survival (%)	Average shoot length (cm)
1	0.5 + 0.5	15	10	66	1
2	1.0 + 0.5	15	7	46	1.6
3	1.5 + 0.5	15	8	53	1.9
4	2.0 + 0.5	15	12	80	2.5

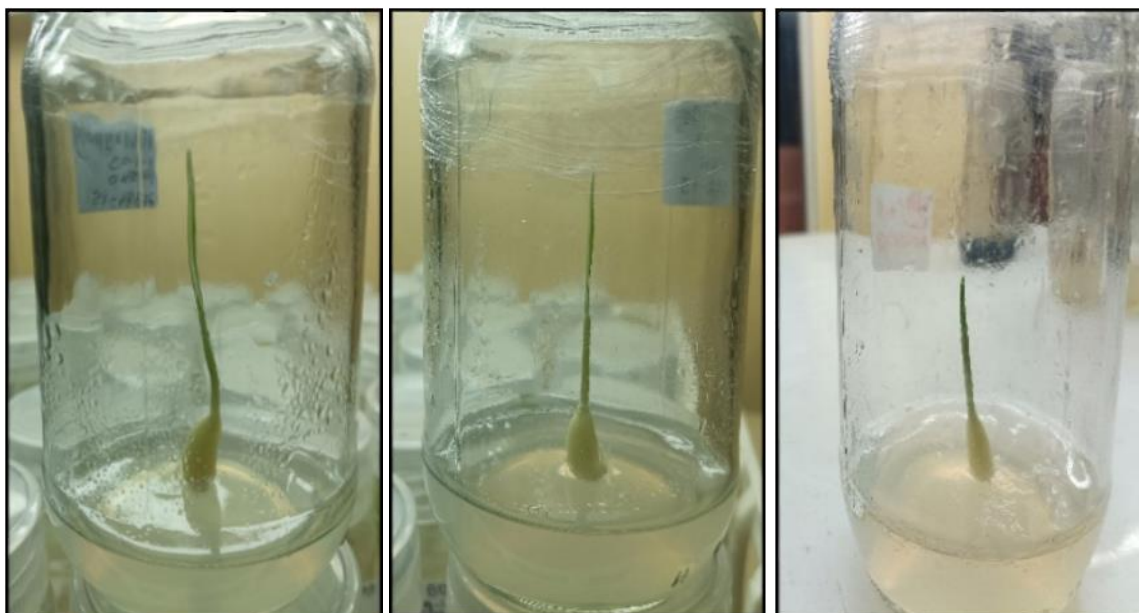


Fig 1: Results of shoot initiation of *Allium sativum* after 7 days using MS Media + BAP 2.0 mg/l + Kn 0.5 mg/l

The cloves at initiation were subjected to shoot elongation on different media. In the present study, the average number of shoots formed at all BAP and Kn concentrations. The best

Shoot elongation: The growth was observed at the 1.5 mg/l BAP + 0.5Kn mg/l concentrations, where the average shoot length per plant was 6 to 10 cm. The results showed that the shoot length per explant increased with increasing concentrations of BAP and Kn. (Table 2, Fig. no. 2).

Table 2: Observation of various combinations of hormone BAP and Kn on shoot elongation of *Allium Sativum* after 10 days of inoculation.

Sr. No.	BAP mg/l+Kn mg/l	Number of Bottles Inoculated	Number of Bottles Growth Found	Rate of Survival (%)	Average shoot length (cm)
1	1.0 + 0.5	10	5	50	6.2
2	1.5 + 0.5	10	7	70	9.7

**Fig 2:** Results of shoot elongation of *Allium sativum* using MS Media + BAP 1.5 mg/l + Kn 0.5 mg/l

Rooting: The shoots were inoculated into media containing various IBA concentrations for root induction. A high concentration of IBA led to improved root induction. MS medium supplemented with 1.5 mg/l IBA showed an

average number of roots of five roots with an average length of 2 ± 1.10 cm. IBA is a root-inducing growth regulator that has been used alone or in combination for root initiation in many cultures. (Table 3, Fig.no.3).

Table 3: Observation of various combinations of IBA on root induction of *Allium sativum* after 15 days of inoculation.

Sr. No.	IBAmg/l	Number of Bottles Inoculated	Number of Bottles Growth Found	Rate of Survival (%)	Average root length (cm)
1	1.0	10	4	40	2.5
2	1.5	10	6	60	3.9

**Fig 3:** Results of root induction with varying concentrations of MS+ IBA 1.5 mg/l

Conclusion

As this herb is an important medicinal plant, and due to its low seed germination percentage and poor seed viability,

vegetative propagation methods are not efficient for conserving this rare species. Large numbers of garlic cloves can be produced through *in vitro* propagation techniques.

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