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An improved method for callus culture and In *vitro* propagation of garlic (*Allium sativum* L.)

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ABSTRACT

This review highlights the recent development and achievements made for the multiple shoots regeneration and callus induction of Allium sativum. Shootlets were regenerated from nodal explants of stem through auxiliary shoot proliferation. The inductions of multiple shoots from nodal segments were highest in MS medium supplemented with 1.0 mg/l Kn. For rooting different concentration of IBA were used and highest rooting was recorded on MS medium supplemented with 2.0 mg/l IBA. The rooted Plantlets were hardened initially in culture room conditions and then transferred to misthouse. Leaf petiole explants were used for the purpose of callus induction. Best growth was observed in MS medium supplemented with 0.25 mg/l 2, 4-D+ 0.5 mg/l Kn and 0.25 mg/l 2,4-D+ 0.5mg/l BAP.

Key Words:- *Allium sativum* , Shoot proliferation, Callus, Garlic.

INTRODUCTION

Allium sativum L., commonly known as garlic, belongs to a member of the onion family (Alliaceae). Garlic has beenused throughout the ages for both culinary and medical purpose. Extensive research work has been carried out on the health promoting and medicinal properties of garlic. *A.sativum* has shown a variety of biological activities including antioxidant, cancer prevention, liver protection, immunomodulation and reduction of cardiovascular disease risk factors[1]. Garlic is characterized by medicinal properties due to the content of over 2000 biologically active compounds[2]. Garlic has an unusually high concentration of sulfur containing compounds. Sulfur compounds, including allicin (thio-2-propene-1-sulfinic acid S-allyl ester) were confirmed to be the main active components in the root bulb of the garlic plant[3]. Allicin has the wide range of biological and pharmacological activities, such as anticoagulation, antihypertensive, antimicrobial, antibiotic, antiparasitic, antimycotic, antiviral, antitumoral, anti-oxidant, anti-aging, antiplatelet, detoxifies heavy metals, fibrinolusis, hypolipidaemic (lipid-lowering) and immune enhancer and modulator[4]. The micropropagation of garlic is by division of the individual cloves of its bulbs. Because garlic almost never produces fertile seeds, it must be propagated vegetatively. As the garlic is vegetatively propagated, the health status of the crop is affected by both primary and secondary virus infection which accumulates in each crop cycle. Almost all garlic seed used is contaminated with one or more pathogens, mainly viruses that play a main role in yield reduction and quality, also reducing the storage longevity of the harvested bulbs .With the aim to improve the health quality of garlic seeds, virus-free stocks tissue culture is considered as an alternative tool.

Therefore, the use of shoot meristem with basal portion as explants for micropropagation of garlic is more suitable than other source of explants.

MATERIAL AND METHODS

The branches (about 5-6 cm) of shoots of *Allium sativum* plants were collected from the Herbal Garden, Kota. The branches with node explants were washed in running tap water and then washed again thoroughly by adding a few drops of Tween-20 to remove the superficial dust particles as well as fungal and bacterial spores. They were surface sterilized with 0.1% HgCl2 for 5 min followed by rinsing them five times with double distilled water inside the Laminar Air flow chamber. Nodal segments (with a single axillary bud) about 0.5-0.8 cm were prepared aseptically and were implanted vertically on MS medium prepared with specific concentrations of BAP, Kn (1.0-5.0 mg/l) singly or in combination were used for shoot proliferation. Same experiments were repeated for shoot multiplication. The medium containing 3% sucrose was solidified with 0.8% agar (Qualigens). The *p*H of the media was adjusted to 5.9±0.02 with 1 N NaOH or 1 N HCl solutions prior to autoclaving. Media poured in culture vessels were steam sterilized by autoclaving at 121°C and 15 psi for 15-20 min. The cultures were incubated under controlled conditions of temperature (25±2°C), light (2000- 2500 lux for 16 h/d provided by fluorescent tubes) and 60-70% humidity. For each experiment a minimum of 7 replicates were taken and experiments were repeated thrice. Observations were recorded after an interval of 3 wk. Once culture conditions for shoot induction from explants were established, the shoots produced *in vitro* were sub cultured on fresh medium every 3 wk. The nodal and shoot tip explants were inoculated in various concentrations of BAP and Kn. Among these, the maximum number of shoots (3.42±0.39) and maximum shoot length 7.54±0.31cm was observed on MS media fortified with 1.0 mg/l Kn. Rooting of elongated shoots was attempted under *in vitro* conditions. Auxins (IBA) alone in different concentrations (1.0-5.0 mg/l) were incorporated in the agar (0.8%) solidified medium containing 1/4 MS salts and 1.0% sucrose. The *in vitro*-rooted plantlets were transferred to culture bottles 1/4th filled with Soilrite composition (soil: sand: peat moss) and irrigated with 1/4 MS salt solution. These bottles were kept in controlled environmental conditions of culture room. After 3 wk of growth, the plantlets were transferred to misthouse for further growth. Leaf petiole explants were used for the purpose of callus induction. Explants were surface sterilized by using standard sterilization method. Explant were inoculated in various concentrations and combination of 2,4-D and BAP, Kn. Best growth was observed in MS medium supplemented with 0.25 mg/l 2, 4-D+ 0.5 mg/l Kn and 0.25 mg/l 2,4-D+ 0.5mg/l BAP. Best growth of callus was obtained in 0.25 mg/l 2, 4-D+ 0.5 mg/l Kn (callus diameter- 4.6).

RESULTS AND DISCUSSION

The nodal explants inoculated on MS medium containing BAP and Kn in the range 1.0-5.0 mg/l showed enhanced shoot proliferation. Kn at its 1.0 mg/l concentration evoked best response. Shoots after their initial proliferation on medium containing 1.0 mg/l Kn were sub-cultured on same fresh medium after every 21 days. Incorporation of BAP or Kn into MS medium supported multiplication of shoots in culture, Kn proved to be a better choice than BAP and the maximum number of shoots 3.42±0.39 , shoot length 7.54±0.31 cm were obtained on its 1.0 mg/l concentration (Table 1, Fig.1- A, Fig. 2).

The full or half strength of MS medium without any PGR was failed to induce rooting of regenerated shoots. However, shoots were capable to induce root when cultured on medium containing auxins. Auxin in different concentration induced rooting when incorporated in the medium containing ¼ of MS salts.

The best rooting response, however, was observed on medium containing 2.0mg/l IBA, where roots measuring 1.68±0.32 cm (average) were formed (Table 1, Fig. 1-B, Fig. 2). *In vitro* rooted plantlets were initially hardened in culture room conditions where leaves expanded. After 3 weeks, the plantlets were shifted to mist house. There was an increase in length of shoots and new leaves emerged which expanded quickly. Leaf petiole explants were used for the purpose of callus induction. Highest diameter of callus was observed on MS Medium fortified with 0.25 mg/l 2,4-D+ 0.5 mg/l Kn (callus diameter 4.6 cm) and 0.25 mg/l 2,4-D+ 0.5mg/l BAP (callus diameter 3.4 cm), (Table 2, Fig. C).

Table-1: Effect of cytokine (BAP and Kn) on shoot proliferation from nodal shoot explants of *Allium sativum*

Hormone Concentration (mg/ l)	Hormone Concentration (mg/ l)	Hormone Concentration (mg/ l)	Response (%)	Number of Shoot/explant (mean±SD)	Shoot length (in cm) (mean±SD)	Number of Root/explant (mean±SD)	Root length (in cm) (mean±SD)
BAP	**Kn**	**IBA**					
1.0	-	-	75	2.24±0.51	6.30±0.26	-	-
2.0	-	-	85	2.48±0.36	6.47±0.29	-	-
3.0	-	-	70	2.75±0.36	6.15±0.24	-	-
4.0	-	-	60	2.37±0.40	5.70±0.41	-	-
5.0	-	-	45	1.81±0.38	4.92±0.51	-	-
-	1.0	-	90	3.43±0.39	7.84±0.31	-	-
-	2.0	-	85	2.32±0.39	6.80±0.39	-	-
-	3.0	-	84	1.85±0.27	4.71±0.29	-	-
-	4.0	-	70	1.57±0.40	5.60±0.41	-	-
-	5.0	-	60	1.28±0.36	3.60±0.28	-	-
-	-	1.0	60	-	-	2.90±0.73	2.43±0.33
-	-	2.0	65	-	-	3.65±0.51	1.68±0.32
-	-	3.0	50	-	-	1.41±0.52	1.66±0.08
-	-	4.0	40	-	-	1.33±0.37	0.92±0.10
-	-	5.0	30	-	-	1.07±0.19	0.61±0.05

Medium: MS+ additives; mean± SD, n= 7 replicates

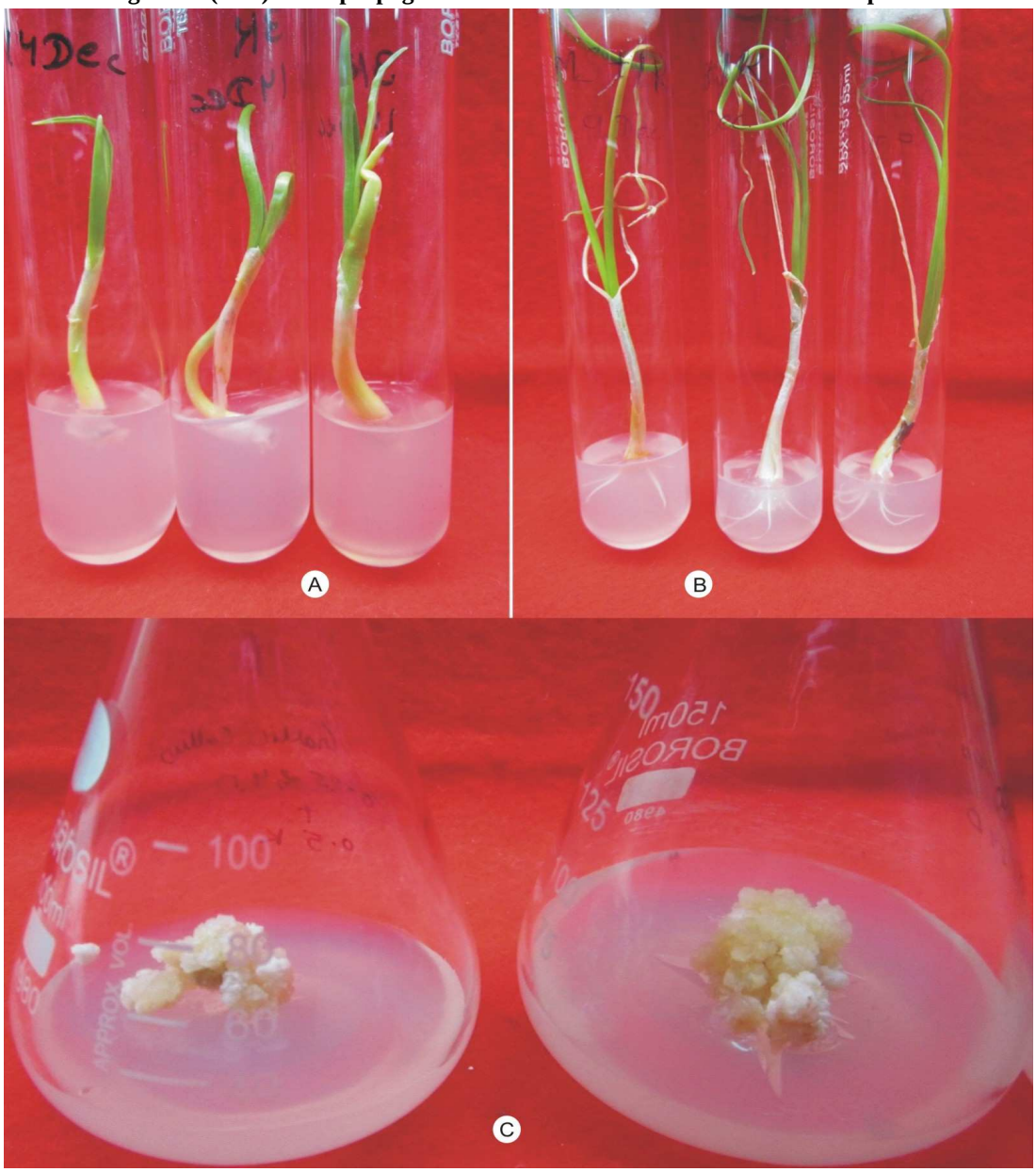
Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

Table-2: Effect of different Hormones on Callus proliferation and Morphology

Hormone Conc.(mg/l)	Callus diameter after 7 weeks subculture (cm)	Callus proliferation Scoring	Color of callus	Morphology of callus
2,4-D + Kn				
0.25 + 0.3	2.6	++	Whitish	Friable
0.25 + 0.4	3.5	+++	Light green	Compact
0.25 + 0.5	4.6	++++	Whitish green	Nodular
2,4-D + BAP				
0.25 + 0.3	2.4	+++	Whitish	Friable
0.25 + 0.4	2.1	++	Whitish green	Trodden
0.25 + 0.5	3.4	++++	Whitish green	Nodular

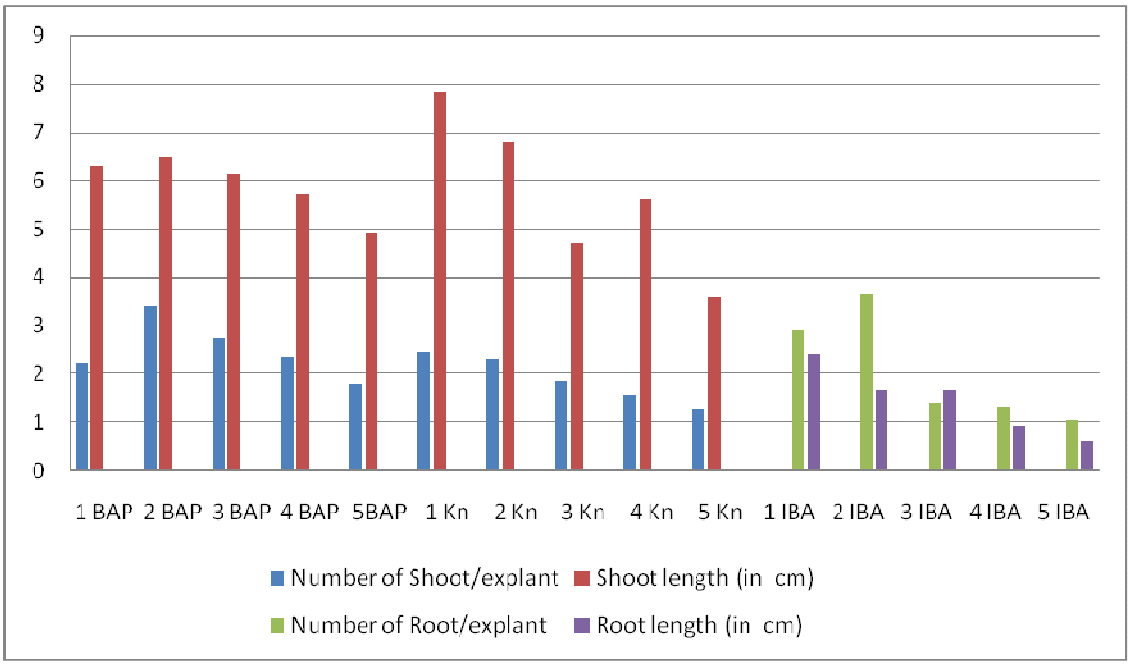
'++++' Intense
'+++' Moderate
'++' Meager

Figure-1: (A-C) Micropropagation of *Allium sativum* from nodal shoot explants



A. Shoot multiplication on MS medium supplemented with 1.0 mg/l Kn, B. Root multiplication on MS medium supplemented with 1.0 mg/l IBA, C. Callus induction from leaf on MS medium supplemented with 0.25 mg/l 2,4-D +0.5 mg/l Kn, & Callus induction from leaf petiole on MS medium supplemented with 0.25 mg/l 2,4-D +0.5 mg/l BAP.

Figure-2 : Effect of cytokine (BAP, Kn and IBA) on shoot, root proliferation from nodal shoot explants of *Allium sativum*



CONCLUSION

The seedling derived explants, being juvenile, are frequently used for micropropagation, as they are easy to establish in culture. In *Allium sativum* MS medium containing 1.0 mg/l Kn was the best for culture initiation. We have found that *Allium sativum* culture grew better on MS medium in comparison to other media. In *Allium sativum* 1.0 mg/l Kn was most suitable for shoot multiplication. Best shooting response was observed on media containing 1.0 mg/l Kn (Average number of shoots 3.42±0.39) and (Average shoot length 7.54±0.31 cm).

IBA (Auxin) has been widely used as root induction hormone under *in vitro* and *in vivo* condition. We also found positive role of IBA during *in vitro* rooting. In *Allium sativum*, 2.0 mg/l IBA proved to be the best for in vitro rooting. The *in vitro* rooted plants were hardened first under controlled conditions of culture room and then shifted to mist house where they exhibited and hence, growth and 90% survival. Most responsive callus induction was observed on MS medium supplemented with 0.25 mg/l 2, 4-D +0.5 mg/l Kn and 0.25 mg/l 2, 4-D +0.5 mg/l BAP.

Results mentioned above revealed shoot tip with basal portion have potential to induce multiple shoots and roots when cultured on the medium containing MS, NAA ,IBA, IAA, BAP, Kinetin; shoot initiation start on all treatments but BAP & Kinetin show good response, after 3 weeks highest shoots found on Kinetin containing MS medium. Roots are emerged on MS medium containing IBA, IAA, and Kinetin. After 3 weeks highest number of roots found on Kinetin. So kinetin is best for shoot-root initiation & proliferation.

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