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REGENERATION OF *ALLIUM SATIVUM* L. BY SHOOT TIP WITH BASAL PORTION

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ABSTRACT

Allium sativum L. belongs to a member of the onion family (Alliaceae) and has been used for both culinary and medicinal purpose. Extensive research works have been carried out on the health promoting and medicinal properties of garlic. A. sativum has shown the wide range of biological and pharmacological activities including antioxidant, cancer prevention, liver protection, immunomodulation and redution of cardiovascular disease risk factor. The propagation of garlic is by division of individual cloves of its bulbs. Because garlic never produces fertile seeds, it must be propagated vegetatively. Vegetative propagation of garlic is infected by a number of virues and pathogens that caused 70% yield reduction. To improve the health quality of garlic seeds, virus-free stock tissue culture is considered as an alternative tool. A study is carried out to develop a protocol of shoot-root induction and proliferation of garlic. Shoot meristem with basal portion were cultured on Murashige and Skoog (MS) medium containing various growth regulators. Shoot initiation was found in with all medium composition but highest in kinetin supplemented medium. Root induction was found in IBA, IAA, and Kinetin but highest& earliest in kinetin supplemented medium.

Key Words: Medicinal plant, *Allium sativum L.*, garlic, Micro propagation, Shoot initiation, roots proliferation

INTRODUCTION

Allium sativum L., commonly known as garlic, belongs to a member of the onion family (Alliaceae). Garlic has been used throughout the ages for both culinary and medical purpose **[1]**. 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 **[2-4]**. Garlic is characterized by medicinal properties due to the content of over 2000 biologically active compounds **[5]**. 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 **[6]**. 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 **[3, 7-9]**.

The propagation 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

Garlic bulb separated into single cloves. The outer dry, papery bulb scales of the cloves were removed. Healthy cloves were surface-sterilized, short tip with basal portion excised from the cloves aseptically. Explants was surface-sterilized by treat with soap solution for 10-15 min, after that treat with choremphenecol & Bovistin one by one, after treatment with Antifungal & Antibacterial, Explants sterilized in tween -20 (2 drop in 100 ml D/W), for 20 min with frequent agitation, and then transfer in LF. In LF bench Explants transfer in Hgcl2 (1%) Solution and then 70% alcohol and finally Explants placed in sterilized tissue-paper and this explants ready to inoculate in MS-media with different hormone concentration. Isolated, sterilized shoot tip with basal portion were inoculated aseptically on MS medium. Two kinds of cytokinins BAP and Kn with different

concentrations (o.s., 1, 2, 3 mg i/l) were used singly in MS basal medium. In addition two cytokinins, 4 kinds of auxins NAA (1, 2, 3 mg/L), IBA (o.s, 1, 2, 3 mg/L), IAA (1,2mglL) 2,4D (o.5, 1, 2 mg/L) were tested for culture. Similar combination was used for BAP + NAA. In all cases 3% sucrose and 1 g/L agar were used with pH 5.8. The medium was autocloved at 15 ib/sq. inch pressure and at 121 C for 20 min. The culture tubes were placed in a growth chamber at 25c and light period 14-16 hrs. (2000-3000 lux) Lastly the regenerated plantlets were transplanted on sterile soil with 1:1 soil and sand.

RESULTS AND DISCUSSION

Shoot and Root Initiation

Shoot initiation was observed within one week in all treatments (1mg/LNAA, 2mg/LNAA, 3mg/LNAA,0.5mg/L IBA, 1 mg/L IBA, 2mg/L IBA,3 mg/L IBA,1 mg/L IAA, 2mg/L IAA, 1mg/L BAP, 2mg/L BAP, 3mg/L

BAP,0.5mg/LKin,1mg/LKin,2mg/LKin,3mg/ LKin), but Root initiation start in some treatments and absent in all concentration of NAA and BAP. This is shown in **Table 1 Figure 1-7** below.

Shoot initiation started in all treatments but earliest and healthy shoot in NAA (1mg/L,2mg/L,3mg/L) & BAP (1mg/L,2mg/L,3mg/L). In comparison to all treatments shoot initiation was low in IBA. Root initiation started in some treatments MS plain, IBA (0.5mg/ L , 1mg/L), IAA (1mg/L,2mg/L), Kin (0.5mg/L,1mg/L,2mg/L,3mg/L) but some treatments IBA (2mg/L,3mg/L), NAA (1mg/L,2mg/L,3mg/L)& BAP (1mg/L, 2mg/L, 3mg/L) showed negative response. Root initiation took more time in comparison to shoot initiation.

Shoot and Root Proliferation

After 21 days it was observed that number of shoots & length of shoot was highest in Kin (0.5mg/L, 1mg/L, 2mg/L, 3mg/L). BAP (1mg/L, 2mg/L, 3mg/L) also showed good response. Highest number of Roots proliferated in all concentration of Kinetin. Result can be seen in **Figure 8**.

Table 1: Effect of different Phytoharmone supplemented to MS medium for in vitro shoot-root initiation and proliferation of garlic from shoot tip with basal portion

Shoot and root Initiation				Shoot and Root proliferation			
Phytoharmone (mg/ L)	Days taken to shoot initiation	Day taken To root initiation	Frequency of explants Response (%)	Number after 21 days		Length (cm) after 21 days	
				Shoot	Root	Shoot (cm)	Root (cm)
Ms	4-8	60-70	70	2	1	7-8	3-4
NAA(1)	3-5	absent	90	2	Absent	8-10	-
NAA(2)	4-5	absent	80	3	absent	10-15	-
NAA(3)	3-5	absent	90	2	absent	8-9	-
IBA(0.5)	5-7	28-25	55	1	2-3	5-7	4-5
IBA(1)	5-7	30-40	60	1	2-3	7-8	4-5
IBA(2)	6-7	absent	65	2	absent	8-9	-
IBA(3)	4-7	absent	60	2	absent	9-10	-
IAA(1,1)	5-7	40-50	66	2	3-4	7-8	4-5
IAA(2)	5-7	50-60	50	2	2-3	7-9	5-6
BAP(1)	3-4	absent	80	3	absent	10-12	-
BAP(2)	3-4	absent	85	3	absent	11-14	-
BAP(3)	3-4	absent	80	2	absent	17-18	-
Kn(0.5)	4-6	10-15	90	3	7-8	16-18	5-6
Kn(1)	4-6	10-15	100	4	8-9	17-20	7-8
Kn(2)	4-7	10-12	92	3	14-15	13-14	5-6
Kn(3)	4-6	10-12	90	3	8-9	12-13	6-7

Figure 1: Shoot initiation and proliferation in Kinetin supplemented MS Medium

Figure 2: Root initiation and proliferation in Kinetin supplemented MS Medium

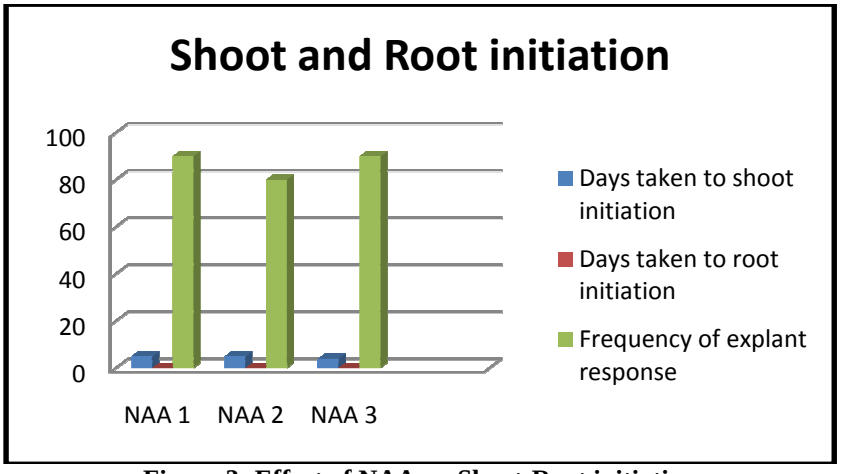

Figure 3: Effect of NAA on Shoot-Root initiation

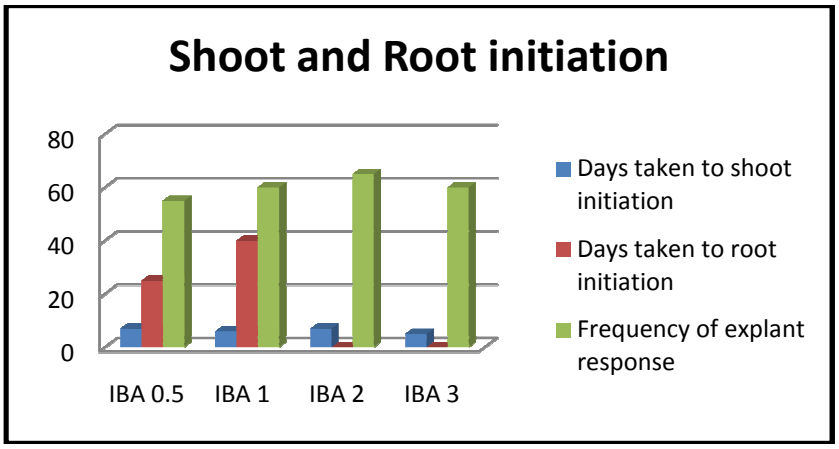

Figure 4: Effect of IBA on Shoot-Root initiation

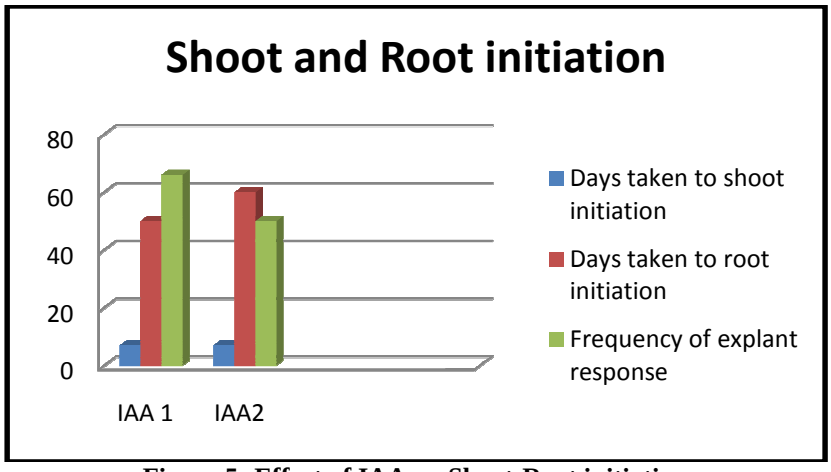

Figure 5: Effect of IAA on Shoot-Root initiation

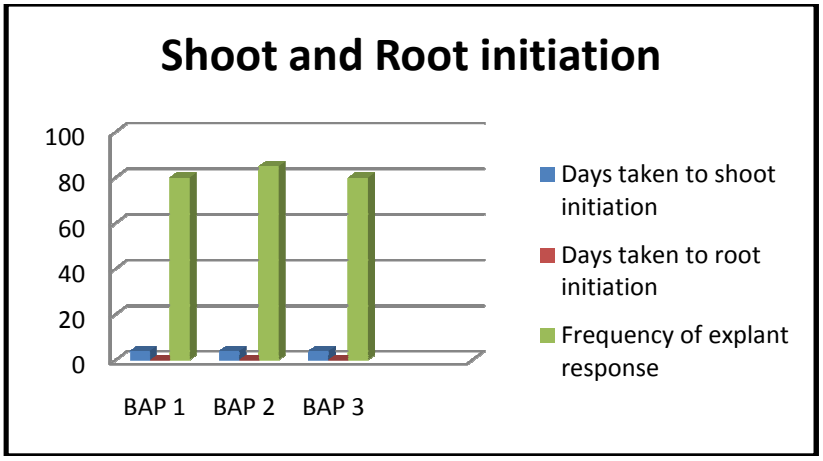

Figure 6: Effect of BAP on Shoot-Root initiation

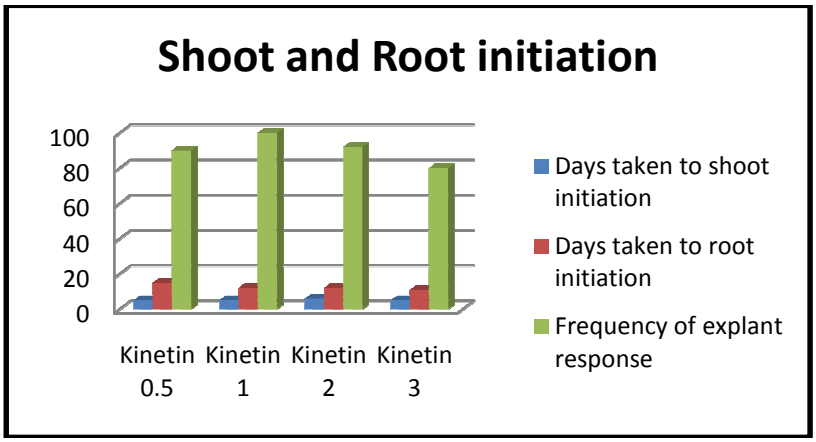

Figure 7: Effect of Kinetin on Shoot-Root initiation

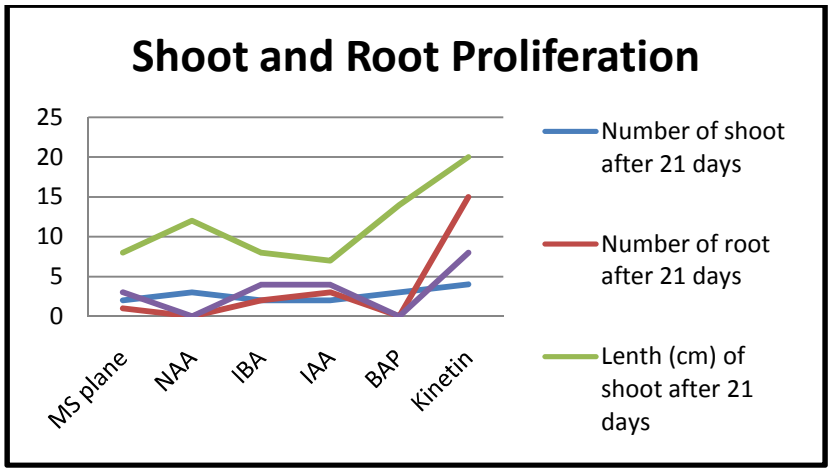

Figure 8: Effect of different Phytohormones on shoot and root proliferation

CONCLUSION

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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