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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effects of Plant Growth Regulators on the Usage of *In Vitro* Stem Disc Culture for Mass Seedling Production in Kahramanmaraş Garlic (*Allium sativum* L.)*

Kahramanmaraş Sarımsağında (*Allium sativum* L.) Kitlesel Fide Elde Etmek Amacıyla *In Vitro* Gövde Diski Üretim Tekniğinin Kullanımında Bitki Büyüme Düzenleyicilerinin Etkileri

Ayşe GÜLÇEBİ[1], İrfan Ersin AKINCI[2*]

Abstract

Anatolia is one of the important production areas of garlic. Garlic production is conducted by using the head or the cloves inside the heads, which are actually the consumed part of the garlic, as propagation material. However, due to the use of cloves, which are the most valuable part of the market, as reproduction material, the profit is reduced by about 10%. The study aims to provide an alternative propagation material to reduce the losses resulting from this practice in local Kahramanmaraş garlic production. For this purpose, generating plantlets directly from stem discs of the garlic cloves *in vitro* and the effects of different growth regulators have been studied. For this, the plant growth regulators added to MS media as BAP (1.0, 1.5 and 2.0 mg l^{-1}), GA_3 (0.5, 1.0 and 1.5 mg l^{-1}), 2-IP (0.75, 1.00 and 1.25 mg l^{-1}); kinetin (1.0, 2.0 and 3.0 mg l^{-1}) and TDZ (0.75, 1.00 and 1.25 mg l^{-1}) were tested. In the study number of explants, number of infected explants, number of healthy explants, number of developed explants, healthy explant rate, developed explant rate, number of callused explants, callus growth rate, number of proliferated explants, proliferation rate, proliferation number, number of rooted explants, rooting rate and number of roots were investigated. However, shoot ratios, shoot numbers, and callus formation were the main focus. The highest rates of proliferation were found in 2-IP (53.8%, 45.5%, and 40.0% at 1.00, 0.75, and 1.25 mg l^{-1} dosages, respectively) and Kinetin (35.3% at 2.00 mg l^{-1}). The maximum shoot number was reached with 2-IP at the dose of 1.00 mg l^{-1} as 1.9 shoot/explants. Kinetin at 3.00 mg l^{-1} and 2IP at 1.25 mg l^{-1} were the other successful applications with 1.8 shoots. This study indicated promising results to obtain plantlets directly from the clove's stem discs and including them into seedling production for the mass production of garlic.

Keywords: Stem disc culture, BAP, GA_3, 2-IP, Kinetin, TDZ, Garlic

[1]Ayşe Gülçebi, Kocaeli Büyükşehir Belediyesi, MIDB, Tarımsal Hizmetler Şube Müdürlüğü, Kocaeli. Turkey, E-mail: gulcebi_ayse@hotmail.com OrcID: 0000-0002-2187-6170.
***Sorumlu Yazar/Corresponding Author:** İrfan Ersin Akıncı, Kahramanmaras Sutcu İmam University, Faculty of Agriculture, Department of Horticulture, Kahramanmaraş, Turkey. E-mail: akinci.ie@ksu.edu.tr OrcID: 0000-0001-6664-5758.
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*Bu çalışma Ayşe GÜLÇEBİ'nin Yüksek Lisans tezinden özetlenmiştir.

Öz

Anadolu sarımsağın önemli üretim alanlarından birisidir. Sarımsak üretimi, sarımsağın tüketilen kısmı olan baş veya başların içindeki dişlerin üretim materyali olarak kullanılmasıyla gerçekleştirilmektedir. Ancak, pazar bakımından en değerli kısmı olan dişlerin üreme materyali olarak kullanılması nedeniyle kazanç yaklaşık %10 oranında azalmaktadır. Araştırmanın amacı Kahramanmaraş yöresel sarımsak üretiminde, dişlerle yapılan üretim sonucu ortaya çıkan kayıpları azaltmak için alternatif çoğaltma materyali sağlanmasıdır. Bu amaçla sarımsak dişlerinin gövde disklerinden in vitro'da doğrudan bitkicik elde edilmesine ve farklı bitki büyüme düzenleyicilerin etkilerine çalışılmıştır. Bunun için MS besi ortamına eklenen bitki büyüme düzenleyicilerden BAP (1.0, 1.5 ve 2.0 mg l^{-1}), GA_3 (0.5, 1.0 ve 1.5 mg l^{-1}), 2-IP (0.75, 1.00 ve 1.25 mg l^{-1}), Kinetin (1.0, 2.0 ve 3.0 mg l^{-1}) ve TDZ (0.75, 1.00 ve 1.25 mg l^{-1}) test edilmiştir. Çalışmada eksplant sayısı, enfekte eksplant sayısı, sağlıklı eksplant sayısı, gelişmiş eksplant sayısı, sağlıklı eksplant oranı, gelişmiş eksplant oranı, nasırlı eksplant sayısı, kallus büyüme hızı, çoğalan eksplant sayısı, çoğalma hızı, çoğalma sayısı, köklenen eksplant sayısı, köklenme oranı ve kök sayısı incelenmiştir. Ancak daha çok sürgün oranları, sürgün sayıları ve kallus oluşumu üzerinde durulmuştur. En yüksek kardeşlenme oranları 2-IP (1.00, 0.75 ve 1.25 mg l^{-1}'de sırasıyla % 53.8, % 45.5 ve % 40.0) ve Kinetin (2.00 mg l^{-1}'de % 35.3)'de sağlanmıştır. En fazla kardeş sayısına 1.9 adet kardeş/eksplant ile 1.00 mg l^{-1} 2-IP dozunda ulaşılmıştır. Kinetin'nin 3.00 mg l^{-1} ve 2-IP'nin 1.25 mg l^{-1} dozları 1.8 sürgün ile başarılı sonuçlar alınan diğer uygulamalar olmuştur. Bu çalışma, sarımsağın yoğun üretiminde, dişlerin gövde disklerinden doğrudan bitkicik elde edilmesinde ve bunların üretime fide olarak dahil edilmesinde umut verici sonuçlara işaret etmektedir.

Anahtar kelimeler: Gövde disk kültürü, BAP, GA_3, 2-IP, Kinetin, TDZ, Sarımsak

1. Introduction

In recent years, climatic conditions, improper agricultural practices, and problems regarding production policy have caused various challenges in garlic production and the demand for garlic cannot be met (Turfan, 2022). The reproduction material problem is one of them.

The production of garlic includes one-stage cultivation where plants are obtained by planting the cloves directly into the field. Cloves separated from the heads are planted as seeds in autumn and spend the winter as small plants. These plants develop and form heads at the beginning of summer. The production cycle is completed when the head matures and the harvesting begins. When varieties with the weight of 1-1.5 g of cloves are preferred, 30-50 kg seed-cloves are needed per decare. If varieties with large cloves are desired to be used, 80-100 kg cloves per decare are required (Şalk et al., 2008). On the other hand, in the case of using medium-sized cloves in planting, 50-60 kg of cloves are used per decare in single-row planting, or 75-90 kg of cloves per decare in multi-row plantings (Günay, 2005). Garlic production is conducted by using the head or the cloves inside the heads, which is actually the consumed part of the garlic, as propagation material. Important economic losses are occurred for garlic producers due to the fact that growth from cloves is the preferred propagation method. These losses correspond approximately 10% of the garlics (80-100 kg cloves per decare) on the market.

Since garlic production cannot be conducted using true seeds, it relies completely upon asexual propagation. Using seedlings may be considered to solve the problem of propagation material in garlic production and also reduces the huge financial loss resulting from using cloves that are considered as consumption material. But the production of garlic using seedlings has not still reached a sufficient level. Accordingly, alternative methods should be employed to reduce these losses. *In vitro* techniques under laboratory conditions offer a conductive alternative to increase the production of garlic seedlings in a short time, and at a level to meet the demand without causing as much loss as in the case of using cloves as production material.

Globally, garlic plantlets are produced via *in vitro* tissue culture methods. However, the practical use of these plantlets, namely seedling production is still very limited (Haque et al., 1997; Xue et al., 1991; Garcia and Vargas, 2000). In the study by Haider et al. (2015), the highest percentage of plantlet regeneration was observed in the genotype G124 for the basal disc explants (63.33%) in MS medium supplemented with 2 mg l^{-1} NAA + 1 mg l^{-1} BAP. The survival rate of the plantlets after acclimatization varied from 40% in G123 to 70% in G121. Among the different phytohormone concentrations and combinations, MS basal medium without any growth regulators (M0) was found optimal for shoot-tip initiation (96% explants development) and plantlets elongation (56.26 mm) in garlic. For shoot proliferation, the M1 culture medium containing 1 mg l^{-1} BAP and 0.25 mg l^{-1} NAA provided the best results, giving a multiplication rate of 1.7 plantlets/explant. Shoots on M0 culture medium formed bulblets earlier. Multiple bulblets per explants were obtained from medium M22 containing 2 mg l^{-1} Kinetin and 0.1 mg l^{-1} NAA. Separated bulblets were transferred individually on bulbification media. Non-dividable bulblet was developed in various sizes.

Bulblet acclimatization step needs to be well studied for high quality cloves production. This efficient, optimized *in vitro* protocol were found to be successfull for large multiplication of virus-free garlic cultivars (Ayed et al., 2018).

This study has been attempted to determine the possibilities for the reproduction of garlic, which is a very important vegetable for production and consumption, by using tissue culture techniques intensively and in a short time. For this purpose, the proliferation and rooting status of garlic stem disc explants were investigated in *in-vitro* culture media, which were created by adding different growth regulators and different concentrations. It was aimed to develop rapid and mass seedling production possibilities for the garlic cultivation in Kahramanmaraş and its surroundings. Thus, preventing the increased economic and production losses. The study includes findings that may be beneficial in providing practical ways of obtaining seedlings for garlic producers in Türkiye and globally.

2. Materials and Methods

Local garlic (*Allium sativum* L.) plants grown extensively in Kahramanmaraş (Center and Pazarcık) were used as plant material; the cloves of the plants were the explant sources for this study.

The research was initiated with the disinfection of garlic cloves to be used as explants in order to prevent disease-induced contamination: the shells of the garlic cloves were peeled, washed three times with tap water, kept in 70% ethanol for 5 minutes, and in 5% sodium hypochlorite for 5 minutes, respectively. After the sterilization, the explants were washed 5-6 times with sterile distilled water; the water was removed by keeping them between drying papers for 3-5 minutes (modified from Gad El-Hak et al., 2011).

MS medium was used in the experiment (Murashige and Skoog, 1962). Study topics were plant growth regulators and different concentrations of these PGR's which were suggested by previous researchers (Haque et al., 1997; Myers and Simon, 1998; Myers and Simon, 1999; Fereol et al., 2002; Haque et al., 2003; Kyte et al., 2013) (*Table 1*).

MS nutrient medium was supplemented with plant growth regulators and adjusted to pH 5.7, and sterilized. Then tubes with a diameter of 2.5 cm and a length of 15 cm were filled with 10 ml of these nutrient media and placed in a sterile cabinet.

Table 1. Plant growth regulators used in the research

Plant Growth Regulator	Concentration (mg l^{-1})		
	Low	Middle	High
BAP	1.0	1.5	2.0
GA_3	0.5	1.0	1.5
2-IP	0.75	1.00	1.25
Kinetin	1.0	2.0	3.0
TDZ	0.75	1.0	1.25

Basal discs of garlic cloves were used to achieve the proliferation in the experiment. The discs were removed in a sterile cabinet via sterilized forceps and scalpel, and were planted in tubes containing MS nutrient media with different growth regulators and concentrations added as experiment subjects (*Figure 1*).

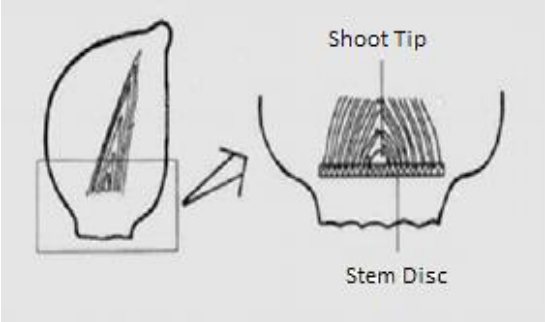

Figure 1. Plant parts were used as explants and cultured in tubes (modified from Ayabe and Sumi, 1998)

The explants were cultured in a climatic chamber at 22±2°C and 3000 lux light for 16 hours of light and 8 hours of darkness (Martin-Urdiroz et al., 2004). The experiments were continued for 45-60 days after planting the explants in the nutrient medium. When the experiment was concluded, observations and measurements were made on the basis of each application, and the data were evaluated according to the following criteria.

- Number of explants: total, infected, healthy, developed
- Healthy and developed explant rates
- Number of callused explants, callus growth rate
- Number of proliferated explants, proliferation rate, number of proliferation
- Number of rooted explants, rooting rate, number of roots

Each tube contained an explant and 48 tubes for BAP, and 36 tubes for GA_3, 2-IP, Kinetin, and TDZ were evaluated for each application. Statistical analyses were not applied in the experiments due to infection-related deaths and disruptions in plant growth.

3. Results and Discussion

This study aimed to reach a large number of materials with high regeneration potential, in other words, with rapid multiplication from explants. Accordingly, many criteria have been evaluated in the study. But proliferation rate, number of proliferations per explant, and callus formation potential were emphasized to interpretation of the results in terms of rapid and mass seedling production possibilities for the garlic cultivation.

3.1. BAP

Table 2 shows growth performances of 48 explants taken from basal discs of garlic cloves in MS nutrient media supplemented with three doses of BAP.

Table 2. The effect of BAP on explant development, callus development, proliferation, and rooting in garlic cultured in vitro

Doses (mg l^{-1})	Explants (number)	Infected explants (number)	Healthy explants (number)	Developed explants (number)	Healthy explant rate (%)	Developed explant rate (%)
1.0	48	12.0	36.0	28.0	75.0	58.3
1.5	48	10.0	38.0	31.0	79.2	64.6
2.0	48	16.0	32.0	29.0	66.7	60.4

Doses (mg l^{-1})	Callused explants (number)	Callus growth rate (%)	Proliferated explants (number)	Proliferation rate (%)	Proliferation (number)
1.0	7.0	25.0	5.0	17.9	1.6
1.5	9.0	29.0	6.0	19.4	1.8
2.0	8.0	27.6	7.0	24.1	1.6

Doses (mg l^{-1})	Rooted explants (number)	Rooting rate (%)	Roots (number)
1.0	10.0	35.7	2.1
1.5	11.0	35.5	2.0
2.0	16.0	55.2	2.4

Although identification techniques were not performed, there were infections we suspect caused by different harmful microorganisms in this study as in many cases in tissue cultures.

Concerning the number of healthy explants that were obtained by subtracting the number of infected explants from the total number of explants, 1.5 mg l^{-1} dose with 38.0 explants provided more explants than the other two doses. And it was possible to continue on the path with sufficient healthy explants at doses 1.0 mg l^{-1} (with 36.0 explants) and 2.0 mg l^{-1} (with 32.0 explants) of BAP. This is also supported by the healthy explant ratio data, which is calculated as the percentage rate of the number of healthy explants to the total explants. The highest developed explants were determined with 31 at 1.5 mg l^{-1}; followed by the doses 2.0 mg l^{-1} with 29.0 explants and 1.0 mg l^{-1} with 28.0 explants. This result was also emphasized by the ratio, which describes how much the growing explants are compared to the total explants in the study.

Although BAP did not offer a significant difference in the callus formation capabilities of explants, the highest values were reached with 9 callused explants and 29.0% callus growth rate (CGR) at the 1.5 mg l^{-1} dose. This application was followed by 2.0 mg l^{-1} (with 8 explants and 27.6% CGR) and 1.0 mg l^{-1} (with 7 explants and 25.0% CGR) doses. Among the BAP doses, the highest number of proliferated explants and the proliferation rates were in 2.0 mg l^{-1} with 7.0 and 24.1%, respectively. The dose was followed by 1.5 mg l^{-1} BAP dose (with 6.0 and a rate of 19.4%); the dose of 1.0 mg l^{-1} took the last place (with 5.0 and 17.9%). In terms of the proliferation number per explant, the most successful application was 1.5 mg l^{-1} with 1.8 while the amounts in 1.0 mg l^{-1} and 2.0 mg l^{-1} doses were 1.6.

It is understood that the enhancement in BAP caused an increase in number of rooted explants and in the rooting rate values. Hence, the highest number of rooted explants and the rooting rate were achieved on 2.0 mg l^{-1} with 16.0 and 55.2%, respectively. Number of roots and rooting rate values were recorded, respectively, as 11.0 roots and 35.5% in 1.5 mg l^{-1} and 10.0 roots and 35.7% in 1.0 mg l^{-1}. As with the number of rooted explants and the

rooting rate, the highest number of roots per explant was found at 2.0 mg l^{-1} (2.4 roots), followed by 1.0 mg l^{-1} (2.1 roots) and 1.5 mg l^{-1} (2.0 roots).

When the effects of BAP are evaluated on the proliferation rate and the proliferation number per explant, data showed that the doses of 1.5 and 2.0 mg l^{-1} provided better results. However, they gave a similar number of proliferation, 2.0 mg l^{-1} dose was slightly better in terms of proliferation rate. Haque et al. (2003) cultivated Bangladesh local garlic in MS nutrient medium containing combinations of different growth regulators and successfully obtained shoots in medium containing 2.0 mg l^{-1} BAP and 0.2 mg l^{-1} NAA. On the other hand, in a study by Ayabe and Sumi (1998) in which they cultured the stem discs of garlic cloves *in vitro* to obtain virus-free plants, they successfully used LS (Linsmaier and Skoog) medium supplemented with 0.1 mg l^{-1} NAA and 0.1 mg l^{-1} BA for reproduction. The differences in results between this study and study by Ayabe and Sumi (1998) at this point may be attributed to nutrient medium used and/or combined effect of NAA and nutrient medium.

Although the callus formation rate did not differ significantly between BAP doses, it can be stated that the best callus formation was achieved with a dose of 1.5 mg l^{-1}. A dose of 2.0 mg l^{-1} of BAP was also notable. Consistent with our results Myers and Simon (1998; 1999) found that the application of 2.66 mg l^{-1} BA provided the highest shoot formation rate.

When all the data obtained from BAP evaluated together, it can be stated that even though the use of BAP alone provided higher values in studied parameters, better results might be achieved from the combined use of some other cytokinins and auxins, as noted by Myers and Simon (1999) and Ayabe and Sumi (1998). It would be more appropriate to use auxins during the explant rooting phase of the study.

3.2. Gibberellic acid

Table 3 shows the effects of gibberellic acid on explant development, callus development, proliferation, and rooting in garlic.

Table 3. The effect of gibberellic acid on explant development, callus development, proliferation, and rooting in garlic cultured in vitro

Doses (mg l^{-1})	Explants (number)	Infected explants (number)	Healthy explants (number)	Developed explants (number)	Healthy explant rate (%)	Developed explant Rate (%)
0.5	36	9.0	27.0	21.0	75.0	58.3
1.0	36	7.0	29.0	22.0	80.6	61.1
1.5	36	10.0	26.0	18.0	72.2	50.0

Doses (mg l^{-1})	Callused explants (number)	Callus growth rate (%)	Proliferated explants (number)	Proliferation rate (%)	Proliferation (number)
0.5	3.0	14.3	4.0	19.0	1.5
1.0	4.0	18.2	5.0	22.7	1.6
1.5	6.0	33.3	3.0	16.7	1.7

Doses (mg l^{-1})	Rooted explants (number)	Rooting rate (%)	Roots (number)
0.5	8.0	38.1	1.4
1.0	9.0	40.9	1.9
1.5	7.0	38.9	1.7

Table 3 shows that the experiment started with 36 explants but concluded with 27.0 explants at 0.5 mg l^{-1}; with 29.0 explants at 1.0 mg l^{-1}; and with 26.0 explants at 1.5 mg l^{-1} doses of GA_3

GA_3 showed the most significant effect on callus formation in garlic at a dose of 1.5 mg l^{-1}. In this application, the numbers of callus forming explants were 6.0 and the callus growth rate was 33.3%. These properties were recorded as 4.0 and 3.0 callused explants, and 18.2% and 14.3% callus growth ratios at 1.0 and 0.5 mg l^{-1} doses, respectively. In terms of the number of explants and proliferation rate, the most successful GA_3 application was 1.0 mg l^{-1} as 5.0 explants were proliferated and the rate reached to 22.7%. At 0.5 and 1.5 mg l^{-1} doses of GA_3, 4.0

and 3.0 proliferated explants, and 19.0% and 16.7% of proliferation rates realized, respectively. The data obtained in the number of proliferations per explant varied depending on the doses. While the most successful application was 1.5 mg l^{-1} GA_3 dose (with 1.7 proliferation), 1.0 mg l^{-1} (with 1.6 proliferation) and 0.5 mg l^{-1} (with 1.5 proliferation) GA_3 doses formed the following ranks.

The effect of GA_3 on the number of rooted explants was less pronounced. The highest number of rooted explants was observed at 1.0 mg l^{-1} dose with 9.0 explants; this was followed by 0.5 mg l^{-1} with 8.0 explants and 1.0 mg l^{-1} with 7.0 explants. Rooting rate and number of roots in three GA_3 doses were listed as 1.0 mg l^{-1} (40.9% and 1.9 roots), 1.5 mg l^{-1} (38.9% and 1.7 roots), and 0.5 mg l^{-1} (38.1% and 1.4 roots).

Evaluating the effectiveness of GA_3 on callus formation revealed that 1.5 mg l^{-1} GA_3 application resulted in superior data in comparison to the other dosages. It was demonsrated that GA_3 showed the highest proliferation rate and proliferated explant by the dosages of 1.0 mg l^{-1} and 0.5 mg l^{-1}, whereas 1.5 mg l^{-1} dosage was found to be more effective at increasing proliferation per explant.

Similar results were obtained in the single-use of GA_3 in the MS environment by previous reports. Nasim et al. (2010) observed that, among the many hormone concentrations being studied, the highest rate of somatic embryo formation occurred in MS medium with 0.5 mg l^{-1} GA_3 supplement. Bekheet (2006), who use different doses of GA_3 + BA and BA + NAA combinations to shoot stimulation in garlic under *in vitro* conditions, emphasized that the GA_3 together with other growth regulators added to the MS environments were more effective in the promotion of the mentioned characteristics.

Although GA_3 had positive effects on proliferation and callus formation, it may be speculated that more favorable results can be obtained from the combining use of GA_3 and other growth regulators under *in vitro* conditions for the mass production of garlic.

3.3. 2-IP

The findings related to explant-callus development, and proliferation and rooting are presented in the *Table 4*.

Table 4. The effect of 2-IP on explant development, callus development, proliferation, and rooting in garlic cultured in vitro

Doses (mg l^{-1})	Explants (number)	Infected explants (number)	Healthy explants (number)	Developed explants (number)	Healthy explant rate (%)	Developed explant rate (%)
0.75	36	20.0	16.0	11.0	44.4	30.6
1.00	36	17.0	19.0	13.0	52.8	36.1
1.25	36	24.0	12.0	10.0	33.3	27.8

Doses (mg l^{-1})	Callused explants (number)	Callus growth rate (%)	Proliferated explants (number)	Proliferation rate (%)	Proliferation (number)
0.75	3.0	27.3	5.0	45.5	1.6
1.00	2.0	15.4	7.0	53.8	1.9
1.25	2.0	20.0	4.0	40.0	1.8

Doses (mg l^{-1})	Rooted explants (number)	Rooting rate (%)	Roots (number)
0.75	4.0	36.4	1.3
1.00	5.0	38.5	1.8
1.25	7.0	70.0	1.6

Table 4 indicates that our results did not presented any significant improvement regarding the effect of 2-IP on developed explant numbers. However, in reference to the dosage, the highest number of explants, 19.0, was found in 1.00 mg l^{-1} 2-IP treatment.

According to the number of callus forming explants and the callus formation data, 0.75 mg l^{-1} dosage resulted in higher amounts for those properties with 3.0 callused explants and 27.3% callus formation rate. The group ranking second consited of, in descending order, dosages of 1.25 mg l^{-1} and 1.00 mg l^{-1}, with the number of callus

forming explants of 2.0 and 2.0, respectively, and with callus formation rate of 20.0% and 15.4%, respectively. For the proliferated explants, the highest number, 7.0, was found in 1.00 mg l^{-1} 2-IP application, followed by 5.0 proliferated explants with 0.75 mg l^{-1} and 4.0 proliferated explants with 1.25 mg l^{-1} dosages, respectively. Proliferation performances of explants was found to be similar to proliferation rates. It was determined that 1.00 mg l^{-1} dosage produced best results with 53.8% proliferation rates, followed by dosages of 0.75 mg l^{-1} and 1.25 mg l^{-1} with the proliferation rates of 45.5% and 40.0%, respectively. The highest number of proliferation was 1.9 at 1.00 mg l^{-1} dose, while 1.25 mg l^{-1} with 1.8 and 0.75 mg l^{-1} with 1.6 were placed in the following rows.

With regards to the 2-IP application to the explants in the experiment, it was observed that the maximum root formation, that is, 7.0 rooted explants and highest rooting rate, that is, 70.0%, were obtained from the 1.25 mg l^{-1} dosage. As second and third ranking dosages, 1.00 mg l^{-1} and 0.75 mg l^{-1} produced 5.0 explants and 38.5% rooting, and 4.0 explants and 36.4% rooting, respectively. Regarding the number of roots per explant, the application with the best results was 1.00 mg/l with 1.8 roots, whereas the dosage of 1.25 mg l^{-1} with 1.6 roots and the dosage of 0.75 mg l^{-1} with 1.3 roots formed the following rows.

An overall evaluation of the impact of 2-IP in callus growth rate revealed that the highest callus formation rate was achieved at the dosage of 0.75 mg l^{-1} by 27.3%. In terms of proliferation rates and the number of proliferations per explant, it was observed that the best results were obtained from 1.0 mg l^{-1} dosage.

Garcia and Vargas (2000), who tested different combinations of 2-IP, BA, and Kinetin as well as NAA and IAA in MS medium in their study to obtain cloves from shoot tip culture of garlic (*A. sativum*) obtained the best results for proliferation from the combination of 0.5 mg l^{-1} 2-IP and 0.2 mg l^{-1} NAA. They determined that the highest proliferation rates were achieved by the combination of 2-IP + NAA. However, reports form Mohamed-Yasseen et al. (1994) were in line with our work, pointing out that 2-IP was able to produce more shoots when used alone.

Despite the promising results of this study on practical usability of garlic clove discs for rapid and mass production of planting material in garlic cultivation, when the results presented here is discussed with the earlier studies, it may have postulated that the promoting effects of PGRs are better expressed when different combinations of PGRs are tested at appropriate doses than the use of 2-IP alone to achieve this goal.

3.4. Kinetin

The effects of Kinetin dosages on explant development, callus development, proliferation, and rooting were summarized in *Table 5*.

Table 5. The effect of kinetin on explant development, callus development, proliferation, and rooting in garlic cultured in vitro

Doses (mg l^{-1})	Explants (number)	Infected explants (number)	Healthy explants (number)	Developed explants (number)	Healthy explant rate (%)	Developed explant rate (%)
1.0	36	8.0	28.0	20.0	77.8	55.6
2.0	36	6.0	30.0	17.0	83.3	47.2
3.0	36	11.0	25.0	15.0	69.4	41.7

Doses (mg l^{-1})	Callused explants (number)	Callus growth rate (%)	Proliferated explants (number)	Proliferation rate (%)	Proliferation (number)
1.0	5.0	25.0	5.0	25.0	1.4
2.0	3.0	17.6	6.0	35.3	1.7
3.0	2.0	13.3	4.0	26.7	1.8

Doses (mg l^{-1})	Rooted explants (number)	Rooting rate (%)	Roots (number)
1.0	8.0	40.0	2.1
2.0	9.0	52.9	2.4
3.0	7.0	46.7	2.6

Table 5 indicates the best growth, regarding the distribution of percent healthy explants to the developed explants, was at the dose of 1.0 mg l^{-1} Kinetin with 20.0 developed explants.

The most promising Kinetin dose for the number of explants forming callus and the rate of callus formation was 1.0 mg l^{-1} (5.0 explants and 25.0%, respectively). This was followed by 2.0 mg l^{-1} (3.0 explants and 17.6%, respectively) and 3.0 mg l^{-1} (2.0 explants and 13.3%, respectively) dosages.

The highest proliferation performance was found at the dosage of 2.0 mg l^{-1} with 6.0 explants and 35.3% proliferation rate. This was followed by 1.0 mg l^{-1} dosage with 5.0 number of explants and 25% proliferation rate, and 3.0 mg l^{-1} dosage produced the lowest number of explant (4.0) and proliferation rate (26.7%). Alternatively, however, 3.0 mg l^{-1} dosage resulted in higher proliferation figures (1.8) over those of the 2.0 mg l^{-1} (1.7) and 1.0 mg l^{-1} (1.4) Kinetin dosages.

The highest rooting values in garlic explants were obtained from the 2.0 mg l^{-1} of Kinetin with the 9.0 rooted explants and 52.9% rooting rate. Interestingly, though 1.0 mg l^{-1} of Kinetin application resulted in superior data on number of rooted explant in comparison to the 3.0 mg l^{-1} of Kinetin, it resulted in the lowest rooting rate (40.0%) and root number (2.1), indicating the beneficial effect of increasing Kinetin dosages up to 2.0 mg l^{-1} in rooting paramaters.

Data revealed that the best results in callus formation rate (25%) and callused explant number (5.0) were from the 1.0 mg l^{-1} dosage. The highest proliferation rate (25%) and the number of proliferation per explant (6.0) was recorded in the dose of 2.0 mg l^{-1}, whereas the number of proliferation per explant were highest with 3.0 mg l^{-1}. It may be speculated that the proliferation rates and proliferation numbers varied according to level of growth after proliferation formation was achieved. The findings obtained a study with grape and discussions of these findings with the results of the previous literature has supported that callus regeneration ratio and quality depend on the explant material and media. MS medium including BAP (1 mg L-1) + 2,4-D (0.1 mg L-1) was recommended for callus regeneration in node explants of Sultana grape (Pehlivan et al., 2017)

In a study conducted on proliferation in onion and garlic, Mukhopadhyay et al. (2005) reported that the combined use of 0.93 mg l^{-1} Kinetin and 1.07 mg l^{-1} NAA resulted in more successful results than other Kinetin and NAA combinations. These reports further confirmed the significant Kinetin effect at developing more shoots and roots in both onion and garlic.

In our study Kinetin conferred the highest positive effects on proliferation and callus formation, which are important components of mass production of garlic. However, it was understood, in the light of the literature on the subject that Kinetin should be used together with other growth regulators to maximize growth, and the application of auxin + cytokinin combinations can be more beneficial in proliferation and callus formation.

3.5. TDZ

Effects of TDZ application *in-vitro* culture of garlic are presented in *Table 6*.

Table 6 shows that the highest number of healthy explants with 29 was recorded in 0.75 mg l^{-1} TDZ, while it was least in 1.00 mg l^{-1} with 25.0. 0.75 mg l^{-1}. TDZ also produced the highest developed explant rate (52.8%) and number of developed explant (19.00), followed by the dosages of 1.00 mg l^{-1} and 1.25 mg l^{-1} with the number of developed explant of 15.0 and 13.0 and with the developed explant rate of 41.7%, and - 36.1%, respectively.

TDZ with 1.25 mg l^{-1} of dosage provided the highest number of callused explant (6.00) and the highest callus growth rate (46.2%), but it failed to produce proliferation. Number of proliferated explants and proliferation rate seemed to decrease with the increasing TDZ dosages. It appears that TDZ did not confer any advantage over the numbers of proliferated explants and proliferation rates.

Considering the effects of TDZ dosages on the rooting performances of garlic explants, 1.00 mg l^{-1} dosage produced the highest number of rooted explants (9.0) and rooting rate (60.0%). This dose was followed by 1.25 mg l^{-1} with the values of 7.0 and 53.8%, respectively. The lowest rooting rate, that is, 31.6% and lowest rooted explant number, that is 6.6, were obtained from the 0.75 mg l^{-1}. The maximum number of roots (2.7) was recorded in 1.25 mg l^{-1} and this was followed by 1.00 mg l^{-1} (2.6) and 0.75 mg l^{-1} (2.2) TDZ dosages.

In a study of Mohamed-Yasseen et al. (1994) on garlic, 2.2 and 7.5 shoots were obtained from whole and sliced cloves, respectively. However, these results realized when 0.1 µM NAA was added to the MS medium with 0.15 µmol TDZ. Alizadeh et al. (2013) have postulated that TDZ had a favorable effect on rooting when it was used alone on mesocotyl axes of garlic *Allium tuberosum* cloves. However, it failed to produce similar success in terms of proliferation and callus formation, pointing out that 1.0 mg l^{-1} dose of TDZ + 0.8 and 1.00 mg l^{-1} doses of 2.4 D should be used together for proliferation.

Table 6. The effect of TDZ on explant development, callus development, proliferation and rooting in garlic cultured in vitro

Doses (mg l^{-1})	Explants (number)	Infected explants (number)	Healthy explants (number)	Developed explants (number)	Healthy explant rate (%)	Developed explant rate (%)
0.75	36	7.0	29.0	19.0	80.6	52.8
1.00	36	11.0	25.0	15.0	69.4	41.7
1.25	36	9.0	27.0	13.0	75.0	36.1

Doses (mg l^{-1})	Callused explants (number)	Callus growth rate (%)	Proliferated explants (number)	Proliferation rate (%)	Proliferation (number)
0.75	4.0	21.1	2.0	10.5	1.0
1.00	2.0	13.3	1.0	6.7	1.0
1.25	6.0	46.2	0.0	0.0	0.0

Doses (mg l^{-1})	Rooted explants (number)	Rooting rate (%)	Roots (number)
0.75	6.0	31.6	2.2
1.00	9.0	60.0	2.6
1.25	7.0	53.8	2.7

These findings show that application of TDZ alone, which causes the least proliferation and callus formation among the plant growth regulators used in our study, can not deliver the desired results for regeneration and rapid reproduction in mass production.

4. Conclusions

From the viewpoint of the results presented here, it seems unlikely that the studied dosages of plant growth regulators would be sufficient to produce seedlings from stem disc of cloves for the mass production of garlic. This further confirmed earlier report on the effect of growth regulators on rate and number of proliferation in garlic and onion.

Nonetheless, our findings suggest that, with the manipulation of dosages beneficial effects of the studied plant growth regulators can translate into significant increases in proliferation and callus formation in stem disc of garlic cloves, and sufficient level of quantity and quality may be achieved to provide seedlings.

In conclusion, this study indicates promising results in *in vitro* shoot formation from stem discs of gloves, therefore, production of seedlings for mass production of garlic. However, further experiments with varying dosages and plant growth regulators, especially with cytokinin and auxin groups, in combinations, may be useful to fully judge on the effectiveness of generating plantlets directly from stem discs of the garlic cloves *in vitro*.

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References

Alizadeh, B., Royandazagh, S. D., Khawar, K. M. and Ozcan, S. (2013). Micropropagation of garlic chives (*Allium Tuberosum* Rottl. Ex Sprang) using mesocotyl axis. *The Journal of Animal & Plant Sciences,* 23(2): 543-549.

Ayabe, M. and Sumi, S. (1998). Establishment of a novel tissue culture method, stem-disc culture, and its practical application to micropropagation of garlic (*Allium sativum* L.). *Plant Cell Reports,* 17: 773-779.

Ayed, C., Bayoudh, C., Rhimi, A., Mezghani, N., Haouala, F. and Dridi, B. A. M. (2018). *In vitro* propagation of Tunisian local garlic (*Allium sativum* L.) from shoot-tip culture. *Journal of Horticulture and Postharvest Research,* 1(2): 75-86.

Bekheet, S. A. (2006). A synthetic seed method through encapsulation of *in vitro* proliferated bulblets of garlic (*Allium sativum* L.). *Arab Journal of Biotechnology,* 9(3): 415-426.

Fereol, L., Chovelon, V., Causse, S., Michaux-Ferriere, N. and Kahane, R. (2002). Evidence of a somatic embryogenesis process for plant regeneration in garlic (*Allium sativum* L.). *Plant Cell Reports,* 21(3): 197-203.

Gad El-Hak, S. E. H., Ahmed, K. Z., Moustafa, Y. M. M. and Ezzat, A. S. (2011). Growth and cytogenetical properties of micro-propagated and successfully acclimatized garlic (*Allium sativum* L.) clones with a modified shoot tip culture protocol. *Journal of Horticultural Science & Ornamental Plants,* 3(2): 115-129.

Garcia, E. and Vargas, T. (2000). Micropropagation Clonal Masivade Variedades de ajo (Allium sativum) Con Fines Comerciales. Memorias del X. Congreso Halo Latinoamericano de Etnomedicina. 217-218.

Günay, A. 2005. Vegetable Cultivation. Volume 1, Meta Press, İzmir.

Haider, S. R., Hossain, M. R., Rahman, S., Sultana, S., Quddus, T., Chakraborti. M., Hoque, A., Shahriar, H. and Haque, A. (2015). *In vitro* plantlet regeneration of four local garlic (*Allium sativum*) accessions of Bangladesh. *British Biotechnology Journal,* 8(3): 1-12.

Haque, M. S., Wada, T. and Hattori, K. (1997). High frequency shoot regeneration and plantlets formation from root tip of garlic. *Plant Cell Tissue and Organ Culture,* 50: 83-89.

Haque, M. S., Wada, T. and Hattori, K. (2003). Shoot regeneration and bulblets formation from shoot and root meristem of garlic cv. Bangladesh local. *Asian Journal of Plant Sciences,* 2: 23-27.

Kyte, L., Kleyn, J., Scoggins, H. and Bridgen, M. (2013). Plants from Test Tubes: An Introduction to Micropropagation. Timber Press, China.

Martin-Urdiroz, N., Garrido-Gala, J., Martin, J. and Barandiaran, X. (2004). Effect of light on the organogenic ability of garlic roots using a one-step *in vitro* system. *Plant Cell Reports,* 22: 721-724.

Mohamed-Yasseen, Y., Splittstoesser, W. A. and Litz, R. E. (1994). *In vitro* shoot proliferation and production of sets from garlic and shallot. *Plant Cell, Tissue and Organ Culture,* 36: 243-247.

Mukhopadhyay, J., Sengupta, P., Mukhopadhyay, S. and Sen, S. (2005). *In vitro* stable regeneration of onion and garlic from suspension culture and chromosomal instability in solid callus culture. *Scientia Horticulturae,* 104:1-9.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum,* 15: 473-497.

Myers, J. M. and Simon, P. W. (1998). Continuous callus production and regeneration of garlic (*Allium sativum* L.) using root segments from shoot-tip-derived plantlets. *Plant Cell Reports,* 17: 726-730.

Myers, J. M. and Simon, P. W. (1999). Regeneration of garlic callus affected by clonal variation, plant growth regulators and culture conditions over time. *Plant Cell Reports,* 19: 32-36.

Nasim, S. A., Mujib, A., Kapoor, R., Fatima, S. and Aslam J. M. (2010). Somatic embryogenesis in *Allium sativum* L. (cv. Yamuna Safed 3): Improving embryo maturation and germination with PGRs and carbohydrates. *Anales de Biología,* 32: 1-9.

Pehlivan, E.C., Kunter, B., Daneshvar Royandazagh, S. (2017). Choise of Explant Material and Media for in vitro Callus Regeneration in Sultana Grape Cultivar (*Vitis vinifera* L.). 2nd International Balkan Agriculture Congress, May 16-18. *Journal of Tekirdag Agricultural Faculty,* Special Issue, 30-34.

Şalk, A., Arın, L., Deveci, M. and Polat, S. (2008). Private Vegetables. Onur Grafik Matbaa ve Reklam Hizmetleri, İstanbul.

Turfan, N. (2022). Comparison of bulb yield, some bioactive compound and elemental profile of Taşköprü garlic (*Allium sativum* L.) grown in greenhouse and open field conditions. *Journal of Tekirdag Agricultural Faculty,* 19(2): 248-261.

Xue, H. M., Araki, H. and Yakuwa, T. (1991). Varietal difference of embryogenic callus induction and plant regeneration in garlic (*Allium sativum* L.). *Plant Tissue Culture Letters,* 8(3): 166-170.