

EFFECT OF PHYTOHORMONES ON THE FORMATION OF CALLUS TISSUES AND THE DEVELOPMENT OF SHOOTS FROM THEM IN SEEDLESS PUNICA GRANATUM L. (MOLLAR DE ELCHE)

Urazboev Azizbek Narzullo ogli ¹, Olimov Avazbek Bobosher ogli ¹, Valieva Shoirabduvositovna ²

¹ Master's student of the Fruit Growing program, Joint Education Faculty, at the Tashkent State Agrarian University and the Northwest A&F University of China.

² Senior Lecturer, Department of Fruit Growing and Viticulture, Faculty of Fruit, Vegetable and Viticulture, Tashkent State Agrarian University.

<https://doi.org/10.5281/zenodo.15545895>

Abstract. This study investigated the effect of phytohormones on callus induction and shoot regeneration in seedless *Punica granatum* L. ('Mollar de Elche'), aiming to develop an efficient in vitro propagation protocol. The results revealed that a combination of the cytokinin BAP and the auxin NAA significantly influenced callus formation and shoot development. The highest callus formation (90.2%) and shoot regeneration (5.3 ± 0.87 shoots per callus) were achieved with 2.0 mg/L BAP and 0.1 mg/L NAA. Lower BAP concentrations, particularly 0.1 mg/L, were less effective in both callus induction and shoot production. Shoot development was optimized at 1.0 mg/L BAP combined with 0.1 mg/L NAA, yielding the highest number of shoots per explant (81). Higher concentrations of BAP (2.0 mg/L) negatively impacted shoot proliferation, especially at elevated NAA levels (1.0 mg/L). Overall, this study highlights the importance of a balanced combination of BAP and NAA for efficient clonal propagation of 'Mollar de Elche' pomegranate, contributing to its large-scale production for commercial cultivation.

Keywords: *Punica granatum* L., Mollar de Elche, micropropagation, Callus formation, Shoot regeneration, BAP, NAA.

Annotatsiya. Ushbu tadqiqot *Punica granatum* L. (granat)ning “Mollar de Elche” navining in vitro mikropopulyatsiyasini ishlab chiqish maqsadida o'tkazilgan. Tadqiqotda, cytokinin BAP va auxin NAA ni kombinatsiyalash orqali kallus hosil bo'lishi va undan o'simlik o'sishi uchun eng samarali sharoitlar aniqlangan. Natijalar shuni ko'rsatdiki, 2.0 mg/L BAP va 0.1 mg/L NAA kombinatsiyasi kallus hosil bo'lishi (90.2%) va o'simlik o'sishi (5.3 ± 0.87 o'simlik bir kallusdan) bo'yicha eng yuqori natijalarga olib keldi. Kamroq BAP miqdorlari, ayniqsa 0.1 mg/L, kallus hosil qilish va o'simlik o'sishini samarali tarzda rag'batlantirmadi. O'simliklarning eng yaxshi rivojlanishi 1.0 mg/L BAP va 0.1 mg/L NAA kombinatsiyasida kuzatildi, bunda har bir eksplantidan o'rtacha 81 dona o'simlik hosil bo'ldi. BAP miqdori 2.0 mg/L ga oshirilganida esa o'simlik o'sishi kamaygan, ayniqsa yuqori NAA (1.0 mg/L) miqdorida. Ushbu tadqiqot natijalari, granatning “Mollar de Elche” navining klonal ko'paytirish uchun BAP va NAA ni muvozanatli qo'llashning muhimligini ko'rsatadi, bu esa navning tijorat maqsadlarida keng miqyosda yetishtirilishi uchun samarali bo'lishi mumkin.

Kalit so'zlar: *Punica granatum* L., Mollar de Elche, mikroko'paytirish, kallus hosil bo'lishi, kurtaklar regeneratsiyasi, BAP, NAA.

Аннотация. Настоящее исследование посвящено микропропагации безсемянного граната сорта “Mollar de Elche” (*Punica granatum* L.) in vitro. Целью исследования было

определение оптимальных условий для образования каллюса и развития побегов с использованием комбинации цитокинина BAP и ауксина NAA. Результаты показали, что комбинация 2,0 мг/л BAP и 0,1 мг/л NAA дала наибольшую частоту образования каллюса (90,2%) и наибольшее количество побегов ($5,3 \pm 0,87$ побега на каллюс). Низкие концентрации BAP, особенно 0,1 мг/л, оказались менее эффективными для формирования каллюса и развития побегов.

Лучшее развитие побегов было зафиксировано при комбинации 1,0 мг/л BAP и 0,1 мг/л NAA, где в среднем образовывалось 81 побег на эксплант. Высокие концентрации BAP (2,0 мг/л) привели к снижению роста побегов, особенно при высоких концентрациях NAA (1,0 мг/л). Результаты исследования подчеркивают важность сбалансированного применения BAP и NAA для эффективного клонового размножения сорта “Mollar de Elche”, что может способствовать его широкомасштабному выращиванию в коммерческих целях.

Ключевые слова: *Punica granatum* L., Mollar de Elche, микроразмножение, образование каллюса, регенерация побегов, BAP, NAA.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a fruit with rich historical significance, cultivated for thousands of years and valued for its wide range of uses and varietal diversity (Preece & Moersfelder, 2016; Chater et al., 2018; Morton, 1987). Native to regions extending from Iran to the northern Indian Himalayas, it is now mainly grown in Mediterranean and Middle Eastern regions (Jbir et al., 2008; Melgarejo et al., 2009). According to Levin (2006), the primary, secondary, and tertiary centers of origin, alongside five major genetic diversity centers, include the Middle East, Mediterranean, East Asia, the Americas, and South Africa. As a temperate climate species, pomegranate thrives in areas with long, hot summers that support optimal fruit maturation.

While pomegranate can be propagated from seed, commercial production typically uses hardwood cuttings ranging from 25 to 50 cm to limit genetic variability, which is a common issue with seed-grown plants. Traditional methods of propagation face challenges like low seed viability, seasonal constraints, and slow vegetative growth. Although air layering can serve as an alternative, grafting tends to yield inconsistent results (Morton et al., 1987). Consequently, conventional breeding practices are often slow and labor-intensive. To address these issues, biotechnological techniques, including tissue culture and molecular genetics, are increasingly incorporated into breeding programs (Lichtenstein & Draper, 1985).

Various in vitro regeneration methods have been successfully developed for pomegranate, utilizing explants such as leaf tissues (Murkute et al., 2002), cotyledons (Kanwar et al., 2010), and anthers (Moriguchi et al., 1987). The combination of NAA and BAP has proven effective in regenerating shoots in several fruit tree species (Zimmerman & Swartz, 1994). Cytokinins, when combined with auxins, promote shoot regeneration (Nike et al., 1999), with auxin, cytokinin, and ethylene synthesis and activity being closely interrelated (Klee & Romano, 1994).

The highest number of shoots per explant was achieved on MS medium containing 1.8 mg/L BAP, 0.9 mg/L NAA, 1.0 mg/L silver nitrate, 30 mg/L adenine sulfate, and 30% sucrose. When different concentrations of BAP and NAA were tested individually, the MS medium produced superior results, while WPM medium showed a lower proliferation rate.

Among modern pomegranate cultivars, ‘Mollar de Elche’ is particularly prized in Spain for its sweet taste, soft arils, and unique sensory characteristics (Cano-Lamadrid et al., 2017). It is renowned worldwide for its remarkable flavor and high antioxidant content, making it one of the most popular pomegranate varieties (Manera et al., 2013). The soft seeds further enhance its appeal, contributing to its status as a "superfruit."

Due to its seedlessness, ‘Mollar de Elche’ is primarily propagated via vegetative methods, which, although common in commercial production, are labor-intensive, slow, and dependent on specific seasonal conditions (Krul & Mowbray, 1984). Micropropagation offers a more efficient alternative, enabling the large-scale production of genetically uniform plants in a controlled setting.

This study aims to develop an efficient sterilization and in vitro culture protocol for the seedless pomegranate cultivar ‘Mollar de Elche,’ with the goal of facilitating its clonal propagation through micropropagation techniques.

MATERIALS AND METHODS

This study investigates the in vitro micropropagation of the seedless pomegranate cultivar ‘Mollar de Elche’ (*Punica granatum* L.), focusing on optimizing key steps for clonal propagation using artificial nutrient media. The primary aim is to establish an efficient tissue culture protocol that maximizes explant survival, ensures genetic consistency, and enhances shoot regeneration, which could facilitate the large-scale propagation of this cultivar. The research was conducted in 2024 at the scientific-experimental stations of the Scientific Research Institute of Horticulture, Viticulture, and Winemaking named after Academician Mahmud Mirzayev.

The plant material used for the study included young, actively growing meristematic shoot tips from ‘Mollar de Elche’ pomegranate trees cultivated in calcareous soils. To ensure high regeneration potential, explants were harvested from healthy, soft shoot segments during the vegetative growth period from March to April.

A multi-step sterilization protocol was employed to eliminate microbial contamination and ensure clean in vitro cultures.

Sterilization success was evaluated by monitoring contamination levels over a 14-day incubation period.

The sterilized explants were then cultured on Murashige and Skoog (MS) medium, which is widely used for plant tissue culture due to its balanced nutrient composition. MS medium supports robust shoot proliferation thanks to its ideal ratio of macronutrients, especially nitrogen and potassium, which are essential for growth and development.

For callus induction and proliferation, explants were transferred onto MS medium containing various concentrations of cytokinin 6-Benzylaminopurine (BAP) and auxin Naphthalene acetic acid (NAA). Specifically, BAP concentrations of 0.1, 1.0, 2.0, and 5.0 mg/L and NAA concentrations of 0.01, 0.1, and 1.0 mg/L were tested. All media also included 200 mg/L activated charcoal, 6 g/L agar, and 30 g/L sucrose. The pH of the medium was adjusted to 5.66 using sodium hydroxide (NaOH) and hydrochloric acid (HCl), followed by sterilization in an autoclave at 121°C for 30 minutes.

The cultures were incubated at 24±1°C with a 16/8 photoperiod in a plant growth chamber. After three weeks, the explants were subcultured onto fresh medium with the same hormone concentrations for further callus proliferation. The callus induction frequency, morphology, and number of shoots per callus were recorded as key indicators of the success of the propagation process.

For shoot regeneration, calli were transferred onto MS media containing 200 mg/L activated charcoal and BAP at concentrations of 0.1, 0.5, 1.0, and 2.0 mg/L. Shoot formation was evaluated three weeks after subculturing. The data collected included the number of shoots formed per callus and their elongation, which were used to assess the efficacy of different hormone treatments in promoting shoot regeneration.

Through these protocols, this study aims to establish an efficient method for the in vitro propagation of ‘Mollar de Elche’, contributing to the widespread cultivation and commercial availability of this high-quality pomegranate cultivar.

RESULTS AND DISCUSSION

The callus induction and proliferation results for explants grown on various MS media containing NAA (auxin) and different concentrations of BAP (cytokinin) are summarized in Table 1. Callus formation was observed in all experimental groups, except for the control group (hormone-free MS medium), where no callus induction occurred. The data indicate that the combination of NAA and BAP at specific concentrations was effective in inducing callus growth, with varying levels of proliferation observed across the different treatments.

Table 1.

Effect of auxin and cytokinin concentrations on callus induction in seedless *Punica granatum* L. (‘Mollar de Elche’) meristem segments

PGR concentrations (m/g-1L)	Callus formation frequency (%)	Callus morphology	Number of shoots per callus
MS+0.1BAP+0.01NAA	0%	Not observed	0
MS+1.0BAP+0.01NAA	40.1± 1.5%	Light greenish color, compact callus structure	2.3 ± 0.25
MS+2.0BAP+0.01NAA	60.5± 2.3%	Light greenish color, compact callus structure	3.5 ± 0.33
MS+5.0BAP+0.01NAA	35.2± 1.8%	Whitish color, compact callus with transparent structure	2.0 ± 0.45
MS+0.1BAP+0.1NAA	0%	Not observed	0
MS+1.0BAP+0.1NAA	65.6± 2.2%	Light greenish color, compact callus structure	3.8 ± 0.75
MS+2.0BAP+0.1NAA	90.2± 1.3%	Dark green color, compact callus structure and amorphous	5.3 ± 0.87
MS+5.0BAP+0.1NAA	45.7± 3.1%	Light greenish color, compact callus structure	2.6 ± 0.12
MS+0.1BAP+1NAA	0%	Not observed	0
MS+1.0BAP+1NAA	30.1± 1.1%	Whitish color, compact callus with transparent structure	1.7 ± 0.47

MS+2.0BAP+1NAA	70.7± 1.5%	Dark green color, compact callus structure and amorphous	4.1 ± 0.59
MS+5.0BAP+1NAA	25.4± 3.3%	Whitish color, compact callus with transparent structure	1.5 ± 0.83

The table provides a detailed summary of the effect of different concentrations of auxin (NAA) and cytokinin (BAP) on callus induction and proliferation in meristem segments of seedless pomegranate (*Punica granatum* L. cv. 'Mollar de Elche'). The results highlight how varying the concentrations of plant growth regulators (PGRs) influences callus formation frequency, callus morphology, and the number of shoots per callus.

No Callus Formation (0%): MS+0.1BAP+0.01NAA and MS+0.1BAP+0.1NAA, and MS+0.1BAP+1NAA: In all these treatments, no callus was observed, indicating that low concentrations of BAP (0.1 mg/L) combined with either 0.01 mg/L, 0.1 mg/L, or 1.0 mg/L NAA were ineffective for inducing callus formation.

Low to Moderate Callus Formation: MS+1.0BAP+0.01NAA: This treatment resulted in a 40.1% callus formation frequency with a light greenish-colored, compact callus structure. On average, 2.3 ± 0.25 shoots were formed per callus.

MS+2.0BAP+0.01NAA: This combination increased the callus formation frequency to 60.5%, with calli exhibiting a light greenish color and compact structure. It also resulted in an average of 3.5 ± 0.33 shoots per callus.

MS+5.0BAP+0.01NAA: With a 35.2% callus formation frequency, the callus had a whitish color and compact yet transparent structure, producing 2.0 ± 0.45 shoots per callus.

Higher Callus Formation and Improved Shoot Production: MS+1.0BAP+0.1NAA: This treatment showed a significant increase in callus formation, reaching 65.6%, with light greenish, compact calli and an average of 3.8 ± 0.75 shoots per callus.

MS+2.0BAP+0.1NAA: The best results in this study were obtained with this combination, where 90.2% of explants formed calli. These calli had a dark green color, compact structure, and an amorphous appearance, yielding an average of 5.3 ± 0.87 shoots per callus. This treatment resulted in the highest callus formation and shoot production.

MS+5.0BAP+0.1NAA: Although callus formation occurred in 45.7% of explants, the morphology was similar to other treatments, with light greenish, compact calli. The number of shoots per callus was 2.6 ± 0.12 .

Moderate Callus Formation with Less Shoot Production: MS+1.0BAP+1NAA: Callus formation occurred in 30.1% of explants, with whitish, transparent calli. The average number of shoots per callus was 1.7 ± 0.47 , indicating lower shoot regeneration compared to other treatments.

MS+2.0BAP+1NAA: This combination resulted in 70.7% callus formation, with dark green, compact, and amorphous calli. It produced an average of 4.1 ± 0.59 shoots per callus, showing a good balance between callus formation and shoot regeneration.

MS+5.0BAP+1NAA: The callus formation rate was 25.4%, and the morphology was similar to other high BAP treatments, showing whitish, compact calli with transparent structures. The number of shoots per callus was the lowest among the treatments, with 1.5 ± 0.83 shoots.

The best callus induction and shoot regeneration occurred with MS+2.0BAP+0.1NAA, resulting in the highest callus formation frequency (90.2%) and the most shoots per callus ($5.3 \pm$

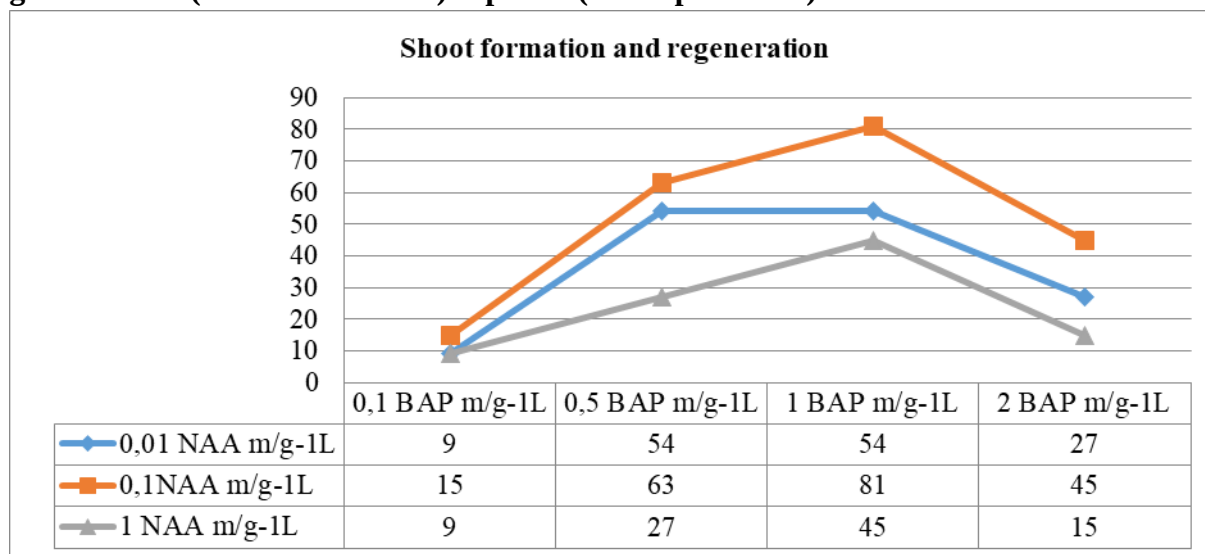
0.87). This combination proved most effective for promoting both high callus induction and shoot proliferation.

The MS+1.0BAP+0.1NAA and MS+2.0BAP+0.01NAA treatments also showed good results, with relatively high callus formation and moderate shoot production.

The study indicates that a balanced combination of BAP and NAA is essential for optimizing callus induction and shoot regeneration in *Punica granatum* L. ('Mollar de Elche'), with higher concentrations of BAP (2.0 mg/L) and moderate levels of NAA (0.1 mg/L) yielding the most successful results for clonal propagation.

Diagram 1.

Effect of cytokinin concentrations on shoot development in seedless *Punica granatum* L. ('Mollar de Elche') explants (in 4 replications)



The diagram shows the effect of different cytokinin concentrations (BAP) in combination with various levels of auxin (NAA) on shoot development in explants of seedless pomegranate (*Punica granatum* L. cv. 'Mollar de Elche'). The number of shoots developed per explant is presented for four concentrations of BAP (0.1, 0.5, 1.0, and 2.0 mg/L) combined with three different levels of NAA (0.01, 0.1, and 1.0 mg/L).

At 0.01 mg/L NAA:

- 0.1 BAP mg/L: Only 9 shoots per explant were observed, indicating a relatively low shoot production.

- 0.5 BAP mg/L: A significant increase in shoot number to 54 shoots per explant, suggesting that moderate levels of BAP can promote shoot development at this low concentration of NAA.

- 1.0 BAP mg/L: The shoot number remained constant at 54, showing that this concentration of BAP is optimal for shoot formation when combined with 0.01 mg/L NAA.

- 2.0 BAP mg/L: A decrease in shoot development to 27 shoots per explant, indicating that higher concentrations of BAP might inhibit shoot proliferation at this low auxin level.

At 0.1 mg/L NAA:

- 0.1 BAP mg/L: The shoot production increased to 15 shoots per explant, though still relatively low compared to higher concentrations of BAP.

- 0.5 BAP mg/L: A sharp increase to 63 shoots per explant, indicating a strong positive effect of 0.5 mg/L BAP on shoot development at this moderate level of NAA.

- 1.0 BAP mg/L: The shoot number increased further to 81 shoots per explant, suggesting that this concentration of both BAP and NAA is highly effective for shoot regeneration.

- 2.0 BAP mg/L: The shoot number decreased to 45 per explant, indicating that while higher BAP levels initially increase shoot development, they may begin to reach a threshold where the growth is less favorable.

At 1.0 mg/L NAA:

- 0.1 BAP mg/L: Only 9 shoots per explant were observed, showing that low levels of BAP are ineffective at this higher auxin concentration.

- 0.5 BAP mg/L: The number of shoots decreased to 27, suggesting that while moderate BAP levels can support shoot development, they are less effective when combined with higher concentrations of NAA.

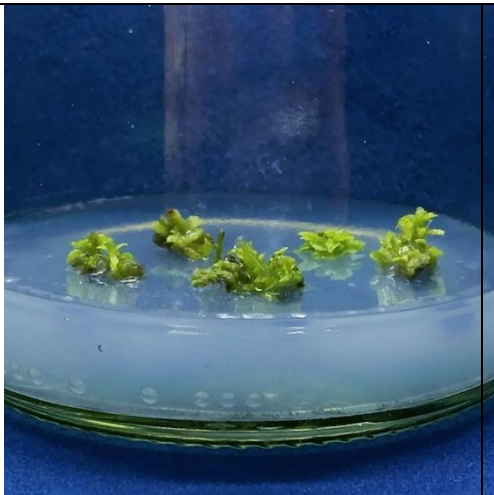
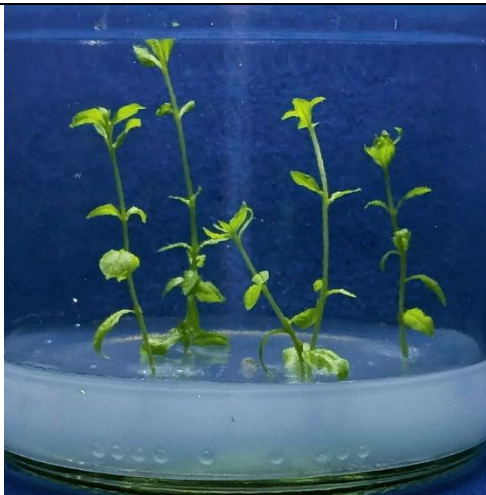
- 1.0 BAP mg/L: A noticeable increase to 45 shoots per explant, which shows that the combination of 1.0 BAP and 1.0 NAA is favorable for shoot proliferation, though not as optimal as the 1.0 BAP and 0.1 NAA combination.

- 2.0 BAP mg/L: The number of shoots decreased to 15, suggesting that high concentrations of BAP may be detrimental to shoot development when NAA is at a high concentration.

Optimal Cytokinin Concentration: The highest number of shoots per explant was observed at 1.0 BAP with 0.1 NAA, producing 81 shoots per explant, indicating that moderate levels of both BAP and NAA (particularly 1.0 mg/L of BAP with 0.1 mg/L NAA) provide the most effective combination for shoot proliferation.

Effect of High BAP: Higher concentrations of BAP (2.0 mg/L) generally led to decreased shoot development, particularly at higher concentrations of NAA (1.0 mg/L), suggesting an inhibitory effect at higher cytokinin concentrations.

A balanced combination of 1.0 mg/L BAP with 0.1 mg/L NAA appears to be the most effective for promoting optimal shoot development in seedless pomegranate explants.

	
Callus formation in MS medium supplemented with activated charcoal and 2 m/g-1L BAP, 0.1 m/g-1L NAA	Shoots developing on MS medium containing activated charcoal and 1 m/g-1L BAP, 0.1 m/g-1L NAA

This study aimed to evaluate the effect of different concentrations of auxin (NAA) and cytokinin (BAP) on callus induction and shoot development in seedless *Punica granatum* L. ('Mollar de Elche'). The results, as summarized in the tables and diagram, provide important insights into how different combinations of plant growth regulators (PGRs) influence both callus formation and shoot proliferation.

The first part of the study focused on optimizing callus induction in meristem segments. The results showed that the addition of NAA and BAP significantly affected callus formation, with varying degrees of success based on their concentrations.

No Callus Formation (0%): The absence of callus formation in treatments like MS+0.1BAP+0.01NAA and MS+0.1BAP+1NAA confirms that low concentrations of BAP (0.1 mg/L) combined with NAA, whether low or high, are ineffective in inducing callus growth. This suggests that both low auxin and cytokinin levels are insufficient to trigger the necessary cellular responses for callus formation. This could be due to the lack of stimulation for cell division and growth, which is required to initiate callus tissue.

Low to Moderate Callus Formation: The MS+1.0BAP+0.01NAA and MS+2.0BAP+0.01NAA treatments showed increasing success in callus induction, with 40.1% and 60.5% callus formation, respectively. The results indicate that moderate concentrations of BAP (1.0–2.0 mg/L) in combination with low NAA levels (0.01 mg/L) are sufficient to stimulate moderate callus formation. Calli produced under these conditions exhibited light green, compact structures, which are desirable for further proliferation and shoot development.

Higher Callus Formation and Improved Shoot Production: The MS+1.0BAP+0.1NAA and MS+2.0BAP+0.1NAA treatments provided the most favorable results. The highest callus formation (90.2%) and the highest number of shoots per callus (5.3 ± 0.87) were observed with MS+2.0BAP+0.1NAA. This combination proved most effective for both callus induction and shoot proliferation, confirming the importance of higher BAP concentrations (2.0 mg/L) and moderate NAA levels (0.1 mg/L) in promoting cellular division and shoot formation. The dark green, amorphous callus morphology indicates robust growth and a high degree of cell differentiation, ideal for micropropagation.

Moderate Callus Formation with Less Shoot Production: Interestingly, the combination of MS+1.0BAP+1NAA led to a reduced callus formation frequency (30.1%) and fewer shoots per callus (1.7 ± 0.47), despite a higher concentration of NAA. This suggests that higher concentrations of NAA (1.0 mg/L) could suppress shoot development, possibly by inhibiting cell differentiation or promoting excessive callus growth at the expense of shoot regeneration. These results point to the need for a balanced approach when using both auxins and cytokinins to achieve optimal results.

The second part of the study, as depicted in Diagram 1, explored the effects of cytokinin concentration (BAP) on shoot development at various NAA concentrations. The results from this diagram offer a more detailed view of the relationship between cytokinin levels and shoot regeneration.

At 0.01 mg/L NAA: The results demonstrate that low levels of BAP (0.1 mg/L) produced very few shoots (9 per explant), while increasing the concentration of BAP (0.5 and 1.0 mg/L) significantly improved shoot number (54 per explant). However, at 2.0 mg/L BAP, the shoot production dropped to 27 per explant. This suggests that while BAP is essential for promoting shoot development, very high concentrations of BAP may have an inhibitory effect on shoot growth, possibly by promoting callus overproduction or causing an imbalance in the hormonal environment. This is consistent with studies showing that high cytokinin levels can inhibit shoot elongation and favor callus formation instead (Ganguly et al., 2014).

At 0.1 mg/L NAA: The number of shoots increased substantially at 0.5 mg/L BAP (63 shoots), and peaked at 1.0 mg/L BAP (81 shoots), indicating that this combination is highly effective for shoot regeneration. However, at 2.0 mg/L BAP, there was a reduction in shoot

production to 45 per explant, once again pointing to the potential inhibitory effect of higher BAP concentrations.

At 1.0 mg/L NAA: The results at this higher auxin concentration show that low BAP concentrations (0.1 and 0.5 mg/L) were ineffective for promoting shoot development, with only 9 and 27 shoots per explant, respectively. However, at 1.0 mg/L BAP, the shoot production increased significantly to 45 shoots per explant, suggesting that this combination of cytokinin and auxin supports moderate shoot regeneration. Again, the 2.0 mg/L BAP resulted in a decrease in shoot production, confirming the inhibitory effects of high cytokinin levels.

Based on the results, the most effective combination for both callus induction and shoot proliferation in seedless pomegranate (*Punica granatum* L. cv. ‘Mollar de Elche’) is MS medium with 2.0 mg/L BAP and 0.1 mg/L NAA. This combination led to the highest callus formation frequency (90.2%) and the highest number of shoots per callus (5.3 ± 0.87). This outcome emphasizes the importance of using moderate auxin and higher cytokinin concentrations to achieve optimal in vitro regeneration for clonal propagation.

Low levels of BAP (0.1 mg/L) combined with any concentration of NAA failed to induce callus formation or shoot development.

Moderate BAP concentrations (1.0–2.0 mg/L) in combination with 0.1 mg/L NAA were the most effective for both callus induction and shoot proliferation.

The highest BAP concentration (2.0 mg/L) had a negative effect when paired with higher NAA concentrations (1.0 mg/L), underscoring the importance of fine-tuning the auxin-cytokinin ratio.

CONCLUSION

This study investigated the effect of phytohormones on callus induction and shoot regeneration in seedless *Punica granatum* L. ('Mollar de Elche'), aiming to develop an efficient in vitro propagation protocol. The results revealed that a combination of the cytokinin BAP and the auxin NAA significantly influenced callus formation and shoot development. The highest callus formation (90.2%) and shoot regeneration (5.3 ± 0.87 shoots per callus) were achieved with 2.0 mg/L BAP and 0.1 mg/L NAA. Lower BAP concentrations, particularly 0.1 mg/L, were less effective in both callus induction and shoot production.

Shoot development was optimized at 1.0 mg/L BAP combined with 0.1 mg/L NAA, yielding the highest number of shoots per explant (81). Higher concentrations of BAP (2.0 mg/L) negatively impacted shoot proliferation, especially at elevated NAA levels (1.0 mg/L). Overall, this study highlights the importance of a balanced combination of BAP and NAA for efficient clonal propagation of 'Mollar de Elche' pomegranate, contributing to its large-scale production for commercial cultivation.

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