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Winter Forcing of Apple (Malus domestica Borkh.) Cuttings for Bud Induction to Enhance Explant Availability in Tissue Culture Applications

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ABSTRACT

This study aimed to evaluate the effects of PGRs, specifically benzyladenine (BA) and gibberellic acid (GA_3), on winter forcing of apple buds, bud break characteristics, and subsequent tissue culture performance of six apple (Malus domestica Borkh.) cultivars. One-year-old semi-woody cuttings of Golden Delicious, Red Delicious, Barwary, Anna, Dwarf Apple and Granny Smith were subjected to cold pre-treatments (4°C) with BA and GA_3 at four combinations: 0:0, 0:10, 10:0, and 10:10 mg.l-1. Results showed that all the treatments significantly reduced the days to bud break, with the 10:10 mg.l-1combination producing the fastest response (22 days), compared to 28.67 days in control. Bud break percentage and bud vigor were also significantly enhanced, particularly under the combined BA plus GA_3 treatment, reaching up to 100% bud break in Red Delicious and Anna. Bud vigor improved from weak (control) to strong under the optimal treatment in most cultivars. Sustainability of flushed buds on MS medium was highest under the combined treatment, with Red Delicious, Barwary and Granny Smith showing 65–70% viability, while Dwarf Apple remained poorly responsive. To improve explant survival, antioxidants (citric and ascorbic acid) and activated charcoal (1.5 g.l-1) were tested. Activated charcoal significantly enhanced survival rates, reaching 100% in Granny Smith and 95% in Barwary, compared to 3.3% in the untreated control. Cultivar differences were evident throughout the study. In conclusion, combining BA and GA_3 with activated charcoal provides an effective strategy for year-round micropropagation of apple, with cultivar-specific responses warranting tailored protocols.

***Keywords*:** Apple (Malus domestica), Bud break, Plant growth regulators (BA and GA_3), Tissue culture initiation and Activated charcoal.

INTRODUCTION

Apple, (*Malus domestica* Borkh.), is a fruit tree of the rose family (*Rosaceae*), one of the most widely cultivated tree fruits. Apples are mainly grown for sale as fresh fruit, though apples are also used commercially for vinegar, juice, jelly, applesauce, and apple butter and are canned as pie stock [1]. The planting and improvement of apple varieties depend mainly on the effective collection, preservation, and utilization of plant materials [2]. Plant Material Collection for apples encompasses diverse techniques aimed at conserving genetic diversity, supporting breeding programs, and ensuring sustainable production systems and tissue culture is the beat means to achieve this goal [3].

Kurdistan Region of Iraq is very famous for apple growing because of the suitable soil, environment and most delicious varieties. The problem facing apple production in this area is that most of the apple varieties are degraded and being susceptible to diseases and insects. Finding a way to renew these varieties will definitely serve as a lifeline rope to enhance the production of this most economical fruit tree in Kurdistan Region of Iraq. Propagation of woody plants by conventional methods necessarily limits the rate of output and makes the end product expensive. Tissue culture can overcome this problem since it has been reported that may acquire higher rooting capability after continuously subculturing *in vitro* [4]. Plant tissue culture techniques provide a fast and dependable method for the production of a large quantity of uniform plantlets in a short time throughout the year [5] The improvement of apple cultivation is dependent on the supply of healthy and high-quality plant material of rootstocks and cultivars. However, in order to

obtain cultivars homogeneous, reduced in growth and with a high and regular fruit production the use of cloned rootstocks is highly demanded [6].

Forcing dormant cuttings under controlled conditions to induce early bud break is a promising strategy to avoid seasonal limitations. This approach enables the timely collection of suitable explants, thereby improving the continuity of tissue culture programs [7].

Artificial dormancy breaking or "forcing" of winter cuttings is a controlled pre conditioning process applied to cuttings of dormant apple buds for artificially breaking of dormancy and promoting early bud flushing. It has been investigated as a strategy to overcome seasonal limitations. The use of plant growth regulators such as BA and GA_3 has been reported to motivate bud break and growth under chilling and post-chilling conditions. Their application in dormant cuttings may improve bud flushing, leading to greater availability of suitable explants for tissue culture [8].

This study aimed to examine the effect of BA and GA_3 pretreatments during cold storage on the bud break performance of dormant apple cuttings from six local cultivars. The objective was to determine optimal treatments for trustworthy explants production under off-season conditions in the Kurdistan Region of Iraq.

Materials and Methods

One-year-old, semi-woody dormant shoots were collected in mid-winter from mature apple trees in several villages around Duhok (Latitude: ~ 37.11° N and Longitude: ~ 43.10° E) including; Bidehe, Mayi, Derishke, Ashawa, and Qadish, Kurdistan Region, Iraq. The cultivars used in the study were: Golden Delicious, Red Delicious, Barwary, Anna, Dwarf Apple and Granny Smith. Shoots were trimmed to 25 cm length, each containing 3–5 buds. All cuttings were surface-cleaned and standardized before treatments.

Four treatment combinations of gibberellic acid (GA_3) and benzyladenine (BA) were prepared and applied by soaking the cuttings in solution for 5 weeks at 4°C in a refrigerator:

1. Control (0.0 mg.l^{-1} GA_3 + 0.0 mg.l^{-1}BA)
2. GA_3 (10.0 mg.$l^{-1}$$GA_3$ + 0.0 mg.l^{-1}BA)
3. BA (0.0 mg.$l^{-1}$$GA_3$ + 10.0 mg.l^{-1}BA)
4. GA_3 + BA (10.0 mg.$l^{-1}$$GA_3$ + 10.0 mg.l^{-1}BA)

After pretreatment, cuttings were placed vertically in containers with the solution and maintained in a growth chamber (Figure 1) with the following conditions: temperature: 25 ± 2°C, light regime: 16 hours light / 8 hours dark and relative humidity: 70–80%

Figure (1): Forcing treatment for the six tested apple cultivars with different combinations between GA3 and BA solutions.

After bud flushing, explants were taken from the tested cultivars and submitted to a disinfestations process by washing under tap water for 40 minutes, followed by sterilization with sodium hypochlorite (NaOCl) 2.5% for 10 minutes. Then they were thoroughly washed by sterilized distilled water three times each for 5 minutes under laminar air-flow cabinet conditions.

Before inoculation, and for to reduce phenolic compounds exudation, three treatments were tested by soaking the explants in 100 $mg.l^{-1}$ citric acid and 100 $mg.l^{-1}$ ascorbic acid solution for 30 minutes as anti-oxidant agents. Furthermore, another treatment was tested by adding 1.5 $g.l^{-1}$ activated charcoal to MS medium as adsorbent agent. Then the explants were inoculated on MS medium by culturing three explants in each culture vessel (Each treatment was replicated five times). The cultures were kept in the incubation room at a temperature of 25 ± 2°C, light regime: 16 hours light/ 8 hours dark, and relative humidity 70–80% for four weeks and then the data were recorded. Observations were recorded weekly for 4 weeks: days to bud break, percentage of bud break per cutting, bud vigor (visual score: weak, moderate, strong) and suitability of flushed buds for tissue culture initiation (tested on MS medium).

The experiment followed a factorial completely randomized design (CRD) with ten cuttings for each treatment. Data were analyzed by ANOVA, and means were compared using Duncan's Multiple Range Test ($p \leq 0.05$) [9].

Results and Discussions

During the period of cuttings incubation in the growth regulators solutions, the cuttings were exuding phenolic compounds in different amounts. The best cuttings were Red Delicious, Golden Delicious, Barwary, Anna, Dwarf apple and finally Granny Smith respectively. Table (1) declares that the pretreatments with BA and GA_3 significantly affected the timing of flushing of buds. The control treatment scored 28.67 days on average. All tested BA and GA_3 treatments reduced the number of days needed for bud break reaching 22–23 days. This shows that both GA_3 and BA; either alone or together, have a positive influence on promoting earlier bud breaking. No significant difference was observed among the three BA and GA_3 treatments. A single use of either BA or GA_3 is approximately as effective as combining them, and this suggest flexibility in selection. On the other hand, Golden Delicious performed the best overall, with the least mean bud break time (22.0 days). Other cultivars including Red Delicious, Barwary, Anna, Dwarf Apple, and Granny Smith required more days (23.5–25.5 days), with no significant differences among them. Barwary, Anna, and Granny Smith had slower bud break under control conditions but performed well with the treatments [10]. The reduction in bud break duration under GA_3 and BA treatments can be attributed to their well-established roles in dormancy release and cell division regulation. GA_3 is known to stimulate hydrolytic enzymes that mobilize stored reserves and weaken bud scales, thereby accelerating bud emergence under chilling conditions. Genetic differences between cultivars affect their internal and natural dormancy and responsiveness to PGRs. Also, it can be seen that Golden Delicious is more responsive or less dormant under chilling conditions [11].

Table (1): Days required for bud break of apple cuttings after treating with different BA and GA_3 combinations

Apple Cultivars	Days Required for Bud Break				Cultivar Means
	BA /GA_3 0: 0 $mg.l^{-1}$	BA /GA_3 0: 10$mg.l^{-1}$	BA /GA_3 10: 0 $mg.l^{-1}$	BA /GA_3 10: 10 $mg.l^{-1}$	
Golden Delicious	24 b	22 a	22 a	20 a	22.0 a
Red Delicious	28 d	22 a	23 ab	24 b	23.5 b
Barwary	31 e	23 ab	23 ab	21 a	24.5 b
Anna	30 e	23 ab	23 ab	22 a	24.5 b
Dwarf Apple	29 d	24 b	24 b	25bc	25.5 b

Granny Smith	30 e	21 a	22 a	23 ab	24.0 b
BA/ GA_3 Means	28.67 b	22.5 a	22.83 a	22.5 a	

Table (2) shows how various BA and GA_3 combinations affect the percentage of bud break per cutting in the six tested apple cultivars. The higher percentage means more buds successfully ends dormancy, a good outcome for propagation and tissue culture purposes. The control treatment recorded the lowest bud break (20%) on average. All pre-treatments significantly enhanced bud break; This declares that both BA and GA_3 have strong effects individually, but their combination is clearly more effective. The 10:10 BA:GA_3 treatment is the most effective for maximizing bud break across the tested cultivars. Red Delicious and Anna showed the highest response reaching 100% under BA+GA_3, showing high responsiveness. Golden Delicious and Granny Smith also showed strong responses reaching 80–90% with the PGRs. Dwarf Apple had the lowest average bud break with only 45%, even with PGRs treatments. Red Delicious is the most responsive cultivar overall (Figure 2), while Dwarf Apple is the least responsive under the tested conditions. The combination of 10:10 BA:GA_3 produced the highest bud break percentage across the all-tested cultivars. Anna and Granny Smith, were dramatically improved by PGRs treatment (from 10–20% in control to 90–100% in treated ones). In Barwary and Dwarf Apple, the response to PGRs treatments was more moderate, declaring a need for further optimization treatments. The treatments significantly enhance bud break percentage in winter-forced apple cuttings. The combination of BA + GA_3 (10 $mg.l^{-1}$ each) is the most effective and synergistic across all cultivars. Red Delicious and Anna are highly responsive to PGRs forcing and are suitable for tissue culture initiation or early season propagation. Dwarf Apple may require alternative approaches or higher doses for effective bud break in future. The dramatic improvement in Anna and Granny Smith from 10–20% in the control to 90–100% under BA + GA_3 demonstrates that these cultivars exhibit strong hormonal responsiveness once their dormancy barrier is artificially reduced. This behavior aligns with findings that certain cultivars with intermediate dormancy benefit markedly from exogenous cytokinin–gibberellin combinations, which can compensate for insufficient natural chilling in warmer climates [8]. Therefore, the hormonal pretreatments used in this study represent an effective tool for off-season explant production, especially in regions like the Kurdistan Region of Iraq, where winters may not consistently meet chilling requirements.

Table (2): Percentage (%) of bud break per cutting of apple cultivars after treating with different BA and GA_3 combinations

Apple Cultivars	Bud Break (%)				Cultivar Means
	BA /GA_3 0: 0 $mg.l^{-1}$	BA /GA_3 0: 10$mg.l^{-1}$	BA /GA_3 10: 0 $mg.l^{-1}$	BA /GA_3 10: 10 $mg.l^{-1}$	
Golden Delicious	20	70	80	80	62.5
Red Delicious	20	80	80	100	70.0
Barwary	30	50	60	80	55.0
Anna	10	50	90	100	62.5
Dwarf Apple	20	50	50	60	45.0
Granny Smith	20	60	90	90	65.0
BA/ GA_3 Means	20.0	60.0	68.33	85.0	

Figure (2): The best flushed buds from Red Delicious apple cuttings treated with 10 mg.l^{-1} BA+ 10 mg.l^{-1} GA3.

Table (3) presents a qualitative assessment of bud vigor including the strength and health of emerging buds, in six apple cultivars after treatment with different combinations of BA and GA_3. The control treatment (0: 0 mg.l^{-1}) showed a weak bud vigor across all cultivars. GA_3 alone (0:10 mg.l^{-1}) improved vigor slightly, especially in Red Delicious, Anna, and Granny Smith. BA alone (10:0 mg.l^{-1}) did not consistently improve vigor; results were mostly weak. The combined treatment of BA and GA_3 (10:10 mg.l^{-1}) clearly resulted in the strongest bud vigor, especially in Red Delicious, Barwary, Anna, and Granny Smith (Strong), while Golden Delicious and Dwarf Apple reached Moderate. Thus, the combination of BA and GA_3 (10:10 mg.l^{-1}) provides a significant effect, by enhancing bud vigor (Kaur *et al.*, 2021). Red Delicious, Barwary, Anna, and Granny Smith responded very well to the combined treatment of the PGRs. Golden Delicious and Dwarf Apple showed less improvement, reaching only moderate vigor even under optimal conditions. So, some cultivars have inherently lower vigor potential or require different PGRs or cultural conditions for full expression situation. Bud vigor improves notably when BA and GA_3 are applied together (10:10 mg.l^{-1}), showing higher benefits. BA or GA_3 alone are may be insufficient to significantly improve bud vigor. Red Delicious, Barwary, Anna, and Granny Smith showed the strongest response and are ideal candidates for tissue culture or vegetative propagation based on vigor potential. Golden Delicious and Dwarf Apple may need modified protocols for improving vigor, such as adjusted concentrations, extended chilling, or supplementary treatments.

Hormonal regulation of bud vigor is strongly influenced by the balance among GA, cytokinin, and ABA during bud activation. Combined BA + GA_3 applications efficiently shift the ABA/GA_3 balance toward growth promotion, often accompanied by enhanced expression of genes involved in carbohydrate mobilization and early bud development (Li *et al.*, 2023). This mechanism likely underlies the strong vigor responses in certain cultivars [3].

Table (3): Bud vigor of apple cuttings after treating with different BA and GA_3 combinations

Apple Cultivars	Bud Vigor			
	BA /GA_3 0: 0 mg.l^{-1}	BA /GA_3 0: 10 mg.l^{-1}	BA /GA_3 10: 0 mg.l^{-1}	BA /GA_3 10: 10 mg.l^{-1}
Golden Delicious	Weak	Weak	Weak	Moderate
Red Delicious	Weak	Moderate	Weak	Strong
Barwary	Weak	Weak	Moderate	Strong
Anna	Weak	Moderate	Weak	Strong
Dwarf Apple	Weak	Weak	Weak	Moderate
Granny Smith	Weak	Moderate	Weak	Strong

Table (4) assesses the percentage of buds that flushed (from forced cuttings) that remained viable and sustained growth on MS medium, a key indicator of success in tissue culture initiation. The control treatment yielded 0% sustainability, confirming the need for PGR treatment for tissue culture readiness (Ma and Chen, 2021). GA_3 and BA individually enhanced bud sustainability, but BA was more effective than GA_3 alone. The combination of both PGRs (10:10 mg.l^{-1}) significantly improved sustainability; achieving more than three times the success rate compared to single PGR applications. The combined BA and GA_3 treatment is critical for obtaining healthy, viable buds for successful *in vitro* culture initiation. Red Delicious (Figure 3) had the highest overall performance in sustainability and showed strong responses to BA and GA_3 (Mustafa and Ahmad, 2022). Dwarf Apple was the least responsive cultivar, even under optimal treatment giving only 10% at 10:10 mg.l^{-1}. These results indicate that cultivar genotype plays a crucial role in determining the success of bud conversion to tissue culture explants. Barwary, Red Delicious, and Granny Smith are the best selections under these conditions. It can be concluded that PGRs treatment, especially the combined BA and GA_3 at 10:10 mg.l^{-1}, is very necessary to improve the sustainability of flushed buds on MS medium. BA alone is more effective than GA_3 alone, but the combination is more effective, greatly enhancing explant viability. Red Delicious, Barwary, and Granny Smith are the most promising cultivars for tissue culture initiation based on bud sustainability. Dwarf Apple is poorly responsive and may need alternative protocols (e.g., different hormone concentrations or explant sources).

The dramatic improvement in bud sustainability under BA + GA_3 treatments over threefold relative to single-hormone applications reinforces the principle that hormonal synergy is crucial for both dormancy release and subsequent tissue culture success. The BA/GA_3 combination likely enhances the ABA/GA3 balance, increases antioxidant enzyme activity, and promotes cell viability during the initial culture stage [3].

Table (4): Sustainability of flushed buds for tissue culture initiation tested on MS medium of apple cultivars after treating with different BA and GA_3 combinations

Apple Cultivars	Sustainability of flushed buds for tissue culture (%)				Cultivar Means
	BA /GA_3 0: 0 mg.l^{-1}	BA /GA_3 0: 10mg.l^{-1}	BA /GA_3 10: 0 mg.l^{-1}	BA /GA_3 10: 10 mg.l^{-1}	
Golden Delicious	0.0	15	20	50	21.25
Red Delicious	0.0	20	35	70	31.25
Barwary	0.0	20	40	70	32.5
Anna	0.0	15	35	50	25.0
Dwarf Apple	0.0	0.0	10	10	5.0
Granny Smith	0.0	20	30	65	28.75
BA/ GA_3 Means	0.0	15.0	28.33	52.5	

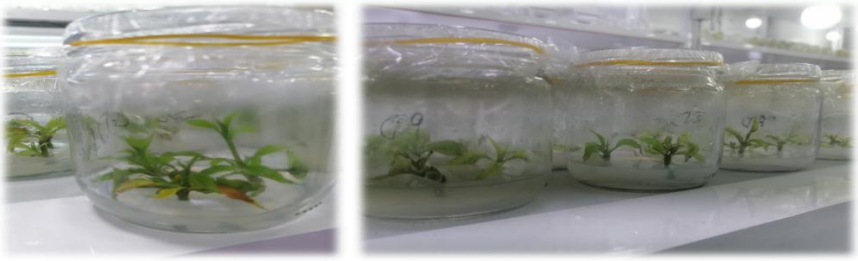

Figure (3): Red Delicious explants grown on MS medium enriched with 1 mg.l^{-1} BA after four weeks in culture under incubation room conditions: temperature: 25 ± 2°C, Light regime: 16 hours light/ 8 hours dark and Relative humidity: 70–80%.

Table (5) shows how anti-oxidant agents and adsorbent treatments affect the survival rate of apple explants *in vitro* over four weeks on MS medium. Explants usually suffer from browning due to oxidation of phenolic compounds, so treatments like citric acid, ascorbic acid, and activated charcoal are used to alleviate this issue. The control treatment had extremely low survival (an average 3%), confirming the severity of oxidative damage in untreated explants group. The citric plus ascorbic acid treatment greatly improved survival (12× better than control), demonstrating the effectiveness of antioxidant pre-treatments. Activated charcoal recorded the best results, about doubling survival rates as compared to antioxidants alone (Figure 4). Activated charcoal is highly positive at preventing phenolic browning and supporting explant survival during early culture stages. Granny Smith and Barwary recorded the highest survival rates overall and responded exceptionally to activated charcoal. Anna and Dwarf Apple showed the lowest survival, even with the treatments, indicating cultivar sensitivity or high phenolic content. It is very clear that cultivar genotype differences play a great role in survival. Barwary and Granny Smith are excellent choices for *in vitro* propagation. Anna and Dwarf Apple require further optimization work. Phenolic oxidation is a major limiting factor for apple explant survival *in vitro*. Activated charcoal (1.5 g.l^{-1}) is the most positive treatment, offering a 100% survival in Granny Smith and >90% in Barwary. Citric plus Ascorbic acids improve survival but are less effective than activated charcoal [12]. Barwary and Granny Smith are highly suitable for tissue culture, while Anna and Dwarf Apple are more challenging and may need specialized protocols in future.

Activated charcoal, however, is known to be one of the most powerful tools for controlling phenolic oxidation because it adsorbs toxic compounds directly from the medium while simultaneously promoting nutrient balance and improving hormonal stability (He *et al*., 2022). The extremely high survival rates recorded in Granny Smith (100%) and Barwary (>90%) confirm that charcoal effectively protects sensitive explants from oxidative injury, consistent with previous reports describing its role in enhancing shoot survival and reducing medium discoloration in apple and pear explants [2].

Overall, the findings clearly demonstrate that controlling phenolic oxidation is essential for apple micropropagation and that activated charcoal remains the most effective strategy for ensuring explant survival across diverse cultivars.

Table (5): Survival rates of apple cultivar explants grown on MS medium after treating with citric acid, ascorbic acid and activated charcoal after four weeks in culture

Apple Cultivars	Survival Rate (%)			
	Control	Citric acid+ ascorbic acid (100+ 100 mg.l^{-1})	Activated Charcoal (1.5 g.l^{-1})	Cultivar Means
Golden Delicious	5	40	70	38.33
Red Delicious	5	40	75	40.00

Barwary	5	55	95	51.67
Anna	0.0	25	50	25.00
Dwarf Apple	0.0	20	45	21.67
Granny Smith	5	60	100	55.00
Treatment Means	3.33	40.00	72.5	

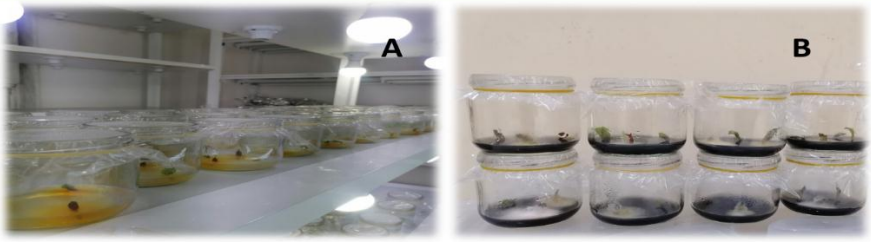

Figure (4): Culture initiation stage of apple explants grown on MS medium for testing buds' sustainability in tissue culture:
A. Phenolic compounds exudation cultures
B. Getting rid-off phenolic compounds by adding 1.5 $g.l^{-1}$ activated charcoal to the culture medium.

Conclusion

The pretreatments of BA and GA_3 pretreatments accelerated bud break in winter-forced apple cuttings, reducing the period from 29 days (control) to about 22 days, with Golden Delicious responding quickest. The combined BA + GA_3 (10:10 mg L^{-1}) treatment gave the highest bud break percentage, vigor, and in-vitro sustainability, showing a clear synergistic effect. In tissue culture, Red Delicious, Barwary, and Granny Smith exhibited the best viability, while activated charcoal (1.5 g L^{-1}) greatly improved survival by preventing phenolic browning. Cultivars varied in responsiveness: Barwary, Red Delicious, and Granny Smith performed best, whereas Anna and Dwarf Apple were least responsive and may need protocol adjustments. Overall, using BA + GA_3 at 10 mg L^{-1} each, selecting the top-performing cultivars as explant sources, and adding activated charcoal are recommended for optimal bud break and early culture success.

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الإجبار الشتوي لعقل التفاح Malus domestica Borkhلتحفيز البراعم وتوفير الأجزاء النباتية لتطبيقات الزراعة النسيجية

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قسم البستنة، كلية علوم الهندسة الزراعية، جامعة دهوك، دهوك، العراق.

الخلاصة
هدفت هذه الدراسة إلى تقييم تأثير منظمات النمو النباتية، وبشكل خاص البنزيل أدنين (***BA)*** وحامض الجبرياليك (***GA_3)*** على الإجبار الشتوي للبراعم في التفاح وخصائص تفتح البراعم، والأداء اللاحق في الزراعة النسيجية لستة أصناف من التفاح (***Malus domestica Borkh.).*** وقد تم استخدام عقل شبه خشبية بعمر سنة واحدة للأصناف: غولدن ديليشس، ريد ديليشس، برواري، آنا، التفاح القزم، وغراني سميث، وتم تعريض هذه العقل إلى معاملات تمهيدية بالبرودة (4°م) باستخدام ***BA*** و***GA_3*** بأربع تراكيز مركبة: 0:0، 0:10، 10:0، و10:10 ملغم.لتر$^{-1}$. أظهرت النتائج أن جميع المعاملات خفضت بشكل معنوي عدد الأيام اللازمة لتفتح البراعم، حيث سجل التركيب 10:10 ملغم.لتر$^{-1}$ أسرع استجابة (22 يوماً) مقارنة بـ 28.67 يوماً في معاملة المقارنة. كما تحسن كل من نسبة تفتح البراعم وقوة البراعم بشكل معنوي، خصوصاً تحت المعاملة المركبة ***BA*** مع ***GA_3***، حيث وصلت نسبة التفتح إلى 100% في صنفي ريد ديليشس وآنا. كما تحسنت قوة البراعم من ضعيفة (في معاملة المقارنة) إلى قوية تحت المعاملة المثلى في معظم الأصناف. وكانت استدامة البراعم المتفتحة على وسط ***MS*** الأعلى تحت المعاملة المركبة، حيث أظهرت أصناف ريد ديليشس وبرواري وغراني سميث نسبة بقاء تراوحت بين 65–70%، في حين ظل صنف التفاح القزم ضعيف الاستجابة. ولتحسين بقاء الأجزاء النباتية، تم اختبار مضادات الأكسدة (حامض الستريك وحامض الأسكوربيك) مع الفحم النشط (1.5 غم.لتر$^{-1}$). وقد حسّن الفحم النشط من نسب البقاء بشكل كبير، حيث وصلت إلى 100% في غراني سميث و95% في برواري، مقارنة بـ 3.3% في المعاملة غير المعالجة. وقد ظهرت فروقات واضحة بين الأصناف طوال فترة الدراسة. واستُنتج في الختام أن الجمع بين ***BA*** و***GA_3*** مع الفحم النشط يُعد إستراتيجية فعّالة للإكثار الدقيق على مدار السنة للتفاح، مع التأكيد على ضرورة تطوير برامج اكثار دقيق مخصصة لكل صنف.

الكلمات المفتاحية: التفاح، تفتح البراعم، منظمات النمو النباتية، زراعة الأنسجة النباتية، مرحلة النشوء، الفحم النشط.