# Effect of Culture Media on Micropropagation of *Pyracantha Fortuneana* Plant by Tissue Culture

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## ABSTRACT

The experimental trial was conducted in the Plant Tissue Culture Laboratory, throughout two years (2022 – 2023). It was intended to find out the well-defined protocol easily for *in vitro* propagation of *Pyracantha fortuneana* Roem. In this respect, buds of the plant were surface sterilized with 1.5 % sodium hypochlorite for 20 min. Seeds were sterilized with a mixture of 1.5 % sodium hypochlorite for 30 min. For establishment stage, MS medium at full strength without activated charcoal gave the highest values of shoot length and number of leaves for buds. While, MS full-strength medium with 0.5 mg/l IBA was the best medium for seeds. For multiplication stage, BA at 1.0 mg/l and 1.0 mg/l Kin formed the highest number of shoots for buds and seeds. For *in vitro* rooting, the highest number of roots/cluster was found at 1.0 mg/l IBA and 0.5 mg/l Kin for buds or 3.0 mg/l IBA and 1.0 mg/l Kin for seeds. The highest percentage of plantlets acclimatized in plastic pots filled with peatmoss alone under greenhouse conditions for Pyracantha buds or seeds.

**Keywords**: *Pyracantha*- Micropropagation- IBA- WPM- CHU.

## INTRODUCTION

*Pyracantha* is a genus of evergreen shrubs in the family Rosaceae. They are native from southwest Eastern Europe to Southeast Asia (Potter et al., 2007). The genus *Pyracantha* includes 11 species as *P.fortuneana* with small white flowers (Chari et al., 2020). The flowers are produced during late spring and early summer. Fruit red pomes, develop in late summer and mature in late autumn (Yang and Lee, 2019). Pyracantha is used in landscape purposes (Jocou and Gandullo, 2019). This species is cultivated as an ornamental garden plant, as the shrub is resistant to cold and drought (Weber , 2017). Micropropagation has been used as an important tool for overcoming the problems caused by heterogeneous seed production (Nunes et al., 2018). Due to the demand for this shrub, the slow propagation of *Pyracantha* under natural conditions, using micropropagation is a suitable method for production of this shrub.

The commercial micropropagation for woody plants is more difficult than herbaceous plants due to mortality of the plantlets at acclimatization (Sahari Moghaddam et al., 2022). The success of the mass production methods is depends on several factors, such as the genotype, media type and plant growth regulators (Baladeh and Livani, 2021). The various studies have shown that different PGR have significant roles in increasing the micropropagation efficiency of trees or shrubs (Gaidamashvili and Benelli, 2021, Zare Khafri et al., 2021 and Kucharska et al., 2020). Nonetheless, several woody plant species are successfully micropropagated (Kudělková et al., 2017).

Explants of *Pyracantha fortuneana* was treated with 1% sodium hypochlorite for 20 min to give the highest survival percentages and lowest contamination percentages. 3/4 strength of MS medium supplemented with 1 or 3mg/l BAP and 0 or 3 mg/l Kin gave the highest number of shoots during multiplication stage. MS medium supplemented with 3 mg/l IBA gave the highest rooting percentage and number of roots at rooting formation stage (Thabet et al., 2010). The best medium for establishment stage of *Pyracantha coccinea* was that containing 6.6 mM BA for shoots. The highest number of shoots during multiplication stage was obtained on medium containing 1.5 m M IBA. The highest rooting percentage was obtained on the medium with 6.9 mM 2,4-D. The best medium for rooting of shoot was MS at quarter salt-strength containing 93 mM IBA (Dong et al., 2017).

Surface sterilization of axillary *Pyracantha angustifolia* buds was performed with a 70.23% success on MS medium supplemented with 0.5 mg/l gibberellic acid ($GA_3$). Explants were then cultured on MS, WPM, and LS media enriched with different concentrations of BAP, 0.3 mg/l $GA_3$ and 0.1 mg/l IBA. The highest multiplication coefficient was obtained for the MS medium

supplemented with 2.5 mg/l BAP. The formed shoots were transferred to rooting media (MS, WPM, and LS) containing different concentrations of IBA with 0.1 mg/l BAP. MS medium supplemented with 1-1.5 mg/l IBA was the most effective in stimulating rooting. The plantlets were transferred into pots filled with perlite and peat moss in a 2:1(v/v), acclimatized to greenhouse conditions (Deltalab, et al., 2022).

The present study aimed to evaluate the micropropagation of different explants (buds and seeds), different media type such as basal MS at full, half or quarter strength, WPM, B5 and CHU media and different growth regulators such as IBA, NAA, BA, Kin and activated charcoal on the *in vitro* propagation of *Pyracantha fortuneana* plant.

## MATERIALS AND METHODS

Our experiments were carried out during the period from 2022 to 2023 in Plant Tissue Culture Laboratory at the Horticulture Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Plant materials were obtained from *Pyracantha fortuneana* Roem shrub grown in El-Zohria Botanical Garden. Explants were collected from the terminal bud and fruits with seeds. Terminal buds were taken in the form of 0.5 cm-long cuttings. The leaves of shoot were removed and cultured with 1 bud.The culture media were MS at different strengths (full, half and quarter strength), WPM, B5 and CHU.

**Explants Disinfection:**

The explants were washed carefully by soapy water for ten minutes. Next, for better washing and removal of inhibitory substances, the explants were placed under tap water for an hour. After preliminary washing, all the explants were placed in sodium hypochlorite (NaOCl) at a concentration of 0.5, 1.0 and 1.5 % (v/v) for 10, 20 and 30 min. All disinfection steps were performed under a laminar flow hood cabinet with three drops of Tween-80, which were added to increase the contact surface of the disinfectant liquid with the explants. Then, the explants were washed five times in sterile distilled water, each time for one min. To remove the parts of the explants that was damaged in the disinfection treatments, the bases of the terminal bud were cut off and the covers of fruit to culture the seeds. All explants were cultured on MS medium free hormones. After one month, survival, contamination and mortality percentages of explants were recorded.

**Establishment of *in vitro* explants:**

Each of the three disinfected buds or seeds were placed inside 350 ml capacity jars containing 50 ml medium of MS basal medium (Murashige and Skoog, 1962) medium at full strength containing 3% (w/v) sucrose and 0.7% (w/v) agar augmented for two weeks in surface sterilization.

For establishment stage, buds were cultured on different media type such as basal MS at full, half, quarter strength, WPM (McCown, and Lloyd 1981), B5 (Gamborg et al., 1968) and CHU (1942) augmented with 0.0 and 1.0 g/l Activated Charcoal (AC). Explants were incubated at 24 ± 2 °C, thereafter, at the same temperature with a 16/8 h light/dark regime with a light intensity of 2000 lux provided by white fluorescent tubes. After one month, the shoot length (cm) and number of leaves were calculated for buds. Whereas , seeds were cultured on MS medium at full strength supplemented with IBA and NAA at 0.0, 0.5, 1.0, 2.0 or 4.0 mg/l and 0.0 or 1.0 g/l Activated Charcoal (AC). Shoot length (cm), number of leaves/seed, number of roots/seed and root length (cm) were calculated for seeds. The formed shoots were used for the next experiments.

**Shoot proliferation:**

For shoot proliferation stage, the formed shoots (from buds or seeds) were cultured on MS medium supplemented with BA at 0.0, 0.5, 1.0 or 2.0 mg/l and Kin at 0.0, 1.0, 2.0, 3.0 or 4.0 mg/l. Shoots were taken in a single bud with 0.5 cm in length and 4-5 leaves. This stage was repeated three times every one month by sub-culturing on the same freshly prepared medium of each treatment. Each treatment consisted of three jars with three shoots in each jar. Data recorded for number of shoots/shoot, shoot length (cm) and number of leaves/shoot were calculated after three subcultures for shoots formed from buds.

Data recorded for number of shoots/seed, shoot length (cm) and number of leaves/seed

were calculated after three subcultures for shoots formed from seeds.

**Root formation:**

The shoots produced from all experiments were transferred to control medium supplemented with Activated Charcoal to release from different growth regulators. Shoots from buds or seeds were cultured on MS medium supplemented with different concentrations of IBA at 0.0, 1.0, 3.0 or 5.0 mg/l and Kin at 0.0, 0.5 or 1.0 mg/l were added to root formation. Shoots were taken in cluster (three shoots) with 2.5 cm in length and 2-3 leaves for each shoot. Each treatment consisted of three jars with three shoots in each jar. Data recorded for number of shoots/cluster, shoot length (cm), number of leaves/ cluster, number of roots/ cluster and root length (cm) was calculated after six weeks for clusters formed from buds or seeds.

**Acclimatization stage:**

The rooted plantlets from the rooting medium were taken out from the culture jars and the remains of the culture medium were removed from the roots. The plantlets were placed in mixtures of peat moss, perlite and sand at 1:0:0, 0:1:0, 0:0:1, 1:1:1, 1:1:0, 1:0:1 and 0:1:1 (v/v) in 10 cm plastic pots , they were irrigated with solution of 0.2 Topsin-M-70 fungicides and covered by transparent polyethylene bags then transferred to the greenhouse. Each treatment consisted of three pots, one cluster (three plantlets) in each pot. Data recorded for acclimatization percentage, number of shoots/ cluster, plantlet height (cm) and number of leaves/ plantlet was calculated after three months for plantlets formed from buds. The plantlets were enriched with nutrients containing 1 g/l of N.P.K every one week from the fifth week of transferring to greenhouse. Data recorded for acclimatization percentage, number of shoots/cluster, plantlet height (cm) and number of leaves/cluster was calculated after three months of acclimatization for plantlets formed from buds or seeds.

**Experimental design and statistical analysis:**

A factorial *in vitro* experiment was conducted in a completely randomized design with three replications. Analysis of variance was used to show significance of statistical differences between treatments using the L.S.D. at probability level (5%) **(**Snedecor and Cochran, 1989).

## RESULTS AND DISCUSSION

**Effect of sodium hypochlorite (NaOCl) and soaking periods (min) on surface sterilization of** *Pyracantha fortuneana* **buds:**

The results represented in **Table (1)** indicate that the use of sodium hypochlorite for surface sterilization of terminal buds had significant effects when 1.5 % was used as compared with the lowest concentration (0.5 and 1.0 %). The highest percentage of survival buds (56.67 %) was recorded when immersion the buds in 1.5 % NaOCl. This treatment decreased contaminated and mortality buds to 20.0 and 23.33 %, respectively.

Noteworthy, surface sterilization with NaOCl for 30 min led to 46.67 % of survival, 36.67 % contamination and 16.67 % mortality of buds. This period (30 min) gave the highest value of survival and the lowest one of contamination as compared with the other periods (10 and 20 min).

Regarding, the interactions between sodium hypochlorite and sterilization period, data indicated that the highest percentage of survival buds and the lowest percentage of contamination and mortality (70.0, 10.0 and 20.0 %, respectively) were observed when buds were immersed for 20 min in 1.5 % sodium hypochlorite. The results obtained here are in harmony with that those obtained elsewhere when clorox and soaking period were used on *Amphilophium paniculatum* (El-Shamy, 2015a).

**Effect of sodium hypochlorite (NaOCl) and soaking periods (min) on surface sterilization of *Pyracantha* seeds:**

The results recorded in **Table (2)** demonstrated also that the use of sodium hypochlorite gave good results for surface sterilization of seeds, giving the highest survived seeds at 1.5 % (56.67 %) and the lowest contamination percentage (43.33 %).

Results of soaking periods represented in **Table (2)** show that soaking periods for 30 min gave the highest survived seeds and the lowest contamination percentage during surface sterilization. Soaking period for 30 min gave 53.33 % survival and 46.67 % contamination.

The interaction between sodium hypochlorite and soaking periods was best for surface sterilization of seeds which gave an increase in the survived seeds percentage and a decrease in contaminated seeds percentage. Sodium hypochlorite at 1.5 % for 30 min gave 70.0 % survival and 30.0 % contamination.

**Table (1). Effect of different concentrations of NaOCl (%) and soaking periods (min) on survival, contamination and mortality percentages on surface sterilization of *Pyracantha fortuneane* plant buds.**

| NaOCl (%) | Soaking periods (min) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | Mean (A) | 10 | 20 | 30 | Mean (A) | 10 | 20 | 30 | Mean (A) |
| | Survival (%) | | | | Contamination (%) | | | | Mortality (%) | | | |
| 0.5 | 10.00 | 20.00 | 30.00 | 20.00 | 90.00 | 80.00 | 70.00 | 80.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.0 | 20.00 | 40.00 | 50.00 | 36.67 | 80.00 | 50.00 | 30.00 | 53.33 | 0.00 | 10.00 | 20.00 | 10.00 |
| 1.5 | 40.00 | 70.00 | 60.00 | 56.67 | 40.00 | 10.00 | 10.00 | 20.00 | 20.00 | 20.00 | 30.00 | 23.33 |
| Mean (B) | 23.33 | 43.33 | 46.67 | | 70.00 | 46.67 | 36.67 | | ns | ns | ns | |
| $LSD_{0.05}$ for | | | | | | | | | | | | |
| NaOCl (A) | | | 15.35 | | | | 16.25 | | | | 12.5 | |
| Periods (B) | | | 15.35 | | | | 16.25 | | | | NS | |
| (AxB) | | | 26.58 | | | | 28.15 | | | | 21.65 | |

**Table (2). Effect of different concentrations of NaOCl (%) and soaking periods (min) on survival and contamination percentages on surface sterilization of *Pyracantha fortuneane* seeds.**

| NaOCl (%) | Soaking periods (min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | Mean (A) | 10 | 20 | 30 | Mean (A) |
| | Survival (%) | | | | Contamination (%) | | | |
| 0.5 | 10.00 | 30.00 | 40.00 | 26.67 | 90.00 | 70.00 | 60.00 | 73.33 |
| 1.0 | 20.00 | 40.00 | 50.00 | 36.67 | 80.00 | 60.00 | 50.00 | 63.33 |
| 1.5 | 40.00 | 60.00 | 70.00 | 56.67 | 60.00 | 40.00 | 30.00 | 43.33 |
| Mean (B) | 23.33 | 43.33 | 53.33 | | 76.67 | 56.67 | 46.67 | |
| $LSD_{0.05}$ for | | | | | | | | |
| NaOCl (A) | | | 14.97 | | | | 27.33 | |
| Periods (B) | | | 14.97 | | | | 27.33 | |
| (A x B) | | | 25.93 | | | | 25.93 | |

**Effect of media type and activated charcoal (AC) on establishment stage of *Pyracantha* buds:**

Results represented in **Table (3)** and **Fig (1)** show that the highest value of shoot length (5.33 cm) and the highest number of leaves (24.50 leaves) was successfully achieved for buds cultured on MS medium at full strength. The lowest values of shoot length (2.83 cm) and the lowest number of leaves (13.33 leaves) was achieved for those cultured on CHU medium.

Results showed that the best data of Pyracantha buds was obtained from culture on MS medium without activated charcoal. The highest mean of shoot length (4.19 cm) and the highest number of leaves (19.33 leaves) was recorded from culture on medium without activated charcoal.

For the interaction between media type and activated charcoal, all buds cultured on media showed healthy signs and new leaves started to develop on them. MS medium at full strength without activated charcoal gave the highest values of shoot length and number of leaves, giving 5.50 cm and 26.00 leaves, respectively.

On the other hand, results showed that the best characters was achieved when WP medium supplemented with activated charcoal was used on *Amphilophium paniculatum* shoot (El-Shamy, 2015a).

**Table (3). Effect of media type and activated charcoal (AC) during establishment stage on mean of shoot length (cm) and number of leaves at *Pyracantha fortuneane* buds.**

| Media strength | AC (g/l) | | | | | |
|---|---|---|---|---|---|---|
| | 0.0 | 1.0 | Mean (A) | 0.0 | 1.0 | Mean (A) |
| | Shoot length (cm) | | | Number of leaves | | |
| Full MS | 5.50 | 5.17 | 5.33 | 26.00 | 23.00 | 24.50 |
| Half MS | 4.67 | 4.33 | 4.50 | 19.33 | 18.33 | 18.83 |
| Quarter MS | 3.67 | 3.33 | 3.50 | 19.00 | 17.00 | 18.00 |
| WPM | 4.67 | 4.00 | 4.33 | 18.67 | 16.67 | 17.34 |
| B5 | 3.67 | 3.33 | 3.50 | 17.33 | 16.33 | 16.83 |
| CHU | 3.00 | 2.67 | 2.83 | 15.67 | 11.00 | 13.33 |
| Mean (B) | 4.19 | 3.81 | | 19.33 | 17.06 | |
| $LSD_{0.05}$ for | | | | | | |
| Media (A) | | 0.64 | | | 0.83 | |
| AC (B) | | 0.38 | | | 0.48 | |
| (A x B) | | 0.94 | | | 1.17 | |

**Effect of IBA or NAA and activated charcoal (AC) on establishment stage of Pyracantha seeds.**

Results at establishment stage in **Table (4)** and **Fig (1)** show that all NAA treatments gave no positive response at establishment stage. All seeds cultured on medium supplemented with NAA had not significantly progressed to plantlets, as seeds started to die. The best concentration of IBA was 0.5 mg/l that produced the highest shoot length (4.50 cm), number of leaves/seed (5.33 leaves), number of roots/seed (0.50 root) and root length (1.17 cm). Seeds culture on medium with IBA at 1.0, 2.0 or 4.0 mg/l, death was clearly realized in all of the replicates.

For activated charcoal, results demonstrated that there was no plantlet formation with activated charcoal during the establishment stage of seeds. Medium without activated charcoal was better than that with activated charcoal in all characters. For the interaction between auxins concentrations and activated charcoal, it showed that the best concentration was 0.5 mg/l IBA without activated charcoal, that produced the highest shoot length (9.00 cm), number of leaves/seed (10.67 leaves), number of roots/seed (1.00 root) and root length (2.33 cm).

**Table (4). Effect of different concentrations of IBA or NAA (mg/l) and activated charcoal (AC) during establishment stage on mean of shoot length (cm), number of leaves and roots/seed and root length (cm) at *Pyracantha fortuneane* seeds.**

| Auxins (mg/l) | AC (g/l) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.0 | 1.0 | Mean (A) | 0.0 | 1.0 | Mean (A) | 0.0 | 1.0 | Mean (A) | 0.0 | 1.0 | Mean (A) |
| | Shoot length (cm) | | | Number of leaves/seed | | | Number of roots/seed | | | Root length (cm) | | |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.50 IBA | 9.00 | 0.00 | 4.50 | 10.67 | 0.00 | 5.33 | 1.00 | 0.00 | 0.50 | 2.33 | 0.00 | 1.17 |
| 1.00 IBA | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2.00 IBA | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 4.00 IBA | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.50 NAA | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.00 NAA | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2.00 NAA | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 4.00 NAA | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Mean (B) | 1.00 | 0.00 | | 1.19 | 0.00 | | 0.11 | 0.00 | | 0.20 | 0.00 | |
| $LSD_{0.05}$ for | | | | | | | | | | | | |
| Auxins (A) | | 0.28 | | | 0.16 | | | 0.16 | | | 0.20 | |
| AC (B) | | 0.13 | | | 0.08 | | | 0.08 | | | 0.09 | |
| (A × B) | | 0.39 | | | 0.23 | | | 0.23 | | | 0.28 | |

**Effect of BA and Kin on multiplication stage of *Pyracantha* buds:**

For BA concentrations, results represented in **Table (5)** and **Fig (1)** demonstrates that there was no new shoot formation at control BA treatment. Increasing of shoot formation was positively correlated with increasing of BA concentrations. BA caused a significant increase in number of shoots/shoot to 12.20 shoots at 1.0 mg/l BA then it decreased to 10.87 shoots at 2.0 mg/l BA. The highest shoot length (4.30 cm) and number of leaves/shoot (21.53 leaves) was recorded at control BA treatment. The highest shoot length and number of leaves/shoot was recorded at control BA treatment for the main shoot that was cultured for this stage.

Results were showed that the number of shoots/shoot and shoot length decreased with the increase of Kin concentrations. The highest number of shoots/shoot and shoot length (15.08 shoots and 3.18 cm, respectively) was recorded at 1.00 mg/l Kin treatment. Although the highest number of leaves/shoot (21.50 leaves) was recorded at 3.00 mg/l Kin.

For the interaction between BA and Kin, BA and Kin, results proved to be superior in number of shoots/shoot and number of leaves/shoot. BA at 1.00 mg/l was preferred with different concentrations of Kin because it gave the highest number of shoots/shoot and number of leaves/shoot (26.00 shoots at 1.00 mg/l Kin and 26.00 leaves at 3.00 mg/l Kin, respectively) which was suitable for the multiplication stage. While, the highest shoot length was recorded 5.33 cm at control treatment. BA which was observed to be better than Kin in number of shoots of *Philodendron bipinnatifidum* (El-shamy, 2015b).

**Table (5). Effect of different concentrations of BA and Kin (mg/l) on number of shoots/shoot, shoot length (cm), number of leaves/shoot in multiplication stage of *Pyracantha fortuneane* buds.**

| BA (mg/l) | Kin (mg/l) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.0 | 1.0 | 2.0 | 3.0 | 4.0 | Mean (A) | 0.0 | 1.0 | 2.0 | 3.0 | 4.0 | Mean (A) | 0.0 | 1.0 | 2.0 | 3.0 | 4.0 | Mean (A) |
| | Number of shoots/shoot | | | | | | Shoot length (cm) | | | | | | Number of leaves/shoot | | | | | |
| **0.0** | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | **1.00** | 5.33 | 4.33 | 4.00 | 3.67 | 4.17 | **4.30** | 25.33 | 22.33 | 20.67 | 19.33 | 20.00 | **21.53** |
| **0.5** | 7.67 | 14.67 | 12.33 | 11.00 | 5.67 | **10.27** | 1.67 | 2.67 | 2.50 | 2.33 | 2.00 | **2.23** | 18.00 | 17.33 | 17.33 | 19.33 | 19.33 | **18.26** |
| **1.0** | 13.00 | 26.00 | 10.67 | 6.00 | 5.33 | **12.20** | 2.67 | 2.83 | 2.83 | 3.17 | 1.83 | **2.67** | 16.67 | 19.67 | 21.33 | 26.00 | 18.67 | **20.47** |
| **2.0** | 15.33 | 18.67 | 10.00 | 5.67 | 4.67 | **10.87** | 2.40 | 2.90 | 2.50 | 2.37 | 1.67 | **2.37** | 18.67 | 19.33 | 20.67 | 21.33 | 17.33 | **19.47** |
| **Mean (B)** | **9.25** | **15.08** | **8.50** | **5.91** | **4.17** | | **3.02** | **3.18** | **2.96** | **2.88** | **2.41** | | **19.67** | **19.67** | **20.00** | **21.50** | **18.83** | |
| **$LSD_{0.05}$ for** | | | | | | | | | | | | | | | | | | |
| **BA (A)** | 0.54 | | | | | | 0.25 | | | | | | 0.54 | | | | | |
| **Kin (B)** | 0.60 | | | | | | 0.28 | | | | | | 0.60 | | | | | |
| **(A x B)** | 1.20 | | | | | | 0.56 | | | | | | 1.21 | | | | | |

**Effect of BA and Kin on multiplication stage of Pyracantha seeds:**

Data recorded in **Table (6)** and **Fig (1)** demonstrates that the highest number of shoots/seed was showed at medium supplemented with 1.0 mg/l BA which gave 16.33 shoots. But, results showed that the highest number of leaves/seed and shoot length was achieved in medium without BA which gave 14.47 leaves and 3.67 cm, respectively.

For Kin concentrations, results showed that the highest number of shoots/seed was recorded when medium supplemented with Kin was used at 1.00 mg/l. While, the highest number of leaves/seed and shoot length was recorded at medium control without Kin. The highest number of leaves/seed and shoot length was recorded 12.58 leaves and 2.79 cm, respectively at zero Kin. For the interaction between BA and Kin on multiplication stage of seeds showed that there were the same trends. BA at 1.00 mg/l and 1.00 mg/l Kin gave the highest number of shoots/seed which gave 20.00 shoots. While, the highest number of leaves/seed and shoot length (16.00 leaves and 4.00 cm, respectively) was recorded at zero-level concentration of BA and Kin (control). BA is effective for shoot multiplications in *Pyracantha angustifolia* when used with high concentrations (2.5 mg/l), as higher contents of this cytokinin

inhibited callus formation (Vujović et al., 2020).

**Table (6):** Effect of different concentrations of BA and Kin (mg/l) on number of shoots/seed, shoot length (cm) and number of leaves/seed at multiplication stage of *Pyracantha fortuneane* seeds.

| BA (mg/l) | Kin (mg/l) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.0 | 1.0 | 2.0 | 3.0 | 4.0 | Mean (A) | 0.0 | 1.0 | 2.0 | 3.0 | 4.0 | Mean (A) | 0.0 | 1.0 | 2.0 | 3.0 | 4.0 | Mean (A) |
| | Number of shoots/shoot | | | | | | Shoot length (cm) | | | | | | Number of leaves/shoot | | | | | |
| **0.0** | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | **1.00** | 4.00 | 3.83 | 3.67 | 3.50 | 3.33 | **3.67** | 16.00 | 14.67 | 14.33 | 14.00 | 13.33 | **14.47** |
| **0.5** | 18.67 | 12.67 | 11.33 | 13.33 | 5.00 | **12.20** | 2.83 | 2.00 | 1.83 | 1.80 | 1.83 | **2.06** | 14.33 | 11.67 | 11.00 | 10.33 | 10.00 | **11.47** |
| **1.0** | 11.33 | 20.00 | 14.67 | 17.33 | 18.33 | **16.33** | 2.17 | 2.67 | 2.50 | 2.33 | 2.00 | **2.33** | 9.33 | 11.33 | 11.33 | 13.00 | 9.33 | **10.87** |
| **2.0** | 10.00 | 15.33 | 15.00 | 15.67 | 16.67 | **14.53** | 2.17 | 2.33 | 1.87 | 1.67 | 1.50 | **1.91** | 10.67 | 12.33 | 13.33 | 10.00 | 8.00 | **10.87** |
| **Mean (B)** | **10.25** | **12.25** | **10.50** | **11.83** | **10.25** | | **2.79** | **2.71** | **2.47** | **2.33** | **2.17** | | **12.58** | **12.50** | **12.50** | **11.83** | **10.17** | |
| **$LSD_{0.05}$ for** | | | | | | | | | | | | | | | | | | |
| **BA (A)** | 0.93 | | | | | | 0.16 | | | | | | 0.64 | | | | | |
| **Kin (B)** | 1.04 | | | | | | 0.18 | | | | | | 0.71 | | | | | |
| **(A×B)** | 2.08 | | | | | | 0.35 | | | | | | 1.43 | | | | | |

## Effect of IBA and Kin on rooting stage of Pyracantha buds:

Results presented in **Table (7)** and **Fig (1)** demonstrate that 3 mg/l IBA gave the highest number of shoots/cluster, shoot length, number of leaves/cluster and root length (3.33 shoots, 4.66 cm, 18.89 leaves and 2.50 cm, respectively). IBA induced the formation of roots on cluster when compared with the remaining treatments. IBA at zero-level concentration gave no response for rooting formation. It was noted that the number of shoots/cluster was more or less stable at 0.0, 1.0 and 3.0 mg/l IBA which gave 3.33 shoots.

For Kin concentrations, results indicated that the highest shoot length, number of leaves/cluster, number of roots/cluster and root length were formed at 0.5 mg/l Kin which gave 4.56 cm, 17.83 leaves, 2.17 root and 2.48 cm, respectively. The highest number of shoots/cluster was recorded at 1.00 mg/l Kin which gave 3.75 shoots.

The interaction between the different concentrations of IBA and Kin showed that number of shoots/cluster was more or less stable at 0.0, 1.0 and 3.0 mg/l IBA and 1.0 mg/l Kin, giving 4.00 shoots. The longest shoot was found in clusters cultured on medium plus 1.0 mg/l IBA and 0.5 mg/l Kin (4.83 cm). While, medium supplemented with 3.0 mg/l IBA and 0.0 or 1.0 mg/l Kin gave the highest number of leaves/cluster (19.0 leaves). The highest number of roots/cluster was found when the clusters were cultured on medium with 1.0 mg/l IBA and 0.5 mg/l Kin (3.67 roots). The longest root length was recorded when the clusters were cultured on medium supplemented with 3.0 mg/l IBA and 0.5 mg/l Kin (3.83 cm).

These findings go in line with those reviewed by Sulusoglu and Cavusoglu, (2013), Mahipal et al. (2016) and Dinesh et al. (2019) on IBA is usually the more effective auxin for rooting than other auxins for several other species.

**Table (7). Effect of different concentrations of IBA and Kin (mg/l) on mean number of shoots/cluster, shoot length (cm), number of leaves/cluster, number of roots/cluster and root length (cm) at rooting stage of *Pyracantha fortuneane* buds.**

| IBA (mg/l) | Kin (mg/l) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.0 | 0.5 | 1.0 | Mean (A) | 0.0 | 0.5 | 1.0 | Mean (A) | 0.0 | 0.5 | 1.0 | Mean (A) | 0.0 | 0.5 | 1.0 | Mean (A) | 0.0 | 0.5 | 1.0 | Mean (A) |
| | Number of shoots/cluster | | | | Shoot length (cm) | | | | Number of leaves/cluster | | | | Root length (cm) | | | | Number of roots/cluster | | | |
| **0.0** | 3.00 | 3.00 | 4.00 | **3.33** | 4.33 | 4.17 | 3.50 | **4.00** | 16.67 | 16.00 | 15.00 | **15.89** | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 | 0.00 | **0.00** |
| **1.0** | 3.00 | 3.00 | 4.00 | **3.33** | 4.67 | 4.83 | 4.00 | **4.50** | 16.33 | 19.33 | 15.00 | **16.89** | 2.33 | 3.67 | 0.00 | **2.00** | 3.40 | 3.57 | 0.00 | **2.32** |
| **3.0** | 3.00 | 3.00 | 4.00 | **3.33** | 4.73 | 4.73 | 4.50 | **4.66** | 19.00 | 18.67 | 19.00 | **18.89** | 3.33 | 2.00 | 0.00 | **1.78** | 3.67 | 3.83 | 0.00 | **2.50** |
| **5.0** | 2.00 | 3.33 | 3.00 | **2.78** | 2.67 | 4.50 | 4.33 | **3.83** | 14.67 | 17.33 | 15.67 | **15.89** | 0.00 | 3.00 | 0.00 | **1.00** | 0.00 | 2.50 | 0.00 | **0.83** |
| **Mean (B)** | **2.75** | **3.08** | **3.75** | | **4.10** | **4.56** | **4.08** | | **16.67** | **17.83** | **16.17** | | **1.42** | **2.17** | **0.00** | | **1.77** | **2.48** | **0.00** | |
| **$LSD_{0.05}$ for** | | | | | | | | | | | | | | | | | | | | |
| **IBA (A)** | 0.16 | | | | 0.21 | | | | 0.95 | | | | 0.26 | | | | 0.19 | | | |
| **Kin (B)** | 0.14 | | | | 0.18 | | | | 0.82 | | | | 0.23 | | | | 0.16 | | | |
| **(A×B)** | 0.28 | | | | 0.36 | | | | 1.64 | | | | 0.46 | | | | 0.33 | | | |

## Effect of IBA and Kin on rooting stage of Pyracantha seeds:

Data recorded in **Table (8)** and **Fig (1)** demonstrate that 3 mg/l IBA gave the highest number of shoots/cluster, shoot length,

number of leaves/cluster, number of roots/cluster and root length which gave 3.78 shoots, 4.24 cm, 20.22 leaves, 3.56 roots and 3.89 cm, respectively.

For Kin concentrations, results revealed that the highest number of shoots/cluster was obtained on medium supplemented with 1.0 mg/l Kin (3.75 shoots). While, the highest shoot length, number of leaves/cluster, number of roots/cluster and root length which gave 4.08 cm, 17.08 leaves, 2.33 roots and 3.04 cm, respectively at 0.5 mg/l Kin.

For the interaction between IBA and Kin on rooting stage of seeds showed that the highest number of shoots/cluster, number of leaves/cluster and number of roots/cluster which gave 4.33 shoots, 21.33 leaves and 5.67 roots, respectively at 3.0 mg/l IBA and 1.0 mg/l Kin. The longest shoot was found on medium supplemented with 1.0 mg/l IBA and 0.5 mg/l Kin (4.50 cm). The longest root was found on medium supplemented with 3.0 mg/l IBA alone without Kin which gave 4.67 cm.

On the other hands, medium supplemented with NAA was tested, but did not form rooting. The best medium for rooting of *Pyracantha coccinea* shoot was MS at quarter salt-strength containing 93 mM IBA (Dong et al., 2017).

**Table (8). Effect of different concentrations of IBA and Kin (mg/l) on mean number of shoots/cluster, shoot length (cm), number of leaves/cluster, number of roots/cluster and root length (cm) at rooting stage of *Pyracantha fortuneane* seeds.**

| IBA (mg/l) | Kin (mg/l) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.0 | 0.5 | 1.0 | Mean (A) | 0.0 | 0.5 | 1.0 | Mean (A) | 0.0 | 0.5 | 1.0 | Mean (A) | 0.0 | 0.5 | 1.0 | Mean (A) | 0.0 | 0.5 | 1.0 | Mean (A) |
| | Number of shoots/cluster | | | | Shoot length (cm) | | | | Number of leaves/cluster | | | | Root length (cm) | | | | Number of roots/cluster | | | |
| **0.0** | 3.00 | 3.00 | 3.67 | **3.22** | 4.17 | 4.00 | 3.33 | **3.83** | 18.33 | 18.00 | 16.67 | **17.67** | 0.00 | 1.67 | 0.00 | **0.56** | 0.00 | 4.17 | 0.00 | **1.39** |
| **1.0** | 3.00 | 4.00 | 4.00 | **3.67** | 4.33 | 4.50 | 3.83 | **4.22** | 17.33 | 20.67 | 17.00 | **18.33** | 3.67 | 3.00 | 0.00 | **2.22** | 3.17 | 3.00 | 0.00 | **2.06** |
| **3.0** | 3.00 | 4.00 | 4.33 | **3.78** | 4.23 | 4.33 | 4.17 | **4.24** | 20.00 | 19.33 | 21.33 | **20.22** | 1.67 | 3.33 | 5.67 | **3.56** | 4.67 | 3.33 | 3.67 | **3.89** |
| **5.0** | 2.00 | 3.00 | 3.00 | **2.67** | 3.17 | 3.50 | 3.00 | **3.22** | 9.00 | 10.33 | 9.33 | **9.56** | 0.00 | 1.33 | 0.00 | **0.44** | 0.00 | 1.67 | 0.00 | **0.56** |
| **Mean (B)** | **2.75** | **3.50** | **3.75** | | **3.98** | **4.08** | **3.58** | | **16.17** | **17.08** | **16.08** | | **1.33** | **2.33** | **1.42** | | **1.96** | **3.04** | **0.92** | |
| **$LSD_{0.05}$ for** | | | | | | | | | | | | | | | | | | | | |
| **IBA (A)** | 0.24 | | | | 0.16 | | | | 0.95 | | | | 0.33 | | | | 0.82 | | | |
| **Kin (B)** | 0.21 | | | | 0.14 | | | | 0.82 | | | | 0.28 | | | | 0.72 | | | |
| **(A × B)** | 0.42 | | | | 0.28 | | | | 1.65 | | | | 0.56 | | | | 1.44 | | | |

**Effect of peatmoss, perlite and sand on acclimatization stage of Pyracantha buds:**

Data recorded in **Table (9)** and **Fig (1)** show that the plantlets of Pyracantha were successfully survived when they were cultured in greenhouse conditions after dipping them in fungi and bacteria antiseptic and then cultured in a mixture of mixtures peatmoss, perlite and sand. After four weeks, no abnormalities in physical appearance were observed on the cultured plantlets. The highest percentage of plantlets acclimatized, number of shoots/cluster, plantlet height (cm) and number of leaves/cluster (93.33 %, 3.00 shoots, 4.67 cm and 35.0 leaves, respectively) were achieved in plantlets cultured in pots containing peatmoss alone for plantlets formed from buds.

**Table (9). Effect of different mixtures of peatmoss, perlite and sand on acclimatization percentage (%), mean number of shoots/cluster, mean plantlet height (cm) and mean number of leaves/cluster during acclimatization stage of *Pyracantha fortuneane* buds.**

| Peatmoss | Perlite | Sand | Acclimatization (%) | Number of shoots / cluster | Plantlet height (cm) | Number of leaves/ cluster |
|---|---|---|---|---|---|---|
| **1** | **0** | **0** | 93.33 | 3.00 | 4.67 | 35.00 |
| **0** | **1** | **0** | 40.00 | 1.00 | 2.00 | 6.00 |
| **0** | **0** | **1** | 46.67 | 1.00 | 2.33 | 7.00 |
| **1** | **1** | **1** | 78.33 | 2.33 | 3.00 | 16.67 |
| **1** | **1** | **0** | 86.67 | 3.00 | 4.33 | 28.00 |
| **1** | **0** | **1** | 90.00 | 3.00 | 4.33 | 33.33 |
| **0** | **1** | **1** | 63.33 | 1.00 | 2.33 | 7.33 |
| **$LSD_{0.05}$** | | | 10.57 | 0.39 | 1.67 | 7.65 |

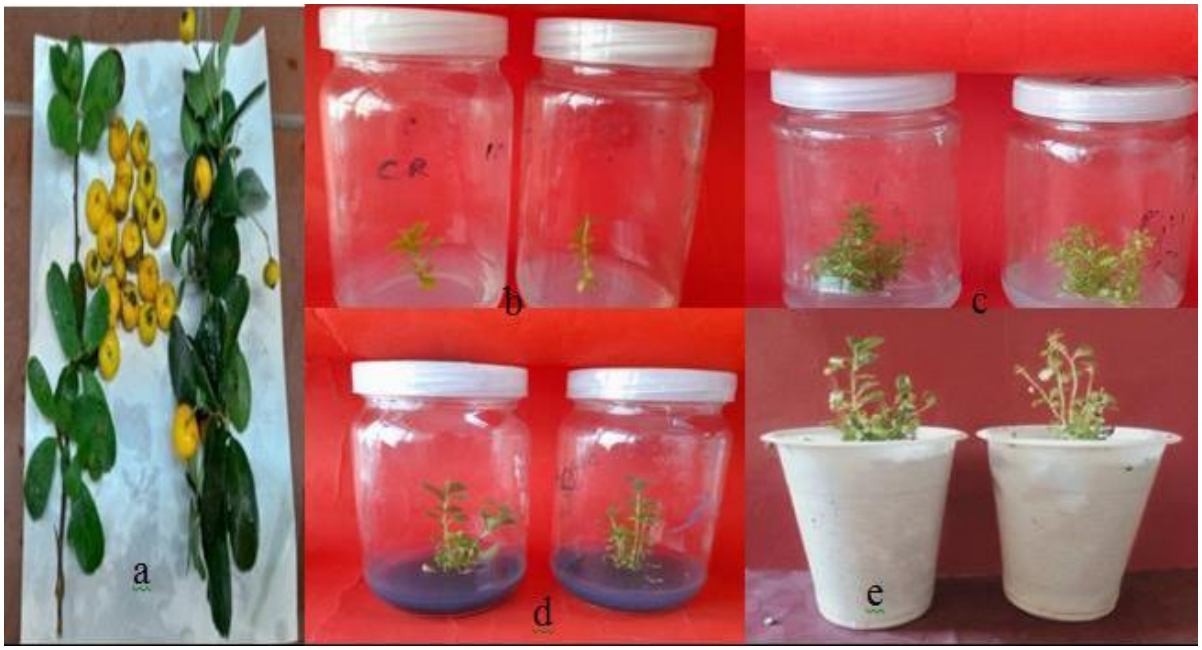


**Fig (1).** *In vitro* micropropagation of *Pyracantha fortuneane*
a. Explants-b. Establishment stage-c. Multiplication stage-d. Rooting stage.-e. Acclimatization stage
Effect of treatments on buds (left) to seeds (right)

**Effect of peatmoss, perlite and sand on acclimatization stage of Pyracantha seeds:**

Data calculated in **Table (10)** and Fig (1) demonstrates that the plantlets grew from seeds showed the same trend as in buds. The highest percentage of plantlets acclimatized, number of shoots/cluster, plantlet height (cm) and number of leaves/cluster (91.67 %, 3.00 shoots, 4.67 cm and 26.67 leaves, respectively) was achieved at cultured plantlets in pots containing peatmoss alone.

**Table (10). Effect of different mixtures of peatmoss, perlite and sand on acclimatization percentage (%), mean number of shoots/cluster, mean plantlet height (cm) and mean number of leaves/ cluster during acclimatization stage of *Pyracantha fortuneane* seeds.**

| Peatmoss | Perlite | Sand | Acclimatization (%) | Number of shoots/cluster | Plantlet height (cm) | Number of leaves/ cluster |
|---|---|---|---|---|---|---|
| **1** | **0** | **0** | 91.67 | 3.00 | 4.67 | 26.67 |
| **0** | **1** | **0** | 53.33 | 1.33 | 1.67 | 4.33 |
| **0** | **0** | **1** | 71.67 | 1.67 | 2.00 | 5.67 |
| **1** | **1** | **1** | 85.00 | 2.67 | 3.67 | 16.67 |
| **1** | **1** | **0** | 85.00 | 3.00 | 4.67 | 22.67 |
| **1** | **0** | **1** | 80.00 | 3.00 | 3.67 | 20.00 |
| **0** | **1** | **1** | 73.33 | 2.33 | 2.17 | 11.00 |
| **LSD $_{0.05}$** | | | 4.41 | 0.63 | 1.74 | 3.24 |

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## الملخص العربي

# تأثير بيئات الزراعة على الإكثار الدقيق لنبات *Pyracantha fortuneane* بواسطة زراعة الأنسجة

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تم إجراء التجربة فى معمل زراعة الأنسجة النباتية لمدة عامين (2022-2023) بهدف عمل بروتوكول مناسب لإكثار نبات *Pyracantha fortuneana*. وفي هذا الصدد تم تعقيم براعم النبات بمحلول هيبوكلوريت الصوديوم NaOCl بنسبة 1.5٪ لمدة 20 دقيقة. كما تم تعقيم البذور بمحلول هيبوكلوريت الصوديوم NaOCl بنسبة 1.5٪ لمدة 30 دقيقة. وفي مرحلة التأسيس كانت بيئة موراشيجى وسكوج (MS) بكامل قوتها وبدون الفحم المنشط هي الأنسب وقد أعطت أعلى القيم فى طول الأفرع وعدد الأوراق بالنسبة للبراعم. بينما كانت بيئة موراشيجى وسكوج (MS) ذات القوة الكاملة مع 0.5 ملجم/لتر إندول حمض البيوتريك (IBA) هى البيئة الأفضل بالنسبة للبذور. وفى مرحلة التضاعف فإن البيئة المضاف إليها البنزيل أدينين (BA) بتركيز 1.0 ملجم / لتر و 1.0 ملجم/لتر كينتين (Kin) أعطت أعلى عدد من الأفرع للبراعم والبذور. وفي مرحلة التجذير وجد أن أعلى عدد للجذورتم الحصول عليه عند تركيز 1.0 ملجم/لتر إندول حمض البيوتريك (IBA) بالإضافة إلى 0.5 ملجم/لتر كينتين (Kin) بالنسبة للبراعم، أما بالنسبة للبذور كان أفضل تركيز هو 3.0 ملجم/لتر إندول حمض البيوتريك (IBA) و1.0 ملجم/لتر كينتين (Kin). وقد تم أقلمة النباتات والحصول على أعلى نسبة نجاح في أصص بلاستيكية مملوءة بالبيت موس فقط للنباتات الناتجة من البراعم والبذور لنبات البيراكانثا.