International Research Journal of Innovations in Engineering and Technology (IRJIET)

ISSN (online): 2581-3048

Volume 8, Issue 10, pp 39-46, October-2024

https://doi.org/10.47001/IRJIET/2024.810007

Effect of Some Treatments on Seed and Vegetative Propagation of Hawthorn (*Crataegus* spp.) in Northwestern Syria

[1]Rasha OBIDEEN, [2]Dr. Amin ALHASAN, [3]*Prof. Rida DRAIE

[1,2,3]Faculty of Agricultural Engineering, Idlib University, Syria

*Corresponding Author's E-mail: rida.draie@idlib-university.com

Abstract **- This research was conducted in Idlib Governorate, located in northwestern Syria, during the years 2021, 2022 and 2023. The two types of wild hawthorn, Monogyna hawthorn (*Crataegus monogyna*), and Aronia hawthorn (*Crataegus azarolus var. aronia*) were propagated by seeds and vegetatively. Two types of treatments were used for sexual propagation by seeds, namely soaking with gibberellin at concentrations of 1000 and 2000 ppm and intervals of 24 and 48 hours, and soaking with sulfuric acid at concentrations of 50% and 98% and intervals of one and two hours. The two treatments were followed by warm stratification for two months, followed by cold stratification for 3 months, while the control treatment was subjected to stratification only. Vegetative propagation was done by cuttings that were dipped in several concentrations of IBA, where concentrations of 4000, 6000, and 8000 ppm were used.**

The results showed that seed propagation achieved better results than vegetative propagation. The treatment of Monogyna seeds with gibberellin at a concentration of 2000 ppm and during the 48-hour soaking period significantly outperformed all other treatments, with a germination rate of 73.33%. The treatment of Monogyna cuttings at a concentration of 8000 ppm of IBA significantly outperformed the rest of the experimental treatments, with a rooting rate of 16.67%. The Monogyna type significantly outperformed the Aronia type in seed germination rate and rooting rate of cuttings, with values of 40 and 20.42% for seed germination and 8.42 and 5.17% for rooting of cuttings for the two types, respectively. Finally, alternating (warm and cold) seed stratification, treatment with gibberellin or sulfuric acid, and the use of seeds stored in the refrigerator for a full year, played an important role in increasing the germination rate of hawthorn seeds.

Keywords: Hawthorn, Propagation, GA3, IBA, Seeds, Cuttings.

I. INTRODUCTION

Hawthorn trees are important in soil conservation, preventing erosion, and combating desertification, in addition to their economic, horticultural, ornamental, and medicinal importance. Hawthorn belongs to the genus *Crataegus*, which belongs to the subfamily Pomoideae and the family Rosaceae (Tutin et al., 1990; Phipps et al., 1991; Lippert 1995). Despite the spread of hawthorn in the wild, there are difficulties in propagating it in nurseries, and these difficulties have been the most important factor in limiting its spread.

Seeds of the genus *Crataegus* show both internal (related to immature embryos) and external (related to the properties of the seed coat) dormancy. Thus, germination is related to several factors, including fruit maturity, external and internal dormancy, seed storage conditions, the thickness of the inner fruit coat, conditions related to embryo maturity, and the period of stratification. Propagation of hawthorn by seeds is difficult, since the seeds do not germinate easily in their natural state, and therefore require special treatments, and may remain dormant for 2-3 years (Bujarska-Borkowska, 2006, 2007). The reason for the external dormancy of hawthorn seeds is the presence of a hard and thick seed coat, which negatively affects their permeability. The thickness of the seed coat varies between hawthorn species, and even within the same species (Gokturk et al., 2017). Inappropriate storage temperature can often result in low germination, damage to seeds, and loss of viability (Schmidt, 2000). It is preferable to place seeds in a refrigerator at 4 °C immediately after harvesting ripe fruits. The potential energy of seeds differs between freshly harvested seeds and seeds stored in cold storage for a long time (Qian et al., 2009). Cold storage reduces the levels of abscisic acid (ABA) within the seeds, which stimulates germination (Yang et al., 2008). Higher germination rates can be achieved with long storage periods compared to short storage periods (Ahmadloo et al., 2015). This storage is much better than freezer storage, as the temperature and relative humidity are maintained properly, which maintains the viability and germination capacity of the seeds for a relatively longer period (Ahmadloo et al., 2017).

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Gokturk*et al.* (2021) indicated that storing *C. azarolus* seeds for 10 months at 15°C, then treating them with sulfuric acid for 3 hours, and ash solution for 4 days, gave the best results.

Seed stratification is the best procedure to accelerate germination, as important physiological and biochemical changes occur during the stratification period, from a gradual increase in enzymatic activity that converts complex reserves into simple reserves, and growth promoters gradually increase and growth inhibitors in seed tissues represented by abscisic acid gradually decrease, and at the end of the stratification period, the seeds acquire the ability to germinate (Wurzer, 2000). Many researchers have recommended warm and cold stratification treatments to remove germination obstacles caused by immature hawthorn seed embryos, to break internal dormancy and achieve seed germination (Morgenson, 2000; Rutar et al., 2001; Persson et al., 2006; Bujarska-Borkowska, 2007; Göktürk and Yıldırım, 2020). Mohammed (2023) confirmed that cold stratification alone is not sufficient to break the dormancy state in hawthorn, while warm stratification for some time before cold stratification is good for breaking dormancy and achieves better results. The duration of periodic stratification (warm and cold) varies according to the species.

Seed scientists have used different methods and techniques to break seed dormancy, and many studies have investigated the effect of external growth regulators in overcoming internal seed dormancy. During breaking seed dormancy by stratification, a gradual increase in the content of gibberellin is observed, while a gradual decrease in the content of growth inhibitors is observed (Younis, 2009). Physiological dormancy in seeds is closely related to the proportion of inhibitors, especially abscisic acid, and growth regulators, especially gibberellin, which contributes to overcoming physiological dormancy in seeds with dormant embryos (Hartmann et al., 2010; Ahmadloo et al., 2017). Miransari and Smith (2014) reported that gibberellin can remove dormancy caused by the seed coat, by regulating proteins, weakening the endosperm, and expanding the embryo cell, and that the outer seed coat cell walls play a very important role in increasing mechanical resistance that can prevent embryo expansion, but the outer seed coat walls are weakened by hydrolytic enzymes, or by modifying the composition of the cell wall, however, the embryo expansion potential increases after treatment with gibberellin, and cold and warm stratification. Ahmadloo et al. (2017) found in their study that gibberellin improves the germination of *C. pseudoheterophylla* seeds, where the highest germination rate (59.7%) was recorded in seeds without an inner coat that were treated with gibberellin at a concentration of 3000 ppm and stored either in the laboratory or refrigerator (32.7-35.3%). The germination rate reached 7.7 and 9% when treated with gibberellin at a concentration of 2500 and 3000 ppm, respectively. This stimulating effect of gibberellin can be attributed to the decrease in the effect of the woody coat surrounding the seed and the increase in the activity of cytokinin in overcoming the activity of germination inhibitors. The highest germination rates were obtained in hawthorn *Crataegus* × *sinaica*, using gibberellin treatment at a concentration of 1000 ppm with a germination rate of 31.66% (Sümbül et al., 2024).

Mechanical and chemical scarifications are recommended to overcome external dormancy. In their study, Göktürk and Yıldırım (2020) obtained the highest germination rate of *C. monogyna* species at 64.98% from sulfuric acid treatment. Good germination results were obtained after treating it with 3% sulfuric acid and after seed stratification for 60-150 days at 1-5 °C (Olmez et al., 2009). Chemical scarification cannot be considered an alternative to warm stratification that precedes cold stratification. It was found that using sulfuric acid before cold stratification had unfavorable consequences for germination, but using it before warm stratification, and then performing cold stratification, shortened the warm stratification period and improved the germination rate (Bujarska-Borkowska, 2002; 2006; 2007). In a study conducted on hawthorn, it was treated with several levels of acidic treatments, and as a result, the sulfuric acid treatment with a concentration of 95% outperformed the other treatments in the first year, but with time the results of the treatments become more convergent (Zindi and Khraman, 2015). Some researchers confirmed that cold stratification after acid treatment or soaking in hot water is effective for both seeds with hard shells and dormant embryos (Hartman et al., 2002; Bektaş et al., 2017). Christensen (1992) indicated that clean and fresh hawthorn seeds need to be stratified or treated with concentrated sulfuric acid, and then stratification for 5 months at a temperature of 4 °C is the best method for germination. A study on the propagation of hawthorn species found in southern Syria proved that soaking seeds with sulfuric acid (*C. azarolus* var. *aronia*, and *C.* × *sinaica*Boiss. ssp. *sinaica* followed by warm stratification at 2°C for two months, followed by cold stratification for 3 months, contributed to increasing the percentage of seed germination (Younis, 2009). In a study of Yahyaoglu et al. (2006) conducted on several types of hawthorn seeds, cold stratification was carried out with immersion in sulfuric acid, and the highest germination percentage (17.5%) was in *C. monogyna* ssp. *azarella* grown in a greenhouse after immersion in sulfuric acid for 120 minutes, with cold stratification for 90 days.

Vegetative propagation of hawthorn species is not a useful method due to the difficulty of rooting, as their rooting rate is very low (Hummer and Janick, 2009). Moreover, even if hawthorn is successfully propagated under laboratory

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conditions, root production is insufficient (Güney et al., 2020). At present, propagation is done using seeds, followed by grafting methods for new plants (Çalışkan and Karaman, 2018; Kacal et al., 2022). Singh (2002) reported that IBA is a rooting hormone that is widely used to promote rooting in stem cuttings or air layering, as it promotes early rooting, bud formation, and vegetative growth, and allows the permanence of the seedlings. In the study conducted by Jamal and Al-Issa (2004) on the rooting of some cuttings of hawthorn *C. azarolus*, it was shown that treating the woody cuttings of the species *C. azarolus* with IBA at a concentration of 4000 ppm, and planting them in a sandy medium, led to raising the rooting percentage to 20%, and the same for the cuttings planted in the Turb medium with fermented organic fertilizer. Hartmann et al. (2010) mentioned that he achieved success in rooting by 35% by applying 8000 ppm of IBA and 2000 ppm of NAA on young hawthorn cuttings.

Consequently, we find that despite the forestry, horticultural, and medicinal importance of the hawthorn plant, it suffers from difficulty in propagation, in conjunction with the continuous encroachments it is exposed to, which has been reflected in its spread in nature. Therefore, the goal of this research was to determine the best methods and treatments for propagating hawthorn and caring for seedlings, thus securing the necessary plant material for expanding its cultivation, whether wild or orchard.

II. MATERIALS AND METHODS

2.1 Plant Material

Seed propagation of wild species spread in Syria, represented by Monogyna (*C.monogyna*) and Aronia (*C. azarolus* var. *aronia*), was carried out. Fully ripe fruits were collected in the 2021 season, the pulp was removed, and then the seeds were dried in a shaded and ventilated place and stored in the refrigerator at a temperature of 4 °C for the year 2022. Treatments were carried out on them, and then they were warmly and then coldly stratified, to be planted in 2023. As for the cuttings, the Monogyna hawthorn cuttings were obtained from the town of Al-Janoudiya in the Al-Jisr area, while the Aronia hawthorn was obtained from Ariha area.

2.2 Research Location

The seeds and cuttings were planted in Idlib city on a house balcony, which is a sunny and ventilated place. They were planted in foam trays containing peatmoss.

2.3 Sexual reproduction

2.3.1 Seeds treatment: The flotation test was performed, and then the seeds were sterilized with a 10% Kaptan solution before treatment. Then the following treatments were performed:

- **Factor 1 (Genotype):** The treatments were performed on seeds taken from the two wild types of hawthorns, *C. monogyna* and *C. azarolus var. aronia.*
- **Factor 2 (Substance):** Seeds were treated with gibberellin and sulfuric acid.
- **Factor 3 (Concentration):** Two concentrations of each treatment material were used, where 1000 and 2000 ppm concentrations of gibberellin, and 50% 98% concentrations of sulfuric acid were used.
- **Factor 4 (Period):** The seeds were soaked for two different periods within each treatment, as the seeds were soaked in gibberellin for 24 and 48 hours, and the seeds were soaked in sulfuric acid for one and two hours.

For the control, seeds taken from the year 2021 were used, stored for a year in the refrigerator and stratified, and without any chemical treatment (soaking in water only). Table 1 shows the concentration, length of period, and type of treatments for the genotypes used in the experiment.

Table 1: Concentration, Period, and type of treatments for the genotypes used in the experiment

Genotype	Treatment	Concentration	Soaking Period	
			A	B
Aronia	Control	0	24 hours	48 hours
	Gibberellin	1000 ppm		
		2000 ppm		
	Sulfuric Acid	%50		
		%98		
Monogyna	Control	0	1 hour	2 hours
	Gibberellin	1000 ppm		
		2000 ppm		
	Sulfuric Acid	%50		
		%98		

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2.3.2 Seeds Stratification: The seeds were placed in a sand container, in layers, and were permanently moistened. An alternating stratification process was carried out for all treatments in two stages:

- Warm stratification: It started on 11/1/2022 until 1/1/2023, where warm stratification began at room temperature approximately 20-25 °C.
- Cold stratification: It started on 1/1/2023 until 1/4/2023, where stratification was carried out in the refrigerator at a temperature of 4 °C.

2.3.3 Seeds Cultivation: The two studied species were treated, with three replicates for each treatment. After treatment, the seeds for all treatments were planted in foam trays and placed in the place designated for cultivation.

2.4 Asexual reproduction

2.4.1 Cuttings treatment: After preparing the mind, two experimental factors were applied to it:

- **Factor 1 (Genotype):** Treatments were performed on cuttings taken from the two wild hawthorn species, *C. monogyna* and *C. azarolus var. aronia.*
- **Factor 2 (Concentration):** Three concentrations of IBA hormone were used, namely 4000, 6000, and 8000 ppm, in addition to the control without any chemical treatment.

2.4.2 Cuttings cultivation: The cuttings were collected and wrapped in a damp cloth until planting the next day. Ten cuttings of each type were planted, with three replicates for each treatment. Then, cuttings of 30 cm in length were taken, and their bases were immersed to a depth of 1 cm for 5 seconds in the previously prepared IBA solution. The cuttings were then left for 20 minutes so that the alcohol evaporated from their bases and the hormone could penetrate inside them. After that, the cuttings were sterilized with a 1% Kaptan solution, and then planted in the peatmoss medium.

2.5 Experimental design and statistical analysis

The experiment used a completely randomized design (CRD). The results were tabulated, and the means were compared by calculating the least significant difference (L.S.D.) at a significance level of 5%, using the GenStat-12 statistical analysis program.

III. RESULTS AND DISCUSSION

3.1 Sexual propagation: The results of sexual propagation were obtained for both wild Monogyna and Aronia genotypes, and then were tabulated in Table 2.

Table 2: Germination percentage in experimental treatments

Genotype	Treatment	Concentration	Soaking Period	
			A	B
Aronia	Control	0	10	
	Gibberellin	1	16.67	20
		2	23.33	26.67
	Sulfuric Acid	1	10	13.33
		2	36.67	16.67
Monogyna	Control	0	13.33	
	Gibberellin	1	16.67	23.33
		2	46.67	73.33
	Sulfuric Acid	1	26.67	50
		2	30	53.33

	Means				
	Genotypes	**Treatments**	**Concentrations**	**Periods**	**General**
Control		11.67	11.67		
1	20.42	30.84	22.09	25.84	30.21
2	40	29.60	38.34	34.58	
L.S.D. (5%)	7.17	5.65	7.48	6.48	10.43

It is noted from Table (2) that the Monogyna genotype was significantly superior to Aronia in germination percentage in the average of the total treatments, as the germination percentage was 40% and 20.42% in the Monogyna and Aronia genotypes, respectively, and the L.S.D. value was equal to 7.17 at the 5% significance level. As for the treatments, both gibberellin and sulfuric acid treatments were significantly superior to the control, without any significant differences between the two treatments. The germination percentage values were 11.67%, 30.84% , and 29.60% in the control, gibberellin, and sulfuric acid treatments, respectively, and the L.S.D. value was equal to 5.65 at the 5% significance level.

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https://doi.org/10.47001/IRJIET/2024.810007

Regarding the treatment concentrations, both the first and second concentrations were significantly superior to the control (0), and the second treatment concentration was also significantly superior to the first concentration. The germination percentage values in the tested concentrations were 11.67%, 22.09%, and 38.34% in the control, first, and second concentrations, respectively. The L.S.D. value was 7.48 at a significance level of 5%. As for the duration of the treatment periods, the second treatment period was significantly superior to the first period, with germination percentage values of 25.84% and 34.56%, respectively, and the L.S.D. value was 6.48 at a significance level of 5%.

Regarding the interaction between the experimental factors (genotype, treatment, concentration, and soaking period), the treatment of the Monogyna genotype with gibberellin at the second concentration (2000 ppm) and during the second soaking period (48 hours) outperformed all other interactions, with a germination rate of 73.33%, while the lowest germination rate value was in the control treatment of the Aronia genotype and also the treatment of the Aronia genotype with sulfuric acid at a concentration of 50% and a soaking period of one hour. The L.S.D. value was equal to 10.43 at the 5% significance level, and the overall average germination rate for all treatments and interactions was 30.21%.

This result is like what was reached by Younis (2009), where the treatment with sulfuric acid was better than the effect of the soaking treatment with gibberellin and had a positive effect in improving germination in the *C. azarolus* var. *aronia* species, where the germination rate reached 65% when soaking in concentrated sulfuric acid for an hour, then warm stratification for 3 months, then cold stratification for 3 months. As for *C. monogyna* var. *monogyna*, the gibberellin treatment was better than the sulfur treatment, in which the germination rate reached 20% when soaking with gibberellin at a concentration of 2000 ppm for 24 hours, then warm stratification for a month, then cold stratification for 3 months. This result is also like the study by Zindi and Khraman (2015) on the germination of *C. azarolus* seeds, which proved the superiority of the sulfuric acid treatment at a concentration of 95% over other treatments. Our study is also like the results of the study Mirzadeh-Vaghefi et al. (2013), conducted on seeds of three hawthorn species *C. babakhanloui*, *C. Aminii*, and *C. persica*, in which the seeds were soaked with gibberellin at a concentration of 1500 ppm, and the germination percentage reached 28, 32, and 17%, respectively. It was noted through this study that the seedlings resulting from gibberellin were stronger and grew better than the seedlings resulting from sulfuric acid, due to the role of gibberellin in improving growth.

The effect of treatments (sulfuric acid and gibberellin) is attributed to the role of sulfuric acid in softening and smoothing the thick, hard coat surrounding the seeds, as Aronia seeds have a thicker coat than Monogyna seeds. As for the role of gibberellin in increasing the germination rate, it allows the embryo to elongate and the roots to form faster. We also conclude from this study that warm stratification and then cold stratification played an important role in increasing the germination rate of hawthorn seeds. This is attributed to the role of warm stratification in mitigating the effect of this coat and increasing the permeability of water to the inside of the seed, as it stimulates the secretion of the enzyme alpha-amylase, which converts starch into reducing sugars, thus raising the osmotic pressure and increasing the seed's absorption of water and food, which causes it to swell and increase in size. As for cold stratification, it played a role in reducing the protective effect of the seeds, reducing the percentage of growth inhibitors, and increasing the percentage of cytokinin and growth-stimulating substances. This is consistent with what previous studies have confirmed about the importance of warm stratification followed by cold stratification in increasing the germination rate of hawthorn seeds (Gordon and Rowe, 1982; Morgenson, 2000; Rutar et al., 2001; Persson et al., 2006; Bujarska-Borkowska, 2007; Göktürk and Yıldırım, 2020). Storing seeds in the refrigerator at 4°C for a full year also played a role in increasing the germination rate, as seeds with a hard coat absorbed more water after 12 months of storage at the refrigerator temperature, indicating that water penetrated the seed coats during this time and began to soften, which contributed to increasing the germination rate. This was also confirmed by Ahmadloo et al. (2017) in their study on the germination of hawthorn seeds.

3.2 Sexual propagation: After planting the cuttings, some of them started to form roots and give green shoots. The results of vegetative propagation were obtained for both Aronia and Monogyna genotypes. The readings were taken and statistically analyzed as shown in Table 3.

Table 3: Rooting percentage of the cuttings tested in the experiment

Genotype / Concentration	Aronia	Monogyna	Mean
0	0.00	0.00	0.00
4000	3.33	6.67	5.00
6000	6.67	10.33	8.50

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8000	10.67	16.67	13.67	
Mean	5.17	8.42	**General Mean**	**6.80**
L.S.D. (5%)	**Genotypes**	**Concentrations**	**Genotypes × Concentrations**	
	1.42	2.64	3.39	

It is noted from Table (3) that the treatments of all tested concentrations were significantly superior to the control in the percentage of rooted cuttings, and increasing the concentration used of rooting hormone (IBA) led to significant differences between treatments. The third concentration (8000 ppm) was significantly superior to the second concentration (6000 ppm), which in turn was significantly superior to the first concentration (4000 ppm), and all concentrations used were significantly superior to the control treatment (water immersion only). The rooting percentage values were 0%, 5%, 8.5%, and 13.67%, respectively (the L.S.D. value was 2.64 at the 5% significance level). As for the two studied genotypes, Aronia and Monogyna, the Monogyna hawthorn genotype was significantly superior to the Aronia genotype with rooting percentages of 8.42% and 5.17%, respectively (the L.S.D. value was 1.42 at the 5% significance level). As for the interaction between the experimental factors, the treatment of the Monogyna genotype cuttings with a concentration of 8000 ppm of IBA was significantly superior to all other interactions and experimental treatments with a rooting percentage of 16.67%. The lowest value for the rooting percentage was in both control treatments for the Aronia and Monogyna genotypes with a rooting percentage of 0%.

These results are consistent with the results of the study conducted by Jamal and Al-Issa (2004) in which the rooted percentage of cuttings reached 20% when treated with IBA at a concentration of 4000 ppm. This result is also close to the result of Hartmann et al. (2010) conducted on hawthorn cuttings, as they achieved a rooting success of 35% by applying IBA at a concentration of 8000 ppm, and NAA at a concentration of 2000 ppm on young hawthorn cuttings.

IV. CONCLUSION

Experimental results showed that it is generally difficult to propagate hawthorn vegetatively and by seeds, but propagating hawthorn by seeds achieved better results than propagating it by cuttings. As for seed propagation, the Monogyna genotype significantly outperformed Aronia genotype in germination percentage, and both treatments with gibberellin and sulfuric acid significantly outperformed the control without any significant differences between the two treatments. The second concentration of the treatment also significantly outperformed the first concentration, and the first concentration in turn significantly outperformed the control. The second soaking period also significantly outperformed the first period in the percentage of germinated hawthorn seeds. In consequence, the treatment of the Monogyna genotype with gibberellin at the second concentration (2000 ppm) and during the second soaking period (48 hours) outperformed all other interactions, with a germination percentage of 73.33%. As for vegetative propagation, the third concentration was significantly superior to the second concentration, which in turn was significantly superior to the first concentration. All concentrations used were significantly superior to the control treatment (water immersion only) in the percentage of rooted cuttings. The hawthorn Monogyna genotype was significantly superior to the Aronia genotype. In general, the treatment of Monogyna genotype cuttings with a concentration of 8000 ppm of IBA was significantly superior to the rest of the treatments. Finally, the alternating stratification (warm and cold) of hawthorn seeds, with treatment with gibberellic acid or sulfuric acid, in addition to using seeds stored in the refrigerator for a full year, played an important and major role in increasing their germination percentage.

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ISSN (online): 2581-3048
Volume 8, Issue 10, pp 39-46, October-2024
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Citation of this Article:

Rasha OBIDEEN, Dr. Amin ALHASAN, Prof. Rida DRAIE. (2024). Effect of Some Treatments on Seed and Vegetative Propagation of Hawthorn (*Crataegus* spp.) in Northwestern Syria. *International Research Journal of Innovations in Engineering and Technology - IRJIET*, 8(10), 39-46. Article DOI https://doi.org/10.47001/IRJIET/2024.810007

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