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Effects of external gibberellin on germination of wall-spray cotoneaster seeds stratified with and without fruit under dry-cold conditions

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Abstract: Wall-spray cotoneaster (*Cotoneaster horizontalis* Decne.) seeds show deep dormancy and have a very low germination rate. Wall-spray cotoneaster seeds were dry cold stratified (+4°C) either in the fruit or removed from the fruit for different periods (0, 30, 60, 90, 120 and 180 days) and then treated with different GA_3 doses (0, 500, 1000 and 2000 ppm) and sown in peat medium in bottom heated (+24°C) trays in the greenhouse in the current study. The effects of GA_3 treatment on germination (emergence) rate, living plant rate (%), plant height (cm) and leaf number were determined. The highest germination rate (35.00%) was obtained from the seeds treated with 2000 ppm GA_3 without cold stratification. These control seeds were kept in fruit between October and May under room temperature. The germination rate in the seeds treated with 1000 ppm GA_3 after stratification in dry cold at +4°C for 60 days in the fruit was only 25.00%. The GA_3 applications did not affect the germination rate of the seeds that were taken out of the fruit and stratified in dry-cold for different periods.

Keywords: Cotoneaster, seed, dry-cold, GA_3, germination

Meyve içinde ve dışında kuru soğukta katlanan yayılıcı dağ muşmulası tohumlarında çimlenme üzerine giberellinin etkisi

Öz: Yayılıcı dağ muşmulası tohumları derin dinlenme gösterir ve çimlenme oranı da çok düşüktür. Bu çalışmada, yayılıcı dağ muşmulası (*Cotoneaster horizontalis* Decne.) tohumları katlanmadan (kontrol) ve 30, 60, 90, 120 ve 180 gün +4°C'de meyve içinde ve meyveden çıkarılarak kuru soğukta katlandıktan sonra 0ppm (kontrol) ile 500, 1000 ve 2000 ppm GA_3 ile muamele edilmiştir. Soğukta katlanarak gibberellin uygulanan tohumlar +24°C'lik alttan ısıtmalı tavalardaki torf ortamına ekilerek çimlenme (çıkış) oranı, yaşayan bitki oranı (%), bitki boyu (cm) ve yaprak sayısı tespit edilmiştir. En yüksek çimlenme oranı %35.00 ile soğukta katlanmadan 2000 ppm GA_3 uygulandıktan sonra ekilen tohumlardan elde edilmiştir. Bu tohumlar oda şartlarında ekim-mayıs arasında meyve içinde bekletilmiştir. Meyve içinde 60 gün +4°C'lik kuru soğukta katlandıktan sonra 1000 ppm GA_3 uygulandıktan sonra ekilen tohumlardaki çimlenme oranı ise %25.00'e kadar çıkabilmiştir. Meyveden çıkarılarak kuru-soğukta farklı sürelerde katlanan tohumlarda çimlenme üzerine GA_3 uygulamaları ise etki etmemiştir.

Anahtar kelimeler: Cotoneaster, çekirdek, kuru-soğuk, GA_3, çimlenme

1. Introduction

Cotoneaster species (*Rosaceae* family) constitute a very important part of ornamental plants in shrub form. They have attractive plant characteristics and are used in landscape planning. *Cotoneaster* plants can be tall or show horizontal or sprayed growth with multi-colored fruits and leaves. Their leaves turn red, orange or yellow in autumn. There are nearly 400 species in the *Cotoneaster*, which includes plants native to temperate climates in Asia. The wall-spreading *Cotoneaster* (*Cotoneaster horizontalis* Decne.), has horizontal branches and grows 50-60 cm height. Although it is native to western China, it is widely used in outdoor arrangements in Türkiye and many countries in Europe due to its blue-green leaves in summer and its dense fruits in winter and fall. It is one of the most valuable shrubs, often used in rock gardens, on roadside slopes, small hillsides and flowering herb gardens. *Cotoneaster* is also considered as a ground cover and used for creating fences, shelter for wild birds and rodents and

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fruiting plants for their feeding. It is good for wind blocking, solitary plant in the landscape by forming abundant flowers, barrier and corridor forming plants for roadsides and area isolation in urban landscapes (Buffin, 2005; Ölmez et al., 2006; Ölmez et al., 2007; Lonnee et al., 2011). It is an attractive landscape plants in all seasons with its plant, flowers and fruits, is easy to maintain, can take different forms with pruning and prefers sunny places all day long. *Cotoneaster* fruits contain one to five seeds (Slabaugh & Shaw, 2008). Cotoneaster seeds have very hard endocarp and seed coats, they physically show deep and double dormancy and the embryo remains physiologically dormant over years (Baskin & Baskin, 2004; Hartmann et al., 2014). According to studies, cold-stratification for up to 24 months may be required for seed propagation of *Cotoneaster* (Bujarska-Borkowska & Suszka, 2019). Hot and/or cold stratification for 2-3 months and scarification with intense sulfuric acid before cold stratification is required for germination of cotoneaster seeds (Tilki, 2013). Aygün et al. (2011) stated that germination rate was just 41.00% of *Cotoneaster horizontalis* seeds stratified at 60 days in wet and cold conditions. They also found that gibberellins and sulfuric acids did not affect the germination rate.

Seed propagation is becoming increasingly popular in ornamental plants. Because propagation by cuttings is much more expensive. There are differences between plant species in terms of seed viability and the storage period of seeds can be short. It is very important for seedling producers that ornamental plants used in landscaping can be propagated by seeds and intensively. It is important to eliminate dormancy and germination prevent substances in the seed propagated plants. Hard and thick seed coats also prevent seed germination. Many factors in the seed endosperm, embryo or coats are effective on germination. They may inhibit germination and affect seed development, gas exchange and water mobility in different ways. To eliminate these factors scarification, cold storage, hot or cold-water applications, acid treatment and plant growth regulators can be used (Karam et al., 2001; Persson et al., 2006; Slabaugh et al., 2008; Liu et al., 2010; Lonnee et al., 2011; Nadeem et al., 2013; Tilki, 2013; Hartmann et al., 2014). While some *Cotoneaster* seeds can show high germination under cold and white light (Tilki, 2013), pre-sowing gibberellic acid treatments gave similar results to the light effect. In addition, since *Cotoneaster* seeds have a hard shell, keeping them in a cool environment and treatment with surface abrasives may affect the germination rate. According to Tilki (2013), some researchers obtained 67.00% germination rate after stratified *Cotoneaster horizontalis* seeds in cold for 11 months and treating them with sulfuric acid. Researchers reported that *Cotoneaster* seeds have a long dormancy with low germination rate. Their seeds have also double dormancy due to hard and impermeable seed coats and embryo physiology (Aygün et al., 2011; Hartman et al., 2014; Zare, 2019). *Cotoneaster* fruits for seed collection can be collected in early fall and after leaf fall by hand scraping or shaking. Fruit firmness and color can be used for maturity (Slabaugh & Shaw, 2008). Hartmann et al. (2014) stated that *Cotoneaster* species have fleshy fruits and the seed is preserved in dry fruits. Seeds of some *Cotoneaster* species can emerge without resting if they are stratified for 115 days and can germinate in a warm environment. It is stated that even in seeds stratified for about three months, there is conditional dormancy and they can germinate better at temperatures of 15-25°C. In some studies, it was also found that *Cotoneaster* seeds that were not stratified or kept in the cold for 60 days were not affected by the ambient temperature and did not germinate. It is also reported that when *Cotoneaster* seeds are kept in sulfuric acid for 1.5 hours and then stratified in a humid environment at +4°C for 3-4 months, the negative effects of impermeable shells on germination may decrease (Hartmann et al., 2014). According to Zare (2019), *Cotoneaster nummularioides* seed germination was 18.30% after sulfuric acid-potassium nitrate treatment and 4 months cold stratification. He revealed that chilling without scarification of the cotoneaster seeds had no germination.

In this study, the effects of dry-cold stratification of wall-spray cotoneaster seeds stratified with or without fruits and gibberellic acid applications on germination were investigated.

2. Materials and Methods

2.1. Obtaining seeds and cold stratification

The seeds of the wall-spray cotoneaster used in the experiment were taken from the fruits of *Cotoneaster horizontalis* Decne. plants. The fruits collected in the first week of November. The large seeds were selected after separation from the pericarp and subjected to a floating test and kept at room temperature for 10 days until they reached 10% moisture content. The 100

seeds were filled into perforated and zip lock bags and kept in cold storage at +4°C for different periods (30, 60, 90 and 120 days). Control and cold stratified seeds were kept in the dark room conditions until sowing. The seeds that will be cold-stratified in the fruit and at +4 °C were transferred to cold storage by filling them into zip lock and perforated bags without removing them from the fruit, and the seeds that will not be cold-stratified in the fruit were kept under dark room conditions until sowing. The fruits that were kept in the cold storage were removed and the seeds were removed from the fruits, washed, dried and filled in perforated and locked bags with holes so that 100 seeds per bag and kept in room conditions and in a dark environment until sowing.

2.2. Gibberellic acid (GA_3) treatment

The seeds of wall-spray cotoneaster, which were removed from the fruit and stratified in dry-cold conditions, were kept in 500, 1000 and 2000 ppm GA_3 solution for 24 hours before sowing. The seeds of the control group (0 ppm), which were not treated with GA_3, were kept in pure water for 24 hours. After treatment, the seeds removed from the GA_3 solution were sown in peat in the greenhouse and in trays with a constant bottom heating temperature of +24°C. The seeds were removed from the fruits after cold stratification. They kept in room conditions for 10 days until they reached 10% humidity. Then they filled in perforated and zip locked bags and kept in 500, 1000 and 2000 ppm GA_3 solution for 24 hours. The seeds of the control (0 ppm GA_3) were kept in pure water for 24 hours. GA_3-treated seeds were sown in trays had peatmoss under greenhouse with a bottom heating temperature of +24°C at the end of April. Fungicides were applied weekly against fungal diseases in the seed sowing trays, irrigation was applied, and the environment was humidified by controlling the misting system with a "leaf wetness sensor" so that the ambient humidity was above 60% after emergence started (Klingaman, 2015).

2.3. Determination of germination rate (%) and transplanting the seedlings

For the beginning of germination in the seeds sown in peat in bottom heated trays under greenhouse, the first date when the cotyledon leaves started to emerge on the peat surface was taken into consideration. After this date, weekly germination (emergence) was determined by counting the seeds that emerged weekly for 6 weeks and total germination rate (%) was determined over all emerged seedlings. At the end of six weeks (Pittcock, 2015), emergence was completed and when the plantlets reached the four-leaf stage, they were removed from the trays and transplanted to the P9 plastic pots (9x9x9 cm dimensions) including garden soil+barnyard manure+sand (1:1:1, v/v) at the end of September. The plants were maintained in the greenhouse for 6 months and the survival rate was determined. Plant height and number of leaves were also determined weekly.

2.4. Statistical analyses

The experiment, which was established in 3 replications with 100 seeds in each replicate in bottom-heated (+24°C) trays in the greenhouse, was designed according to the "split plots experimental design divided by blocks of coincidence". Here, the stratification type (inside the fruit, outside the fruit) was randomly allocated to the main plots, cold storage period (0, 30, 60, 90, 120 and 180 days) to the sub-plots and GA_3 doses (0, 500, 1000 and 2000 ppm) to the sub-sub-plots. Angle ($\arcsin\sqrt{x}$) transformation was applied to the "%" data obtained from the experiment and statistical analyses were performed on these data. Statistical analyses of the data obtained from the experiment were performed using SPSS V25.0 software (SPSS Inc., USA) based on 3-factor analysis of variance (ANOVA). Differences between the mean values were evaluated by Duncan Multiple Range Test. In the statistical evaluation of the results, the significance level between the differences was expressed as significant at $P<0.05$ level.

3. Results and Discussion

The results of the effects of stratification type, duration and GA_3 doses on germination and survival rates (%), seedling height and number of leaves in wall-spread cotoneaster seeds are given in Table 1. According to the findings, the effects of all factors on the criteria examined in the seeds of spreading cotoneaster kept in the cold with the fruit or removed from the fruit were found to be significant. The best germination rate (35.00 %) observed from 2000 ppm GA_3 applied seeds which were not cold stratified but kept inside the fruits under room conditions between October and May till sowing. It was determined that the germination in the seeds which were kept in dry cold at +4°C for 60 days without removing from the fruit and then treated with 1000 ppm GA_3 could reach up to 25.00% (Table 1).

As the stratification period in fruit increased, the efficiency of GA_3 application decreased. In the seeds of wall-spray cotoneaster that were kept in fruit and under room conditions until planting, i.e. that were not treated with cold, 0 ppm GA_3 application showed 27.33% germination success, while there was no germination in the 500 ppm GA_3 application. The germination which was 17.33% at 1000 ppm increased to 35% at 2000 ppm. This situation proves the research results indicating that the germination in wall-spray cotoneaster seeds is irregular. In wall-spray cotoneaster seeds, the efficiency of GA_3 application decreased as the stratification period in the fruit and under cold increased, only the seeds cold-stratified for 30 and 180 days and no GA_3 applied gave germination results (16.33% and 22.67%, respectively) and 1000 ppm GA_3 application showed 25.00% germination in cold-stratified seeds for 60 days. In cotoneaster seeds kept in the fruit and in the cold for 120 days, only 11.66% germination was achieved with 500 ppm GA_3 application. The remaining seeds kept in the fruit and in the cold and applied with GA_3 did not germinate (Table 1). After being removed from the fruit and cold stratified, the germination in the seeds to which GA_3 was applied did not increase, and only the control groups germinated. Accordingly, the germination in the seeds that were not kept in the cold outside the fruit and that were not applied GA_3 was 5.33%. While the germination in the seeds that were cold stratified for 30 days outside the fruit was 3.33% at 0 ppm. The seeds that were cold stratified for 60 and 120 days without any GA_3 application showed a germination success of 2.33%. The seeds that were cold stratified for 180 days outside the fruit and that were not applied GA_3 had the lowest germination rate with 1.67%. It was also determined that most of the seeds that were kept in the cold outside the fruit and for different periods and applied GA_3 did not germinate at all (Table 1). When the average values were taken into consideration, it was determined that the seeds that were not stratified reached the highest values in terms of germination, survival rate, plant height and leaf number in terms of stratification period. It was determined that 60 days of cold stratification came second in germination and survival rates, and 120 days of cold storage came second in plant height and leaf number (Figure 1a). When GA_3 doses were considered, 0 ppm GA_3 application as control dose came first in all criteria, while 1000 ppm GA_3 followed, but the results obtained were very low (Figure 1b). In the stratification type, it was determined that germination and survival rates and plant height were better in seeds stratified in the fruit, while leaf number gave similar results in both stratification types (Figure 1c).

According to the stratification type, duration and GA_3 applications, all plants that germinated and emerged to the soil surface and were transplanted after the formation of true leaves had the same survival rate. Accordingly, the highest survival rate, as in the germination rate, was obtained from the seeds kept in the fruit and kept in room conditions until planting (October-May) and to which 0 ppm GA_3 was applied, with 35.00%. The lowest survival rate, with 1.67%, was obtained from the seeds kept outside the fruit and kept dry-cold in +4°C for 180 days and again to which 0 ppm GA_3 was applied. The highest value in terms of plant height was determined as 25.67 cm in the plants that grew from the seeds kept outside the fruit and kept in cold for 60 days and not to which GA_3 was applied. The number of leaves reached the highest value with 28.33 in the seeds kept in the fruit and kept in room conditions and to which 2000 ppm GA_3 was applied (Table 1). In our study, it was found that the hard and impermeable shells in the seeds of the wall spreading cotoneaster had a negative effect on germination, and the germination success was very low even after the seeds were stratified in the cold and GA_3 was applied. The fact that the germination rates obtained from previous germination experiments involving chemical scarification were slightly higher than the results of our study. This may be since we did not erode the hard coats of the seeds with acids. Therefore, it may be necessary to use scarified chemicals together with GA_3 application in addition to dry or moist cold stratification in spreading cotoneaster. Because species in the genus *Cotoneaster* produce seeds with hard and impermeable shells, and the embryo in these seeds has double dormancy due to its physiological characteristics. Seed germination of this species can be increased by wet cold stratification, scarify with acid and soaking in hot water (Slabaugh & Shaw, 2008; Lonnee et al., 2011). The addition of some commercial activators to wet and cold soaking can also increase germination (Hartmann et al., 2014). The duration of pre-treatments affecting germination in seeds may vary according to plant species, seed type and the environment. Region and years in which the seeds were taken due to shell thickness differences and the degree of resting in the

embryo is also important. Because some researchers applied concentrated sulfuric acid to the seeds of spreading cotoneaster for 90 min and kept at +2°C cold and wet. On the other hand, germination in cotoneaster seeds is inconsistent and rapid germination is very difficult to achieve (Tilki, 2013). Slabaugh & Shaw (2008) showed that increasing the germination rate in cotoneaster seeds depends on soaking them in sulfuric acid for 3 hours and then keeping them in hot water at +27°C for 48 hours. Zare (2019) resolved the dormancy problem of Cotoneaster seeds under the combined effects of scratches shell and cold stratification.

The germination rate in cotoneaster seeds varied depending on the activators used. Bujarska-Borkowska & Suszka (2019) obtained 48% germination on stratified the wall-spray cotoneaster seeds in hot-cold environments and scarified them with sulfuric acid. In our experiment, where scarifying was not used, the germination rate was up to 35.00% when 2000 ppm GA_3 was applied to the seeds that were not stored in the fruit and in the cold. On the other hand, Slabaugh & Shaw (2008) germinated wall-spray cotoneasters in sand and found that the seeds that were applied at 30°C during the day and 20°C at night germinated at a rate of 30.00% in 100 days. Zare (2019) resolved the problem dormancy Cotoneaster under the combined effects of scratches shell and cold stratification, but the highest germination rate was just 18.20% and lower than our findings. However, Aygün et al. (2011) found 41.00% germination rate of Cotoneaster horizontalis seeds and this may cause from cold and wet stratification.

Table 1. Variation of germination rate (%), survival rate (%), plant height (cm) and number of leaves in wall-spray cotoneaster seeds stored with or without fruit and kept in dry cold according to stratification duration and GA_3 application.

Stratification type	Stratification day	GA_3 (ppm)	Germination rate (%)	Mortality rate (%)	Plant height (cm)	Leaf number
With fruit	0	0	27.33 b	27.33 b	21.66 bc	16.33 cde
		500	0.00 e	0.00 e	0.00 f	0.00 f
		1000	17.33 cd	17.33 cd	12.33 e	14.66 de
		2000	35.00 a	35.00 a	16.00 de	28.33 a
	30	0	16.33 cd	16.33 cd	16.67 de	19.50 c
		500	0.00 e	0.00 e	0.00 f	0.00 f
		1000	0.00 e	0.00 e	0.00 f	0.00 f
		2000	0.00 e	0.00 e	0.00 f	0.00 f
	60	0	0.00 e	0.00 e	0.00 f	0.00 f
		500	0.00 e	0.00 e	0.00 f	0.00 f
		1000	25.00 bc	25.00 bc	21.67 bc	18.00 cd
		2000	0.00 e	0.00 e	0.00 f	0.00 f
	120	0	0.00 e	0.00 e	0.00 f	0.00 f
		500	11.66 d	11.66 d	19.33 cd	19.33 c
		1000	0.00 e	0.00 e	0.00 f	0.00 f
		2000	0.00 e	0.00 e	0.00 f	0.00 f
	180	0	22.67 bc	22.67 bc	18.33 d	18.33 cd
		500	0.00 e	0.00 e	0.00 f	0.00 f
		1000	0.00 e	0.00 e	0.00 f	0.00 f
		2000	0.00 e	0.00 e	0.00 f	0.00 f
Without fruit	0	0	5.33 de	5.33 de	21.67 bc	20.67 bc
		500	0.00 e	0.00 e	0.00 f	0.00 f
		1000	0.00 e	0.00 e	0.00 f	0.00 f
		2000	0.00 e	0.00 e	0.00 f	0.00 f
	30	0	3.33 e	3.33 e	22.67 bc	23.50 bc
		500	0.00 e	0.00 e	0.00 f	0.00 f
		1000	0.00 e	0.00 e	0.00 f	0.00 f
		2000	0.00 e	0.00 e	0.00 f	0.00 f
	60	0	2.33 e	2.33 e	25.67 a	24.00 b
		500	0.00 e	0.00 e	0.00 f	0.00 f
		1000	0.00 e	0.00 e	0.00 f	0.00 f
		2000	0.00 e	0.00 e	0.00 f	0.00 f
	120	0	2.33 e	2.33 e	19.33 cd	21.50 bc
		500	0.00 e	0.00 e	0.00 f	0.00 f
		1000	0.00 e	0.00 e	0.00 f	0.00 f
		2000	0.00 e	0.00 e	0.00 f	0.00 f
	180	0	1.67 e	1.67 e	14.33 d	14.00 de
		500	0.00 e	0.00 e	0.00 f	0.00 f
		1000	0.00 e	0.00 e	0.00 f	0.00 f
		2000	0.00 e	0.00 e	0.00 f	0.00 f

* There is no statistical difference between the data shown with the same letter in the columns, $p<0.05$.

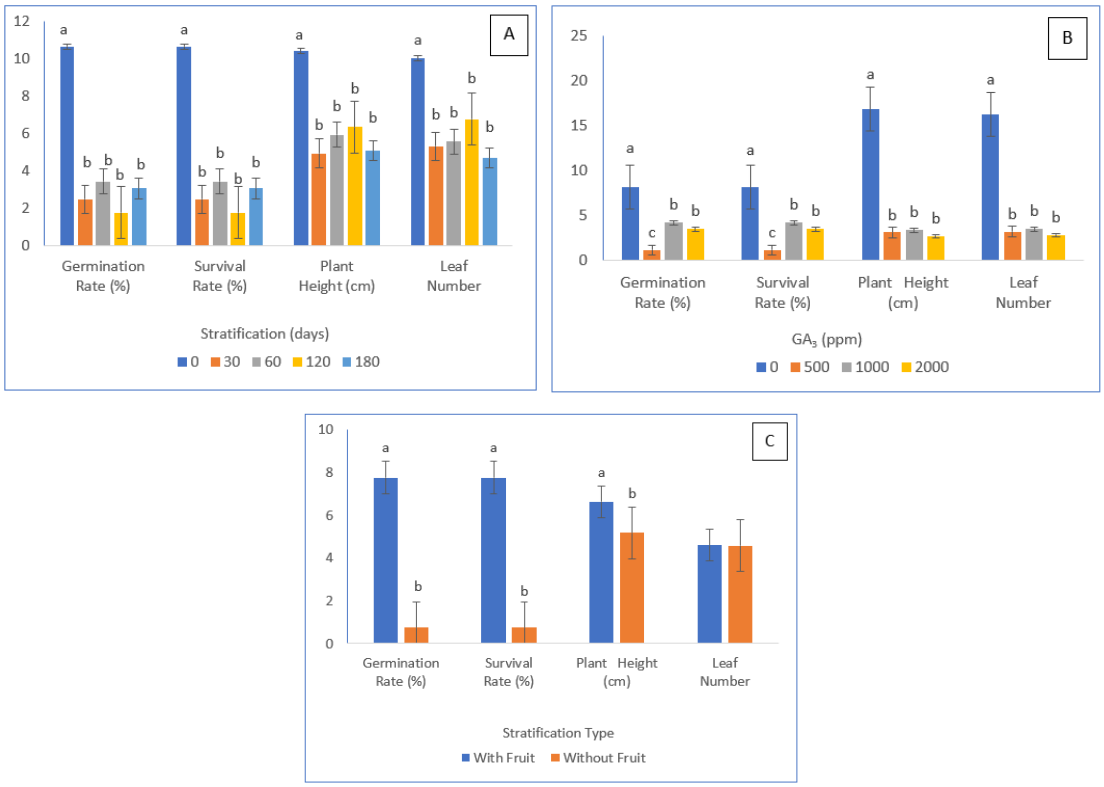

Figure 1. Changes of germination rate (%), survival rate (%), plant height (cm) and leaf number in wall-spray cotoneaster seeds stored in dry-cold with or without fruit, according to stratification period (A), GA_3 doses (B) and stratification type (C).

As can be seen, the germination rate in wall-spray cotoneasters is unstable and inconsistent. The low germination rate obtained in our study may also be due to environmental conditions, plant growth and development, and the characteristics of the place where the seeds were collected. Because environmental conditions can affect the end of seed dormancy and germination capacity. The most promising way to understand this phenomenon is to examine not only morphological characters but also physiological diversity of seeds together with plant taxonomy, ecology and geographical factors (Hartmann et al., 2014). Important factors include photoperiod, temperature and light intensity in seed maturation. Germination rates in species of the *Cotoneaster* genus may vary depending on the species, and it is stated that stratification of seeds in cold-hot conditions, abrasion of the seed coat, soaking in hot water and light in the germination environment may also be effective (Slabaugh and Shaw 2008; Lonnee et al., 2011; Tilki, 2013; Hartmann et al., 2014). Cotoneaster seeds have thick and very hard shells and stratification and chemical abrasion are carried out in cold and humid environments. In addition, double dormancy and excessive germination inhibitory substances prevent their germination (Hartman et al., 2014). On the other hand, some Cotoneaster species can germinate in light and GA_3 treatment can create a light effect (Tilki, 2013). This situation is also stated by Slabaugh & Shaw (2008) and they state that the ambient temperature should be high for germination. In this experiment, germination success may have decreased because the seeds were kept in dry cold. However, the germination rate is low in dispersing cotoneasters (Slabaugh & Shaw, 2008) and the pretreatments for germination, their duration, the thickness of the seed coats and the degree of embryonic dormancy should also be taken into account (Liu et al., 2010; Hartmann et al., 2014).

4. Conclusion

Wall-spray cotoneaster (*Cotoneaster horizantalis* Decne.) is an ornamental plant in the *Rosaceae* family and widely used in outdoor arrangements. In recent years, it has been necessary to study this species generatively and vegetatively in order to respond to the increasing plant demands. For this purpose, the effectiveness of GA_3 treatment in addition to dry cold stratification of seeds in and out of the fruit on germination and seedling quality in seed propagation, which is a rapid and intensive propagation method, was investigated. The highest germination rate in wall-

spray cotoneaster seeds was obtained with 35.00% in those kept in the fruit, not treated with cold but treated with 2000 ppm GA_3. The germination rate in all other applications remained below this value, and in fact, there was no germination in most applications. The germination and survival rate of seeds that were not treated with GA_3 were higher than the others among the seeds that were stratified both with the fruit and by removing them from the fruit. The survival rate in germinated and transplanted plants gave the same result in all applications. According to the average values, no or 60 days stratification, no GA_3 or and 1000 ppm GA_3 treatment and stratification the seed inside the fruits has come to the fore. All the results showed that the germination success of the wall-spray cotoneaster, especially without using different scarification of the hard and impermeable shells, may be very low. Therefore, after treatment with some plant growth regulators to break the dormancy in spreading cotoneaster (*Cotoneaster horizontalis* Decne.) seeds, several applications such as seed shell corrosive chemicals, light, germination medium ingredients, pH and EC value, temperature and humidity should be investigated.

Conflict of interest

The authors declare no conflicts of interest.

Authorship contribution statement

H.Ç: Methodology, Planning and conducting the experiment, performing statistical analyses, writing-original draft preparation, writing- reviewing and editing. Ö.K.K: Conducting the experiment, taking observations and measurements.

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