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Effects of seed sowing methods on emergence and seedling growth of some *Rhododendron* species

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ABSTRACT
This study was conducted to determine the effects of different seed sowing methods on emergence rate, densities of seedling emergence and seedling growth of five *Rhododendron* species (*R. ponticum* L., *R. luteum* Sweet, *R. caucasicum* Pallas, *R. simirnowii* Trautv and *R. ungernii* Trautv). Four different sowing methods were tested, manual sowing in a row (control), mixing with plant agar, mixing with Murashige and Skoog Basal medium (MS) and mixing with stream sand. The highest seed emergence rates were obtained by manual sowing in a row (control) with *R. ponticum* species (76 %) followed by *R. luteum* (66.6 %), *R. smirnowii* (55.33 %), *R. ungernii* (24 %) and *R. caucasicum* (15.33 %), respectively. Similarly, the values closest to ideal seed distribution for homogenous seedling density were obtained by hand-sowing in row method. The highest seedling height was obtained in MS medium with *R. ponticum* (2.11 cm), followed by *R. luteum* (3.08 cm), *R. smirnowii* (4.07 cm), and *R. ungernii* (1.39 cm), while the effect of the seeding method on seedling growth of *R. caucasicum* species was not significant. To conclude, the best homogeneous distribution of seedling emergence densities of *Rhododendron* species was obtained by a controlled manual seeding method. However, the seed sowing with MS mixing increased significantly seedling growth in all *Rhododendron* species.

Keywords:
Rhododendron
Seed
Sowing medium
MS
Agar
Sand

Farklı tohum ekim yöntemlerinin orman gülü (*Rhododendron* ssp.) tohumlarının çıkışları ve fide gelişimi üzerine etkileri

ÖZET
Bu araştırma, orman gülü türlerinin (*Rhododednron ponticum* L., *R. luteum* Sweet, *R. caucasicum* Pallas, *R. simirnovii* Trautv ve *R. ungernii* Trautv) tohumlarınına uygulanan farklı tohum ekim yöntemlerinin çıkış süresi, fide çıkış sıklıkları ve bitki büyümesine olan etkilerini belirlemek amacıyla yürütülmüştür. Ekim yöntemi olarak, elle sıraya ekme (kontrol), plant agar ile karıştırma, MS ile karıştırma ve dere kumu ile karıştırma olmak üzere dört farklı tohum ekim yöntemi denenmiştir. Araştırmamız sonucunda, en yüksek tohum çıkışı oranları *R. ponticum* türünde % 76, *R. luteum* % 66.6, *R. smirnovii* % 55.33, *R. ungernii* % 24 ve *R. caucasicum* % 15.33 olarak elle sıraya ekme (kontrol) uygulamasından elde edilmiştir. Benzer şekilde, homojen fide sıklığı bakımından da ideal tohum dağılımına en yakın değerler elle sıraya ekme yönteminden elde edilmiştir. Fide boylarında en iyi sonuç *R. ponticum* (2.11 cm), *R. luteum* (3.08 cm), *R. smirnovii* (4.07 cm), *R. ungernii* (1.39 cm) türlerinde MS ortamından alınmışken, *R. caucasicum* türünde tohum ekim yönteminin fide gelişimi üzerine etkisi olmamıştır. Sonuç olarak orman gülü türlerinin generatif yöntemle çoğaltılmasında fide çıkış sıklıklarının homejen dağılması için elle kontrollü ekim yönteminin en iyi sonucu verdiği halde, MS ile karıştırarak yapılan tohum ekim yönteminin tüm *Rhododendron* türlerinde fide gelişimini dikkat çekici bir oranda arttırdığı belirlenmiştir.

Anahtar Sözcükler:
Rhododendron
Tohum
Ekim ortamı
MS
Agar
Kum

1. Introduction

Rhododendron contains quite different sizes and shapes including low height ground cover to medium-sized shrub or tree. Some cultivars form a rounded habitat, while others may be in the form of an open shrub or an upright growing tree (Cullen, 2005; Shen et al., 2015; Francon et al., 2017; Li et al., 2018). Some species are deciduous, while others are evergreen. *Rhododendron* species have a wide range of use due to their unique characteristics. These plants can be used as an accent plant, especially in outdoor landscape planning, as border plants alone or along with other shrubs, and tall ones as curtain plants. Natural and cultivated *Rhododendron* species, and cultivars obtained by various breeding methods decorate the gardens in many countries with their showy flowers (Hay et al., 2006; Weia et al., 2018).

Rhododendron taxa, a precious ornamental plant, can be reproduced by generative or vegetative methods similar to many other cultivated plants. *Rhododendron* taxa in nature, which have high germination ability and produce many seeds depending on the species, are produced with their seeds and underground stems. The seedlings can be propagated using modern techniques such as cutting, grafting, layering methods and tissue culture (Altun, 2011; Elmongy et al., 2018).

Cross-pollination occurs in *Rhododendron* due to its hermaphrodite flower structure. Pollination is generally done by bees. However, recent reports indicated that some insects, birds and squirrel breeds are also effective in pollination (Georgian et al., 2015). Therefore, intermediate species and their hybrid individuals and pure species can be encountered in the natural flora (Milne et al., 1999; Altun and Çelik, 2016; Zhang et al., 2017). Reproduction with seed, easy and natural method, is a suitable method to obtain new hybrid plants in breeding studies or obtain rootstocks in grafting studies.

The fruit of *Rhododendron* is a septicite capsule (Yıldız and Aktoklu, 2010). The quite small seeds in the fruits can be broad-winged, narrow-winged or without wings. The average 1000-seed weight of *Rhododendron* species in Turkey was reported between 0.142 g and 0.067 g (Altun, 2011). The seeds do not need any chilling requirements due to the higher germination and emergence rates, depending upon species (Ryabova and Zueva, 1993; Sakharova, 1993; Arocha et al., 1999; Glenn et al., 1999; Basnet, 2005; Vologdina, 2006; Altun, 2011). Seeds need light to germinate (Vologdina, 2006); therefore, seeds must be sown superficially during planting and not be covered.

The main problems in generative reproduction of small seed plants are irregular emergence, seed losses during planting and seedling losses in transplanting. *Rhododendron*, which has very small seeds, cannot be planted homogenously in seed reproduction and a homogeneous emergence cannot be obtained in seedling emergence. Seedling losses occur in very dense seedlings during transplanting. The classical manual spreading method is used to sow *Rhododendron* seeds despite mixing the seeds with various materials such as sand or sawdust to minimize the losses in many plant species with small seeds and obtain a homogeneous emergence. The effects of many factors such as photoperiod (Li et al., 2012; Chen and Sheng, 2017), different mediums, temperatures, seeding depths (Vologdina, 2006; Jin et al., 2007), seed sowing times (Tewari and Tewari, 2019), various stress factors (Chang et al., 2015; Pan et al., 2018), pre-treatments (Vipasha and Kaler, 2018), and seed preservation temperatures and durations (Juan et al., 2014) were investigated on the germination and emergence of *Rhododendron* seeds. However, the studies conducted to determine the effects of different seed sowing methods on the emergence rates and seedling emergence homogeneity of *Rhododendron* seeds are lacking in the literature. In the current study, different alternative seed sowing methods were tested in *Rhododendron* reproduction. This study was conducted to investigate the effects of different sowing methods on seed emergence rate, homogeneity of the interrow and intra row seedling distances and seedling growth of five natural *Rhododendron* species collected from the Black Sea Region of Turkey.

2. Materials and Methods

2.1. Plant material

The seeds of five *Rhododendron* species (*R. luteum* Sweet, *R. ponticum* L., *R. ungernii* Trautv, *R. simirnowii* Trautv, *R. caucasicum* Pallas), collected from natural distribution areas in Artvin province and district boundaries, were used as plant material of the experiment (Table 1).

Table 1. Information of the locations where *Rhododendron* seeds collected
Çizelge 1. Rhododendron tohumlarının alındıkları yerlere ait veriler

Species	Altitude	Coordinate	Location
R. ponticum L.	1652 m	41°08‟914 K /41°46‟205 D	Artvin
R. luteum Sweet	1671 m	41°10‟424 K/ 42°18‟943 D	Şavşat
R. simirnowii Trautv.	1982 m	40°14‟743 K /41°35‟699 D	Murgul
R. ungernii Trautv.	1249 m	41°18‟870 K/ 41°53‟495 D	Borçka
R. caucasicum Pallas	2289 m	41°43‟450 K/ 42°28‟376 D	Şavşat

2.2.Collection of seeds and preparation for the sowing

The seeds were pooled when the capsules ripened and turned brown, before opening and dispersing the seeds, stored into dry pouch paper (Altun, 2011), and transported to the laboratory. The seed capsules were placed in the containers in air-dry room conditions at the laboratory and waited until the capsules were totally opened. The seeds were passed through a 0.850 mm mesh sieve to clean the materials, such as plant parts in the seeds that came from the opened capsules. The cleaned seeds were placed in dry glass jars for storage and kept at 22 ± 2 ℃ under laboratory conditions until the emergence tests commenced.

2.3. Experimental design

Sterile acidic peat (pH=3.5-4.5) was used as a germination medium. Four different seed sowing methods were applied, namely control, Plant agar medium (Duchefa Biochemie), Murashige and Skoog Basal medium (MS) (Sigma) and mixing with stream sand. The classical manual sowing method was applied in the control treatment. The experiments were conducted in four climate chambers (75 x 65 x 60 cm) in the laboratory of the Horticulture Department of Agriculture Faculty in Kırsehir Ahi Evran University. The experimental design was randomized plots with three replications, and each consists of 50 seeds.

2.4. Seed sowing

The seeds were sown in foam containers (35 x 50 cm) placed in climate chambers. The foam containers were filled with sterile acidic peat for each treatment and a smooth surface was obtained by leveling the container surface. Peat was moistened by a hand sprayer and seeds were sown superficially by the methods mentioned above.
The seeds were sown as 4 cm between species and 2 cm between replicates. The containers were slightly irrigated with a hand sprayer after sowing the seeds, and the containers were placed inside the climate chamber. The temperature and humidity values were recorded hourly from November 3, 2016 till December 28, 2016 and seed emergences were counted. The average temperature during emerging was 18.12 °C, and the average humidity was 64.58 %.

The seeds in the control group were placed on small papers and evenly distributed on the peat surface by slightly tapping them In the sowing method with stream sand, 50 seeds were mixed with 5 cc of stream sand. This mixture was equally transferred to the peat surface over the V-shaped cardboard. In the MS medium (Murashige and Skoog, 1962) and Plant Agar (Sağlam, 2009) mixing method, 30 ml substrate was transferred into paper cups. The seeds were added into the paper cups and mixed until obtaining a homogeneous mixture. The nutrient medium + seed mixture was placed on aluminum foil as 30 cm thin strips then, transferred over the peat using aluminum foil.

2.5. Seed emergence trials

The seed emergence was controlled regularly every seven days from seed sowing (Hay et al., 2006). Emerging seeds were counted with a magnifying glass and the data were routinely recorded on the seed emergence sheet. Counting was continued until the number of emerged seeds became constant. The seed emergence rates were calculated in percent.

2.6.Determination of the planting methods effect on seedling density

The distance among seedlings was measured by a caliper and values were obtained after the seed emergence rate became constant. These values were noted on a graph paper and recorded. The seedling locations marked on graph paper were transferred to the computer and schematized using the AutoCAD software. The seedling emergence density was determined visually by comparing the ideal seedling emergence distribution with the seedling emergence schematized by the AutoCAD software.

2.7. Measuring of seedling height

Six months after the seed sowing seedlings were removed, the height (cm) of each seedling was measured using a ruler and average values were calculated.

2.8. Statistical analysis

SPSS 16.0 software was used for statistical analysis. Since the emergence rate values were calculated as percent, the data were normalized using the "ArcSin" transformation. The data obtained were subjected to one-way analysis of variance. The mean values for the treatments were compared by Duncan Multiple Comparison Test. The comparisons and letterings were carried out separately for the species. However, all results were compiled and presented in a single table.

3. Results and Discussion

3.1. Seed emergence rates

The first emergence was observed nine days after sowing in *R. luteum* and *R. caucasicum* species in the MS environment. The first emergence in the control group was observed 13 days after sowing in *R. ponticum* and *R. cacucasicum* species. Similar to the control group, the first emergence in the plant agar group was observed 13 days after sowing in *R. luteum*, *R. caucasicum* and *R. ponticum*, while the first emergence in river sand was observed 18 days after sowing in *R. luteum* and *R. ponticum* species.

The effect of sowing methods on seed emergence of *R. ponticum* and *R. caucasicum* species was not statistically significant (p>0.05), and the seed emergence rates were recorded as 42.66-76.00 and 8.66-15.33 %, respectively (Table 2). In contrast, the effects of sowing methods on seed emergence rates of *R. luteum*, *R. smirnowii* and *R. ungernii* species were statistically significant. The highest emergence rate (66.6 %) of *R. luteum* variety was obtained in the control method (Table 2). Agar (51.33 %) and MS (50.00 %) mediums were included in the same statistical group, while the lowest seed emergence rate (24.66 %) was obtained from the seeds sown by mixing with the stream sand. The highest emergence rate (55.33 %) of *R. smirnowii* seeds was obtained in the control method, followed by the MS medium with an emergence rate of 50.00 %. The stream sand (15.33 %) and agar (14.00 %) methods, which had lower emergence rates than those of control and MS methods, were statistically placed in the same group. The highest emergence rate (24.00 %) of *R. ungernii* seeds was obtained in the control method, and the emergence rates of other methods were relatively low compared to the control method.

Table 2. Effects of treatments on seed emergence rate
Çizelge 2. Uygulamaların tohum çıkış oranına etkisi

Species	Treatments Control (%)	Sand (%)	Agar (%)	MS (%)	SEM	P values
R. ponticum	76.00	51.33	45.33	42.66	5.311	0.091
R. luteum	66.66 a**	24.66 c	51.33 b	50.00 b	4.938	0.001
R. smirnowii	55.33 a**	15.33 b	14.00 b	50.00 a	6.451	0.004
R.ungernii	24.00 a	12.66 b	10.66 b	10.66 b	2.061	0.029
R.caucasicum	15.33	8.66	13.33	14.66	1.424	0.382

* The difference between the averages in the same line and starting with the same letter is statistically insignificant according to Duncan Multiple Comparison Test (P<0.05).

The seed emergence rates may vary depending on many factors such as plant type, location of plant growth, time of seed collection and storage conditions. The highest seed emergence rate in all *Rhododendron* species examined in this study was obtained by manual sowing (control) treatment. Vologdina (2006) reported that seeds of *Rhododendron dauricum* L., *R. mucronulatum* Turcz. and *R. sichotense* Pojark species collected from natural populations are light-sensitive and the emergence rate of all species varied between 73 and 90 %. The highest emergence rate in the control method can be explained by the higher light intensity in the seed bed under the control condition during the germination stage. The lowest seed emergence rate in *R. ponticum* species was obtained in the MS treatment, while in *R. luteum* and *R. caucasicum* species by mixing with stream sand treatment. The emergence rates of *R. fortunei* species in leaf rot + sawdust dust and leaf rot + algae mediums were 38.6 and 38.2 % respectively (Jin et al., 2007). The germination of *R. luteum* was reported as 71.0 % (Sakharova, 1993). The seed emergence rate using manual sowing in row method in winter months and under unheated greenhouse conditions was determined as 78.50 % in *R. poticum*, 76.00 % in *R. luteum*, 64.75 % in *R. smirnowii*, 57.25 % in *R. ungernii* and 55.50 % in *R. caucasicum* species (Altun and Çelik, 2016). The findings on seed emergence were in line with the findings of other researchers, while lower emergence rates were obtained in *R. ungernii* and *R. caucasicum* species compared to the literature. The seed emergence rates may vary between species. Compared to previous reports, the lower emergence rates of both *Rhododendron* species may be related to the genetic characteristics of *Rhododendron* species and their seed qualities. The climate in the seed sampling period was rather extreme than usual, and the seed of many *Rhododendron* species was not sufficiently ripened before the winter (personal observation). Therefore, the two *Rododendron* species' seeds in this study may have been harvested before reaching the the generative maturity.

3.2. Effect of seed sowing methods on seedling density

There were differences between the number of seed emergences in the replications of *Rhododendron* species used. Some of the seeds sown in the replicates did not emerge. Therefore, the emergence rate could not be statistically compared with the ideal emergence density. However, the data were transferred to the digital environment to enable a visual comparison and presented with the ideal seed distribution (ISD). The ideal seed distribution expected was shown schematically next to each treatment container and the emerging seed comparisons were made accordingly.

The seedling emergence locations in the control method transferred to the Autocad software were visually compared to determine the closest seed distribution to the ideal seed distribution (Figure 1). The closest result to the ideal seed distribution in the control group occurred in *R. ponticum* and *R. luteum*. The *R. simirnowii* was the third variety, while the least similar seed distribution was obtained in *R. caucasicum* and *R. ungernii* species. The best seed emergence distribution pattern in mixing with river sand method was obtained in *R. ponticum* species, followed by *R. luteum*, *R.ungernii*, *R. simirnowii* and *R. caucasicum* species, respectively (Figure 2).

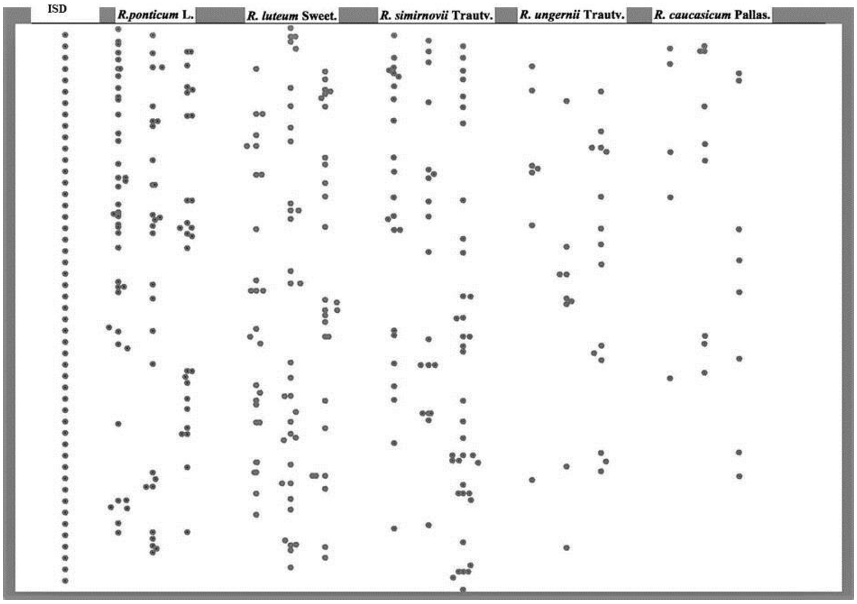

Figure 1. Seed emergence distributions in control method (sowing with manual spreading to the lines).
Şekil 1. Kontrol (Elle Sıraya Serpme Ekim) uygulaması tohum çıkış dağılımları.

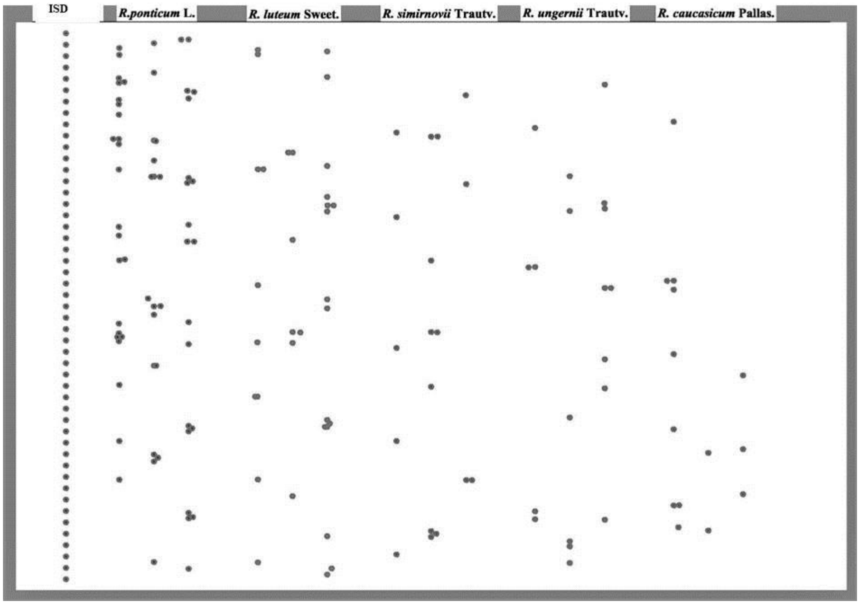

Figure 2. Distributions of seed emergence in the mixing with stream sand (banksand) method.
Şekil 2. Dere kumu ile karıştırılarak ekim uygulaması tohum çıkış dağılımları.

The closest seed emergence distribution in the MS group to the ideal distribution was determined in *R. luteum* species, and followed by *R. ponticum* and *R. simirnowii* species. The seed emergence distribution of *R. caucasicum* was quite far from the ideal distribution.

The seed emergence distribution of *R. ungernii* was determined as the least similar distribution to the ideal distribution (Figure 3). The best seed emergence distribution in the agar medium was found in the *R. luteum* variety, followed by the *R. ponticum*. The species far from the ideal distribution were determined as *R. caucasicum*, *R. ungernii* and *R. simirnowii* (Figure 4).

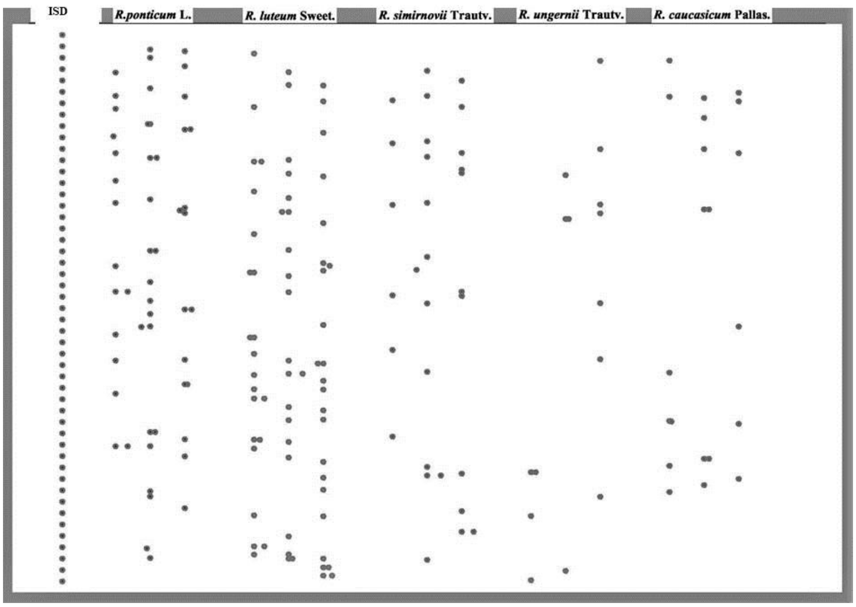

Figure 3. Distribution of seed emergence in the MS method.
Şekil 3. MS uygulaması tohum çıkış dağılımları.

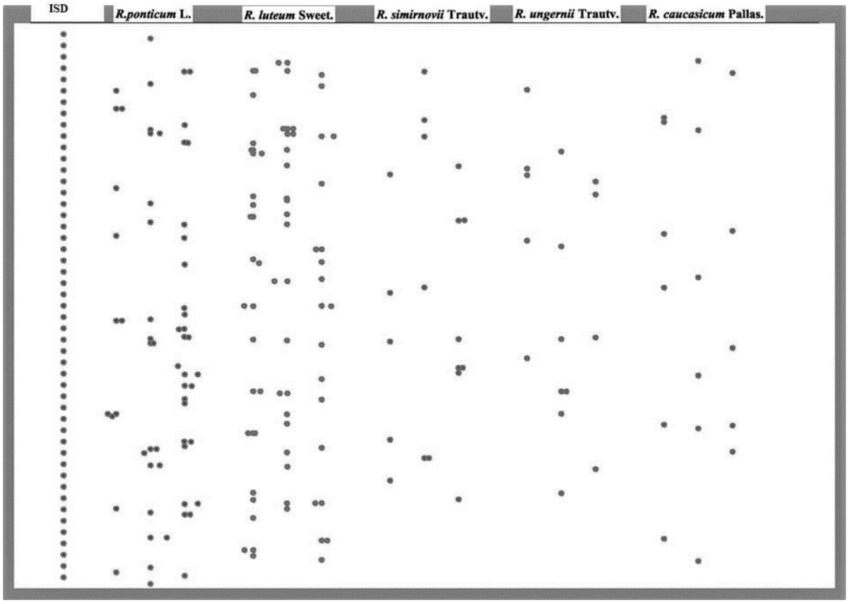

Figure 4. Distribution of seed emergence in the agar treatment.
Şekil 4. Agar ile karıştırılarak ekim uygulaması tohum çıkışlarının dağılımları.

In this study, a new technique, which is not available in the literature, was introduced to compare the seedling density. In this technique, the measured seedling distances were transferred into the AutoCAD software. The seedling density of the ideal distribution for each variety was compared with the AutoCAD images. The examination of the image indicated that the most density in seedling emergence occurred in the control method. The finding may be related directly to the amount of light received by the seeds. For example, some seeds mixing with river sand treatment probably could not receive sufficient light due to the cover of sand grains and therefore could not germinate. The seed emergences in plant agar and MS mediums were higher than the stream sand, but the seedlings were sparsely located and even there were some clusters of seedlings. Plant Agar and MS mediums were prepared hot; therefore, both have to be cooled for seed sowing. Cooling mediums, on the other hand, turn into a solid gel consistency, which may be broken down by mixing. The seeds were added into both media by thoroughly mixing before entirely solidify to obtain a homogeneous seed distribution. The viability of seeds may have been impaired in hot mediums used, and thus the seed emergence may have been influenced and caused sparse seed emergences. Clustering may indicate an incomplete mixture, or the mixture may have carelessly transferred into the germination medium and not homogenously mixed. Since seedlings covered a large part of the germination area, the seedling distribution pattern in the control application which had the highest number of seed emergence was credited as the closest sowing method to the ideal seed distribution.

3.3. *Effects of sowing methods on seedling height*

The effects of seed sowing methods on seedling heights of *R. ponticum*, *R. luteum* and *R. smirnowii* species were statistically very significant ($p<0.01$) (Table 3). In contrast, the effect of treatments on *R.ungernii* and *R. caucasicum* species' seedlings was not statistically significant ($p>0.05$).

Table 3. Effect of treatments on seedling heights
Çizelge 3. Uygulamaların fide boylarına etkisi

Species	Methods				SEM	P values
	Control	Sand	Agar	MS		
R. ponticum	1.02 b*	1.17 b	0.89 c	2.11 a	0.147	0.00
R. luteum	1.44 b*	1.48 b	1.49 b	3.08 a	0.213	0.00
R. smirnowii	1.07 b*	0.98 b	0.95 b	4.07 a	0.409	0.00
R.ungernii	1.00	0.62	0.93	1.39	0.120	0.14
R.caucasicum	1.08	0.80	1.69	1.43	0.141	0.10

*The difference between the averages in the same line and starting with the same letter is statistically insignificant according to Duncan Multiple Comparison Test ($P<0.05$).

The best seedling growth (mean seedling height of 2.11 cm) in *R. ponticum* species was obtained in seeds sown by mixing with MS medium. The mean height of seedlings obtained from seeds sown in control and stream sand treatments, included in the same statistical group, was measured as 1.02 and 1.17 cm, respectively. The lowest effect on the seedling length was recorded in the plant agar medium and the mean seedling height was 0.89 cm.

Similarly, the better seedling heights in *R. luteum* (Figure 5) and *R. smirnowii* species were obtained in MS medium with 3.08 cm and 4.07 cm, respectively. Control (1.44 cm), stream sand (1.48 cm), and plant agar (1.49 cm) methods were statistically in the same group. Similarly, all methods in the *R. smirnowii* species, except for the MS medium, were statistically in the same group. The plant heights obtained from *R. smirnowii* were 1.07, 0.98 and 0.95 cm for control, stream sand and plant agar methods, respectively.

Different seed sowing methods did not have a significant effect ($p>0.05$) on seedling *growth of R. ungernii* and *R. caucasicum* species (Table 3). The seedling height of *R. ungernii* ranged from 0.62 to 1.39 cm, while the seedling height of *R. caucasicum* was between 0.80 cm and 1.69 cm. However, the highest seedling (1.39 cm) in *R. ungernii* was obtained in the MS medium.

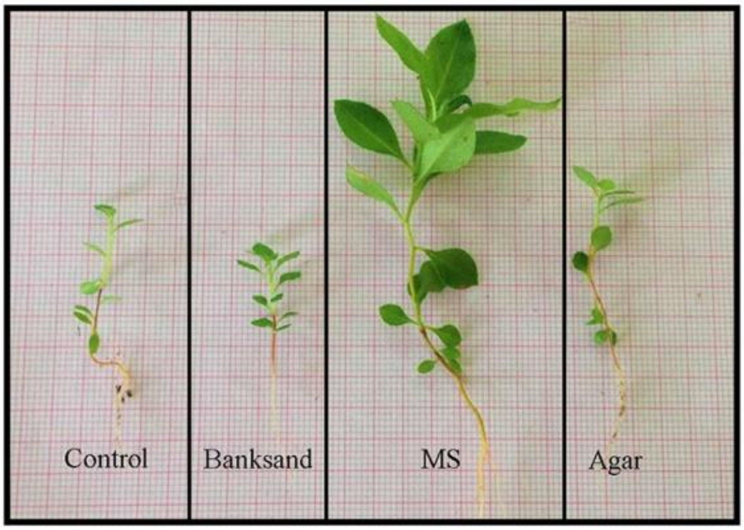

Figure 5. Effects of sowing mediums on plant heights of *R. luteum.*
Şekil 5. Ekim ortamlarının R. luteum'un fide boylarına etkisi.

The results revealed that different seed sowing methods did not affect the seedling growth of *R. ungernii* and *R. caucasicum*. Conversely, the best outcomes in *R. ponticum*, *R. luteum* and *R. simirnowii* species were obtained by applying MS medium. Investigating the effects of various physical and chemical factors and plant growth regulators to increase the uniform seed germination, Tiwari and Chauhan (2007) found that only a few among plant growth regulators and chemicals on seed germination were significant compared to the control. The maximum seed germination was reported in *R. maddenii* and *R. niveum* using 250 µM GA3 added MS medium. Parmar et al. (2015) investigated *in vitro* seed germination and seedling growth of *Coelogyne flaccida* Lindl., an orchid species. They reported that the MS medium enriched with 0.5 mg L^{-1} BAP and 0.5 mg L^{-1} NAA was optimal for the growth of the whole seedlings. The highest germination percentage (60.25 %) was obtained in garden soil + sand + barn manure (1:1:1) medium at a study conducted to reproduce Sand Lily (*Pancratium maritimum* L.) using seeds under in vitro and in vivo conditions (Kanmaz, 2013).

The findings on the very significant effect of mediums on the growth of *Rhododendron* seedlings are similar to those of mentioned above studies. Nutrient-enriched growing medias are ideal for the emergence and rapid growth of *Rhododendron* seedlings. Considering the prolonged growth of rhododendrons, the results of this study indicating the increase of two times larger seedlings than control, are very important in terms of cultivation.

4. Result

The data obtained in this study show that manual sowing was the best method to obtain a more homogeneous distribution between the interrow and intra row distances in the generative reproduction of *Rhododendron* species. However, sowing by mixing with the MS medium gave much better results for seedling growth than the classical method. It increased the seedling growth of all *Rhododendron* species investigated almost two folds compared to other applications. Accelerating the seedling growth is a significant benefit considering the *Rhododendron* species are very slow-growing plants. The present findings gave ideas to solve the problems experienced in the generative reproduction of *Rhododendron* species. Future studies to be conducted to determine the factors affecting the decrease in seed emergence in sowing to the MS media, the causes for the disruption of homogeneity in the distribution of seedlings, and the solutions to eliminate the stated deficiencies will make an essential contribution to the literature.

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