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BOTANY

GA_3 treatments on seed germination in *Rhodothamnus sessilifolius*, an endangered species in Turkey

Tratamientos GA_3 sobre la germinación de semillas en *Rhodothamnus sessilifolius*, una especie en peligro de extinción en Turquía

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ABSTRACT

Rhodothamnus sessilifolius is a species of the Ericaceae family endemic to the Artvin province in NE Turkey. This is in the critically endangered species list and current populations are decreasing, therefore, it requires protection and reproduction. Thus the present study aimed to investigate the effect of various gibberellic acid (GA_3) applications on germination of the *R. sessilifolius* seeds. The effects of various GA_3 concentrations on final germination percentage, mean germination time, germination value, and germination rate were analyzed in a growth cabinet. The study demonstrated that gibberellic acid application positively affected germination. Seed percentages of germination were 2.22 %, 18.89 %, 17.78 %, and 15.56 % in the control group for 100, 500 and 1000 ppm GA_3 concentrations, respectively. First germination was observed in seeds treated with GA_3 after 11 days. There were significant differences ($P < 0.05$) between all measured germination properties. These results are promising for future conservation strategies.

Keywords: Endemic species, germination rate, gibberellic acid, *Rhodothamnus sessilifolius*.

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RESUMEN

Rhodothamnus sessilifolius es una Ericaceae endémica de la provincia de Artvin en el noreste de Turquía. Esta especie se encuentra en la lista de especies en grave peligro de extinción y la población actual continúa disminuyendo, por lo tanto, requiere protección y reproducción. El presente estudio tuvo como objetivo investigar el efecto de varias aplicaciones de ácido giberélico (GA_3) sobre la germinación de las semillas de *Rhodothamnus sessilifolius*. En cabina de crecimiento se analizaron los efectos de varias concentraciones de GA_3 sobre el porcentaje de germinación final, el tiempo medio de germinación, el valor de germinación y la tasa de germinación. El estudio demostró que la aplicación de ácido giberélico afectó positivamente la germinación. Los porcentajes de germinación de la semilla fueron de 2,22 %, 18,89 %, 17,78 % y 15,56 % para el grupo de control y para concentraciones de 100, 500 y 1000 ppm de GA_3, respectivamente. La primera germinación se observó en semillas tratadas con GA_3 después de once días. Hubo diferencias significativas ($P < 0,05$) entre todas las propiedades de germinación medidas. Estos resultados son prometedores para futuras estrategias de conservación.

Palabras clave: Especie endémica, tasa de germinación, ácido giberélico, *Rhodothamnus sessilifolius*.

INTRODUCTION

Turkey hosts one of the richest flora in the world including around 11 707 Pteridophyta and Spermatophyta taxa and around 3649 endemic plant taxa (endemism rate of 31.82 %) (Güner *et al.* 2012). Considering that the continental flora in Europe includes 12 000 Pteridophyta and Spermatophyta and 2750 endemic species and the continent is about fifteen times the size of Turkey, the floral richness of Turkey is quite evident (Ekim *et al.* 2000, Güner *et al.* 2012). The unique flora and habitats in Turkey are under a major threat, especially during the last 30-40 years. It is known that the prevalence of several natural plant species and the abundance rates in these areas have been decreasing every day and certain species are already extinct (Özhatay *et al.* 2003, Ekim *et al.* 2000, Palabaş Uzun 2009). However, the extinction patterns of species have been completely different during recent years. Natural extinction rate with natural disasters, climate change, fungal diseases and insect pests is one or two species per year; however, the number of extinct species caused by the anthropogenic factors is estimated to be higher than the natural extinction (Çepel 2003, Doğan *et al.* 2010, Tsering *et al.* 2010). One of the endangered endemic species in Turkey is *Rhodothamnus sessilifolius* P.H.Davis, an Ericaceae.

R. sessilifolius, is an evergreen shrub of 20-25 cm [10cm] and is a very rare local endemic restricted to Artvin province in Turkey (Davis 1962, Stevens 1978, Terzioğlu and Milne 2002). It was determined that the plant is under critical threat of extinction (CR) based on the IUCN red list criteria. It is known to grow only at two locations in northeastern Anatolia, which are very close to each other. The current population seems to decrease even further. *R. sessilifolius* grows on moist banks and igneous cliff ledges. The main threats include human settlements, overgrazing and harvesting, trampling by goats and over-collection for scientific purposes (Tüfekçioğlu *et al.* 2004, Ekim *et al.* 2014).

In general, *in-situ* conservation is the most basic method of preservation of genetic resources, while *ex-situ* conservation plays a complementary role that ensures the success of the former method (Martin 1986, FAO 1992, Dirik 1994). To design an efficient conservation management, propagation of the species should be investigated. The first stage in plant propagation entails sowing the seeds and germination (Muhyaddin and Wiebe 1989). The determination of the seed germination rate would provide information about best propagation practices to conserve the species both within and outside its natural habitat (Assogbadjo *et al.* 2011). Several forest tree species and woody shrubs seeds do not germinate or germinate on time when they are not pre-treated even under optimum germination conditions. Such mechanisms should be eliminated before germination (Bonner and Vozzo 1987, Bewley and Black 1994, Tilki and Kambur 2010).

The seed germination is regulated by hormonal interactions. Gibberellic acid (GA_3) is an exogenous growth regulator that promotes germination by stimulating the activation of nutrient-mobilizing enzymes (Hartman and Kester 1983). Gibberellic acid plays two important roles during germination. It improves the growth potential of the embryo and eliminates the mechanical restrictions caused by the boll by weakening the tissues around the radicle (Ogawa *et al.* 2003).

The aim of the study was to investigate the effect of GA_3 applications on seed germination of *R. sessilifolius* to ensure the sustainability of the species and to produce quality individuals. Furthermore, final germination percentage, mean germination time, germination value and germination rate were determined.

MATERIAL AND METHODS

R. sessilifolius seed capsules were collected in Artvin province Murgul (2446 m) district during September 2019. The seed capsules were transferred to the laboratory where ripe seeds were manually broken, and the seeds were removed. Because the seeds are so small, before sowing, they were not selected for viability and only healthy seeds were selected (Pulatkan and Kamber 2019). Cold stratification pre-treatment was conducted at 2-4 °C in dark for one month until November 2020 before sowing. Also, seed weight (g) was measured for 8 x 100 = 800 seeds with a precision scale (0.0001 g) (ISTA 1993). Seed capsule width, seed and radicle length were measured with a microscope (Fig. 1).

Seeds were treated with GA_3 (0 (control),100, 500, 1000 ppm) for 24 hours and allowed to germinate in a growth chamber at 25°C in the dark for 28 days. Germination tests were conducted in petri dishes and each trial entailed a single Petri dish with 30 seeds placed on filter paper. Trials were conducted in three replicates (30 x 3). Filter paper was kept moist using distilled water and germinated seeds were removed and counted during each inspection. Seeds with longer than 1 mm radicle length were considered germinated and removed from the petri dish (Rossini-Oliva *et al.* 2009, Vera *et al.* 2010).

Four germination properties were determined: final germination percentage, mean germination time (Bewley and Black 1994), germination value (Djavanshir and Pourbeik

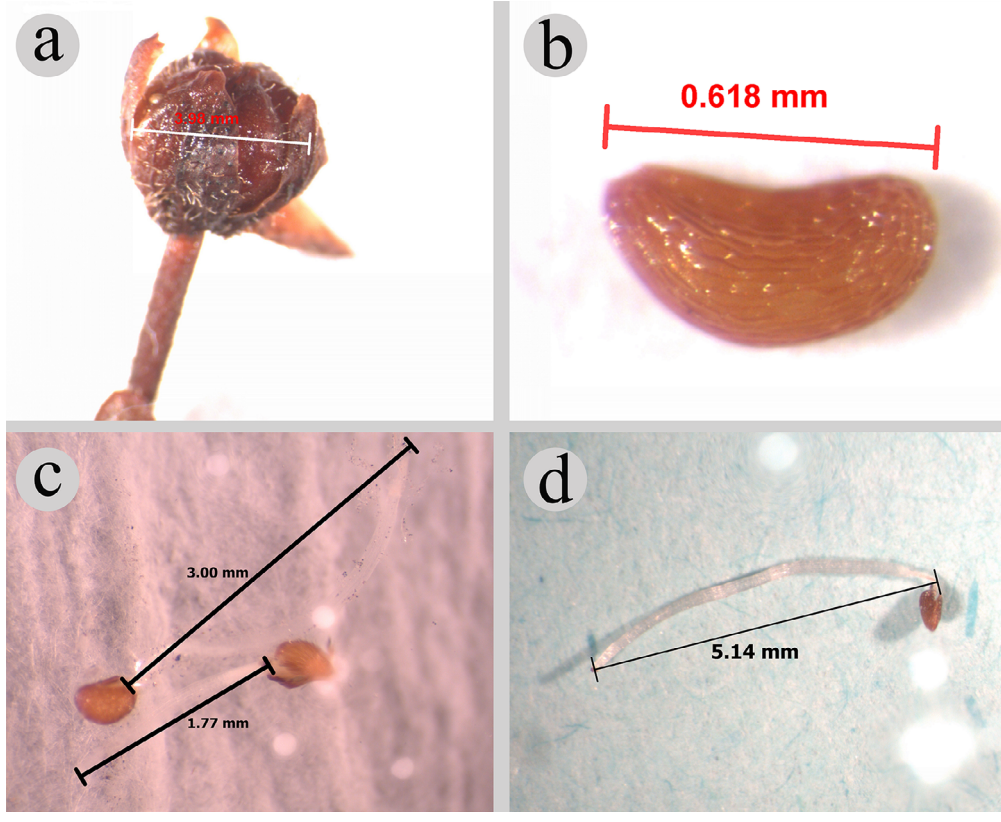

Figure 1. Images of *Rhodothamnus sessilifolius*. **a.** seed capsule, **b.** seed, **c-d.** endocarp germination characteristics.

1976) and germination rate (Saatçioğlu 1971). Lower mean germination time indicates faster germination (Vecino-Bueno *et al.* 2009).

Statistical analyses were conducted with SPSS 23 statistics software. Data were analyzed with one-way analysis of variance (F test). Significant test results were analyzed with the Duncan's test to determine the groups where GA_3 affected the germination.

RESULTS

Measurements demonstrated that the 1000-seed weight was a mean of 0.022 g. The first germination was observed 11 days after sowing. Germination percentage (%) was calculated based on the germination findings and analysis of variance was conducted. The analysis (Fig. 2) revealed a significant ($P < 0.05$) hormone germination percentage (F = 178.802).

The lowest germination percentage was observed in the control group, while the highest was observed in 100 ppm GA_3 treatment. Based on the Duncan's test results, the control treatment created the first group, 1000 ppm GA_3 treatment took place the second group, and the last group contained 100 and 500 ppm GA_3 treatments. Thus, a slight decrease was observed in the germination percentage with the increase in the GA_3 doses (Fig. 2).

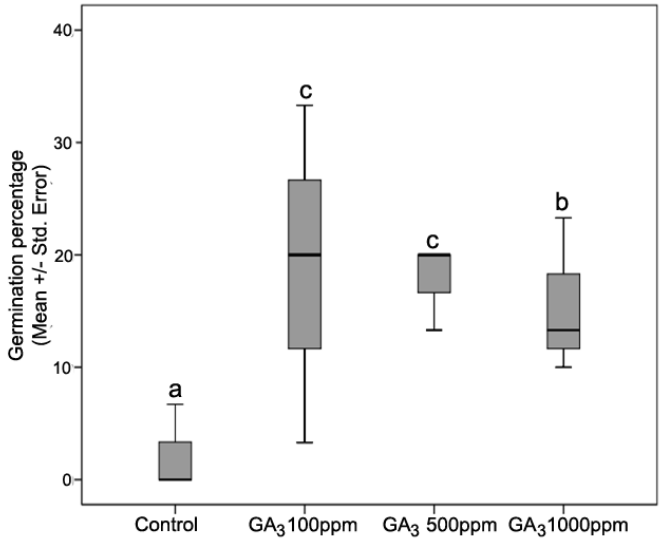

Figure 2. Germination percentages (%), and Duncan's test results pointed by letters above each box. Bars indicate mean, standard error and range per group.

Significant differences were obtained in terms of mean germination time (F: 82.751; $P < 0.01$) and germination value (F: 35.395; $P = 0.000$). In addition, Duncan's test results are presented in Table 1. The mean seed germination time of seeds treated with GA_3 was significantly low, while the highest value was observed in the control group. The longest mean germination time was observed in the control group. On the other hand, the germination rate was the lowest in the control treatment (Fig. 3) and the highest in 100 and 500 ppm GA_3 followed by 1000 ppm GA_3 application. The germination value followed the same pattern of germination percentage and germination rate.

Table 1. Effect of gibberellic acid (GA_3) concentration on germination value and mean germination time. Letters on each data column indicate differences according to Duncan's test ($P < 0.05$).

Treatments	Mean germination time (day)	Germination value
Control	28.00 b	0.05 a
GA_3 100 ppm	18.12 a	7.59 c
GA_3 500 ppm	16.63 a	7.04 c
GA_3 1000 ppm	18.00 a	5.17 b

DISCUSSION

Propagation with seeds is a viable method for the *ex-situ* conservation of *R. sessilifolius,* in this study which investigated the effects of different concentrations of GA_3 on seed germination; it was observed that the highest germination percentage occurred as 18.89 % in GA_3 100 ppm treatment. On the other hand, germination is only the first step in the reinforcement of species population. After germination, studies should be conducted for the adaptation of the species in the land after transplantation. Gibberellins are known to obviate seed requirements associated with various environmental cues, to promote germination, and to counteract the inhibitory effects of abscisic acid (Bewley and Black 1994). Treatments that include gibberellic acid significantly increase germination in *Astroloma xerophyllum* (DC.) Sond. and *Leucopogon polymorphus* Sond. but not in *Croninia kingiana* (F.Muell) J.M. Powell (Just c2018). In the present study, gibberellic acid application significantly increased germination.

Vera *et al.* (2010) reported that *Erica australis* L. seed germination was 100 % when the seeds were treated with

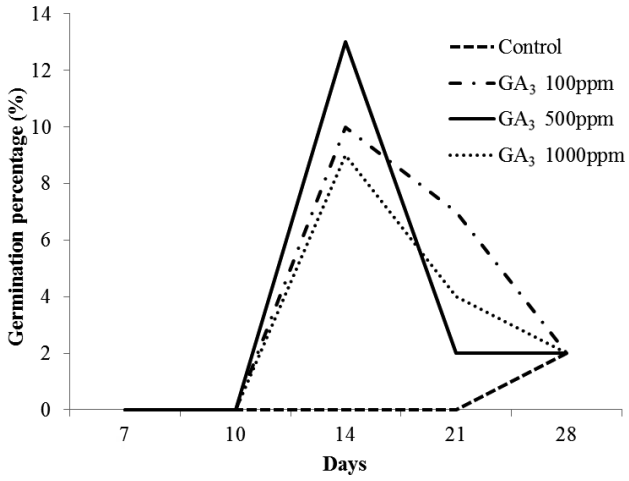

Figure 3. Effect of gibberellic acid (GA_3) concentration on *Rhodothamnus sessilifolius* germination rate along the days.

a minimal GA_3 concentration (25 ppm). The highest germination rate in *Erica andevalensis* Cabezudo & Rivera (41.6 %) was obtained with seeds treated with 400 ppm GA_3 (Rossini-Oliva *et al.* 2009). The present study findings show the highest germination percentage in *R. sessilifolius* 18.89 % in seeds treated with GA_3 (100 ppm) and decreased at 1000 ppm GA_3. In *Leucopogon melaleucoides* A.Cunn. ex DC, O'Brien and Johnston (2004) reported that the highest results were observed in treatment of 1000 ppm.

Cerabolini *et al.* (2004) investigated the effect of various GA_3 doses on the germination of the endemic species *Physoplexis comosa* (L.) Schur and *Primula glaucescens* Moretti. The germination percentage in *Physoplexis comosa* was extremely low without gibberellic acid application (1.7 %), the optimum GA_3 concentration was 100 mg l^{-1} (> 90 % germination), and the lower germination percentage was observed at higher doses (250–500 mg l^{-1}). Similarly, germination percentage was the highest with GA_3 (100 ppm) application. The maximum germination percentage in *Arbutus unedo* L. (85.75 %) was observed when the seeds were soaked in 500 ppm GA_3 for 24h (Smiris *et al.* 2006). In the present study, 500 ppm GA_3 promoted the germination percentage and the highest germination rate.

In *Erica andevalensis* the first germination was observed after seven days with 400 ppm GA_3, and after days with GA_3 200 ppm concentration. Control seeds germinated between 13 and 19 days (Rossini-Oliva *et al.* 2009). Sixteen weeks passed before the first seeds germinated in the control treatment of *Leptecophylla tameiameiae* (Cham. & Schltdl.) C.M.Weiller drupes (Baskin *et al.* 2005). In a study conducted by Romero-Saritama and Cueva-Ojeda (2020), germination started in 20 days in *Pernettya prostrata* (Cav.) DC. and ended on the 35 th day. In the present study, germination was observed in all GA_3 groups in 11 days, while only 2.22 % germination was observed in the control group at 24 days. Maximum germination was observed after only 24 days of incubation in *Astroloma xerophyllum* (Ericaceae) when the seeds were pretreated with GA_3 for 24 h (Turner *et al.* 2009). In contrast, in this study the maximum germination was obtained after only fourteen days.

According to Martin (1946), Ericaceae seeds are very small, dwarf-seed type, and certain genera of this family produce very small undifferentiated embryos, which lead to morphological (Baskin and Baskin 1998) and physiological dormancy (Aparicio 1995, Rossini-Oliva *et al.* 2009). In *R. sessilifolius*, dormancy is broken by cold-stratification and gibberellins.

In conclusion, germination was highest for seeds treated with GA_3. In other studies, germination in seeds treated with GA_3 was also high; thus, germination can be increased by applying different concentrations of GA_3 to the seeds. The present study demonstrated that *ex-situ* propagation of *R. sessilifolius* was possible with seeds and phytohormones were required for successful germination. This species could be propagated with this method and employed in landscape architecture. In addition, it is important to ensure the continuity of the species due to the low number of individuals in the area and the difficulty of collecting seeds (spreading in high rocky areas).

AUTHOR´S CONTRIBUTION

NY and MP original idea, study design, statistical analyses, ınterpret the data and paper writing, GEO field measurement and paper writing.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

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